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Preface

Volume XX of our magazine *Mycologia Montenegrina* is dedicated to the 25th anniversary of vital taxonomy. In 1992, Hans-Otto Baral published a critical study in the journal *Mycotaxon* about the current methods of doing microscopic preparations of mushrooms. By emphasizing this date, we want to remind you of the exceptional importance of methodological practice which, although it has deep roots (see Introduction), came to a broad attention and gained its full meaning only through the aforementioned work. On the other hand, with the articles that we have gathered in this volume and which have been performed according to the propositions of this method, we would like to contribute to its strengthening and expanding. Vital taxonomy is understood as a set of taxonomic methods focused on microscopic processing of all groups of Ascomycota but also Basidiomycota and other organisms with hyaline structures, relating to all of their taxonomically important organs. It is based on experiences of extensive research on living specimens that are often supplemented with the data of dead, usually herbarium specimens, and it implies careful distinction and comparison of living and dead cells when studying fresh or recently collected material. The application of this method brings a more complete and more realistic picture of the explored object and proved superior to traditional herbarium taxonomy. "*In vivo veritas*" is the formula for this method, which has the strength and value of axioms. It performs deepest anthropological experiences in which gnoseological, ontological, ethical, aesthetic and other, particularly ontogenetical and ecological aspects are closely intertwined in the knowledge and description of a new living entity. The fact that it is only a living awareness of the truth, and that the truth lives in the living, reveals this correlation as complementary. Many mycologists from many countries show interest in and respect of practicing vital taxonomy. However, the number of those who are consistently adhering to it is still not satisfactory, especially if we have in mind its advantages over the practice of herbarium taxonomy. They are all, more or less, in close communication with the author of vital taxonomy from whom they receive help and support. In their works we meet high-quality illustrations, whether drawings or photo plates produced during processing collections, being done with high exactness and precision. Together with the style of describing cell contents and separating measurements of dead shrunken cells from the living, it permits to recognize the powerful influence of the author of vital taxonomy, whose creative opus is recognizable to many today, and especially to the younger generation of mycologists, as a guide and paradigm.

Branislav Perić
for Editorial board

Introduction

It is now 27 years ago that I published my comparative study on the current microscopical preparation methods of fungi, generally referred to as 'herbarium taxonomy', and the strongly favoured 'vital taxonomy' (Baral 1992). The knowledge about vital taxonomy, which implies careful distinction between living and dead cells, was not newly introduced in 1992, it was only rediscovered. In fact, this virtually simple method was already practiced about 130–160 years ago when Pringsheim (1858) and De Bary (1887) reported excessive shrinkage of asci and inflation (imbibition) of the ascus wall in Pleosporales and Helotiales, either during forcible spore discharge or when asci die due to preparation pressure. Boudier (1879, 1886, 1914), followed by Lagarde (1906), emphasized the importance of microscopic study of the fresh living fungus, which Boudier consistently practiced in his papers, particularly in his well-known Icones mycologicae (Boudier 1904–1910). Relating to the opening mechanism of asci he wrote in 1879: "It is only by examining the species in a fresh state that any perfection can be attained in a study so difficult as the classification of *Pezizae*. In the dry state these observations are very difficult and often impossible, in consequence of the contracted condition in which the asci are found.", and in 1914 he stated about ascospore contents: "*les descriptions faites sur des échantillons desséchés ou conservés dans l'alcool seront toujours ou risqueront d'être fautives ou incomplètes*".

Some of the later workers also favoured this method. One of them was Velenovský who stated (1940: 16) that "*Die Diagnose der Pilze sollte immer nur nach dem frischen Materiale aufgestellt werden*", not just because of the macroscopical colour change, but also relating to changes in shape and content of the spores. However, in his monograph on Bohemian discomycetes, Velenovský's (1934) drawings reveal that he was a bit careless in distinguishing living and dead cells. In the family *Orbiliaceae*, for instance, he only rarely depicted the spore bodies in the ascospores, and almost never spores inside the asci, which both suggest that in this group he mainly studied dead asci and spores.

As a matter of fact, the low contrast between ascoplasm and ascospores is responsible for the difficulty in seeing spores inside dead asci of small-spored inoperculate discomycetes (see my powerpoint presentation, Baral 2008). Therefore, apothecia containing numerous mature asci have not rarely been considered as immature because the asci seemed to be empty. *Orbiliaceae* are a suitable group, though by far not the only one, to demonstrate that the study of herbarium material alone resulted in the past in a high rate of confusion among the taxa and in a much lower number of recognized species. After we have applied molecular methods to this group, our species concepts were confirmed in many cases, but in some the conventional microscopical approach failed, and the split of taxa without clear morphological traits became necessary.

Another mycologist favouring observation of living cells was Le Gal (1947: 78) who wrote in her thesis on spore ornamentation development in Pezizales that "*seules les observations vitales pouvaient nous donner des résultats satisfaisants*". On the other hand, in her taxonomic study on *Sarcoscypha* (Le Gal 1941) she did not distinguish between living and dead ascospores by overlooking the artificial confluence of oil drops. Therefore, she could not confirm the constant

lipid pattern reported by Boudier (1904–10) as distinctive for *S. coccinea* var. *jurana* on *Tilia*. As a consequence, Le Gal concluded that this variety has no taxonomic relevance.

Despite this historic knowledge about the severe morphological changes that living cells undergo when they are killed with lethal mountants or mechanical pressure during preparation, the methods of herbarium taxonomy, which involve the use of potassium hydroxide (KOH) for swelling the tissues and Melzer's reagent for clearing the cell contents, continue to be practiced, particularly by academic workers.

When adopting the terms *vital taxonomy* and *vital state,* I was aware that the word "vital" may have different meanings within a language. These terms conform with the term *vital staining* (staining in statu vivo, in German *Vitalfärbung*), a method based on the application of basic dyes such as Cresyl blue for staining vacuoles and proving cell viability.

It is a common mistake to believe that the study of a fresh fungus under the microscope necessarily results in the observation of living cells. Fungal cells are highly sensitive to chemicals as well as mechanical influence. Therefore, cautious methods are necessary, which include tap water as mounting medium, no or only slight pressure on the cover slip, and a preference for free-hand sections with a razor blade whenever possible.

On the other hand, the sexual morphs of many species, including their meisporangia (asci or basidia) which are their most sensitive organs, tolerate complete drying in the herbarium for several months or even years. This means that, depending on the group of fungi, a vital taxonomist sometimes works predominantly with herbarium specimens which have more or less recently been collected and were already dry in the field. In this case, application of the term "fresh" makes little sense, since the dry fungus can repeatedly be revived and returned to the dormant state, i.e., rehydrated and dehydrated. The current argument against vital taxonomy, lack of time and facilities when a collection is made, does not apply to such desiccation-tolerant fungi.

One of many examples for the importance of vital taxonomy is the genus *Crocicreas*, which was considered for a long time to be the correct name for members of *Cyathicula* and some of *Allophylaria*. This synonymy was doubted by me (in Baral & Krieglsteiner 1985) based mainly on the *Calycina*-like apical ring structure, the absence of crystals, and lanceolate protruding paraphyses in *Crocicreas*. For a long time the type species of *Crocicreas* was only known in the dead state, but a recent Russian collection documented by N.V. Filippova (pers. comm.) revealed that it sharply differs in lacking refractive vacuolar bodies (VBs) inside the paraphyses, in contrast to the multiguttulate VBs in all typical *Cyathicula* spp. This critical additional difference was later supported by a high distance in rDNA obtained by D. Haelewaters (pers. comm.), resulting in placement of *Crocicreas* and *Cyathicula* in different families (Baral et al. 2015, Johnston et al. 2019).

Numerous workers have adopted the practice of vital taxonomy when doing descriptions and documentations by drawings or digital photography. For example, Dougoud (2013) in his amazing and very helpful guide to the study of discomycetes "limited himself to describing the elements of microscopy, the microchemic reactions and stainings, from live fungi (Baral 1992)". Also Quijada (2015) in his not publicly available thesis gave a detailed illustrated account on the "importancia de la taxonomía vital" in his study of the Helotiales and Orbiliales, and

likewise Kušan (2015) in her thesis on different Helotiales and Pezizales, in collaboration with N. Matočec, questioned the "traditional laboratory methods in mycology" as "insufficiently informative because a large number of important taxonomic characters are irretrievably lost or changed during the material conservation".

Nevertheless, herbarium studies, which often involve the uncritical presentation of both living and dead elements on a photo plate, prevail in international research practice till now. Despite of such frequent use of herbarium specimens, monographic studies on a group of phylogenetically related species, for which the dry specimens and exsiccatae are held in herbaria, became nowadays comparatively rare, a fact regretted already by Korf (1994: 16) when stating that the increasing application of modern techniques like transmission (TEM) and scanning electron microscopy (SEM), molecular analysis of DNA, numerical taxonomy, chemistry, and genetic mating systems has "decreased rather than increased the production of monographs". Regrettably, the increasing application of molecular methods has provoked a disinterest to young mycologists in the study and interpretation of morphology, resulting in often poor quality documentation. Instead of monographs, large papers became trendy which present accidental collections from various groups of fungi thanks to the today's ease of rapid electronic editing and publishing. Although recently collected, they often present some or all elements in the dead state.

A broad knowledge and experience about the aspect of living fungal cells results in a better understanding of the biological function of microstructures and avoids erroneous conclusions and hypotheses, including misinterpretation of spore maturity. It also improves the skills when studying herbarium specimens. The higher number of available characters in vital taxonomic studies permits better delimitation of taxa and interpretation of phylogenetic results. Last not least, vital taxonomy saves time, a common though rarely mentioned experience, expressed by Svrček (1976: 116) in his revision of Velenovský's type specimens of Pezizales: "In fact, the study of dried specimens as such is much more difficult and more time-consuming than work with fresh material."

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