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Commentary:

Epitypification: should we epitypify?

Kevin D. HYDE[†1,2], Ying ZHANG[3]

([1]*International Fungal Research and Development Centre, the Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming 650224, China*)

([2]*Fungal Research Group, School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand*)

([3]*Centre for Research in Fungal Diversity, School of Biological Sciences, University of Hong Kong, Hong Kong, China*)

[†]E-mail: kdhyde1@gmail.com

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Abstract: Epitypification can solve many taxonomic problems and stabilize the understanding of species, genera, families or orders. The aim of this paper is to illustrate how to epitypify. A few examples where taxa have been epitypified are considered and the benefits and disadvantages of epitypification are discussed. We also outline some examples of taxa which need to be epitypified with reasons.

Key words: *Colletotrichum*, GenBank, Molecular phylogeny, Taxonomy, Typification
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INTRODUCTION

What can be done if the material that represents a type species, genus, family or order is lost, is in poor condition, cannot be used to extract DNA, or molecular data cannot be obtained? How do we form concepts of species, genera, families and orders in these cases? Recent publications from the Assembling the Fungal Tree of Life (AFTOL) project have named exemplar genera to conceptualize orders (Hibbett *et al*., 2007). However, the understanding of the genera is included, and thus the understanding of the order, may be interpreted differently by various mycologists, and therefore this approach cannot be recommended. In the case of genera, species descriptions, but not types, have often been used to represent genera (Kodsueb *et al*., 2006). Stylized drawings and wrongly identified collections, however, might mean that the generic concept is understood differently by various mycologists. Fresh isolates are also generally used to obtain DNA data, but one person's understanding of a species may differ from another's; thus the sequences in GenBank and other public databases are surely unreliable (Nilsson *et al*., 2006). This paper reviews the options that can be used to address the above problems. Epitypification is the best option for replacing lost type material or types in poor condition. If DNA cannot be extracted from types, then we need to designate epitypes that are identical to the examined types. When fresh collections are sequenced and those sequences are deposited in GenBank, dried herbarium material and cultures should also be deposited in accessible herbaria culture collections. If these rules are followed then we will be able to avoid GenBank becoming a dustbin for DNA sequences whose names cannot be verified by morphology.

WHAT IS EPITYPIFICATION?

Article 9.7 of the *International Code of Botanical Nomenclature (Vienna Code)* (Mcneill *et al*., 2006) states that "An epitype is a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise

application of the name of a taxon. When an epitype is designated, the holotype, lectotype, or neotype that the epitype supports must be explicitly cited." It has become relatively common to epitypify fungi (Alves *et al*., 2008; Crous *et al*., 2007; Phillips *et al*., 2006; 2007; Shenoy *et al*., 2007b; Than *et al*., 2008) and this paper will examine whether it is a good idea to epitypify fungi and will also point out some of the associated problems.

There are many reasons to epitypify. Often the type material has been lost or is in poor condition. The genus may be relatively well known, e.g., *Massaria inquinans* (Tode) De Not.; however, unless there is a type that can serve to represent the genus, it is possible that each individual mycologist may have a different understanding of the taxon representing this genus. More recently with the advent of molecular phylogenetics, living material is needed for sequencing as in most cases the ancient herbarium specimens cannot be successfully sequenced. Therefore, even if the type material is in relatively good condition, one could argue that the taxon needs epitypifying, so that living material is available for gene research. This is evident in the paper by Hibbett *et al*.(2007). In that paper, each order was represented by 1~6 exemplar genera. The understanding of each genus, however, may differ. Many mycologists may not have seen good type material and their understanding of a taxon/genus/family may be based on literature or stylized drawings of the type or representative (possibly misidentified) collections. The individual understanding of an order may, therefore, be questionable. The AFTOL project has sequenced numerous taxa and derived a framework for modern taxonomy (Aime *et al*., 2006; Binder and Hibbett, 2006; Celio *et al*., 2006; Geiser *et al*., 2006), yet few of the fungi sequenced were types. This could lead to erroneous data being generated and since strains may not have been deposited in culture collections and herbarium material is scarcely available, thus there is no way to repeat the experiment (Shenoy *et al*., 2007a).

HOW TO DECIDE ON AN EPITYPE

According to the definition of an epitype (Mcneill *et al*., 2006), the epitype should be identical to previous type material. Hence before epitypifying a species, the type material should be located, loaned and carefully studied. This should include detailed examination of morphological characters and other data such as host, location, or anamorphic relationship. The next step is to obtain a fresh specimen. The epitype specimen should undoubtedly have all of the characters exhibited in the type and if possible be obtained from the same location and host. Of course this is not always possible. We have recently searched for a fresh collection of *Colletotrichum circinans* (Berk.) Vogl., the cause of smudge in onion (Walker, 1925); however, the original site is now a housing estate. We have also searched for the type of *Colletotrichum falcatum* Went which was described from a small village in central Java (Went, 1893). Amazingly the sugarcane plantation is in its original location (although part has been destroyed for housing) and during a recent trip we were able to obtain a fresh collection.

DNA data obtained from different strains isolated from different locations or environments are often identical. For example, the internal transcribed spacer (ITS) sequences of two isolates of *Botryosphaeria cortices* (Demaree & M.S. Wilcox) Arx & E. Müll. from North Carolina showed no significant difference from those isolated from New Jersey from the same host (Phillips *et al*., 2006). The rDNA (28S, 18S) and RNA polymerase II (RPB2) sequences of *Trematosphaeria pertusa* Fuckel on a dead stump of *Fraxinus excelsior* L. from Deux Sèvres, France, were almost the same with those of the one from Haute Garonne (France) occurring on submerged wood of *Platanus* (Zhang *et al*., 2008). Thus as long as the collections are morphologically identical to the type, a fresh collection from a different location could feasibly be designated as an epitype.

It has recently proven possible to extract DNA and obtain the DNA sequences from old herbarium material with kits (Fredricks *et al*., 2005; O′Gorman *et al*., 2008). This material may not be the type, but can be collections identified by the same mycologist who designated the type, or collections by others from the same host and location. This DNA data is very important to determine the phylogenic status of a particular fungus, especially for those rarely encountered taxa. Furthermore, if it was collected by the original mycologist it is likely to be authentic. As long

as enough DNA data can be obtained from this old material which should be identical to the type, it could also be designated as an epitype.

EXAMPLES OF EPITYPIFICATION

By epitypification, a name can be fixed to a specimen or a culture, which is very important for phylogenetic study of this taxon. A fresh collection of *Botryosphaeria corticis* was made from a commercial field of *Vaccinium corymbosum* L. in the USA and a culture was obtained. Epitypification of *Botryosphaeria corticis* with this specimen was carried out by designating it as the epitype as none of the previous cultures was connected with the type specimen (BPI 598729) (Phillips *et al*., 2006). Because of this, the name of the fungus causing an important disease of blueberry (cane canker) has been stabilized. *Collectotrichum capsici* (Syd.) Butler & Bisby is an economically important taxon that causes anthracnose in chilli. However, molecular data could not be obtained from the type specimen. The authors therefore obtained a fresh collection from chilli in Coimbatore in India and designated this as the epitype of *C. capsici*. More importantly they obtained living cultures and deposited these in public culture collections (as ex-epitype living cultures). The phylogenetic status of this taxon has thus been stabilized and cultures are freely available for future work in this important pathogen and pathogenic genus (Shenoy *et al*., 2007b). Similarly, the phylogenetic characters of *Mycosphaerella* and *Teratosphaeria* have also been clarified by designating the epitypes of the type species of these two genera (Verkley *et al*., 2004; Crous *et al*., 2007).

Some specimens, especially old types, can be very difficult to locate or obtain. For example, the neo-type specimen of *Trematosphaeria pertusa* (type species of *Trematosphaeria*) is kept in Persoon's *exsiccata* in the University of Leiden (L). According to the rule of the herbarium, this specimen could only be studied in the herbarium, and it is very difficult to obtain funding for such. Thus this has been poorly studied (Boise, 1985), and limited morphological characters for this taxon can be found in the protologue. With such a situation, designation of an epitype would be beneficial.

Sometimes, the traditional concept for a species differs from that observed in the type protologue and early descriptions. This is the case with *Massaria inquinans* (Tode) De Not., the type species of *Massaria*, which represents the family Massariaceae. We have been unable to locate the type material of this species. Shoemaker and LeClair (1975) systematically studied the Massariaceae and examined a specimen of *Massaria inquinans* (CLXXX) lodged at University of Uppsala (UPS). Shoemaker and Kokko (1977) reported this taxon to have asci with 8 ascospores with no-constriction at each septum. We have examined the specimen of UPS CLXXX and found it to have asci with 4 ascospores that are constricted at the central septum. This species therefore needs epitypification so that all mycologists have the same basic understanding of this taxon, genus and family (Massariaceae).

We are studying the type specimens of genera of the order Pleosporales and have found that about 20%~30% of herbarium specimens are in bad condition, and little morphological data could be obtained from these types. For example, the type specimen of *Teichospora trabicola* Fuckel (G00110113), syntype of *Metacoleroa dickiei* (Berk. & Br.) Petr. (K(M) 143928), the type specimen of *Leptosphaeria michotii* (Westend.) Sacc. (Belgium, 89509-75) (as *Sphaeria michotii* Westend.), the holotype specimen of *Pleospora scirpicola* (DC.) P. Karst. (as *Sphaeria scirpicola* DC.) (G00110110) are all in poor condition. Even when specimens are in good condition, it is not always possible to evaluate some valuable characters, such as ascospore sheaths, type of ascus dehiscence, and these are characters that might be informative in classification (Eriksson, 1982; Hawksworth, 1994). Therefore fresh material should be located and detailed descriptions of these taxa should be published (after comparison with the type) and designated as epitypes which should include living material for future study.

POSSIBLE PROBLEMS ASSOCIATED WITH EPITYPIFICATION

It is very important that an epitype is identical to the original type; however, since the type may be lost, or in poor condition, it is not always possible to verify

this. Therefore subjective judgement has to be used to decide on a specimen that can be designated as an epitype. Thus, although there may be a chance of error, designating an epitype is a better approach to move forward, than having many interpretations of what characters of the type species of a genus, family or order may comprise. There is a chance that the type may be newly located at a later date; however, this is unlikely. If this did happen, the original type (if in good condition, freely available and DNA can be extracted and sequenced) should represent the species. It is very unlikely, however, that an isolate of the type could be located at a later date.

Colletotrichum acutatum Simmonds is an important pathogen that was introduced by Simmonds (1965) without a type, and validated in 1968 with a broad concept. This was demonstrated by the selection of several type specimens from a range of hosts (Simmonds, 1968). Than *et al*.(2008) considered that Simmonds (1965; 1968)'s broad concept of *C. acutatum* has created uncertainties in the species concept. No viable ex-type cultures of *C. acutatum* were located and further there were no viable cultures of this taxon on Papaya from the type locality. Than *et al*. (2008) therefore designated a specimen of living culture stored at Queensland Department of Primary Industries and Fisheries, Plant Pathology Herbarium, Indooroopilly, Australia (BRIP 28519), as an epitype from Papaya (same host as type) from Yandina (nearby location). Subsequent to this publication, it has been discovered that a culture of *C. acutatum* had been sent by Simmonds to Dingley in New Zealand (and then to ATCC 56816) (Guerber and Correll, 2001). This culture, however, was not derived ex-holotype. The culture Simmonds sent to Dingley is given as culture No. 16633D [p.225 in (Guerber and Correll, 2001)]. This culture was also sent by Simmonds to Herbarium of CABI Bioscience UK Centre (IMI) where it was assigned to IMI 117620 and is a "pink type" [Table 1 in (Than *et al*., 2008)]. The holotype (IMI 117617) was derived from Simmonds culture No. 16741B1, which is a different isolate. ATCC 56816 is therefore an ex-paratype culture of *C. acutatum*.

The designation of an epitype by Than *et al*. (2008) sought to stabilize the status of *C. acutatum*; however, since an ex-paratype culture has been found, this creates an interesting problem. The ex-paratype is poorly characterised and is unavailable to researchers unless the high costs of buying these cultures from American Type Culture Collection (ATCC) are paid. The ex-epitype is well characterised, is freely available (in six public culture collections) and has been sequenced using several genes. We would therefore recommend that the epitype is used to represent this species as it was derived from the same host in the same area and has identical morphology to the type. Others may disagree and thus attempt by Than *et al*.(2008) to stabilize the species may not have worked.

Interestingly, the story does not stop here! *Colletotrichum acutatum* isolates cluster into groups based on restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA), RFLPs of the 900-bp glutamine synthase (GS) intron and sequence analysis of the glutaraldehyde-3-phosphate dehydrogenase (G3PD) intron 2, and the GS intron 2 gene (Guerber *et al*., 2003; MacKenzie *et al*., 2008; Peres *et al*., 2008). These groups might represent phylogenetically distinct species of *C. acutatum sensu lato* (Guerber *et al*., 2003). It will be interesting to establish which group(s) the ex-paratype with pink cultures and the epitype with orange to grey cultures cluster in. There will no doubt be more written on this subject.

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