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Hosts, species and genotypes: opinions versus data

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Abstract. We are currently in the middle of a revolution in fungal taxonomy. Taxonomy is at the crossroads, where phenotypic data must be merged with DNA and other data to facilitate accurate identifications. These data, linked to open access journals and databases, will facilitate the stability of nomenclature in the future. To achieve this, however, plant pathologists must embrace new technologies, and implement these policies in their research programmes.

Additional keywords: Armillaria, *Botryosphaeria*, *Cercospora*, *Cylindrocarpon*, *Cylindrocladium*, *Fusarium*, *Heterobasidium*, MycoBank, *Mycosphaerella*, *Ophiostoma*, *Phaeoacremonium*, *Phomopsis*, *Phytophthora*, *Pyrenophora*, species concepts.

Introduction

Many recent plant pathology meetings have been focused on themes incorporating elements such as ‘back to basics’, ‘meaningful’ or ‘practical’. What this means is that for a large part, the plant pathological community remains in step with its mission, namely to reduce plant disease, feed the masses, and enhance export of produce. An important aspect of global trade and free market access is that importing countries make themselves vulnerable to receiving a range of new and potentially devastating plant pathogenic fungi. Many pathogens can be hidden as endophytes, or as latent pathogens in apparently healthy tissue. A further problem, one that I want to discuss in more detail, is that most of these pathogens are in fact species complexes, and that detection based on morphology, devoid of data other than the visual phenotype, is rather worthless for quarantine purposes. Plant materials frequently harbour well-known pathogens that already occur in the importing regions or countries, but that may represent new genetic diversity or newly introduced mating types, which could be as devastating as the introduction of a completely new pathogen. We are presently living in a global village, where agricultural produce from one country can be served in households in another within days of harvest. Global trade is inextricably linked to the future, and plant pathologists will have to develop new tools to deal with this challenge. Currently, the occurrence of fungi in imported plant materials can be the basis for recommending rejection of shipments, a process that depends on the name linked to the organism. Given the current complexity which I am about to discuss, it may be prudent to rethink this system, and to base decisions on DNA in future, rather than on concepts, or on names without data, as has largely been the case in the past.

In the latest estimate of the number of fungal species that are thought to exist, Hawksworth (2004) retains his opinion that it is around 1.5 million taxa. Furthermore, he states that the current estimate of the number of plant species in existence, 270 000, appears to be too low. This number strongly influences the estimated number of fungal species, so that as the former number rises, so must the latter at a ratio of not less than 5.5 fungal species per plant species. The conclusion from these estimates is that we currently know around 7% of the fungi expected to be in existence. The apparent low number of known fungi is further corroborated by the finding of Suh *et al*. (2005), who have reported

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over 200 new species of yeasts from the gut of a variety of beetles.

One consideration playing a central role in this debate is our definition of species. In brief, what one scientist sees as populations of a single taxon may be accepted by another as different species. Based on a combination of DNA sequence data and mating compatibility, O'Donnell *et al.* (2004) described eight new species within the *Fusarium graminearum* species complex. Using a similar approach, Crous *et al*. (2004*d*) described eight new species within the *Cylindrocladium floridanum* species complex. Where do we draw the line, and how do we define species? These questions are not easy to answer, but they definitively influence estimates of species numbers, and, in practice, our decisions and actions pertaining to quarantine and trade issues.

Discussion

Show me a plant pathogen, and I will show you a species complex

In most cases (especially in developing countries), control strategies and fungicides are recommended to farmers based on disease symptoms observed in the field that are typical for specific diseases. However, we now have a range of molecular techniques that suggest that most, if not all, pathogens represent several closely related sibling or cryptic species.

The genus *Mycosphaerella* (including its anamorphs) contains several thousand species (Corlett 1991; Crous and Braun 2003), which are usually assumed to be host specific. Although recent molecular data have indicated that some species exist that have wider host ranges (Crous *et al*. 2004*c*), this is generally the exception and not the rule. A crop that has been studied rather intensively these past few years is *Eucalyptus*. Eucalypt trees are commonly cultivated via cuttings for the paper and pulp industry. Most of these species are native to Australia and Papua New Guinea. The genus *Eucalyptus* includes 700-odd species, and has in excess of 60 species of *Mycosphaerella* associated with leaf spots and cankers on one or more of these species (Crous *et al*. 2004*b*). What is especially intriguing is that many of the *Mycosphaerella* species have travelled to other continents with their hosts, probably carried as endophytes in asymptomatic material (Crous 1998; Verkley *et al*. 2004). *Mycosphaerella suberosa*, for instance, was first found causing disease in South America (Crous *et al*. 1993), and only later in Indonesia (Crous and Wingfield 1997) and Australia (Carnegie *et al*. 1997), and finally in New Zealand (Dick and Dobbie 2001). Various eucalypt species have in the past been transplanted to other continents, and the *Mycosphaerella* populations that were taken along seem to have genetically diverged within this time. In some cases, these populations have diverged enough to be recognised as distinct species.

Mycosphaerella heimii was first described from Madagascar in 1946 (Bouriquet 1946). *Eucalyptus* plantations were established on Madagascar with seed and cuttings introduced from Indonesia. Not surprisingly, therefore, later collections found this pathogen also to be well represented in Indonesia (Crous 1998). *Mycosphaerella heimii* is morphologically similar to two other closely related species, namely *M. heimioides*, which also occurs in Indonesia, and *M. irregulariramosa*, which occurs in South Africa (Crous 1998). Moreover, a multigene phylogeny of isolates collected on *Eucalyptus grandis* and *E. globulus* in Brazil and Hawaii suggests that the *M. heimii* complex has radiated widely with this crop, and that isolated populations are currently evolving into separate sibling species that are still morphologically similar, but genetically distinguishable.

The genus *Botryosphaeria* is commonly associated with stem cankers, leaf spots and fruit rots of many hosts. Species in this genus are notoriously difficult to identify, and until recently, most taxa were simply referred to as representative of the *B. dothidea*/*ribis* complex. In recollecting these species, and by employing a multigene phylogeny, Slippers *et al*. (2004*a*) were able to distinguish *B. ribis* and *B. dothidea*, as well as the closely related *B. parva* and *B. lutea*. Based on similarities within the ITS ribosomal gene, it was initially suspected that *B. lutea* was the dominant pathogen of numerous hosts in the Southern Hemisphere. However, a multi-gene phylogeny revealed that isolates obtained from *Acacia* in Australia were in fact representative of a new species, *B. australis* (Slippers *et al*. 2004*c*). Further collections and research also revealed that five species occur on eucalypts, with *B. eucalyptorum* and *B. eucalypticola* being common in Western Australia, from whence they have probably been introduced to South Africa (Slippers *et al*. 2004*b*). While investigating the taxa associated with disease on grapevines in South Africa, Van Niekerk *et al*. (2004) also reported 11 species from grapevines, three of which were new to science. Current research findings further suggest that most of the well-established names in *Botryosphaeria* in fact represent several distinct species, and that detailed research would be required to resolve their identities. It would appear that most records of these fungi in the literature have to date been incorrect, and that they in fact represent other, recently described, or as yet undescribed species. Once again, it appears that many of these species have been introduced along with their hosts. Earlier work by Smith *et al*. (1996) has shown that these species commonly occur as endophytes in apparently healthy tissue. Thus, new introductions could, even if the same species is involved, enhance the genetic diversity and fitness of an existing population.

Host specificity v. Mycosphaerella *and the pogo stick hypothesis*

As stated previously, members of the genus *Mycosphaerella* are generally accepted as being host specific. This assumption, however, has rarely been tested experimentally, as few isolates have been available for molecular comparison. For the past few years, the first author has thus been collecting and culturing extensively from hosts such as *Protea*, *Eucalyptus*, *Acacia* and *Musa*.

Mycosphaerella colombiensis was described from eucalypts in Colombia (Crous 1998), and based on DNA sequence data of the ITS region, was also suspected to occur on other hosts. However, once additional loci were sequenced, it was found that isolates from other hosts represented a cryptic species, *M. thailandica*, closely related to *M. colombiensis*, which has adapted to acacias in Thailand, and bananas in the Windward Islands (Crous *et al*. 2004*c*). *Mycosphaerella citri* is an important foliar and fruit pathogen of *Citrus*, causing premature leaf drop, as well as reduced tree vigour, yield and fruit size (Mondal *et al*. 2003). In a recent phylogenetic study (Crous *et al*. 2004*c*), we were able to confirm the presence of this pathogen on two new hosts, *Musa* and *Acacia,* outside the Rutaceae. These species, as well as several other species of *Mycosphaerella* (Table 1), have been isolated, albeit in low numbers and frequencies, from hosts on which they had never been reported. From these results, it would appear that these exotics are acting as catch-crops for some of the major foliar pathogens out there, and that by planting them in new regions, we are providing new host material to drive evolution. Many species of *Mycosphaerella* can grow and sporulate in culture; they do not absolutely require host involvement to complete their life cycles (Crous 1998). Ascospores are aerially dispersed, and it seems that they have the ability, when landing on leaves of plants other than their usual host, to infect dead tissue (usually the result of the primary *Mycosphaerella* pathogen on this host), form ascomata (low ascospore yield suggesting few ascomata to be present), and then be further dispersed, quite likely back to the natural host. In essence, *Mycosphaerella* is acting like a 'pogo stick', jumping to suitable catch crops, in the hope of finding its natural host. This has also been observed for asexual fungi isolated from non-plant substrates that after cultivation and DNA analysis, are shown to represent well-known plant pathogenic species of *Mycosphaerella*. In the few cases in which we encountered a pathogen occurring on a 'non-host', only very few (less than eight) ascospores were retrieved of this species, always in association with a primary *Mycosphaerella* pathogen of this host. What it suggests is that the fungus probably produces a single fruiting body, which again releases very few ascospores. Obviously the non-host pathogen does not like this host, but uses it to produce some progeny to enable onward dispersal. To circumscribe this phenomenon, we use the analogy of a pogo stick, jumping from a dying host to a non-host, in an attempt to find its true host to infect. To explain this we formulate the pogo stick hypothesis as follows:

> 'Host-specific fungal plant pathogens frequently exhibit the ability to colonise non-host tissue or other substrates, forming fruiting bodies that will produce a limited amount of propagules, and enabling them to disperse further, in an attempt to find the host on which they are pathogenic'.

Another aspect to consider by planting exotics like *Citrus*, *Acacia*, *Eucalyptus*, *Protea* etc., is that we are essentially driving evolution by encouraging the adaptation of some well-established pathogens to new hosts. This is an aspect that has never before been reported in *Mycosphaerella*, and holds serious implications not only for the newly planted host, but also for the original host from which it came. The fact that *Mycosphaerella* has the ability to adapt in this manner is startling, and also has serious implications for quarantine and export, as necrotic lesions of obscure host plants could thus harbour major pathogens of economic crops.

The assumption that planting a new crop would not influence the established pathogen structure out there is thus incorrect: these findings suggest that established pathogens also begin to adapt to infect newly introduced hosts. Host specificity as we thought we knew it, is thus untrue. It fact, host/pathogen relationships appear to be always in flux within a dynamic system of constantly evolving new species that begin to arise on whatever we are planting at the time. The results in Table 1 present us with some interesting examples. For most of the species, ITS sequences of isolates of that species range from 100% identical to a similarity as low as 98.7% (for example the *Trimmatostroma abietis* complex). When one includes additional datasets, it is clear that caution should be taken when defining species boundaries. For example, the extra datasets for *M. heimii* are almost all 100% identical, with the calmodulin sequence of only one isolate (CPC 11940) being 95.4% similar. If only ITS and calmodulin were used for this species and a sufficient number of isolates carrying the less similar calmodulin allele were found, one might be tempted to split this species in two based on the sequence data. *Mycosphaerella parva*, isolated from *Protea repens*, has an ITS sequence that is 99.6% similar to *Eucalyptus* isolates. However, when adding four other datasets, we observe more variation. Whether the variation observed for isolates of the same species on different host substrates represents random mutations or an evolution of new species will only become clearer once more isolates are sampled over time. For this, culture collections containing cultures or herbarium material dating back decades might provide an invaluable resource.

Table 1. Isolates included in this study (where available, type strains are indicated in bold)
For each species, the number of nucleotides over which the percentage similarity was calculated is given in the first row, followed by the percentage similarity in the next row(s)

Species and strain information (accession number[A]; substrate; country)	Gene sequenced[B] ITS[C]	EF[C]	TUB[D]	ACT[C]	CAL[C]	HIS[D]
Mycosphaerella nubilosa						
CPC 937 = CBS 116005; *Eucalyptus globulus*; Australia	505	273	534	225	–	379
CPC 11926; *Acacia auriculiformis*; Thailand	99.8%	100%	100%	100%	318	100%
Mycosphaerella citri						
CPC 10522; *Acacia mangium*; Thailand	480	291	–	204	300	388
CBS 116426; *Musa* sp.; Florida	99.8%	92.4%	–	94.1%	96.7%	98.2%
X126; *Citrus* sp.; Florida	99.6%	96.6%	–	97.1%	97.3%	99.0%
Mycosphaerella heimii						
CBS 110682; *Eucalyptus* sp.; Madagascar	480	–	–	216	306	387
CPC 11918; *Acacia auriculiformis*; Thailand	99.4%	–	–	100%	99.3%	100%
CPC 11940; *Acacia* sp.; Thailand	99.4%	–	–	100%	95.4%	100%
CPC 11925; *Acacia auriculiformis*; Thailand	99.6%	–	–	100%	100%	100%
Mycosphaerella communis						
CPC 10440 = CBS 114238; *Eucalyptus globulus*; Spain	507	322	–	238	386	354
CBS 112889; *Protea magnifica*; Australia	99.8%	91.3%	–	89.9%	–	94.9%
Mycosphaerella parva						
CPC 11273; *Eucalyptus globulus*; Spain	478	–	513	228	317	387
CPC 10935; *Eucalyptus* sp.; South Africa	99.6%	–	99.8%	99.6%	100%	99.7%
CPC 2120; *Protea repens*; South Africa	99.6%	–	98.8%	89.9%	85.5%	94.8%
Trimmatostroma abietis *complex*						
CBS 459.93; *Abies* sp.; Germany	469	–	–	229	–	386
CBS 145.97; Sandstone; Germany	98.7%	–	–	–	–	99.5%
CBS 618.84; *Ilex aquifolium*; Germany	99.8%	–	–	95.6%	–	100%
CPC 12033; *Pinus* sp.; Netherlands	98.9%	–	–	93.0%	–	96.4%
Teratosphaeria microspora CPC 1832; *Protea cynaroides*; South Africa	98.7%	–	–	97.4%	–	97.4%
Teratosphaeria microspora CPC 1848; *Protea cynaroides*; South Africa	98.9%	–	–	96.1%	–	97.4%
Taeniolina scripta CBS 539.88; Stone; Germany	99.8%	–	–	95.6%	252	100%
Mycosphaerella marksii						
CBS 110920; *Eucalyptus botryoides*; Australia	483	–	–	241	–	381
CPC 11795; *Vepris reflexa*; South Africa	99.4%	–	–	92.5%	–	99.7%
CBS 115501; *Leucadendron tinctum*; Madeira	99.4%	–	–	92.9%	–	99.5%
CPC 11215; *Eucalyptus comaldulensis*; Bolivia	99.0%	–	–	93.8%	–	95.5%
CPC 10892; *Eucalyptus botryoides*; New Zealand	99.4%	–	–	100%	–	99.5%

[A]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS.
[B]ITS: internal transcribed spacer region, EF: elongation factor 1-α, TUB: β-tubulin; ACT: actin, CAL: calmodulin, HIS: histone H3.
[C]Protocol and primers as described by Crous *et al*. (2004*c*).
[D]Protocol and primers as described by Crous *et al*. (2004*d*).

Intraspecific variation, e.g. clones and mating types, represents a level of complexity that is as significant as interspecific variation

The occurrence of both mating types means that when conditions are favourable, the organism can undergo sexual recombination, which can result in a vast number of new progeny, many of them representing new lineages or clones that could become dominant over time. The most obvious example for us today concerns *Phytophthora ramorum*, which is killing all the *Rhododendron* bushes growing along the streams in the Netherlands, as well as elsewhere in Europe. Contrary to the situation in the USA, one mating type of *P. ramorum* appears to be dominant in Europe (Werres and De Merlier 2003). Given the devastation associated with *P. infestans*, and the renewed problems associated with the later introduction of its opposite mating type, there is now considerable concern about the movement of plant species known to carry *P. ramorum*. The introduction and spread of its opposite mating type would enable it to

adapt more rapidly, thus further enhancing its potential as plant pathogen.

Gonthier *et al*. (2004) recently reported the introduction of a novel population of *Heterobasidium annosum* from eastern North America into the Presidential Estate of Castelporziano, near Rome (Italy). They were able to link these isolates with the corresponding USA isolates thanks to a unique insertion in the mitochondrial ribosomal operon. They also were able to infer that this population was introduced into Italy on wooden transport crates shipped to the 5th USA army, who occupied the estate grounds during World War II.

When we described the hyphomycete genus *Phaeoacremonium* (Crous *et al*. 1996), this went past without much attention. It was only later, when this fungus was shown to be one of the primary causes of Black Goo or Petri Disease of grapevines, that headlines like the 'Grapes of Wrath' and the 'End of the Grapevine Industry' started to appear in popular press. Our recent discovery of the *Togninia* sexual state of *Phaeoacremonium* (Mostert *et al*. 2003), again heralded an important breakthrough, as it was shown that these organisms were recombining, which again explained other data showing that they were rapidly speciating. Several species also cause phaeohyphomycosis in humans, and in a recent study Mostert *et al*. (2005) described nine new species, six of which were associated with opportunistic human infection. A further finding that many of these species also formed teleomorphs in culture (Mostert *et al*., unpublished data), underlines the importance of their mating types and distribution.

In a study of the causal organisms of black foot rot of grapevines, Halleen *et al*. (2004) characterised several species involved in this complex, some of which were shown to have *Neonectria* teleomorphs, and a heterothallic mating strategy. As both mating types of all these species have not yet been reported from different wine growing countries and regions, care should thus be taken with the movement of material and soil, as this could again play a significant role in the ability of these pathogens to rapidly adapt to new cultivars and rootstocks.

Today's introduction forms tomorrow's hybrid on yesterday's host

Rapid evolution is frequently driven by the introduction of plant pathogens into a new environment. This exposes them to new biotic and abiotic influences, such as different climatic conditions, vectors and hosts (Brasier 1995). A newly introduced pathogen will often be subject to novel or episodic selection, which may be influenced by contact between two genetically similar, but previously geographically isolated, pathogens. The coming together of two such separated lineages creates the opportunity for pathogen modification via interspecific gene flow.

Dutch elm disease is caused by *Ophiostoma ulmi* and *O. novo-ulmi* (the latter including two subspecies). These two species can interbreed, resulting in fertile progeny with reduced virulence (Brasier 1977; Kile and Brasier 1990). If *O. novo-ulmi* is introduced into an area, it quickly replaces *O. ulmi* (Brasier 1986). During this period, however, the two species are in close contact, and gene transfer can occur between them, which influences the population structure, and can have beneficial effects on the pathogen. In Oomycetes, this is also a common phenomenon. In the Netherlands, a new *Phytophthora* species that has been found on *Primula* and *Spathiphyllum* is actually a hybrid between *P. cactorum* (endemic species) and *P. nicotianae* (an introduced species) (Man in't Veldt *et al*. 1998). In South Africa, Campbell *et al*. (1999) induced sexual matings between the spot and net type of *Pyrenophora teres*, which causes net blotch of barley. The resulting progeny were found to be able to infect cultivars that were usually only susceptible to the net type, in addition to those only susceptible to the spot type (Campbell and Crous 2003). Hybridisation thus results in a different genetic makeup that influences not only the virulence of the pathogen, but also its host range.

Armillaria root rot is a well-known disease on Proteaceae in different regions in the world, including Australia, California, Hawaii, Kenya, Madeira, New Zealand, Tanzania and Zimbabwe. Although the disease is well known on many plant species in Africa, it has only recently been reported from indigenous Proteaceae in the Kirstenbosch Botanical Garden of South Africa (Denman *et al*. 2000). RFLP profiling of South African isolates indicated that there were two *Armillaria* species present (Coetzee *et al*. 2003). Although it was originally expected that these species would be African taxa, this was shown not to be so. The species present were *Armillaria mellea* and *A. gallica*, and both are known to be native to the Northern Hemisphere; they have clearly been introduced into the gardens. Coetzee *et al*. (2001) established that *A. mellea s. str.* was introduced into the Dutch East India Co. Gardens in the centre of Cape Town approximately 300 years ago. This most likely occurred with citrus plants that were brought to the area from Europe. *Armillaria mellea s. str.* is restricted to the Northern Hemisphere, and its occurrence in the Co. Gardens of Cape Town is the only recorded exception (Coetzee *et al*. 2001). It appears that the fungus has spread from the Co. Gardens, where it sporulates profusely, to the nearby plants in the Kirstenbosch Botanical Garden. A phylogenetic study by Coetzee *et al*. (2003) indicated that the second *Armillaria* species found in the gardens was *A. gallica*, a species possibly introduced with plants from Japan. These findings suggest that such introductions during the early European colonisation of South Africa might have been more common than we previously expected. Although these pathogens were probably introduced several hundred years ago, they were always well confined in a specific garden,

isolated by high buildings from the rest of the Cape fynbos. The recent finding that these pathogens now occur on the foot of Table Mountain is incredibly serious, and these escape events could have devastating effects on the Cape fynbos as it is known today.

> 'If a sparrow flies to a cherry tree, it's a cherry tree sparrow. If the same sparrow sits in an apple tree, it's an apple tree sparrow'

This classic quote of F.C. Deighton explains in a nutshell how plant pathologists have in the past treated the cercosporoid fungi – if two similar looking fungi occurred on two different plant hosts, then two different species names were created. With thousands of names, and hardly any cultures, it was extremely difficult to contradict this line of thought. It was only later, however, when pathologists started cross-inoculating *Cercospora* species on vegetables and getting disease, that some of these concepts began to be questioned (Crous and Braun 2003). There are currently 659 recognised *Cercospora* species, and names of another 281 morphologically identical species are included in the synonymy of *C. apii sensu lato.* Two of the species that belong to the *C. apii* complex, *C. apii* and *C. beticola*, cause Cercospora leaf spot on *Apium graveolens* (celery) and *Beta vulgaris* (sugar beet), respectively. Previous studies have demonstrated that these two species can, in fact, cross infect each other's host. Because of their morphological similarity, ability to cause disease on a wider host range, and DNA similarity based on the ITS region, Crous and Braun (2003) considered them synonymous. However, by employing multi-locus sequence data, AFLP analysis and cultural characteristics, Groenewald *et al*. (2005) were able to recognise additional features, and distinguish *Cercospora* strains occurring on celery and sugar beet. From the data obtained, it was shown that *C. apii* should be treated as a separate entity from *C. beticola.* In this case, therefore, it seems that Deighton was correct, and that these morphologically similar 'sparrows' are indeed two genetically distinct entities. However, when one consults other genera, such as *Phomopsis* for instance, different rules apply.

The genus *Phomopsis* contains more than 800 species, most of which are recorded as being plant pathogenic on stems, leaves, fruit or roots of various plant species (Uecker 1988). As in the case of *Cercospora*, species of *Phomopsis* have mainly been based on host affinity. Recent studies have shown, however, that various species are able to infect a wide variety of hosts (Rehner and Uecker 1994, Uddin *et al*. 1997, Mostert *et al*. 2001), and that host association is no longer sufficient for identification purposes. This effectively means that strains can be only identified to species level if advanced molecular techniques are employed. In a study of species causing Phomopsis cane and leaf spot of grapevines, Van Niekerk *et al*. (2005) identified 15 species. Several of these taxa, however, provide evidence of host switching. For instance *P. amygdali*, a known pathogen of peaches and almonds (Farr *et al*. 1999), and *P. helianthi*, a known pathogen of sunflowers, were also found on grapevines. Several species could not be identified to species level due to the limited molecular data presently available for this group. One of the unknown *Phomopsis* species also included isolates from roses and cranberries. In the case of *Phomopsis*, therefore, it appears that only a few species exhibit any sign of host specificity, and that all known species in this group should thus be treated with caution.

Future

In less than 10 years time DNA data will replace nomenclature

Microorganisms are the key to biodiversity, ecology and life on our planet. As mentioned previously, we currently know an estimated 7% of the fungal species that are thought to exist. Of the currently known number of species, 16% have been cultured, which represents 1.1% of the total estimated number of species (Hawksworth 2004). Most fungal names, thus, rely on the phenotype, and are largely unsupported by DNA data. Of all the plant pathogens that we currently work with, very few have in fact been subjected to molecular characterisation. One possible way to improve this situation is to epitypify known, older names with fresh collections that are linked to cultures and sequences. Usually once this is done, numerous new and undescribed species reveal themselves. As a rule of thumb then, names that are solely based on the phenotype represent species complexes of cryptic species, and not operational units. Names based on phenotypes can usually be keyed out with traditional dichotomous or synoptic keys using a variety of data. Cryptic or molecularly characterised species, however, can be identified in a much more precise manner, using polyphasic identification keys (for examples, visit <http://www.cbs.knaw.nl/databases/index.htm>), and a variety of PCR-based molecular techniques. Because the users of names demand a higher level of accuracy than has been given in the past, DNA-barcoding different species will be the future of taxonomy. In many cases, it may even become necessary to distinguish clones and genotypes, which could call for narrower parameters than those traditionally linked to a name, and thus the genotype, or genetic signature, will become the standard of the future. However, as can be seen from Table 1, the choice of gene and the sample size will play a significant role in defining these species and/or clones. Currently, a major drawback for genera such as *Mycosphaerella* is the lack of good, robust primers that can be used in DNA-barcoding or phylogeny projects. For example, the primers of Carbone and Kohn (1999) that were used here to amplify parts of the elongation factor 1-α, actin and calmodulin genes were

developed for mainly sclerotiniaceous species. Although the authors also tested a few other representative species while designing the primers, they did not include any species from *Mycosphaerella*. The primers currently used for β-tubulin have a very low success rate in *Mycosphaerella* (Table 1). To improve the effectiveness of sequence projects, be it for phylogeny or barcoding, we are in the process of selecting representative *Myosphaerella* and *Cladosporium* species for which sequences covering the entire length of the genes listed in Table 1 will be determined. From these sequences, robust primers will be developed covering the whole gene (where possible) or at least all of the introns. We believe that this will provide us with valuable tools, allowing us to analyse introns, or even genes, previously not available to us.

Building species banks

We are currently in the middle of a revolution in fungal taxonomy. Names are being linked to data. Old names, however, are limited to insufficiently small datasets (mostly phenotypic), and thus they are subject to change. How will we, as plant pathologists and phytomycologists, deal with this process of continuous change?

The answer to this question lies, we believe, in the taxonomic information backbone. Names are valuable only when they are linked to meaningful data. These datasets need to be managed in an intelligent fashion, by linking various databases, and making them readily available. One such initiative is MycoBank <http://www.mycobank.org> (Crous *et al*. 2004*a*), which will link names to their DNA sequence data, pleomorphic states, herbarium specimens, descriptions, illustrations, publications, etc. Names will also get assigned unique identifier codes that will link them to other data. These codes will in effect keep track of all aspects of the species, providing a bioinformatic structure that will eventually support the formation of species banks. An example of the MycoBank system being linked to a journal can be seen at <http://www.cbs.knaw.nl/simonline/index.htm>, in the *Studies of Mycology*, a mycological journal which is now freely available online. In 10 years time, we will probably have reached a stage where we may consider the unique DNA signature and species identifier number to be more relevant and important in species recognition than the name. Field isolates will either fall into the range of DNA variation accepted for a species, or will be detected as new. The correct identification of organisms requires a connection to various types of modern data, which challenges taxonomists to employ more sophisticated polyphasic approaches as a standard protocol. Similarly, plant pathologists are also challenged to deposit the DNA data, voucher specimens and strains of the organisms they are working with in appropriate public databanks. This is the only approach, I believe, that will ensure stability – when names are based on verifiable data, not on opinions.

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