The home stretch for fungal barcoding

In 2009, the plant research community published a paper (Hollingsworth *et al.* 2009) that designated two genes as a barcode for all plants. This means that among the major groups of eukaryotes, only the *Fungi* remain without a formal barcode. This may come as a surprise to many mycologists, who continue to loosely apply the term 'barcode' in numerous papers and projects. Importantly, barcodes are intended to be used as identification markers and may have properties, such as unalignable hypervariable regions, that make them inappropriate as phylogenetic markers. A strict set of criteria for barcodes has been established (<barcodeoflife.org/content/about/what-dna-barcoding>) and GenBank will only apply barcode tags to sequences of the genes or loci accepted as barcodes for a particular taxonomic group by the Consortium for the Barcode of Life (CBOL). Despite its long history of DNA sequencing and diverse ecological interests, the mycological community has not engaged with CBOL and associated organisations with much enthusiasm. At two major conferences in 2010 (the Mycological Society of America in Lexington, Kentucky, and the International Mycological Congress in Edinburgh), we suggested that it is in mycology's best interest to engage and collaborate with the international barcode community, and judging by the response after these sessions, this message was received clearly by a significant number of mycologists.

The road towards adoption of a fungal barcode has already had numerous twists and turns (Seifert 2009). An important milestone was a meeting of 37 mycologists from 12 countries at Front Royal, Virginia, in May 2007. At this meeting, participants were unanimous that the 5.8S nuclear ribosomal gene and its two flanking spacer regions (ITS) was the most likely candidate for a universal fungal barcode, despite some promising but mixed results for the default barcode sanctioned by CBOL, COI (Seifert *et al.* 2007). For a gene other than COI to be granted status as a barcode, CBOL has established a set protocol for validation. For mycologists, this process includes the publication of a proposal comparing the ITS against a set of other candidates. We intend to publish a paper in 2011 and together with an international group of interested mycologists, we are gathering and generating data for this purpose (Eberhardt 2010). Until now we received commitments from colleagues covering more than 40 clusters of five or more sibling species, covering 12 of the main fungal lineages identified in phylogenies generated from 'Assembling the Fungal Tree of Life' (AFTOL) project. The final paper will probably involve more than 50 coauthors from more than 12 countries and we intend to keep participation open to all interested parties until the data gathering deadline is passed in February 2011. More details can be found at the discussion group 'Fungi' on connect.barcodeoflife.net and the project website(<fungalbarcoding.org>).

As we set our sites on this publication, it is also important to look at projects and opportunities that may follow. We hope that the energy now building within the community will accelerate and improve collaborations between like-minded researchers across national borders. One pressing need is to coordinate and accelerate ongoing efforts to barcode authoritatively identified specimens and type cultures in herbaria and culture collections. Equally critical is coping with the vast amount of sequences generated by environmental sequencing projects.

Making a dent in this mountain of

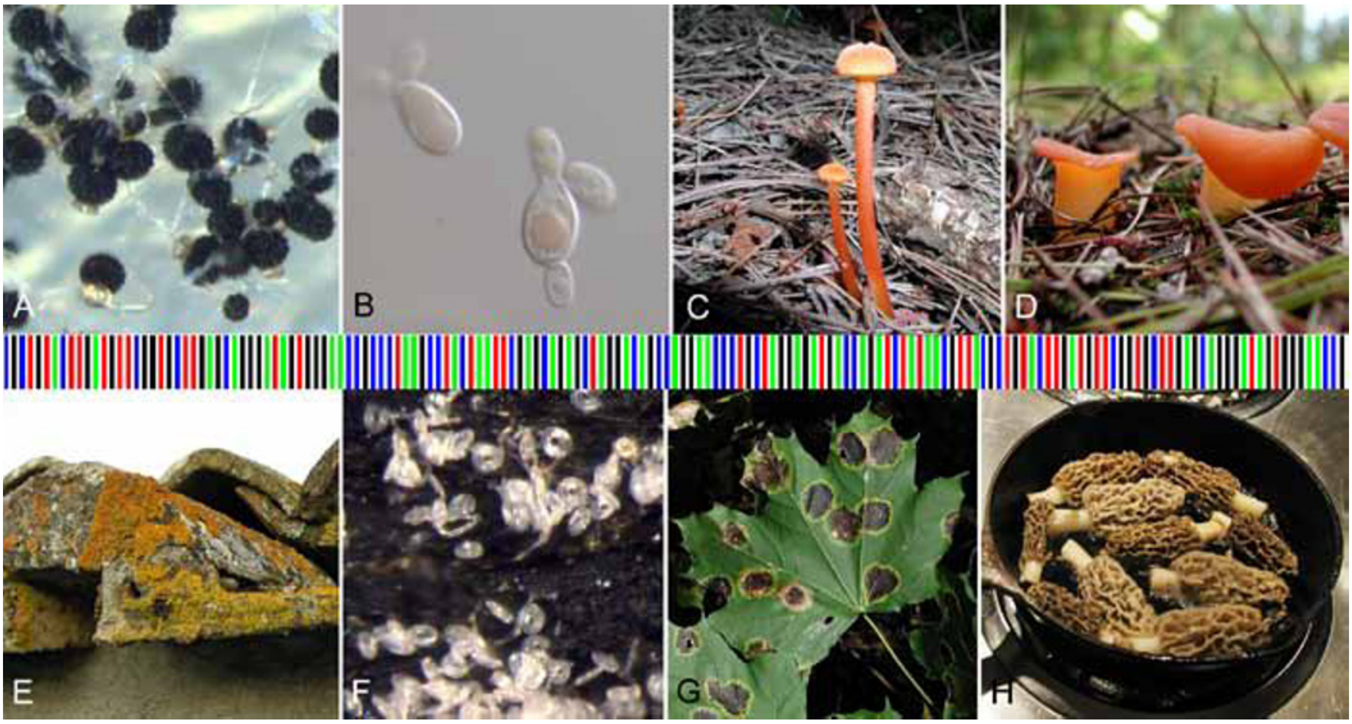

Fig 1. A. Zygospores of *Zygorhynchus heterogamous*. **B.** The yeast *Cryptococcus macerans*. **C.** Basidiomata of *Hygrocybe cantharellus*. **D.** Basidiomata of *Tremiscus helvelloides*. **E.** *Xanthoria parietina* on Walter Gams' roof in Bomarzo, Italy. **F.** Helicoconidia of *Helicomyces roseus*. **G.** Tar spot of sugar maple caused by *Rhytisma acerinum*. **H.** *Morchella elata* ready for further processing. Photos: Keith Seifert.

undescribed fungal diversity will require a monumental community effort to update and curate our sequence databases with accurate sequences tied to well validated samples; barcoding will be central to this. In addition, barcoding should provide excellent opportunities to expand the boundaries of fungal taxonomy by engaging teachers and amateur groups to help collect, locate and identify samples that could eventually be identified or validated by barcoding. Much is possible, but first we urgently need to formally declare a fungal barcode. We must not let this opportunity slip away to finally clear this hurdle.

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Eberhardt U (2010) A constructive step towards selecting a DNA barcode for fungi. *New Phytologist* **187**: 266–268.

Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, *et al.* (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences, USA* **106**: 12794–12797.

Seifert KA (2009) Progress towards DNA barcoding of fungi. *Molecular Ecology Resources* **9**: 83–89.

Seifert KA, Samson RA, Dewaard JR, Houbraken J, Levesque CA, *et al.* (2007) Prospects for fungus identification using C01 DNA barcodes, with *Penicillium* as a test case. *Proceedings of the National Academy of Sciences, USA* **104**: 3901–3906.

Fungi and the Convention on Biological Diversity

Recognizing that fungi are not plants, but need their own conservation strategies, is a first step to proper protection for species like critically-endangered *Pleurotus nebrodensis*, the only mushroom currently on the IUCN red-data list. Photo: David Minter.

The tenth follow-up conference of the parties to the 1992 Rio Convention on Biological Diversity was held in Nagoya, Japan on 18–29 October 2010. For the first time at these meetings, as a result of lobbying from the new International Society for Fungal Conservation (see *Reports* in this issue), and the Cup Fungi, Truffles and their Allies specialist group of the IUCN Species Survival Commission, fungi were explicitly considered independently from animals and plants.

The focus of the lobbying was section E, paragraph 10 of the Global Strategy for Plant Conservation. The wording of that paragraph read, "accordingly the Strategy addresses the Plant Kingdom with main focus on higher plants, and other well-described groups such as Bryophytes and Pteridophytes. This does not imply that these lower groups do not have important ecological functions, nor that they are not threatened. Parties may choose on a national basis to include other taxa, including algae, lichens and fungi". It was pointed out to delegates of the conference that the final sentence of this paragraph gave the mistaken impression that fungi are "lower" plants, that "lichens" are different from fungi, and that strategies for fungal conservation could be treated as an optional extra.

At Nagoya, delegates discussed these concerns and agreed that the wording needed to be changed. The revised and agreed wording reads, "while the Strategy addresses the plant kingdom with main focus on higher plants, and other well-described groups such as bryophytes and pteridophytes; Parties, other governments and other relevant stakeholders may consider developing conservation strategies for other groups such as algae and fungi (including lichen-forming species)".

The revised wording recognizes that lichens are fungi, and that fungi are not plants. It also recognizes the possibility that fungi should have their own separate strategy. In 1992, the Convention established that all groups of living organisms have the right to exist on this planet and to be protected. Up to now, the Convention has not lived up to that promise in respect of the fungi. By explicitly recognizing fungi as different from plants, this re-wording can be seen as a small but significant first step towards getting the convention to honour its promise for the fungi.

The Convention also agreed key targets for 2020, which included commitments to: cut the rate of loss of natural habitats by at least half; to increase terrestrial nature reserves from 13 % to 17 % of the world's land area; increase marine and coastal nature reserves from 1 % to 10 % of the world's seas; restore at least 15 % of the areas where biodiversity is classed as 'degraded'; and safeguard at least 75 % of plant species in collections. For further information see <.cbd.int/>.

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Mycologists go political, with success

A critically important message in this International Year of Biodiversity is to promote the missing "F" word at every opportunity – Fauna, Flora, AND Fungi. This was part of a rallying call to all mycologists delivered in a recent IMC paper by Dave Minter, and through the establishment of the International Society for Fungal Conservation during IMC9. Already, the Society's founding members have demonstrated the power of global coordination to influence a bastion of public broadcasting – the BBC.

On 15 October 2010, the BBC's "The World Tonight" programme focused attention on the biodiversity crisis ahead of the COP 10 meeting in Nagoya. Although an engaging and well-produced 45-min programme, it perpetuated the simplification of biodiversity to "fauna and flora". A call to action went out from Dave, and mycologists globally responded by petitioning the BBC. Dave sent e-mails to 180 people, all the Founder Members of the new International Society for Fungal Conservation. At least 62 messages were sent to the BBC, which came from a minimum of 29 countries: Argentina, Australia, Brazil, Canada, China, Cuba, Ecuador, Egypt, France, Ghana, Greece, India, Italy, Malaysia, Mexico, New Zealand, Philippines, Poland, Puerto Rico, Russia (Novosibirsk oblast, St Petersburg oblast, Tula oblast; i.e. Russia in Asia and in Europe), Serbia, South Africa, Spain, Sweden, Ukraine, UK, United Arab Emirates, USA, and Zimbabwe.

Is this the first ever global political action by mycologists? That appears to be the case. The result was a triumph; within hours the BBC producer Alistair Burnett by way of the BBC editors' blog (<bbc.co.uk/blogs/theeditors/alistair_burnett/>) apologised for the omission, stating:

"The UN's member states are getting together next week ...... to stop the loss of plants, animals and fungi species - or biodiversity - ......Just a note to our listeners who've e-mailed us in the past day pointing out that we failed to mention fungi when describing what biodiversity is, my apologies, we could have been more explicit."

The word "fungi" was hyperlinked to an excellent blog about the fundamental importance of fungi: <biodiversityislife.net/?q=node/382>.

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Mushrooms – the new plastic?

Styrofoam is one of the most egregious offenders in the disposable plastics category, US $ 20 Bn of this material is produced annually and used in products as diverse as building insulation, coffee cups, and packaging; it is said to occupy around 25 % of landfill sites, and will stay there for 100s of years. In the oceans it can break into smaller and smaller globules, but these do not really ever disappear. Gavin MacIntyre and Eben Bayer (Ecovative Design, Green Island, NY, USA), with financial support from the National Science Foundation (NSF), have been developing a way to transform agricultural wastes, especially seed husks, with basidiomycete mycelia to form an entirely new class of materials, which perform much like plastics, but as they are made from crop waste differ in being totally compostable when no longer required. The waste material is transformed into a chitinous polymer, Mycobond™ that can be moulded into different shapes and used in products ranging from packaging to insulation boards for the construction industry (Greensulate™). The inventors have developed a continuous system which cleans, cooks, cools and pasteurizes the waste materials, while continuously inoculating them with the mycelium. The resultant stream of material can be made into almost any shape which self-assembles in a mould. This invention is particularly exciting as there is potential for different locally available materials to be used according to what agricultural wastes are available in a country. The basidiomycete fungi utilized are not, however, named in the released information.

For further information on this most exiting and novel discovery, which has such enormous economic potential and environmental benefits, see the video on <ted.com/talks/eben_bayer_are_mushrooms_the_new_plastic.html>, and the NSF report on <nsf.gov./awardsearch.showaward.do?AwardNumber=0944529>.

White truffles still demand a high price

In times of austerity, it might have been reasonable to expect that luxury and gourmet foods would drop in value. However, at the Fiera Nazionale del Tartufo Bianco d'Alba, the annual truffle fair in the town of Alba in the Piedmont district of northern Italy, which ran from 9 October to 14 November 2010, a single 900 g specimen of *Tuber magnatum*, the Alba White Truffle, was sold to a Hong Kong buyer for a massive 105 000 € (US $ 141 605), i.e. 117 € g^{-1}. The previous record paid for a single truffle was one of 1.5 kg which sold to a Macau casino owner for £ 165 000 (194 330 €, US $ 262 255) in 2007, equating to 129 € g^{-1}.

Fungi on German TV: Focus on black fungi

Sybren de Hoog and Bert Gerrits van den Ende of CBS (Utrecht) featured on Germany's second TV network in an episode of the ZDF science program 'Abenteuer Wissen'. The CBS homepage (<cbs.knaw.nl>) has a link to the original program, as well as a version with English subtitles. De Hoog explained on the background of a brain infection after near-drowning of a child, published by Mursch *et al.* (2006). This concerned a 2-year-old boy who fell into a rainwater container and aspirated polluted water for several minutes. The child was reanimated and did well, but later he became sleepy and fell into a deep coma. Multiple fungal brain abcesses were observed and removed by surgery. The etiologic agent proved to be *Scedosporium apiospermum*, one of the very few species consistently associated with near-drowning fungal encephalitis (Guarro *et al*. 2006), which is almost always fatal. The boy was saved thanks to timely diagnostics and appropriate therapy.

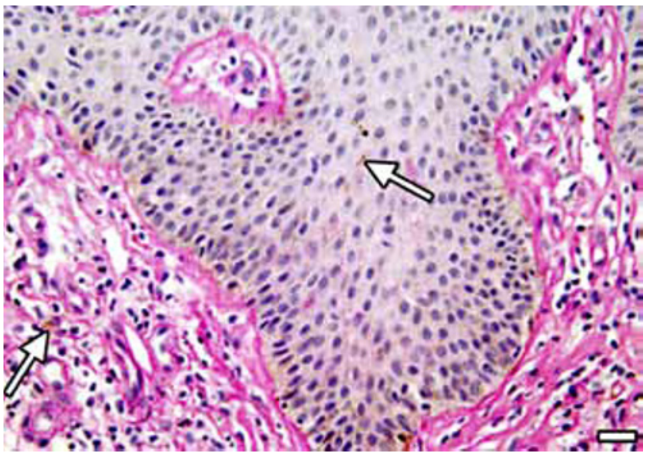
De Hoog and coworkers recently published a special issue of *Medical Mycology* on *Scedosporium* (47 (4), 2009: '*Pseudallescheria* and *Scedosporium*: emerging opportunists', JP Bouchara *et al.*, eds), stemming from a series of Workshops on this group of fungi. They are abundantly present in stagnant, nutrient-rich waters, as well as in poorly aerated industrial and agricultural soils. Alkane pollution is a promotive factor. Investigations are ongoing by the group of Johannes Rainer in Innsbruck to utilize *S. dehoogii* for bioremediation of diesel-polluted sites. The taxonomy of the genus is still under debate (Gilgado *et al.* 2005, Lackner *et al.* 2010), but it is clear that a complex of species is concerned each occupying different slots in an ecological continuum. *Scedosporium apiospermum* has a bias towards virulence, whereas *S. dehoogii* at the opposite end of the spectrum is almost strictly environmental.

There are more fungi inhabiting oils and toxic pollutants. Hans-Michael Fürst demonstrated frequent isolation of *Hormoconis resinae*, not only from hydrocarbon-polluted soils, but also as a monoculture in the oil tanks and machinery of ships (Fürst 2000). Biofouling by this fungus may cause engines to stop, which potentially is quite a threat to aviation. But authorities will never admit that there is a problem, said Dr Fürst. Growth of *Hormoconis* is also promoted by phenol-rich creosote used in wood protection, in which respect there is a striking resemblance to black yeasts (Prenafeta-Boldú *et al*. 2006). Chaetothyrialean black yeasts are massively isolated from treated wood by enrichment with phenolic aromates (Zhao *et al.* 2010). Chaetothyrialean black yeasts differ by combining this feature with oligotrophy (Satow *et al.* 2008), which explains their abundance in steam baths and dishwashers (Zalar *et al.* 2011).

Black yeasts belonging to the orders *Dothideales* and *Capnodiales* are mainly extremophiles, surviving harsh conditions of heat, cold, dryness and solar irradiation. Their evolutionary origin lies far back, when they occupied micro-niches in crevices between crystals in bare rock of deserts, mountains or the Antarctic (Selbmann *et al.* 2005). On German TV, Thomas Warscheid gave a more recent example of their rock-inhabiting life style in the deterioration of the famous Dom of Cologne, Germany. Monuments are crumbling under the slow but effective force of black fungi. Earlier Warscheid observed the same thing happening with the temples of Angkor Wat in Cambodja, as he explained in *The New York*

Processing *Scedosporium* samples, recorded by a ZDF TV team for 'Abenteuer Wissen', a science program on Germany's second network.

Blackish discoloured skin of toes and toe webs with scaling. [from Li *et al.* (2008) *Studies in Mycology* **61**: 131–136].



Skin biopsy stained with HAE; some fungal elements are visible (arrows). Bar = 5 µm. [from Li *et al.* (2008) *Studies in Mycology* **61**: 131–136].

Times of 24 June 2008. The fungi utilize hydrocarbon air pollutants and assimilation products of algae, acidify their environment, and finally turn all buildings black. Aspects of this behaviour were explained in a special issue of *Studies in Mycology* (**61**, 2008: 'Black Fungal Extremes', GS de Hoog & M Grube, eds), while medically significant species were presented in one of *Medical Mycology* (**47**(1), 2009; 'The Dark World of Black Fungi', Vitale *et al*., eds). *Fungal Biology* will also devote a special issue to black yeasts shortly, entitled 'The Emerging Potential of Black Yeasts' (Gunde-Cimerman *et al.,* eds).

Black fungi are remarkable for their odd ecology, which we still have a long way to go towards understanding. In 'Abenteuer Wissen', Axel Brakhage explained his research on *Aspergillus fumigatus*, a ubiquitous compost fungus which is hypothesized to express factors enhancing escape from phagocytosis when confronted with human alveolar macrophages (Behnsen *et al.* 2007). Similar processes are to be expected when black yeasts invade our homes and our bodies from the specialized niches somewhere in nature (Sudhadham *et al.* 2008). They differentially adapt to a highly deviating environment using different combinations of the essential factors determining their natural habitat. Gerrits van den Ende *et al.* (2011) applied the term 'ecological fitting' to this process enabling rapid host and environmental shifts, eventually leading to sympatric speciation. Many extremophiles, however, already occupy their ultimate habitat and don't have an adaptive space around them. They are likely to have entered a dead-end street and are likely to become extinct when climate changes dramatically, as it is expected to do.

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Behnsen J, Narang P, Hasenberg M, Gunzer F, Bilitewski U, *et al.* (2007) Environmental dimensionality controls the interaction of phagocytes with the pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*. *PLoS Pathogens* **3**: e13.

Fürst HM (2000) *Ökologie des Hyphenpilzes Hormoconis resinae und Eigenschaften seines n-Alkan-induzierten P450-Monooxygenasesystems*. Thesis. Technische Universität Berlin.

Gilgado F, Cano J, Gené J, Guarro J (2005) Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. *Journal of Clinical Microbiology* **43**: 4930–4942.

Guarro J, Kantarcioglu AS, Horré R, Rodriguez-Tudela JL, Cuenca Estrella M, *et al.* (2006) *Scedosporium apiospermum*: changing clinical spectrum of a therapy-refractory opportunist. *Medical Mycology* **44**: 295–327.

Lackner M, Gerrits van den Ende AHG, Hoog GS de, Kaltseis J (2010) Barcoding of the therapy-refractory species of *Pseudallescheria* and *Scedosporium*. *Medical Mycology*: in press.

Mursch K, Trnovec S, Ratz H, Hammer D, Horré R, *et al.* (2005) Successful treatment of multiple *Pseudallescheria boydii* brain abscesses and ventriculitis/ependymitis in a 2-year-old child after a near-drowning episode. *Child's Nervous System* **22**: 189–192.

Prenafeta-Boldú FX, Summerbell R, Hoog GS de (2006) Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? *FEMS Microbiology Reviews* **30**: 109–130.

Satow MM, Attili-Angelis D, Hoog GS de, Angelis DF, Vicente VA (2008) Selective factors involved in oil flotation isolation of black yeasts from the environment. *Studies in Mycology* **61**: 157–163.

Selbmann L, Hoog GS de, Mazzaglia A, Friedmann EI, Onofri S (2005) Fungi at the edge of life: cryptendolithic black fungi from Antarctic desert. *Studies in Mycology* **51**: 1–32.

Sudhadham M, Prakitsin S, Sivichai S, Chaiyarat R, Dorrestein GM, *et al.* (2008) The neurotropic black yeast *Exophiala dermatitidis* has a possible origin in the tropical rain forest. *Studies in Mycology* **61**: 145–155.

Zhao J, Zeng J, Hoog GS de, Attili-Angelis D, Prenafeta-Boldú FX (2010) Isolation and identification of black yeasts by enrichment on atmospheres of monoaromatic hydrocarbons. *Microbial Ecology* **60**: 149–156.

Executive Committee and Officers of the International Mycological Association (2006–2010).