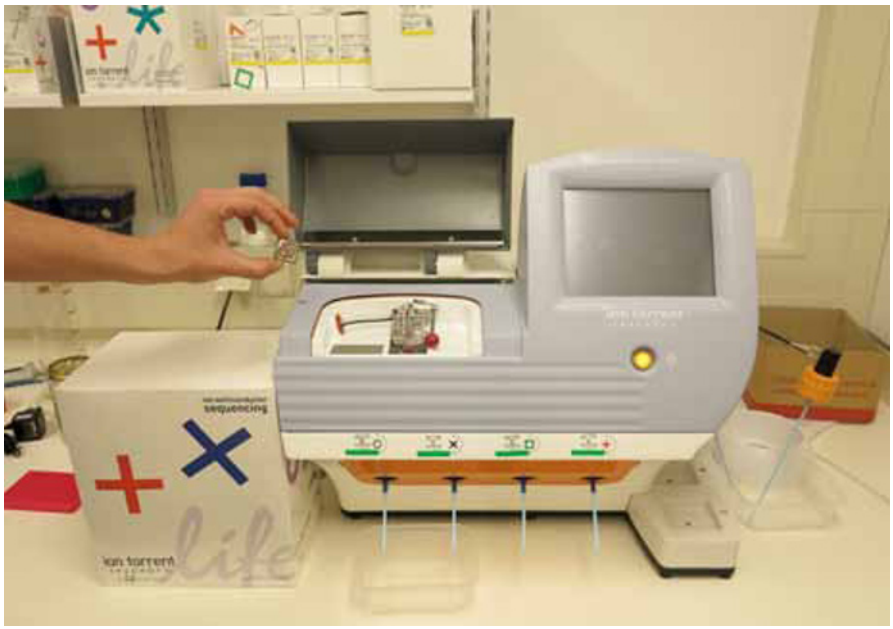
High-throughput sequencing of fungal communities: environmental samples not so species-rich after all?

"*Without awareness of methodological biases, limitations of markers, and bioinformatics challenges, large-scale sequencing projects risk yielding artificial results and misleading conclusions*" (Lindahl *et al*. 2013: 288).



High-throughput sequencing analysis methods enable detailed, semiquanitative analysis of fungal communities in large sample sets and provide ecological information that is far beyond anything that could previously be contemplated. As more and more named fungal sequences become available in public repositories such as GenBank, more sequences recovered can be linked to known species, The procedures do, however, require attention to protocols, and it is vital that users are aware of potential problems as well as prospects. Now, several of the world leaders in fungal community molecular ecology have come together to produce a valuable and pragmatic "user's guide" (Lindahl *et al*. 2013).

The guide proceeds stepwise from sampling to interpretation. In sampling it is necessary to be aware of problems that may arise because of mycelia size and stratified vertical profiles. Freezing of samples is necessary to arrest development; –20 °C will arrest development and preserve DNA, but if RNA analysis is being considered shock-freezing in the field on dry ice or liquid nitrogen and storage at –80 °C has to be arranged. As analyses are necessarily carried out on mg to g-size samples, field samples require homogenization, but even so large differences between repeated extractions from the same sample have been found. Extraction protocols have to be chosen, and which will depend on the nature of the sample, not least the content of humic materials. When it comes to marker and primer selection, none available are considered to meet all desirable requirements, and different ones have come to be used in different phyla – and many discriminate inadvertently against particular fungal taxa. *Glomeromycota* are found to present particular problems. Also critical is the length of the amplified fragments to be considered when primers are chosen. Further, where samples are to be pooled so they can be put through a single run, i.e. multiplexed, the amplicons can be tagged by molecular identifiers specific to each of the samples in several different ways.

Issues relating to the PCR process itself are also addressed. The authors consider that the number of PCR cycles should be minimized to avoid preferential amplification of rare sequences and the creation and propagation of chimeric sequences. The process needs stringent discipline, including negative controls, but PCR products can form even in the controls if the number of cycles is large. Great care is also needed in the purification and pooling of PCR products, but confirmatory sequencing of a few cloned amplicons can add to confidence in the PCR schemes to be used. The available sequencing platforms and bioinformatic methods are explained in some detail, and attention is drawn to issues that can be a cause for concern.

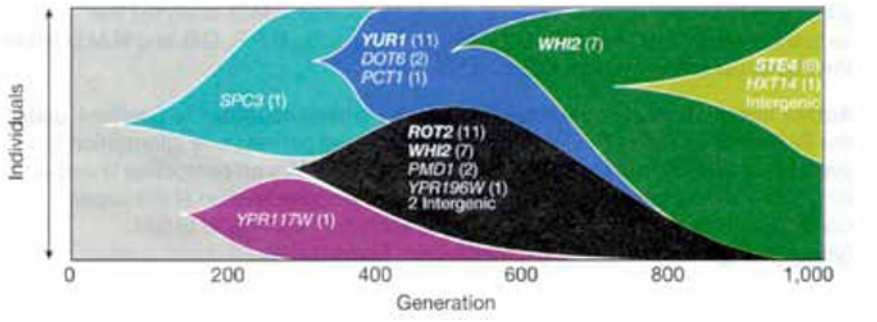
Then there is the question of interpretation. Just what do the results really mean? Singleton sequences, i.e. ones only recovered once, are a particular problem. A common practice is to delete these from the analysis, or delete ones recovered say five or fewer times, but some or all of the low-frequency sequences could represent authentic sequences (i.e. species or Operational Taxonomic Units, OTU's) from the original sample. Further, erroneous singletons increase apparently infinitely with more sequencing effort and this makes it difficult to be confident over estimates of species numbers obtained. The quantification of genomes in a sample may also not reflect accurately the taxonomic makeup in a sample. For example, filamentous species are likely to be under-represented in comparison to those with a yeast-like habit. The authors observe that "early claims of astonishingly high species richness in 454-sequenced amplicons were exaggerated, because of problems in distinguishing technical artefacts from true diversity"(p. 296).

The conclusions of this experienced group of mycologists may be summed up by the last sentence their abstract, reproduced at the start of this item. It is evident that we now have an enormously powerful tool, but need to develop our knowledge on how to interpret such vast data sets. There would also be some advantage in the mycological community exploring whether particular protocols could be developed and their use promoted in order that the results from different groups of researchers could be more meaningfully compared.

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, Kauserud H (2013) Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. *New Phytologist* **199**: 288–299.

Whole-genome whole-population sequencing provides insights into genome sequence evolution

Tracking single mutations through generations is necessary to determine their long-term fates. *Saccharomyces cerevisiae* is an ideal model organism for such studies as its genome has been so thoroughly explored and there is a rapid turnover in populations. Cutting edge molecular technology now provides tools to enable such questions to be explored by following particular mutations through successive populations. Lang *et al.* (2013) used whole-genome whole-population sequencing to examine the dynamics of genome sequence evolution in 40 replicate *S. cerevisiae* populations through a staggering 1000 generations. This proved not to be a matter of single genes moving independently, but cohorts of genes which moved together. Further, there could be multiple clonal cohorts present in a population at the same time and competing with one another. Cohorts arose at different times, could peak at different times, and also decline or be lost at different times. The total number of actual mutations found across 40 populations was an amazing 995, of which 723 fell within coding regions, and 246 (25 %) became fixed. They refer to the phenomenon they found, where some genes are carried along with others in a single cohort, as "hitchhiking".



Dynamics of sequence evolution in *Saccharomyces cerevisiae*, showing the six main cohorts in the population as they proceeded through the generations through. Reproduced from Lang *et al.* (2013).

Recognition of this phenomenon may in part explain cases where apparently non-adaptive mutations persist as they are just by chance hitch-hikers with other genes that are of adaptive value. Once again, studies with yeasts are contributing to a deeper understanding of evolution in action in eukaryotes as a whole.

Lang GI, Rice DP, Hickman MJ, Sodergren E, Weinstock GM, Botstein D, Desai MM (2013) Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations. *Nature* **500**: 571–574.

What is the Chinese Lingzhi *Ganoderma*?

Ganoderma lingzhi cultivated on wood chips in Taiwan, 1996. Photo: D.L. Hawksworth.

It has been known for many years that the *Ganoderma* species used in traditional Chinese medicine, and widely cultivated in China and Taiwan, "Lingzhi", is not *G. lucidum,* but a separate species – hardly surprising when it is appreciated that the type of *G. lucidum* was collected near London, UK. The issue of what name to apply has, however, been a matter of ongoing uncertainty and controversy since the mid-1990s (e.g. Moncalvo *et al.* 1995). However, last year Cao *et al.* (2012) introduced the new species name *G. lingzhi* for the medicinally valued species following a molecular and morphological investigation. While there could have been some value in changing the type of *G. lucidum* through conservation, that would not have been without problems, and the choice of the epithet seems highly appropriate. The name change has been the subject of announcements in two of the main Chinese mycological journals (Yang & Feng 2013, Dai *et al.* 2013), and is evidently now accepted by Chinese mycologists.

Cao Y, Wu S-H, Dai S-C (2012) Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". *Fungal Diversity* **56**: 49–62.

Dai Y-C, Cao Y, Zhou L-W, Wu S-H (2013) Notes on the nomenclature of the most widely cultivated *Ganoderma* species in China. *Mycosystema* **32**: 947–952. [Chinese with English summary.]

Moncalvo JM, Wang HF, Hseu RS (1995) Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences: comparison with traditional taxonomic characters. *Mycological Research* **99**: 1489–1499.

Yang ZL, Feng B (2013) What is the Chinese "Lingzhi" – a taxonomic mini-review. *Mycology* 4: 1–4.

Back to *Pyricularia* for the rice-blast fungus

Pyricularia grisea on *Digitaria* sp.

With the end of dual nomenclature for pleomorphic fungi, the issue of whether the generic name *Pyricularia* Sacc. 1880 should be resurrected to replace the increasingly used *Magnaporthe* R. A. Krause & R. K. Webster 1972 was bound to be controversial amongst plant pathologists. Now fresh molecular phylogenetic studies reported by Luo & Zhang (2013) show that there is no place for argument as the type species, *P. grisea* 1880 and *Magnaporthe salvinii* (Catt.) R. A. Krause & R. K. Webster 1972 (syn. *Leptosphaeria salvinii* Catt. 1879) respectively, prove not to be congeneric after all! *Pyricularia oryzae* Cavara 1892 is, however, confirmed as congeneric with *P. grisea*, so Cavara's name can now be reinstated and adopted. In contrast, *M. salvinii* proves to be conspecific with the type species of *Nakataea* Hara 1930, *Sclerotium oryzae* Catt. 1877 and is now transferred to *Nakataea* by Luo & Zhang. This taxonomy has been confirmed by Japanese researchers (Murata *et al.* 2013) who also concluded *Pyricularia* should be adopted for the rice blast fungus.

This reversion will be welcomed by many plant pathologists, not least as the name *Pyricularia oryzae* is more widely cited in the literature than the 110 year-younger binominal *Magnaporthe oryzae* (Cavara[1]) B. C. Couch 2002. In Google Scholar on 12 December 2013, there were 19 400 hits for *P. oryzae* and 16 600 for *M. oryzae.*

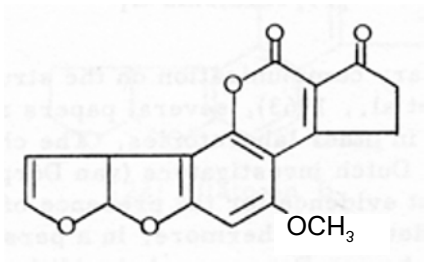
Intriguingly, *M. poae* Landsch. & M. Jacks. 1989, proved to be more closely related to *Gaeumannomyces* than either *Nakataea* or *Pyricularia,* and the new generic name *Magnaporthiopsis* was introduced by Luo & Zhang for that species.

Luo J, Zhang N (2013) *Magnaporthiopsis*, a new genus in *Magnaporthaceae* (*Asocomycota*). *Mycologia* **105**: 1019-1029.

Murata N, Aoki T, Kusaba M, Tosa Y, Chuma I (2013) Various species of *Pyricularia* consitute a robust clade distinct from *Magnaporthe salvinii* and it relatives in *Magoporthaceae*. *Journal of General Plant Pathology*: DOI10.1007/s10327-013-0477-z

[1]"Cavara" is cited in parenthesis here following the recommendations of Hawksworth *et al.* (*IMA Fungus* 4: 53–56, 2013).

Aflatoxin B_1 levels and HIV-positive people



Aflatoxin B_1.

Aflatoxins are amongst the mycotoxins of most concern to human health. Produced primarily by *Aspergillus flavus*, post-harvest, concentrations often build up to and exceed those considered tolerable for human consumption in groundnuts (peanuts), maize, rice, and other cereals stored in hot and damp conditions. One of the effects of aflatoxins is immunosupression, leading to increased susceptibility to disease. As the immunodeficiency virus HIV also causes immune suppression, it might be expected that HIV-positive people might be particularly high-risk.

Jolly *et al.* (2013) studied plasma samples from the blood of 314 HIV-positive people in Ghana. They found that there was a positive association between aflatoxin B_1 albumin adduct levels (AF-ALB) and the viral load in the blood. Further, there was a strong correlation between the HIV viral load and AF-ALB levels, and the viral load could be as much as 2.9 times higher in people with higher AF-ALB levels in the blood.

While cause-and-effect were not demonstrated unequivocally, the results suggest that it would be prudent for HIV-positive patients to avoid possible aflatoxin B_1 contaminated foods.

Jolly PE, Inusah S, Lu B, Ellis WO, Nyarko A, Phillips TD, Williams JH (2013) Association between high aflatoxin B_1 levels and high viral load in HIV-positive people. *World Mycotoxin Journal*: DOI 10.3920.WMJ2013.155.

A second *Batrachochytrium* species causing a lethal chytridiomycosis

The chytrid *Bactrachochytrium dendrobatis*, first recognized taxonomically in 1999, has been involved in the devastating amphibian decline witnessed in both temperate and tropical regions over the last two decades. A staggering 2100 of the 6300 known amphibian species are estimated to be at risk, and over 40 % of the species present have been lost in some regions. However, that single species does not appear to be responsible for all declines, including that of the fire salamander (*Salamandra salamandra*), the population of which has dropped 96 % in The Netherlands since 2010. An *ex situ* conservation programme was initiated for that species, but 49 % of the captive animals died over a period of two months in 2012; death took just seven days. *Bactrachochytrium dendrobatis* could not be detected, but a second species of the genus which was pathogenic and causing ulcerations was recovered.

The new species has been named as *B. salamandrivorans* by Martel *et al.*

Salamandra salamandra, with skin ulcerations caused by *Bactrachochytrium salamandrivorans*. Photo courtesy A. Martel.

(2013), who provide a detailed account of the disease, including transmission electron micrographs of infected skin tissue. Appropriately, the species epithet can be roughly translated as "salamander devouring". It is, however, not known whether the new species was introduced from outside Europe, or whether it can infect other amphibian species. It does, however, clearly merit searching for in cases where amphibian losses are occurring and *B. dendrobatis* is not detected.

It will be interesting to see whether further species of *Bactrachochytrium,* pathogenic or otherwise associated with amphibians, also come to light in the years ahead.

Martel A, Spitzen-van der Sluojs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuy F, Pasmans F (2013) *Bactrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences, USA* **110**: 15325–15329.

Basidiolichens: more numerous than previously suspected

The lichenized basidiomycete *Cora aspera.* Photo R. Lücking.

Lichen-forming basidiomycete fungi are generally considered as something of a novelty, with few species having adopted this life-style. That picture is emerging as simplistic now molecular phylogenetic studies are starting to accelerate. Parmasto (1978) had accepted just one genus of thelephoroid lichens, *Dictyonema,* and relegated *Cora, Corella,* and several other generic names to synonymy. He accepted just five species in the whole complex.

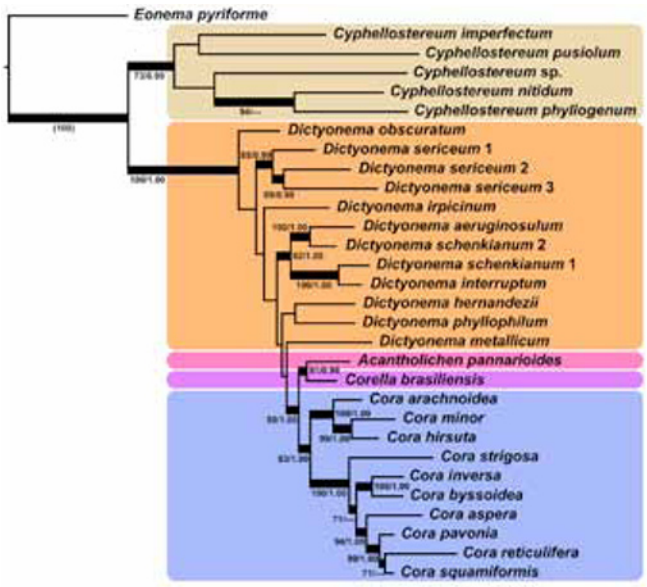
Molecular phylogenetic studies by Dal-Forno *et al.* (2013), using three different genes and fresh collections from the tropics, reveal a very different situation. *Cyphellostereum* is sister to the main lichenized clade, which includes 24 species. Further, the analysis supports up to five genera rather than one. An ancestral character state analysis enabled the authors to elucidate the development of thallus form in the complex. The thallus progresses from appressed and crustose morphs in *Cyphellostereum* and *Dictyonema*, to microsquamulose in *Acantholichen*, and microsquamulose to large-foliose morphs in *Cora* and *Corella*. Increasing complexity of the thallus correlated with the number of nodes from the root to the terminals. The authors speculate that the route from filamentous to foliose morphs may be one along which filamentous lichenized ascomycete genera such as *Cystocoleus* and *Racodium* have yet to travel.

The species recognized here are overlooked rather than cryptic as they can evidently generally be separated by morphological features. From works mentioned as "in press" in this paper, it is clear that yet more species have been found and require names; one such paper is to introduce ten new species. Further, most of the collections so far examined have been from Central and South America, and it would not be unreasonable to find that examination and sequencing of additional material from Asia and the Pacific, for example, would further swell the species number.

Basidiolichens are evidently not just a curiosity to be casually dismissed by both lichenologists and other mycologists, but can potentially shed light on the evolution of thallus types in lichen-forming fungi.

Dal-Forno M, Lawrey JD, Sikaroodi M, Bhattarai S, Gillevet PM, Sulzbacher M, Lücking R (2013) Starting from scratch: evolution of the lichen thallus in the basidiolichen *Dictyonema*. *Fungal Biology* **117**: 584–598.

Parmasto E (1978) The genus *Dictyonema* (*'Thelephorolichenes'*). *Nova Hedwigia* **29**: 99–144.



Best-scoring maximum-likelihood tree of thelephoroid basidiolichens based on a three-gene dataset. Adapted from Dal-Forno *et al.* (2013).