Viewpoints

Glomeromycotina: what is a species and why should we care?

Summary

A workshop at the recent International Conference on Mycorrhiza was focused on species recognition in Glomeromycotina and parts of their basic biology that define species. The workshop was motivated by the paradigm-shifting evidence derived from genomic data for sex and for the lack of heterokaryosis, and by published exchanges in *Science* that were based on different species concepts and have led to differing views of dispersal and endemism in these fungi. Although a lively discussion ensued, there was general agreement that species recognition in the group is in need of more attention, and that many basic assumptions about the biology of these important fungi including sexual or clonal reproduction, similarity or dissimilarity of nuclei within an individual, and species boundaries need to be re-examined and scrutinized with current techniques.

Introduction

'What is a species?' remains a classic question that still tortures many PhD candidates during qualifying exams, but last year this issue resulted in differing interpretations of endemism from a study involving global sampling of Glomeromycotina, the fungi responsible for arbuscular mycorrhizas (Davison *et al.*, 2015; Bruns & Taylor, 2016; Öpik *et al.*, 2016). This discussion brought the question of species concepts back into the forefront of arbuscular mycorrhizal ecology. Glomeromycotina are perhaps the most important group of fungi in terrestrial ecosystems because they are mutualistically associated with roots of most vascular plants. To resolve the issues generated by this controversy, and to highlight the new emerging perceptions of these enigmatic fungi, a workshop was assembled at the recent International Conference on Mycorrhiza (ICOM9) in Prague, Czech Republic by two authors from conflicting sides of the species debate, Tom Bruns (UC Berkeley) and Maarja Öpik (University of Tartu). The workshop included talks by the organizers and by Nicolas Corradi (University of Ottawa), John Taylor (UC Berkeley) and Dirk Redecker (Université de Bourgogne) in order to present a broad spectrum of ideas and results about species and related biology in this group of fungi. The session was well attended by the principals in the field and ample time was allocated for a discussion on Glomeromycotina, their biology, the ways that their species are recognized, and the future approaches to be taken in the field.

Do they have sex?

Sex is the primary means of genetic exchange in eukaryotic organisms, and it is the defining process for biological species concepts (De Queiroz, 2005). Gene flow that is enabled by sex within and between populations is also the basis of genetic concordance that is widely used to phylogenetically recognize species of fungi (Taylor *et al.*, 2006). For these reasons the presence or absence of sex is a critical feature of Glomeromycotina. However, their small size and obligate biotrophy have made them difficult to investigate, and many aspects of their basic biology remain unknown. Until recently it was widely believed that Glomeromycotina lacked sex and that their mycelium was heterokaryotic (i.e. it contained multiple dissimilar nuclei; Sanders, 1999; Croll & Sanders, 2009), and it was theorized that selection on the nuclear content within heterokaryotes might substitute for conventional sex (Angelard *et al.*, 2014; Wyss *et al.*, 2016). If, in this case, selection focused on individual nuclei and not an individual, species recognition could be decoupled from that used in other organisms. However, genomic data have now revealed indirect evidence of sex, produced evidence to counter the hypothesis of heterokaryosis, and placed the phylum back within a group of similar looking fungi where homology may help decipher mating behavior.

Corradi led the discussion on the evidence for sex in Glomeromycotina. The indirect evidence from genomic data includes recombination tracts, intact sets of meiotic genes, and homologues of mating type genes (Corradi & Brachmann, 2017). The proposed mating type genes were found to be surprisingly similar to those in the Basidiomycota, and the alleles (or idiomorphs) are found in separate haploid nuclei present in either homokaryotic or dikaryotic mycelium (i.e. cells that contain only one type of nuclei vs two; Ropars *et al.*, 2016). With this evidence for cryptic sex, Glomeromycotina are in step with other fungi that were long thought to be asexual but with new data were found to exhibit the traits of sexual reproduction (Taylor *et al.*, 2015).

While the evidence for sex is growing, the evidence for heterokaryosis (multiple types of different nuclei/cell) is slowing unraveling. There were a few researchers who were skeptical about heterokaryosis and had reported results that contradicted its presence over a decade ago (Pawlowska & Taylor, 2004; Stukenbrock & Rosendahl, 2005), but now new genomic evidence has begun to sway many others (Corradi & Brachmann, 2017). Strains purported to be heterokaryotic (Boon *et al.*, 2015; Wyss *et al.*, 2016) were re-examined and shown to be haploid and either monokaryotic or dikaryotic (Ropars *et al.*, 2016). Variation within the genomes was found to be quite low and similar to that of other

nonheterokaryotic fungi. The genome size was originally estimated to be quite small – too small it was argued to contain all the gene variants found, thus the variation had to be located between nuclei (Hijri & Sanders, 2005), but assembled genomes and recent flow cytometry analyses (Sędzielewska *et al.*, 2011) have now shown their genomes to be large by fungal standards, and loaded with repeated elements and paralogue gene copies (Lin *et al.*, 2014; Tisserant *et al.*, 2014). No one at the meeting questioned the new evidence, although Ian Sanders (University of Lausanne) pointed out that some data that supports heterokaryosis could still come from species that have not been studied with genomic approaches. The primary example is the *in situ* florescent hybridization evidence of Kuhn *et al.* (2001), a report that is widely cited, but was never universally accepted (Pawlowska & Taylor, 2004; Stukenbrock & Rosendahl, 2005). The consensus at the meeting was that these results now need to be re-examined with modern sequencing techniques and data analysis approaches.

How unique are these fungi?

Bruns discussed the changing views on the phylogeny of Glomeromycotina and how it affects our expectations for these fungi. The early molecular systematic work on Glomeromycotina made these fungi look like a unique phylum that was the sister group to the Dikarya (the Ascomycota and Basidiomycota). This view was initially based on rDNA loci (Schussler *et al.*, 2001) and was later reinforced by a large multigene phylogeny (James *et al.*, 2006), though strong support for the sister group relationship was not provided by these data. Nevertheless, this placement strongly influenced the perception of the group as unique, and it justified the idea that precedents from other fungi did not apply. Although the results conflicted with those from nuclear protein phylogenies (Redecker & Raab, 2006) and the mitochondrial genome data (Lee & Young, 2009), these alternative views were largely ignored. Now nuclear genomic data have yielded a new analysis based on 192 orthologous genes that places Glomeromycotina as a subphylum within Mucoromycota equally distant from the two other subphyla, Mucoromycotina and Mortierellomycotina (Spatafora *et al.*, 2016). However, the relationship among these three subphyla remains unresolved and several taxonomists at the workshop argued that the phylum name Glomeromycota should be retained. Taxon coverage for fully sequenced genomes within Glomeromycotina is currently very sparse, and expanding coverage of basal lineages in this group and in the other subphyla may help resolve these relationships.

This changing perception of phylogeny puts Glomeromycotina back into a more traditional placement within the fungi (Gerdemann & Trappe, 1974), and with this move comes the expectation that they may behave more like their relatives. In particular, the large 'azygospores' that some Glomeromycotina produce are likely to be homologous to zygospores, the site of meiosis in other Mucoromycotina. The problem with these spores is that they are formed from multinuclear hyphae and yield a multinuclear spore – without a single nucleus stage, how can traditional meiosis function? It turns out that this problem is not unique to Glomeromycotina; it is also shared by their relatives in the Mucoromycotina. They too have multinucleate haploid hyphae that fuse, the nuclei disappear into a large zygosporangium, and later yields multiple recombinant spores, but in this case the fungi grow well in culture. As a result, genetic evidence in *Phycomyces* showed decades ago that the recombinant progeny are generally consistent with a single meiosis followed by post-meiotic mitoses (Eslava *et al.*, 1975). Thus, although a single nuclear state is never seen, it must occur in *Phycomyces*, and by homology one can be expected in Glomeromycotina as well.

In addition, the similarity with other fungi again brings into question the heterokaryotic state, which has no precedent in any other fungal group. Indeed, heterokaryosis outside the highly regulated dikaryon in the sexual cycle of Basidiomycota or Ascomycota is typically a lethal condition in other fungi, where somatic incompatibility systems trigger programmed cell death when genotypes differ at any of the highly polymorphic het loci (Aanen *et al.*, 2010). This behavior is consistent with evolutionary biology arguments that nonself fusions are risky endeavors that could lead to nuclear take-overs or parasitism (Rayner, 1991). Although it is possible that members of Glomeromycotina have uniquely evolved around this problem, it is certainly not the simplest explanation for the conflicting results.

Different species concepts and why we need to care

With this background in mind Taylor presented data on species recognition in *Neurospora*, a model member of the Ascomycota. His talk built on the idea that species can be recognized by multilocus sampling of populations, where well supported clades indicate the limits of frequent genetic exchange (Taylor *et al.*, 2006). Applying this concept to *Neurospora* he showed that no single genetic marker, including internal transcribed spacer (ITS region), was sufficient to identify all the species, and that gene flow, even in this wind dispersed fungus, was limited by geographic barriers (Ellison *et al.*, 2011). These results related back to the controversy, which spawned this workshop, concerning the meaning of worldwide distributions of small subunit (SSU)-rDNA-defined species in Glomeromycotina. If the experience with *Neurospora* and other fungi is relevant, then currently recognized Glomeromycotina species are likely to be collections of related species. Bruns & Taylor (2016) argued that comparing these species from the Glomeromycotina to those that are much better resolved in plants and animals is invalid, and leads to the erroneous conclusions that there is little endemism and dispersal limitation. This is one reason why we need to care about species concepts – if species are hugely different categories in different groups, then comparing them across groups leads one astray. While Öpik *et al.* (2016) did not agree that current species in Glomeromycotina are likely to be collections of cryptic phylogenetic species, both groups concede that resolution of this issue will require multilocus data from population-level samples of what we now consider to be single species.

One additional point of general agreement was that different species concepts and methods for species recognition are unavoidable and necessary. In fact, this was a point of agreement even in the exchange of *Science* letters that motivated this workshop (Bruns &

Taylor, 2016; Öpik *et al.*, 2016). For example, when using high-throughput sequence technology for ecological questions we are currently limited to single locus identifications. In addition, the locus needs to have highly conserved priming sites and a high copy number to increase sensitivity in environmental settings. These constraints have largely limited the choices to rDNA loci: SSU, large subunit (LSU) or ITS. Although alternatives have occasionally been proposed (Raab *et al.*, 2005; Borstler *et al.*, 2008; Stockinger *et al.*, 2014), the lack of databases have left the field dominated by nuclear rDNA loci. One could call this the 'utilitarian species concept', but is more correctly referred to as operational taxonomic units (OTUs) or virtual taxa (VT). These are useful because they often correlate with species defined in other ways and as a result capture some of the species signal in a consistent way. In addition, these taxa allow us to compare species behavior across studies and assemble information about host-range, soil preferences, genetics, geographic distributions, and so on, and to test predictions of community ecology about how deterministic and stochastic processes affect species richness and composition and how these parameters affect ecosystem functions. If these utilitarian species lump cryptic phylogenetic species, it may reduce the resolving power of our ecological studies, but as long as they capture enough signal for the needs of the study they serve an absolutely indispensible purpose.

Molecular and morphological species

Currently the best reference database for Glomeromycotina is for the SSU locus (Öpik *et al.*, 2010; Öpik & Davison, 2016). Öpik discussed the use of this database and showed that most of the 'virtual taxa' (or OTUs) are known only from environmental sequences. This fact helps to emphasize how poorly this important group of fungi is known. It also underscores the value of a conservative marker, like SSU, that allows one to place unknown sequences into phylogenetic framework – something that ITS region alone cannot consistently do. Even if the SSU-based species estimates are highly conservative, they often correlate with current morphologically defined species. Operationally, that correlation means that species estimates by the SSU marker have similar, broad resolution to morphological approaches. However, rDNA analysis has the advantage of being faster at large scale and not requiring sporulation to recognize and detect new species. Further, SSU-based species correlated well with a set of ecological factors (Powell *et al.*, 2011). Therefore, the advantage of refined species concepts in ecological settings will depend critically on their ability to expand the set and significance of such correlations. This criterion embraces the idea of Freudenstein *et al.* (2017) that considering the species 'role' in the environment needs to be part of the species recognition process.

Proponents of the LSU and ITS regions discussed the higher resolution of these loci compared to SSU, and pointed out multiple exceptions to the correlation between SSU alone and morphological species. Problems with divergent copies of the ITS within single genomes quelled some of the enthusiasm for that locus. However, this is not a problem that is unique to ITS; all of the rDNA loci, including SSU, have divergent copies (Thiery *et al.*, 2016). The longer read lengths now available with PacBio sequencing platforms were mentioned as a possible solution as all three rDNA loci can be sequenced as a single amplicon (e.g. Schlaeppi *et al.*, 2016). Such a solution had been previously suggested (Öpik & Davison, 2016). However, the increased resolution of PacBio sequencing would be purchased at the price of much lower read depth per sample, and this may limit its use in ecological settings unless technical progress eliminates this trade-off.

How morphologically defined Glomeromycotina species will correlate with species defined by multilocus genetics remains unanswered, but the limitations and difficulties with the current morphological taxonomy were clarified by Redecker. What was clear is that current taxonomy is based on a limited number of relatively subtle spore differences. Furthermore, homologous structures are often not easy to discern because some species can make two or more types of spores, and this will likely complicate the search for the site of meiosis. In most current species descriptions, morphological analysis is supported by sequencing and phylogenetic analysis of a combination of SSU, ITS and LSU. In particular, the rDNA repeat fragment proposed by Krüger *et al.* (2009) is increasingly used and in some studies, complemented with rpb1. Rarely is SSU used alone in current Glomeromycotina taxonomic studies because of its limited resolution. The limited characters and difficulties with traditional morphology paint a picture of morpho-species that are in many cases likely to contain collections of related species that cannot be separated by morphology or ribosomal DNA sequencing alone. In this way they again would be similar to other groups of fungi where the presence of cryptic species are well documented (Taylor *et al.*, 2006), but in Glomeromycotina there are no data to address the issue, yet.

The way forward

Many important questions about the basic biology and species recognition within Glomeromycotina remain unanswered, but the participants of the workshop left with a feeling that the critical problems had been laid out in an open forum. The overall conclusion was that the pervasive assumptions about biology and species limits of arbuscular mycorrhizal fungi need to be re-examined, and results from earlier studies need to be scrutinized and repeated with current genomic methods. The hope for achieving this goal lies in part on the growing repertoire of new molecular and genomic techniques, including comparative analyses of single nuclei, but it also depends on support for the field expanding. To that end, the inescapable conclusion is that it is certainly a great time for new researchers to join this field with fresh ideas and new approaches!

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Author contributions

TDB and MÖ organized the meeting at ICOM9. TDB, NC, DR, JWT and MÖ participated in the meeting and in an extended discussion that led to this manuscript. TDB wrote the draft, and all authors contributed to shaping it into the final version.

Thomas D. Bruns[1]*, Nicolas Corradi[2], Dirk Redecker[3], John W. Taylor[1] and Maarja Öpik[4]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA;
[2]Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5 Canada;
[3]Agroécologie, Université de Bourgogne Franche-Comté, INRA, CNRS, AgroSup Dijon, F-21000 Dijon, France;
[4]University of Tartu, 40 Lai Street, 51005 Tartu, Estonia
(*Author for correspondence: tel +1 510 642 7987; email pogon@berkeley.edu)

References

Aanen DK, Debets AJM, Glass NL, Saupe SJ. 2010. Biology and genetics of vegetative incompatibility in fungi. In: Borkovich KA, Ebbole DJ, eds. *Cellular and molecular biology of filamentous fungi*. Washington, DC, USA: ASM Press, 274–288.

Angelard C, Tanner CJ, Fontanillas P, Niculita-Hirzel H, Masclaux F, Sanders IR. 2014. Rapid genotypic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation. *ISME Journal* **8**: 284–294.

Boon E, Halary S, Bapteste E, Hijri M. 2015. Studying genome heterogeneity within the arbuscular mycorrhizal fungal cytoplasm. *Genome Biology and Evolution* **7**: 505–521.

Borstler B, Raab PA, Thiery O, Morton JB, Redecker D. 2008. Genetic diversity of the arbuscular mycorrhizal fungus *Glomus intraradices* as determined by mitochondrial large subunit rRNA gene sequences is considerably higher than previously expected. *New Phytologist* **180**: 452–465.

Bruns TD, Taylor JW. 2016. Fungal symionts comment on "Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism". *Science* **35**: 826.

Corradi N, Brachmann A. 2017. Fungal mating in the most widespread plant symbionts? *Trends in Plant Science* **22**: 175–183.

Croll D, Sanders IR. 2009. Recombination in *Glomus intraradices*, a supposed ancient asexual arbuscular mycorrhizal fungus. *BMC Evolutionary Biology* **9**: 924–937.

Davison J, Moora M, Öpik M, Adholeya A, Ainsaar L, Ba A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T *et al.* 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**: 970–973.

De Queiroz K. 2005. Ernst Mayr and the modern concept of species. *Proceedings of the National Academy of Sciences, USA* **102**(Suppl 1): 6600–6607.

Ellison CE, Hall C, Kowbel D, Welch J, Brem RB, Glass NL, Taylor JW. 2011. Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *Proceedings of the National Academy of Sciences, USA* **108**: 2831–2836.

Eslava AP, Alvarez MI, Delbruck M. 1975. Meiosis in *Phycomyces*. *Proceedings of the National Academy of Sciences, USA* **72**: 4076–4080.

Freudenstein JV, Broe MB, Folk RA, Sinn BT. 2017. Biodiversity and the species concept-lineages are not enough. *Systematic Biology* **66**: 644–656.

Gerdemann JW, Trappe JM. 1974. The Endogonaceae in the Pacific Northwest. *Mycological Memoirs* **5**: 1–76.

Hijri M, Sanders IR. 2005. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature* **433**: 160–163.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miądlikowska J *et al.* 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**: 818–822.

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist* **183**: 212–223.

Kuhn G, Hijri M, Sanders IR. 2001. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* **414**: 745–748.

Lee J, Young JPW. 2009. The mitochondrial genome sequence of the arbuscular mycorrhizal fungus *Glomus intraradices* isolate 494 and implications for the phylogenetic placement of *Glomus*. *New Phytologist* **183**: 200–211.

Lin K, Limpens E, Zhang ZH, Ivanov S, Saunders DGO, Mu DS, Pang EL, Cao HF, Cha HH, Lin T *et al.* 2014. Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS Genetics* **10**: e1004078.

Öpik M, Davison J. 2016. Uniting species-and community-oriented approaches to understand arbuscular mycorrhizal fungal diversity. *Fungal Ecology* **24**: 106–113.

Öpik M, Davison J, Moora M, Partel M, Zobel M. 2016. Fungal symionts response to comment on "Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism". *Science* **351**: 970–973.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier U, Zobel M. 2010. The online database Maarj*AM* reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223–241.

Pawlowska TE, Taylor JW. 2004. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* **427**: 733–737.

Powell JR, Monaghan MT, Öpik M, Rillig MC. 2011. Evolutionary criteria outperform operational approaches in producing ecologically relevant fungal species inventories. *Molecular Ecology* **20**: 655–666.

Raab PA, Brennwald A, Redecker D. 2005. Mitochondrial large ribosomal subunit sequences are homogeneous within isolates of *Glomus* (arbuscular mycorrhizal fungi, Glomeromycota). *Mycological Research* **109**: 1315–1322.

Rayner ADM. 1991. The challenge of the individualistic mycelium. *Mycologia* **83**: 48–71.

Redecker D, Raab P. 2006. Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* **98**: 885–895.

Ropars J, Toro KS, Noel J, Pelin A, Charron P, Farinelli L, Marton T, Kruger M, Fuchs J, Brachmann A *et al.* 2016. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. *Nature Microbiology* **1**: 1–9.

Sanders IR. 1999. Evolutionary genetics – no sex please, we're fungi. *Nature* **399**: 737–739.

Schlaeppi K, Bender SF, Mascher F, Russo G, Patrignani A, Camenzind T, Hempel S, Rillig MC, van der Heijden MGA. 2016. High-resolution community profiling of arbuscular mycorrhizal fungi. *New Phytologist* **212**: 780–791.

Schussler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.

Sędzielewska KA, Fuchs J, Temsch EM, Baronian K, Watzke R, Kunze G. 2011. Estimation of the *Glomus intraradices* nuclear DNA content. *New Phytologist* **192**: 794–797.

Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A *et al.* 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* **108**: 1028–1046.

Stockinger H, Peyret-Guzzon M, Koegel S, Bouffaud ML, Redecker D. 2014. The largest subunit of RNA polymerase II as a new marker gene to study assemblages of arbuscular mycorrhizal fungi in the field. *PLoS ONE* **9**: e107783.

Stukenbrock EH, Rosendahl S. 2005. Development and amplification of multiple co-dominant genetic markers from single spores of arbuscular mycorrhizal fungi by nested multiplex PCR. *Fungal Genetics and Biology* **42**: 73–80.

Taylor JW, Hann-Soden C, Branco S, Sylvain I, Ellison CE. 2015. Clonal reproduction in fungi. *Proceedings of the National Academy of Sciences, USA* **112**: 8901–8908.

Taylor JW, Turner E, Townsend JP, Dettman JR, Jacobson D. 2006. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. *Philosophical Transactions of the Royal Society B – Biological Sciences* **361**: 1947–1963.

Thiery O, Vasar M, Jairus T, Davison J, Roux C, Kivistik PA, Metspalu A, Milani L, Saks U, Moora M *et al.* 2016. Sequence variation in nuclear ribosomal small subunit, internal transcribed spacer and large subunit regions of *Rhizophagus irregularis* and *Gigaspora margarita* is high and isolate-dependent. *Molecular Ecology* **25**: 2816–2832.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frey NFD, Gianinazzi-Pearson V *et al.* 2014. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis (vol 110, pg 20117, 2013). *Proceedings of the National Academy of Sciences, USA* **111**: 563.

Wyss T, Masclaux FG, Rosikiewicz P, Pagni M, Sanders IR. 2016. Population genomics reveals that within-fungus polymorphism is common and maintained in populations of the mycorrhizal fungus *Rhizophagus irregularis*. *ISME Journal* **10**: 2514–2526.

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