Fungal Genomics & Biology
ISSN: 2165-8056

Gryganskyi and Muszewska, Fungal Genom Biol 2014, 4:1
DOI: 10.4172/2165-8056.1000e116

Editorial Open Access

Whole Genome Sequencing and the Zygomycota

Andrii P Gryganskyi[1]* and Anna Muszewska[2]

[1]Department of Biology, Duke University, Durham, North Carolina, USA
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The zygomycetes (polyphyletic phylum Zygomycota Moreau) include the first terrestrial and "primitive" fungi. The group is an assemblage of six lineages whose status, relative to each other, is undefined: Mucoromycotina, Entomophthoromycota, Kickxellomycotina, Zoopagomycotina, Mortierellomycotina and Glomeromycota. So far, less than a dozen zygomycetes genomes are publically available, a very small proportion of the total number of sequenced genomes – there are ~400 Genomes for Dikarya (Ascomycota and Basidiomycota).

There are several probable reasons for this. Firstly, there are substantially fewer described species of early divergent fungi in comparison with those that have evolved more recently. There are nearly one thousand valid taxa names for zygomycetes (~1% of all known fungi), compared to over 30,000 taxa in each of the Ascomycota and Basidiomycota. However given zygomycetes are understudied, the diversity of this group might be much higher, as was recently shown for Cryptomycota [1]. Another reason there has been less interest in the zygomycetes from a genomic perspective is that dikaryotic fungi play more significant roles in human life. Many of these are important pathogens of humans, domestic animals and agricultural plants, biotechnology agents, edible mushrooms and model organisms. In addition, the representatives of Dikarya that produces fruit bodies drew early attention and were more straightforward to study. Zygomycota have been considered less important, however many of their taxa are ubiquitous and also play vital roles in ecosystems and human life. Here, we introduce subphyla, which have been grouped within Zygomycotas.

Mucoromycotina

Mucoralean fungi are the most numerous and the best known clade in the Zygomycota; nearly 300 species are known. They are common in all soils, rapidly colonizing any easily degradable carbohydrate or protein source; therefore most of them are easy to grow under laboratory conditions. Eight genomes have been sequenced so far – more than in any other clade [2-4]. Several more genomes will be released soon [2,4,5].

Mucoralean genomes are interesting, firstly from a medical perspective. It is notable that the number of mucormycoses with fatal outcomes is growing every year. A major reason for this is the introduction of newer anti-fungals (azoles). These have facilitated successful control of other infective fungal agents (candidioses and aspergilloses), generating new opportunities for Mucoromycotina which can infect humans and which do not respond to these treatments [6]. Additionally, it is likely that some of the mucoralean fungi can be opportunistic pathogens in animals and humans under certain conditions due to their ability to grow at body temperature and dimorphic growth potential [7]. Especially endangered are immunocompromised patients with conditions such as hematological malignancies, neutropenia or uncontrolled ketoacidosis in diabetes. Patients are also vulnerable to infections by direct inoculation via burns, car accidents or nosocomial transmissions [6]. Sequencing pathogenic fungi and their non-pathogenic relatives, to elucidate genetic regions of pathogenicity, would be particularly useful. Such pathogenic and non-pathogenic 'pairs' can be easily found in the genera *Mucor, Lichtheimia*, *Rhizopus* and others. Some of the Mucoromycotina are also important crop pathogens, especially known for post-harvest diseases of sweet potatoes, strawberries and other agricultural plants. However this group can be useful for food production and biotechnological purposes. Many of the mucoralean fungi have been used for centuries for many fermented foods and drinks and nowadays are also used as vigorous producers of various secondary metabolites: enzymes, fatty acids and carotenoids.

Mortierellomycotina

There are nearly 100 known species of these fungi, and aside from the genus *Modicella*, they are easy to grow in culture. As with other zygomycetes, studies using environmental sequencing suggest Mortierellomycotina might contain unculturable and currently undescribed microscopic fungi. All species in this subphylum are common and ubiquitous soil dwellers and saprotrophs, some of them are also plant associates and endophytes [8]. *Mortierella wolfii* is a cattle pathogen which causes abortive infections and in rare cases leads to disseminated systemic infections [9].

Obtaining genomic information would aid efforts to elucidate the structure of this clade, which rDNA data alone are unable to resolve [10]. The current molecular phylogeny of Mortierellomycotina shows that the dominating genus *Mortierella* contains several other genera, which are very different morphologically, but which have similar rDNA. Genus *Mortierella* needs a thorough revision based on at least a multiple gene phylogeny. Genome sequencing of mortierellalean fungi would also elucidate the origin of fungal fruit bodies, which apparently first occurred in this group [11]. Genome data will help us to understand the role of these fungi in natural ecosystems, and to utilize their industrial potential (production of poly-unsaturated fatty acids) more efficiently. Genome and transriptome data are available now for three species: *Mortierella verticillata* [12], *M. elongata* [13], and *M. alpina* [14].

Entomophthoromycota

This is the second largest group of zygomycetes with ca. 300 species, including saprotrophic and entomopathogenic zygomycetes. Only one genome is available: *Conidiobolus coronatus* [15]. Three more are currently being sequenced through the 1000 Fungal Genomes project [16]: *Basidiobolus meristosporus*, *Conidiobolus thromboides* and *Zoophthora radicans*. These three taxa represent three major fungal

***Corresponding author:** Andrii P Gryganskyi, Department of Biology, Duke University, USA, Tel: 919 536 9702; E-mail: apg10@duke.edu

Received January 10, 2014; **Accepted** January 20, 2014; **Published** January 24, 2014

Citation: Gryganskyi AP, Muszewska A (2014) Whole Genome Sequencing and the Zygomycota. Fungal Genom Biol 4: e116. doi:10.4172/2165-8056.1000e116

clades in Entomophthoromycota: saprotrophic Basidiobolomycetes, saprotrophic and facultatively pathogenic Ancylistaceae and the 'core' obligately entomopathogenic Entomophthoraceae [17,18]. Genome information from pathogens of economically important crop pests will provide insight into pathogenicity mechanisms, and could improve the use of these fungi as bio-control agents. Some *Basidiobolus* species are known to infect humans [19]. Besides being of practical interest, sequencing these genomes will help to resolve questions around the origin of terrestrial fungi, because Entomophthoromycota is probably one of the earliest terrestrial fungal groups [20].

Glomeromycota

This clade is famous for arbuscular mycorrhizae (AM) formation and the ~430 MYA old fossils generated by its ancestors. The morphology of these fossilized species is remarkably similar to that seen in current AM fungi [21,22]. All of the ca. 250 described species can grow only on the roots of host plants. This makes harvesting sufficient amounts of biomass for DNA extraction difficult. Genome and transcriptome data are available only for *Rhizophagus irregularis* (formerly *Glomus intraradices*) [23]. In general, the genome assemblies done for this fungus thus far are of poor quality relative to those available for other fungi. The relatively large size of this group's asexual spores (up to 800 um in diameter) and the thousands of genetically identical nuclei they contain [24] means new techniques of genome sequencing from one cell are very promising for the Glomeromycota. Sequencing genomes of glomeralean fungi is of great importance for forestry and agriculture, because at least 80% of vascular plants host AM fungi [24]. Besides being of practical interest, genome information will help place Glomeromycota on the Fungal Tree of Life. Former zygomycetes, they have recently been separated from this group and placed as a sister group to Dikarya based on rDNA phylogeny [25]. However, phylogenies based on multiple orthologs suggest Glomeromycota are closely related to Mortierellomycotina and Mucoromycotina (Bonito; Gryganskyi, unpublished).

Kickxellomycotina and Zoopagomycotina

Despite there being a relatively high number of described taxa in these two related fungal groups (nearly 180 species in each subphylum), only one genome has been sequenced: *Coemansia reversa* [26]. Many of these fungi are parasites of invertebrates, commensal of arthropod guts or saprotrophs. Some of them are of importance as pathogens of insect pests. Sequencing their genomes would be useful from a bio-control perspective. The majority of these fungi are hard to grow in vitro, but several coprophilic species are cultivable on standard media. Several species of this subphylum will be sequenced within 1KFG project [16].

Common Problems in Zygomycete Sequencing

There are several major obstacles hindering Zygomycota genome projects. Everything starts with cultivation, which is feasible mostly for saprotrophs and pathogens with a broad host range (most of Mucoromycotina, genera *Basidiobolus* and *Conidiobolus* (Entomophthoromycota); *Kickxella, Dimargaris* and *Coemansia* (Kickxellomycotina)). Obligate parasites or commensal of arthropods are either unculturable or need special conditions for their growth in the lab. Many of them loose their vigor after several culture transfers, therefore obtaining nucleic acids in sufficient quantities is complicated. Many representatives of entomophthoraleans and some Mortierellas develop 'empty' colonies, which actively grow only at the colony edge. This eventually reduces the output of DNA, even when a relatively large amount of biomass can be harvested.

Another reason for the difficulties in obtaining sufficient quantity and quality of DNA and RNA is perhaps the high activity level of DNAses and RNAses in this group. Common extraction protocols like CTAB-chloroform extraction do not inhibit these enzymes, and nucleic acids after extraction are usually either highly degraded or hardly visible on gels. In order to obtain good quality nucleic acids standard extraction kits need to be modified by adding higher amounts of nuclease inhibitors, reducing of some of the incubation steps and performing additional cleaning to remove protein and phenolic contamination.

The genome sizes, ploidy and karyotypes for most fungi are unknown. It is generally assumed that zygomycetes are always haploid, making the assembly of their genome sequences easier than for diploid organisms. However, this still needs to be proven. Recent research and the discovery of genome duplication events in the zygomycota indicate that: 1) their genomes might be much bigger, on average, than those of other fungi; 2) whole genomes or significant parts of them might be duplicated which might complicate genome assembly [27]. For example, there are some indirect estimates of genome size for two entomophthoralean fungi: *Basidiobolus* (350-750 Mb) and *Entomophaga* (800 Mb) [28]. This is substantially higher than the average genome size of other fungi whose genomes have been sequenced, which is usually between 10 and 60 Mb [29].

The quality of genome data for zygomycetes, as for fungi in general, needs improvement. None of the sequences obtained from zygomycetes are assigned to chromosomes (as in *Saccharomyces cerevisiae* and *Aspergillus nidulans*). As for many fungi, the number of chromosomes is unknown. For some of them, the nuclear chromatin is never condensed during nuclear division. Karyotyping methods based on pulsed field gel electrophoresis often fail to separate fungal chromosomes. For some species, different varieties might have significant variation in chromosome numbers, one example being *Rhizopus arrhizus* [30]. Only a few sequenced genome databases contain separated mitochondrial genomes, in most cases mitochondrial contigs and scaffolds are not annotated. Many zygomycetes have bacterial endosymbionts, consequently their genome projects are in fact metagenome projects!

All these reasons make zygomycete genome sequencing, assembly and annotation complicated and delay genomic studies. Despite this, the number of fungal sequencing projects for this group increases each year, partly because of progress in sequencing and bioinformatics techniques and ongoing reductions in sequencing costs. A number of laboratories are currently sequencing and annotating genomes (Timothy James, Rytas Vilgalys and Kerstin Voigt, personal communication). For example, there is an interesting project underway at Broad Institute to sequence all known zygomycetous pathogens, which have been recorded infecting humans and other mammals. Most of these are mucoralean fungi, but some entomophthoroid fungi are also included [31]. The results of these studies will advance our knowledge of the early diverging fungi and it would be great to see more work take place in this area.

The authors are really thankful to Renee Johansen for her help with the manuscript.

References

1. James TY, Pelin A, Bonen L, Ahrendt S, Sain D et al. (2013) Shared signatures of parasitism and phylogenomics unite cryptomycota and microsporidia. Curr Biol 23: 1548-1553.

2. (1997) Genome Portal version: 7.0.85.5b0f342. DOE Joint Genome Institute.

3. (2005) Fungal Genomics Project. Concordia University.
4. (2004) Mucorales database. Broad Institute.
5. (2009) Genozymes project. In: Genome Canada GQ, and Genome Alberta, editor: Concordia University - Centre for Structural and Functional Genomics.
6. Skiada A, Pagano L, Groll A, Zimmerli S, Dupont B, et al. (2011) Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. Clin Microbiol Infect, 17: 1859-67.
7. Lee SC, Li A, Calo S, Heitman J (2013) Calcineurin plays key roles in the dimorphic transition and virulence of the human pathogenic zygomycete Mucor circinelloides. PLoS Pathog 9: e1003625.
8. Salt GA (1977) The Incidence of Root-Surface Fungi on Naturally Regenerated *Picea sitchensis* Seedlings in Southeast Alaska. Forestry 50: 113-115.
9. Davies JL, Ngeleka M, Wobeser GA (2010) Systemic infection with Mortierella wolfii following abortion in a cow. Can Vet J 51: 1391-1393.
10. Wagner L, Stielow B, Hoffmann K, Petkovits T, Papp T, et al. (2013) A comprehensive molecular phylogeny of the Mortierellales (Mortierellomycotina) based on nuclear ribosomal DNA. Persoonia 30: 77-93.
11. Smith ME, Gryganskyi A, Bonito G, Nouhra E, Moreno-Arroyo B, et al. (2013) Phylogenetic analysis of the genus Modicella reveals an independent evolutionary origin of sporocarp-forming fungi in the Mortierellales. Fungal Genet Biol 61: 61-68.
12. (2007) Origins of Multicellularity Database. Broad Institute.
13. (2012) Mortierella elongata v1.0. DOE Joint Genome Institute.
14. Wang L, Chen W, Feng Y, Ren Y, Gu Z, et al. (2011) Genome characterization of the oleaginous fungus Mortierella alpina. PLoS One 6: e28319.
15. (2012) Conidiobolus coronatus NRRL28638 v1.0. DOE Joint Genome Institute.
16. Spatafora J, Stajich JE (2012) 1000 Fungal Genomes Project. Wordpress.
17. Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, et al. (2013) Phylogenetic lineages in Entomophthoromycota. Persoonia 30: 94-105.
18. Gryganskyi AP, Humber RA, Smith ME, Miadlikowska J, Wu S, et al. (2012) Molecular phylogeny of the Entomophthoromycota. Mol Phylogenet Evol 65: 682-694.
19. Vikram HR, Smilack JD, Leighton JA, Crowell MD, De Petris G (2012) Emergence of gastrointestinal basidiobolomycosis in the United States, with a review of worldwide cases. Clin Infect Dis 54: 1685-1691.
20. Humber RA (2008) Evolution of entomopathogenicity in fungi. J Invertebr Pathol 98: 262-266.
21. Simon L, Bousquet J, Levesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Letters to Nature 363: 67-69.
22. Krings M, Taylor TN, Dotzler N (2013) Fossil evidence of the zygomycetous fungi Persoonia 30: 1-10.
23. (2008) Glomus intraradices DB v. 2.0. INRA.
24. Redecker D, Raab P (2006) Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. Mycologia 98: 885-895.
25. Blaszkowski, Renker C, Buscot F (2006) Glomus drummondii and G. walkeri, two new species of arbuscular mycorrhizal fungi (Glomeromycota). Mycol Res 110: 555-566.
26. (2012) Coemansia reversa NRRL 1564 v1.0. DOE Joint Genome Institute.
27. Ma LJ, Ibrahim AS, Skory C, Grabherr MG, Burger G, et al. (2009) Genomic analysis of the basal lineage fungus Rhizopus oryzae reveals a whole-genome duplication. PLoS Genet 5: e1000549.
28. Henk DA, Fisher MC (2012) The gut fungus Basidiobolus ranarum has a large genome and different copy numbers of putatively functionally redundant elongation factor genes. PLoS One 7: e31268.
29. Gregory TR, Nicol JA, Tamm H, Kullman B, Kullman K, et al. (2007) Eukaryotic genome size databases. Nucleic Acids Res 35: 332-338.
30. Min B-R, Lee TJ, Choi Y-K (1982) Chromosomal Studies on the genus of Rhizopus. Korean Journal of Microbiology 20: 134-146.
31. Bruno VM (2009) Comparative Genome Analysis of Mucormycoses-causing Strains Of From Clinical Settings. Genomic Sequencing Center for Infectious Diseases, University of Maryland.