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SHORT COMMUNICATION

The largest fungal genome discovered in *Jafnea semitosta*

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Abstract *Jafnea semitosta* is an ascomycete (Pyronemataceae, Pezizales) originating from North America and spreading uncommonly in Europe. Its genome size was measured via flow cytometry of fruiting bodies from five localities in the Czech and Slovak Republic. The nuclear 1*C* DNA content was estimated at 3.706 ± 0.011 pg (~3.625 ± 0.011 Gbp) which represents the highest value ever reported for fungi and 100× higher than the average. Generally, the genome inflation in fungi appears to be driven mainly by proliferation of repetitive sequences, but polyploidy should also be considered in further studies on this greatly unexplored topic.

Keywords Ascomycetes · Flow cytometry · Genome size · *Jafnea semitosta*

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Introduction

Genome size data are used in many biological disciplines. Their availability is nevertheless limited, especially in some phyla. For instance, the current Plant DNA *C*-values Database contains entries of around 8500 species (Bennett and Leitch 2012–2017a), of which only 253 are algae (Kapraun et al. 2004–2017), 232 bryophytes (Greilhuber et al. 2010–2017), 128 pteridophytes (Bennett and Leitch 2012–2017b) and 355 gymnosperms (Murray et al. 2012–2017). Similarly, Animal Genome Size Database contains 6222 species, mostly vertebrates (3793; Gregory 2005–2017). Fungi appear to be explored even more poorly in absolute numbers; the Fungal *C*-value Database includes 1940 entries of 1145 species (790 species of Ascomycota, 317 Basidiomycota and 38 species of other phyla; Kullman et al. 2005–2016). It represents around 1.2% of cca 98,000 species of fungi so far described (more than 64,000 belong to Ascomycota and over 31,500 species to Basidiomycota; Kirk et al. 2008). Moreover, many values are based on inappropriate methods, such as DAPI-based cytometry which leads to erroneous estimates where AT:GC ratio differs between the sample and the reference standard (Doležel et al. 1992). Another source of error is the use of inappropriate standard. The genome size of the standard should be known as accurately as possible and should not differ too much from the sample (especially in flow cytometry), among other requirements (Greilhuber et al. 2007). Unfortunately, re-evaluation of some commonly used cytometric standards (either the same genotypes or the same species) has led to significantly different DNA content values than considered previously and caused much confusion (see, e.g., Kullman 2000 vs. Kullman et al. 2005–2016). Because even the whole-genome sequencing may be insufficient for precise genome size estimation

(Doležel and Greilhuber 2010), many of the available data remain questionable. Our understanding of fungal genome size variation and the underlying processes is thus far from sufficient.

In comparison with other eukaryotes, fungal genomes are generally very small. While the nuclear 1*C* DNA content ranges between 0.01 and 152.20 pg in plants (Bennett and Leitch 2012–2017a) and 0.02 and 132.83 pg in animals (Gregory 2005–2017), it is only 0.007 pg (Kullman et al. 2005–2016) to 2.545 pg (Ramos et al. 2015) in fungi, with ~90% of the available fungal data lying within the range 0.01–0.06 pg (Gregory et al. 2007). When including microsporidia, the lower limit falls even to 0.002 pg (Corradi et al. 2010; Pombert et al. 2012). So far, the largest fungal genomes have been discovered in rusts (Pucciniales, Basidiomycota) with the average value of 0.359 pg (Ramos et al. 2015). This order, which is one of the largest ones among fungi, includes more than 7000 species (Kirk et al. 2008) and comprises species with the largest genomes hitherto known—*Puccinia chrysanthemi* having 0.825 pg, *Gymnosporangium confusum* 0.913 pg (Tavares et al. 2014) and *Uromyces bidentis* 2.544 pg (Ramos et al. 2015), so far the largest recognized fungal genome. Extraordinarily large genome sizes were reported also for Pezizales (Ascomycota), which were studied mainly by Weber (1992) via photometric cytometry with DAPI staining. In 85 studied species, she reported the values between 19 a.u. (arbitrary units) in *Rhizina undulata* and 958 or 982 a.u. in *Neottiella rutilans*, using *Morchella esculenta* as a reference standard; these values were converted by Kullman et al. (2005–2016) to 0.016–0.818 pg, based on *Morchella conica* draft genome assembly size of 48.21 Mbp (P. Baldrian unpublished data, available at http://genome.jgi.doe.gov). Nevertheless, the former standardization with *Saccharomyces cerevisiae* resulted in values 0.008–0.430 pg (8.1–421 Mbp; Kullman 2000). Kullman (2002) also reported large values in *Neotiella vivida* and *N. rutilans*—~0.77 and 0.54 pg, respectively. Measurements of *N. vivida* from the Czech Republic showed the size 2.76 pg (Z. Egertová unpublished data). During our preliminary screening of the DNA content in Pezizales, an unexpectedly large genome was detected in *Jafnea semitosta* (Berk. & M.A.Curtis) Korf. The present short paper thus aims to report this discovery, briefly discuss available information on fungal genome size evolution, and point to some pitfalls of fungal flow cytometry.

Materials and methods

Jafnea semitosta (Pyronemataceae, Pezizales) was originally described from North America (Berkeley 1875). In Europe, it was discovered for the first time in Austria as late as in 1996 (Benkert and Klofac 2004). The fungus is suspected of spreading on the continent together with *Juglans nigra* plantations. Reports from the Czech Republic support this hypothesis, as the tree was present at all of the four currently known localities in the country (Antonín and Moravec 2010; Z. Egertová personal observation; V. Halasů personal comm.). Fruiting bodies from three Czech and two Slovak localities were used in this study (Online Resource 1).

Flow cytometry with BD Accuri C6 instrument (BD Biosciences, Franklin Lakes, NJ, USA), propidium iodide staining and internal standardization was used for the nuclear DNA content estimation. A small piece (~8 mm^3) of a fresh *J. semitosta* apothecium was chopped together with leaf tissue of a reference standard in 500 µL of nuclei isolation buffer. The nuclear suspension was filtered through a 42-µm nylon filter, incubated with RNase (final concentration 50 µg/mL) and stained with propidium iodide (final concentration 50 µg/mL). Three independent measurements were made for each locality with each standard. The value in pg was converted to Gbp according to the relation 1 pg = 0.978 Gbp (Doležel et al. 2003). To obtain the most accurate estimates, different standards, buffers and RNase treatments were tested. Five flow cytometric standards were used—*Raphanus sativus* 'Saxa' (2*C* = 1.11 pg), *Solanum lycopersicum* 'Stupické polní rané' (2*C* = 1.96 pg; Doležel et al. 1992), *Glycine max* 'Polanka' (2*C* = 2.5 pg; Doležel et al. 1994), *Zea mays* 'CE-777' (2*C* = 5.43 pg, Lysák and Doležel 1998) and *Pisum sativum* 'Ctirad' (2*C* = 8.76 pg; Greilhuber et al. 2007). Two isolation buffers were tested—Otto solutions and LB01 buffer with 2% polyvinylpyrrolidone 40 and 15 mM β-mercaptoethanol (pH = 7.8; Doležel et al. 2007). Since addition of RNase had an apparent effect on the measurements, different incubation times (0, 5, 15, 30 or 60 min) with RNase at 37 *C* were also tested. Descriptive statistics and *t* tests were computed in NCSS (version 2007; J. Hintze, Kaysville, Utah, www.ncss.com).

Results and discussion

Optimizing the flow cytometry protocol

The results obtained in a series of experiments showed variation influenced by used buffers, incubation conditions and internal standards. Measurements using Otto solutions and centrifugation (as described in Doležel et al. 2007) provided very poor histograms and thus only LB01 buffer was used further. Although measurements with *Raphanus* and *Pisum* as standards were roughly similar to the other results, deviations caused by instrument non-linearity may have played a role in slight instability of the results due to

too small or too large genome size, respectively. *Solanum* provided distinctly lower values than other standards (Online Resource 2), possibly due to cytosolic effects on DNA staining (see, e.g., Noirot et al. 2000). Therefore, only *Glycine* and *Zea* were used for further measurements. It turned out that RNase treatment influenced the resulting DNA content estimates with most standards (Online Resource 2). Although RNA is in general very unstable, its short fragments are also effectively stained with propidium iodide and may affect the measurements (Doležel et al. 2007). To ensure that all RNA is cleaved, we tested incubation with RNase at 37 *C*. Moreover, the heat treatment promotes chromatin decondensation and thus improves DNA staining in heterochromatic regions (Jacobsen et al. 1988; Noirot et al. 2000). DNA content estimates were more or less positively associated with the incubation time, especially for *Glycine* (Online Resource 2), possibly as a result of chromatin decondensation. Nevertheless, the peak quality decreased (and CV increased) after the 30-min or longer incubation, indicating activation of other enzymatic processes and nuclei degeneration. Therefore, 15-min RNase treatment was applied for final measurements as it provided the most stable results and high-quality histograms with CV around 2.5% (Fig. 1). The same incubation conditions were found to be optimal also for microscopic Hypocreales by Veselská et al. (2014) and seem therefore to be well performing across different fungi.

Intraspecific variation in genome size within *Jafnea semitosta*

DNA content (± SE) of fungi from the most intensively studied population Záhlinice was estimated at 3.706 ± 0.011 pg (~3.625 ± 0.011 Gbp). Accessions from other localities did not differ in genome size (equal-variance *t* test *P* values > 0.39; data not shown) and the observed variation (±1% around average) did not exceed the method accuracy. Nevertheless, in two accessions (localities Ivaň and Bratislava) measurements with *Glycine* and *Zea* led to different estimates (±4% around average), although the mean value remained similar to the other accessions (Table 1). This deviation was most prominent in the Bratislava accession, which was represented by a well-developed fruiting body, had been collected one week prior to analysis and transported via post. Similarly, a shift in the sample peak position (+4%) was observed in an even older fruiting body from Močenok (Slovakia) when measured without and with internal standardization, respectively. That resulted in the DNA content value of 3.875 ± 0.030 pg with *Glycine* and 3.896 ± 0.002 pg with *Zea*, and higher CV values (>3.5% after gating), irrespective of the incubation. These measurements were therefore excluded. Hypothetically, metabolic processes in the old fruiting body could have influenced nuclei isolation and staining and thus the results obtained (Noirot et al. 2000), despite the fact that histogram quality did not always decrease. This points to another of the many factors influencing flow cytometric measurements—the age and the freshness of the analyzed material.

Genome diversity and dynamics in fungal genomes

The value of 3.7 pg is by far the highest ever reported for fungi—1.5× higher than the second largest genome in *Uromyces bidentis* (Ramos et al. 2015) and 100× higher than the average in fungi (Gregory et al. 2007). Many mechanisms were proposed to explain genome evolution in fungi. One of them—polyploidy—is still a rather controversial phenomenon in fungi, despite recent advances in fungal genomics. Although ancient polyploidy played an

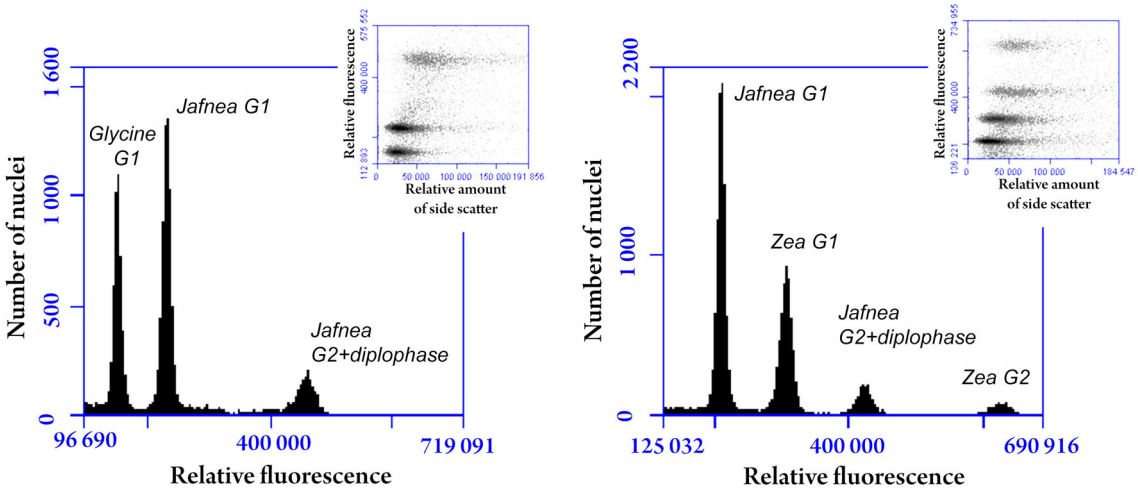

Fig. 1 Examples of FCM histograms and side scatter plots with *Glycine* (*left*) and *Zea* (*right*) as the internal standards

Table 1 Mean estimates of *Jafnea semitosta* 1*C* DNA content and standard errors (SE) in pg from measurements with different standards (*Zea mays* and *Glycine max*)

Locality	*Zea*		*Glycine*		Pooled data (*Zea* + *Glycine*)	
	Mean	SE	Mean	SE	Mean	SE
Ivaň (CZ)	3.6694	0.0323	3.7984	0.0276	3.7339	0.0346
Valtice (CZ)	3.6959	0.0178	3.7184	0.0131	3.7088	0.0107
Záhlinice (CZ)	3.7058	0.0053	3.7072	0.0281	3.7064	0.0110
Bratislava (SK)	3.5564	0.0163	3.8066	0.0134	3.6815	0.0429

Only one fruiting body per locality was studied, except for Záhlinice where three fruiting bodies were analyzed

important role in evolution and radiation of most non-fungal eukaryotes, and even recent polyploidizations are common across the eukaryotic domain, paleopolyploidy is virtually unknown in fungi (reviewed by Campbell et al. 2016). Also, recent polyploidization events and polyploid series were documented in relatively few fungal taxa, mainly in *Saccharomyces* and other yeasts (Albertin and Marullo 2012) and the chytrid fungus *Allomyces* (Wilson 1952). In many ascomycetes, polyploidy was proposed based on meiotic multivalents (Rogers 1968), unexpectedly high chromosome numbers (Uecker 1967), fluorescence intensity of interphase nuclei (Weber and Bresinky 1992) or other indirect approaches (Albertin and Marullo 2012). Nevertheless, to our knowledge, none of these hypothesized polyploids have been confirmed by any systematical study with chromosome counts (see also Campbell et al. 2016). Genome expansion in fungi, particularly in ascomycetes, seems therefore mostly associated with other phenomena, such as the increase in gene numbers, proliferation of mobile elements, expansion of introns, lineage-specific emergence of large subtelomeric islands or supernumerary chromosomes (Martin et al. 2010; Kelkar and Ochman 2011). Interestingly, many of the largest reported genomes belong to obligate parasites, such as Erysiphales (Spanu et al. 2010), Pucciniales (Tavares et al. 2014) or parasitic Pezizales (Kullman 2002). This fact can be explained by accessory genetic elements that can span entire chromosomes and constitute a large part of the genome. These regions exhibit high mutation and recombination rate and provide an ideal 'testing ground' for the evolution of new genes which are necessary for the rapid host–pathogen co-evolution (Croll and McDonald 2012). Alternatively, large-scale genome expansions may be catalyzed by the increasing genetic drift which has many non-adaptive effects, including proliferation of introns and mobile elements. Excessive genome size thus may be a result of decreased effective population size in some point of evolution (Kelkar and Ochman 2011; Martin et al. 2010). *Jafnea semitosta* is a saprotrophic species (Antonín and Moravec 2010) in which nothing is known about its chromosome count or genome structure. The cause of its extraordinary genome size remains unknown. Nevertheless, it seems probable that the genome inflation in *Jafnea* was fast and lineage-specific, since the most closely related genera (according to Perry et al. 2007) exhibit very small genome size; the DNA content was estimated at only 0.072 pg in *Smardaea protea* (Z. Egertová and M. Sochor unpublished data) and even 0.037 pg in a slightly more distant genus *Otidea* (Kullman et al. 2005–2016). Discovery of such a huge genome was therefore quite unexpected in this clade, and further examinations may bring valuable insights into fungal genome evolution.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. List of studied accession of *Jafnea semitosta* for flow cytometric measurements.
Online Resource 2. DNA content estimates (in pg) for different internal standards and RNase treatments (without RNase and with RNase with different incubation time at 37 °C).

References

Albertin W, Marullo P (2012) Polyploidy in fungi: evolution after whole-genome duplication. Proc Biol Sci 279:2497–2509. doi:10.1098/rspb.2012.0434

Antonín V, Moravec J (2010) *Jafnea semitosta* (Ascomycota, Pyronemataceae), first collection in the Czech Republic. Czech Mycol 62:1–11

Benkert D, Klofac W (2004) *Jafnea semitosta* (Ascomycetes, Pezizales), ein amerikanischer Becherling offenbar erstmals in Europa. Osterr Z Pilzk 13:55–59

Bennett MD, Leitch IJ (2012–2017a) Plant DNA *C*-values database (release 6.0, Dec 2012). Available at: http://www.kew.org/cvalues/. Accessed 11 Apr 2017

Bennett MD, Leitch IJ (2012–2017b) Pteridophyte DNA *C*-values database (release 6.0, Dec 2012). Available at: http://www.kew.org/cvalues/. Accessed 11 Apr 2017

Berkeley MJ (1875) Notices of North American fungi. Grevillea 3:145–160

Campbell MA, Ganley ARD, Gabaldón T, Cox MP (2016) The case of missing ancient fungal polyploids. Amer Naturalist 188:602–614. doi:10.1086/688763

Corradi N, Pombert JF, Farinelli L, Didier ES, Keeling PJ (2010) The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. Nat Commun 1:77. doi:10.1038/ncomms1082

Croll D, McDonald BA (2012) The accessory genome as a cradle for adaptive evolution in pathogens. Pl Pathol 8:8–10. doi:10.1371/journal.ppat.1002608

Doležel J, Greilhuber J (2010) Nuclear genome size: Are we getting closer? Cytometry A 77:635–642. doi:10.1002/cyto.a.20915

Doležel J, Sgorbati S, Lucretti S (1992) Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiol Pl 85:625–631. doi:10.1111/j.1399-3054.1992.tb04764.x

Doležel J, Doleželová M, Novák FJ (1994) Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). Biol Pl 36:351–357. doi:10.1007/BF02920930

Doležel J, Bartoš J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry A 51:127–128. doi:10.1002/cyto.a.10013

Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat Protoc 2:2233–2244. doi:10.1038/nprot.2007.310

Gregory TR (2005–2017) Animal genome size database. Available at: http://www.genomesize.com. Accessed 11 Apr 2017

Gregory TR, Nicol JA, Tamm H, Kullman B, Kullman K, Leitch IJ, Murray BG, Kapraun DF, Greilhuber J, Bennett MD (2007) Eukaryotic genome size databases. Nucl Acids Res 35(Database issue):D332–D338. doi:10.1093/nar/gkl828

Greilhuber J, Temsch EM, Loureiro JCM (2007) Nuclear DNA content measurement. In: Doležel J, Greilhuber J, Suda J (eds) Flow cytometry with plant cells: analysis of genes, chromosomes and genomes. Wiley, Weinheim, pp 67–101. doi: 10.1002/9783527610921.ch4

Greilhuber J, Obermayer R, Leitch IJ, Bennett MD (2010–2017) Bryophyte DNA *C*-values database (release 3.0, Dec 2010). Available at: http://www.kew.org/cvalues/. Accessed 11 Apr 2017

Jacobsen PB, Stokke T, Solesvik O, Steen HB (1988) Temperature-induced chromatin changes in ethanol-fixed cells. J Histochem Cytochem 36:1495–1501. doi:10.1177/36.12.2461412

Kapraun DF, Leitch IJ, Bennett MD (2004–2017) Algal DNA *C*-values database (release 1.0, Dec 2004). Available at: http://www.kew.org/cvalues/. Accessed 11 Apr 2017

Kelkar YD, Ochman H (2011) Causes and consequences of genome expansion in fungi. Genome Biol Evol 4:13–23. doi:10.1093/gbe/evr124

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi, 10th edn. CABI, Wallingford

Kullman B (2000) Application of flow cytometry for measurement of nuclear DNA content in fungi. Folia Cryptog Estonica 36:31–46

Kullman B (2002) Nuclear DNA content, life cycle and ploidy in two *Neottiella* species (Pezizales, Ascomycota). Persoonia 18:103–114

Kullman B, Tamm H, Kullman K (2005–2016) Fungal genome size database. Available at: http://www.zbi.ee/fungal-genomesize. Accessed 4 Oct 2016

Lysák MA, Doležel J (1998) Estimation of nuclear DNA content in *Sesleria* (Poaceae). Caryologia 52:123–132. doi:10.1080/00087114.1998.10589127

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, Porcel B, Rubini A, Amicucci A, Amselem J, Anthouard V, Arcioni S, Artiguenave F, Aury J-M, Ballario P, Bolchi A, Brenna A, Brun A, Buée M, Cantarel B, Chevalier G, Couloux A, Da Silva C, Denoeud F, Duplessis S, Ghignone S, Hilselberger B, Iotti M, Marçais B, Mello A, Miranda M, Pacioni G, Quesneville H, Riccioni C, Ruotolo R, Splivallo R, Stocchi V, Tisserant E, Viscomi AR, Zambonelli A, Zampieri E, Henrissat B, Lebrun M-H, Paolocci F, Bonfante P, Ottonello S, Wincker P (2010) Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464:1033–1038. doi:10.1038/nature08867

Murray BG, Leitch IJ, Bennett MD (2012–2017) Gymnosperm DNA *C*-values database (release 5.0, Dec 2012). Available at: http://www.kew.org/cvalues/. Accessed 11 Apr 2017

Noirot M, Barre P, Louarn J, Duperray C, Hamon S (2000) Nucleus–cytosol interactions - A source of stoichiometric error in flow cytometric estimation of nuclear DNA content in plants. Ann Bot (Oxford) 86:309–316. doi:10.1006/anbo.2000.1187

Perry BA, Hansen K, Pfister DH (2007) A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). Mycol Res 111:549–571. doi:10.1016/j.mycres.2007.03.014

Pombert JF, Selman M, Burki F, Bardell FT, Farinelli L, Solter LF, Whitman DW, Weiss LM, Corradi N, Keeling PJ (2012) Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. Proc Natl Acad Sci USA 109:12638–12643. doi:10.1073/pnas.1205020109

Ramos AP, Tavares S, Tavares D, Silva MC, Loureiro J, Talhinhas P (2015) Flow cytometry reveals that the rust fungus, *Uromyces bidentis* (Pucciniales), possesses the largest fungal genome reported—2489 Mbp. Molec Pl Pathol 16:1006–1010. doi:10.1111/mpp.12255

Rogers JD (1968) *Xylaria curta*: cytology of the ascus. Canad J Bot 46:1337–1340. doi:10.1139/b68-182

Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stüber K, van Themaat EVL, Brown JKM, Butcher SA, Gurr SJ, Lebrun M-H, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M, Brewer MT, Cadle-Davidson L, Cadle-Davidson MM, Collemare J, Cramer R, Frenkel O, Godfrey D, Harriman J, Hoede C, King BC, Klages S, Kleemann J, Knoll D, Koti PS, Kreplak J, López-Ruiz FJ, Lu X, Maekawa T, Mahanil S, Micali C, Milgroom MG, Montana G, Noir S, O'Connell RJ, Oberhaensli S, Parlange F, Pedersen C, Quesneville H, Reinhardt R, Rott M, Sacristán S, Schmidt SM, Schön M, Skamnioti P, Sommer H, Stephens A, Takahara H, Thordal-Christensen H, Vigouroux M, Weßling R, Wicker T, Panstruga R (2010) Powdery mildew fungi reveal tradeoffs in extreme parasitism. Science 330:1543–1546. doi:10.1126/science.1194573

Tavares S, Ramos AP, Pires AS, Azinheira HG, Caldeirinha P, Link T, Abranches R, Silva MC, Voegele RT, Loureiro J, Talhinhas P (2014) Genome size analyses of Pucciniales reveal the largest fungal genomes. Frontiers Pl Sci 5:422. doi:10.3389/fpls.2014.00422

Uecker FA (1967) *Stephensia shanori*. I. Cytology of the ascus and other observations. Mycologia 59:819–832. doi:10.2307/3757194

Veselská T, Svoboda J, Růžičková Ž, Kolařík M (2014) Application of flow cytometry for genome size determination in *Geosmithia*

fungi: a comparison of methods. Cytometry A 85:854–861. doi:10.1002/cyto.a.22500
Weber E (1992) Untersuchungen zu Fortpflanzung und Ploidie verschiedener Ascomyceten. J Cramer, Berlin-Stuttgart
Weber E, Bresinky A (1992) Polyploidy in discomycetes. Persoonia 14:553–563
Wilson CM (1952) Meiosis in *Allomyces*. Bull Torrey Bot Club 79:139–160. doi:10.2307/2481932