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The use of flow cytometry for fungal nuclear DNA quantification

Pedro Talhinhas[1] | Rita Carvalho[1] | João Loureiro[2]

[1]LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

[2]Department of Life Sciences, Centre for Functional Ecology, University of Coimbra, Coimbra, Portugal

Correspondence
Pedro Talhinhas, LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.
Email: ptalhinhas@isa.ulisboa.pt

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Abstract

Genome size information is sparse across fungi, with information being available for less than 2000 species. So far, most records have been obtained using static, microscope-based cytometry methods or derived from genome sequencing projects. Flow cytometry is now considered the state-of-the-art method for obtaining genome size measurements, and appropriate methods and DNA standards are available, enabling the analysis of most genome size ranges in a rapid, robust and inexpensive way. The average fungal genome size is 60 Mbp, but sizes vary across phylogeny, ranging from 2.2 (*Encephalitozoon romaleae*) to 3706 Mbp (*Jafnea semitosta*). In several fungal clades, genome size expansion seems to accompany evolution either to plant mutualism or to plant parasitism (particularly biotrophy), and fungi that interact with plants seem to have larger genomes than saprobes and those that interact with animals. Whereas flow cytometry for nuclear DNA quantification is routinely employed in plant sciences for genome size and ploidy studies, its use in fungal biology is still infrequent. Appropriate standards, methods and best practices are described here, with the aim of stimulating a more generalized and widespread use of flow cytometry for fungal genome size measurement.

KEYWORDS
DNA standards, flow cytometry, genome size, mycology, nuclear extraction

1 | INTRODUCTION

The employment of flow cytometry (FCM) for the analysis of fungi has gained momentum in recent years [1], but it still lags behind its use in plants. Fungi may be uni- or pluricellular (yeast-like or filamentous), or both, they may be amenable for axenic cultivation, or not, they can be micro- or macroscopic, they can undergo simple to complex life cycles (including the differentiation of various spore types), and they can have diverse nuclear cycles and types of physiology. All these traits must be considered when defining the scientific objectives and methodological approaches for the use of FCM in fungi. In general terms, the use of FCM in pseudofungi (namely Oomycota) tends to follow that in fungi. Cell viability and nuclear DNA amount are the most common uses of FCM in fungi, although applications in detection/diagnosis and physiology/morphology have also been reported [1, 2]. FCM deployment for analysis of fungi frequently arises within applied fields, such as medical mycology, plant pathology, the fermentation industry, and environmental sciences, but fundamental studies on fungal biology and physiology have also been produced. Although testing viability and/or cell death in fungi is also relevant for drug development in the medical mycology and pharmaceutical industries [1, 3] or for screening growth conditions in fermentation systems [4], in this review particular attention will be given to the use of FCM for nuclear DNA content measurements. This is particularly relevant for the correct planning of downstream genome sequencing initiatives, but also for ploidy and cell cycle studies [2].

2 | SAMPLE PREPARATION

The availability of sufficient fungal biomass for nuclear extraction depends on the type of substrate used by each fungus and on the

growth habit, and low amounts of biomass can be problematic. Nuclei can be released from filamentous fungi using the razor blade chopping procedure with a standard buffer (e.g., Woody Plant Buffer; Loureiro et al. [5]), as shown by Bourne et al. [6]. For filamentous fungi amenable to axenic cultivation, that procedure yields copious amounts of nuclei even from minute quantities of mycelia (e.g., References [6, 7]). Obligate parasitic fungi (i.e., fungi that can only be grown on tissues of their host organisms) pose additional difficulties, as they are not amenable for axenic cultivation. Tavares et al. [8] adapted the protocol of Bourne et al. [6] to fungi in the order Pucciniales (rust fungi), which are obligate biotrophic parasites of various plant species. The protocol involves chopping fungal hyphae present in infected plant tissues, releasing both fungal and plant nuclei. A parallel analysis of uninfected plant material enables clear discrimination of plant and fungal nuclei populations. As for cultivable filamentous fungi, this protocol yields large amounts of fungal nuclei even from small-sized infections (<20 mm^2 plant tissue area), evidencing the fact that fungal cells are much smaller than plant cells (besides being bi-nucleated for most life stages in the Pucciniales) (Figure 1).

Fungi also have unicellular life stages, either because they form spores, or they have dimorphic life cycles, or they are unicellular (yeast-like) throughout their life cycle. Analyzing unicellular structures poses additional difficulties, since the chopping procedure is hardly effective in releasing nuclei from such small cells. Tavares et al. [8] used a mortar and pestle to grind rust fungi urediniospores in the presence of a buffer, although this generates considerable amounts of debris making this approach only feasible for nuclei having large DNA contents. Otherwise, the protocol by Veselská et al. [9], based on a fixation with methanol:glacial acetic acid, DMSO, Triton-X 100, and EDTA in combination with Tris-$MgCl_2$ buffer, is more appropriate for measuring genome size of nuclei obtained from fungal spores, although being more laborious than the chopping procedure. This protocol was modified by Vondrák et al. [10] to be used on apothecia of lichen fungi in the genus *Blastenia*, combining the above-mentioned fixation procedure with chopping (and subsequent incubation) in Tris-$MgCl_2$ buffer. Additionally, Sabatinos and Forsburg [11] developed a protocol for yeast cells, fixing them in 70% ethanol and incubating on sodium citrate buffer with RNase. This incubation period must be optimized for each organism, ranging between minutes to overnight. Although more laborious and time-consuming than the chopping protocol, the Sabatinos and Forsburg [11] protocol enables analysis of small nuclei such as those of *Saccharomyces* spp. Ethanol fixation had previously been shown to be useful for the analysis of mithramycin-stained *Saccharomyces cerevisiae* nuclei [12]. Alternatively, the methanol:glacial acetic fixation-based protocol by Veselská et al. [9] can also be used for the analysis of *S. cerevisiae*.

Beyond sharing a common eukaryotic cell structure and composition with animals and plants, fungal cells are surrounded by a cell wall similarly to plants. In fungal genome size estimation using FCM, background noise due to cell wall debris frequently poses greater difficulties than seen with plants, due to the smaller fungal genome sizes. Reducing the working concentration of propidium iodide can mitigate this problem, substantially reducing background noise while still enabling an adequate staining of nuclei. Trials suggest that a 4x dilution of the recommended propidium iodide concentration (i.e., using a final concentration of 12.5 μg/ml) can be used in fungal genome size estimation in a range of ascomycetes and basidiomycetes (data not shown).

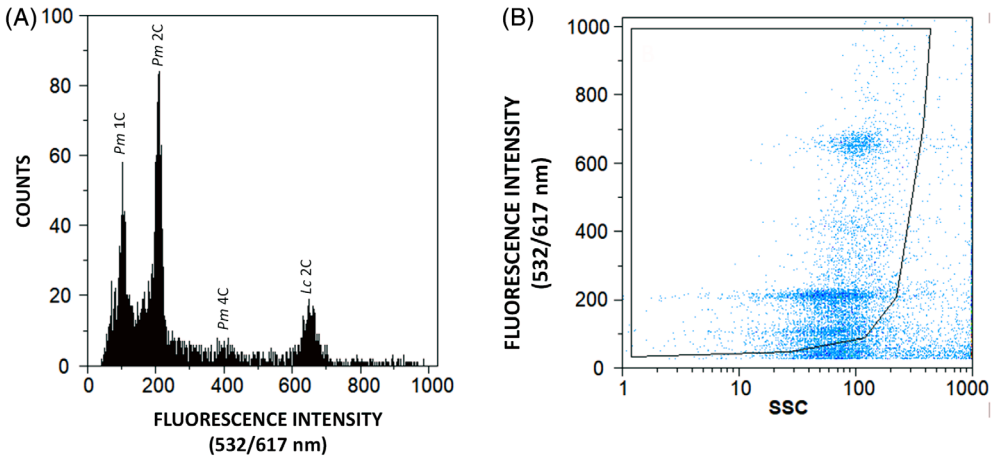

FIGURE 1 Flow cytometric analyses of relative fluorescence intensities of propidium iodide-stained nuclei simultaneously isolated from *Puccinia malvacearum* (Pm) teleutosporic sori and the surrounding host plant, *Lavatera cretica* (*Lc*), leaf tissues. (A) Depicts a uniparametric flow histogram of relative fluorescence intensities, whereas (B) illustrates the gating applied to the biparametric dot-plot of side scatter (SSC) versus fluorescence intensity to exclude as much as possible partial nuclei and other debris. Analysis was performed using a CyFlow space flow cytometer (Sysmex, Norderstedt, Germany) equipped with a 30 mW green solid-state laser emitting at 532 nm for optimal propidium iodide excitation and emission at 617 nm (532/617 nm). The fungal 2C population includes diploid nuclei formed upon karyogamy and the 4C population corresponds to replicated diploid nuclei that will subsequently undergo meiosis while basidia differentiate [Color figure can be viewed at wileyonlinelibrary.com]

3 | STANDARDS FOR FUNGAL DNA AMOUNT ESTIMATION

Precise fungal genome size estimations are frequently hampered due to a lack of adequate within-range DNA standards. Small-sized plant or animal standards have been employed, such as *Raphanus sativus* [6], *Arabidopsis thaliana* [7], or chicken erythrocytes [13]. Even then, fungal genomes have shown to be several times smaller than the standards used. While fungal species with large genomes can be compared with plant or animal nuclei, as in the case of the Pucciniales [8, 14] or of *Jafnea semitosta* [15], most fungal species are not reliably comparable to eukaryotes other than fungi. Veselská et al. [9] selected *S. cerevisiae* strain BY4743aα (24.1 Mbp/1C) and *Aspergillus fumigatus* strain CEA10 (29.2 Mbp/1C) as DNA standards based on genome sequence information, considering the average genome size of the fungal kingdom to be 37 Mbp (and the median 28 Mbp). However, above-average fungal genomes are not adequately covered by these standards, which led Talhinhas et al. [16] to select and validate three fungal species as additional DNA standards: *Inonotus hispidus* isolate LPV629 (41 Mbp/1C); *Colletotrichum acutatum* isolate PT812 (68 Mbp/1C); *Cenococcum geophilum* isolate 844.1 (203 Mbp/1C). In most cases intra-specific genome size variability is not characterized but cannot be disregarded, and therefore it is important to use specific fungal strains as DNA standards. Considering that fungal genome sizes range between 2.19 (for *Encephalitozoon romaleae*; Pombert et al. [17]) and 3706 Mbp (for *J. semitosta*; Egertová and Sochor [15]), with an overall average upgraded to 44.2 Mbp by Ramos et al. [14], taken together these standards enable adequate analysis of the vast majority of fungal species (with the larger genomes being covered by plant or animal DNA standards). To this end, Carvalho et al. [18] have validated the nuclei of the plant *Rhamnus alaternus* (664 Mbp/2C) as a standard, useful for the analysis of fungal species with high amounts of DNA.

4 | FUNGAL GENOME SIZES

As for plant species, a centralized fungal genome size database is available online (http://www.zbi.ee/fungal-genomesize; Kullman et al. [19]). This database compiles information from the literature along with data from submitters, either arising from genome sequencing initiatives or from direct genome size measurements, and currently contains a total of 2412 entries. Nevertheless, data arising from static microscope-based cytometry methods or derived from genome sequencing projects are the most common, while only less than 5% of records were obtained using FCM (Figure 2). These records represent ca. 1770 fungal species from 640 genera, 370 families and 140 orders, leaving another 44 orders (23% of all fungal orders) without information for any species. In another 51 orders, there is information only for one species. Genome size estimation in fungi therefore still needs much input, particularly in an era of proliferating fungal genome sequences, often without prior knowledge on the respective genome sizes which may lead to inadequate sequencing strategies. For example, attempts to sequence the genome of the Pucciniales *Hemileia vastatrix*, the causal agent of Coffee Leaf Rust, were

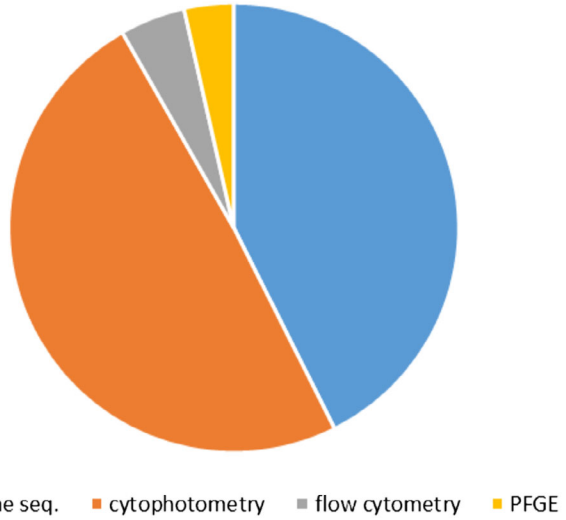

FIGURE 2 Relative proportion of measuring methods of fungal genome sizes recorded in the fungal genome size database (http://www.zbi.ee/fungal-genomesize; as of September 2, 2020); cytophotometry—static microscope-based cytometry methods; PFGE—pulsed-field gel electrophoresis [Color figure can be viewed at wileyonlinelibrary.com]

hampered by assumptions of a genome size of ca. 300 Mbp [20], which ultimately turned out to be of ca. 800 Mbp as revealed by FCM [8].

Currently, the average fungal genome size is 59.6 Mbp with a median of 39.5 Mbp, although these figures have shifted profoundly over the last 10 years reflecting the still volatile nature of such information. Genome sizes in fungi vary according to the phylogeny (Figure S1), with an average size of 74.3 Mbp in the Basidiomycota and 50.6 Mbp in the Ascomycota. Although less represented, other phyla stand out: Microsporidia, 3.9 Mbp; Chytridiomycota, 48.1 Mbp; Zoopagomycota, 61.9 Mbp; Mucoromycota, 77.6 Mbp. In the Basidiomycota, the Pucciniales represent a group with expanded genome sizes (average 466 Mbp), strongly contrasting with neighboring taxa such as the Microbotryomycetes (23.3 Mbp) or the Cystobasidiomycetes (24.8 Mbp). Still in the Basidiomycota, while the Ustilaginomycotina and the Tremellomycetes present average genome sizes of 18.7 and 23.9 Mbp respectively, the Agaricomycetes attain an average value of 60.4 Mbp.

In the Ascomycota, the Taphrinomycotina and the Saccharomycotina present values of 12.2 and 13.7 Mbp respectively, while several families present above-average values: Dermateaceae (61.5 Mbp); Rutstroemiaceae (65.4 Mbp); Sarcosomataceae (66.2 Mbp); Helvellaceae (73.0 Mbp); Erysiphaceae (84.6 Mbp); Tuberaceae (95.0 Mbp); Pyronemataceae (214 Mbp). Whereas in some fungal phylogenetic lineages genome sizes are stable, in other parts the phylogenetic divergence processes seem to have shaped strong shifts in genome size, suggesting a relationship. In the early days of the proliferation of fungal genome sequences, Spanu [21] speculated on the coincidence between the acquisition of biotrophy in plant pathogenic fungi and an expanded genome, taking the Pucciniales and the Erysiphales as the key examples. While little progress was attained in the estimation of genome sizes in the Erysiphales, a large number of Pucciniales species had their genome sizes determined, reinforcing this view [8]. Similarly, specialized *Geosmithia* fungi living in

TABLE 1 Genome size variation across fungal species according to the type of interaction with the substrate (homogeneous groups obtained using the Tukey HSD test, $p < 0.05$); analysis based on the fungal genome size database (http://www.zbi.ee/fungal-genomesize; data retrieved in September 2, 2020)

Interaction	Genome size (Mbp)	*n*	Homogeneous groups
Commensalism	40.99	73	a
None (non-living substrate)	53.17	1079	a
Mutualism	72.53	157	a
Parasitism	75.71	432	a

association with bark beetles tend to have higher DNA content than generalist *Geosmithia* spp. [22]. While no differences concerning genome size are found between the type of interaction established between each fungal species and its typical substrate (Table 1), fungi that interact with plants have genomes significantly larger than those interacting with animals or other fungi or saprobes (Table 2).

Across fungal species that interact with plants, pathogens (parasitism) and mutualists have larger genomes than saprobes (commensalism) (Table 3). Moreover, among plant pathogenic fungi, biotrophic fungi have larger genomes than necrotrophic (and hemibiotrophic) fungi (Table 4), although data for biotrophs is strongly based on Pucciniales.

Fungal genome sequencing projects have shown that larger genomes tend to contain higher proportions of non-coding regions, including repetitive and transposable elements. Such sources of genetic variability may be relevant for the intimate dialog established between mutualists and biotrophs with their plants hosts and would therefore explain the larger genome of these fungi as compared to animal pathogens and saprobes.

The employment of FCM for genome size measurement in fungi is still insufficient and in two thirds of the fungal orders genome size information is available for only one species or none. However, appropriate methods for nuclei isolation and adequate DNA standards are available for most fungi as described here. Considering the vast number of species for which the genome size is not known and putative relation of genome size with fungal phylogeny and substrate usage, conditions are now met for a wider use of FCM on fungal genome size estimation.

5 | CONCLUSION/BEST PRACTICES

The use of FCM in fungi, namely for nuclear DNA quantification, is still underexplored, as most taxa have not had their genome sizes determined and most of those that had, such estimations were not done using FCM. Some protocols are available and have been optimized for certain groups of fungi, namely yeasts [11], lichens [10], culturable filamentous fungi [6] and obligate biotrophic fungi [8], based either on ethanol/methanol:acetic acid fixation of nuclei [10, 11] or on the release of nuclei onto a stabilizing buffer [6, 8]. However, much is still to be explored, with life-style and small genome sizes posing challenges to the generalization of the available protocols to

TABLE 2 Genome size variation across fungal species according to the substrate (homogeneous groups obtained using the Tukey HSD test, $p < 0.05$); analysis based on the fungal genome size database (http://www.zbi.ee/fungal-genomesize; data retrieved in September 2, 2020)

Interacts with	Genome size (Mbp)	*n*	Homogeneous groups
Fungi	28.59	40	a
Animals	33.72	117	a
Non-living substrate	53.17	1080	a
Plants	83.21	504	b

TABLE 3 Genome size variation across fungal species that are known to interact with plants according to the type of interaction (homogeneous groups obtained using the Tukey HSD test, $p < 0.05$); analysis based on the fungal genome size database (http://www.zbi.ee/fungal-genomesize; data retrieved in September 2, 2020)

Interaction	Genome size (Mbp)	*n*	Homogeneous groups
Commensalism	41.31	71	a
Mutualism	72.84	156	b
Parasitism	99.79	277	b

TABLE 4 Genome size variation across fungal species that are known to parasite plants according to the type of parasitism (homogeneous groups obtained using the Tukey HSD test, $p < 0.05$); analysis based on the fungal genome size database (http://www.zbi.ee/fungal-genomesize; data retrieved in September 2, 2020)

Interaction	Genome size (Mbp)	*n*	Homogeneous groups
Necrotrophy (and hemibiotrophy)	44.57	169	a
Biotrophy	228.40	84	b

understudied taxa. Bearing in mind that much more is to be learnt, the following best practices can be issued:

- unspecific fluorescence associated to phenolic compounds in cell walls creates background noise, and strategies to avoid or remove them are fundamental for the successful analysis of fungal nuclei;
- gating strategies that remove background noise can prove to be efficient in improving nuclear fluorescent signal;
- young actively growing fungal cultures (mycelial or yeasts) are preferred over old cultures or spores, as cell walls in young hyphae/cells generate less background noise;
- propidium-iodide concentration can be adjusted in order to find an optimal balance between adequate nuclei staining (depends on the number of nuclei in the sample and its DNA content) and low staining of background noise;
- most fungal taxa have not been analyzed by FCM and, thus it is likely that the protocols (including the available FCM buffers) need to be optimized for some of these groups.

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AUTHOR CONTRIBUTIONS

Pedro Talhinhas: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; writing-original draft; writing-review & editing. **Rita Carvalho:** Formal analysis. **João Loureiro:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; writing-original draft; writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Pedro Talhinhas https://orcid.org/0000-0001-6694-7502
Rita Carvalho https://orcid.org/0000-0002-5847-9814
João Loureiro https://orcid.org/0000-0002-9068-3954

REFERENCES

1. Bleichrodt R-J, Read ND. Flow cytometry and FACS applied to filamentous fungi. Fungal Biol Rev. 2019;33:1–15. https://doi.org/10.1016/j.fbr.2018.06.001.
2. D'Hondt L, Hofte M, Van Bockstaele E, Leus L. Applications of flow cytometry in plant pathology for genome size determination, detection and physiological status. Mol Plant Pathol. 2011;12:815–28. https://doi.org/10.1111/j.1364-3703.2011.00711.x.
3. Assaraf MP, Ginzburg C, Katan J. Weakening and delayed mortality of *Fusarium oxysporum* by heat treatment: flow cytometry and growth studies. Phytopathology. 2002;92:956–63. https://doi.org/10.1094/PHYTO.2002.92.9.956.
4. Schwinn M, Durner D, Wacker M, Delgado A, Fischer U. Impact of fermentation temperature on required heat dissipation, growth and viability of yeast, on sensory characteristics and on the formation of volatiles in Riesling. Aust J Grape Wine Res. 2019;25:173–84. https://doi.org/10.1111/ajgw.12386.
5. Loureiro J, Rodriguez E, Doležel J, Santos C. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. Ann Bot. 2007;100:875–88.
6. Bourne EC, Mina D, Gonçalves SC, Loureiro J, Freitas H, Muller LAH. Large and variable genome size unrelated to serpentine adaptation but supportive of cryptic sexuality in *Cenococcum geophilum*. Mycorrhiza. 2014;24:13–20. https://doi.org/10.1007/s00572-013-0501-3.
7. Pires AS, Azinheira HG, Cabral A, Tavares S, Tavares D, Castro M, et al. Cytogenomic characterization of *Colletotrichum kahawae*, the causal agent of coffee berry disease, reveals diversity in minichromosome profiles and genome size expansion. Plant Pathol. 2016;65:968–77. https://doi.org/10.1111/ppa.12479.
8. Tavares S, Ramos AP, Pires AS, Azinheira HG, Caldeirinha P, Link T, et al. Genome size analyses of Pucciniales reveal the largest fungal genomes. Front Plant Sci. 2014;5:422. https://doi.org/10.3389/fpls.2014.00422.
9. Veselská T, Svoboda J, Růžičková Ž, Kolařík M. Application of flow cytometry for genome size determination in *Geosmithia* fungi: a comparison of methods. Cytometry A. 2014;85A:854–61. https://doi.org/10.1002/cyto.a.22500.
10. Vondrák J, Frolov I, Košnar J, Arup U, Veselská T, Halıcı G, et al. Substrate switches, phenotypic innovations and allopatric speciation formed taxonomic diversity within the lichen genus *Blastenia*. J Syst Evol. 2020;58:295–330. https://doi.org/10.1111/jse.12503.
11. Sabatinos SA, Forsburg SL. Measuring DNA content by flow cytometry in fission yeast. Methods Mol Biol. 2009;521:449–61. https://doi.org/10.1007/978-1-60327-815-7_25.
12. Galbraith DW, Harkins KR, Knapp S. Systemic endopolyploidy in *Arabidopsis thaliana*. Plant Physiol. 1991;96:985–9. https://doi.org/10.1104/pp.96.3.985.
13. Hosny M, Gianinazzi-Pearson V, Dulieu H. Nuclear DNA contents of eleven fungal species in Glomales. Genome. 1998;41:422–8. https://doi.org/10.1139/g98-038.
14. Ramos AP, Tavares S, Tavares D, Silva MC, Loureiro J, Talhinhas P. Flow cytometry reveals that the rust fungus, *Uromyces bidentis* (Pucciniales), possesses the largest fungal genome reported—2489 Mbp. Mol Plant Pathol. 2015;16:1006–10. https://doi.org/10.1111/mpp.12255.
15. Egertová Z, Sochor M. The largest fungal genome discovered in *Jafnea semitosta*. Plant Syst Evol. 2017;303:981–6. https://doi.org/10.1007/s00606-017-1424-9.
16. Talhinhas P, Tavares D, Ramos AP, Gonçalves S, Loureiro J. Validation of standards suitable for genome size estimation of fungi. J Microbiol Methods. 2017;142:76–8. https://doi.org/10.1016/j.mimet.2017.09.012.
17. Pombert JF, Selman M, Burki F, Bardell FT, Farinelli L, Solter LF, et al. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. Proc Natl Acad Sci USA. 2012;109:12638–43. https://doi.org/10.1073/pnas.1205020109.
18. Carvalho R, Silva E, Loureiro J, Ramos AP, Talhinhas P. Characterisation of *Puccinia hemerocallidis* causing the first outbreak of daylily rust in Europe. Rev Ciên Agrár. 2018;41:110–5. https://doi.org/10.19084/RCA.17075.
19. Kullman B, Tamm H, Kullman K. Fungal genome size. Database. 2005; http://www.zbi.ee/fungal-genomesize/.
20. Cristancho MA, Botero-Rozo DO, Giraldo W, Tabima J, Riaño-Pachón DM, Escobar C, et al. Annotation of a hybrid partial genome of the coffee rust (*Hemileia vastatrix*) contributes to the gene repertoire catalog of the Pucciniales. Front Plant Sci. 2014;5:594. https://doi.org/10.3389/fpls.2014.00594.
21. Spanu PD. The genomics of obligate (and nonobligate) biotrophs. Annu Rev Phytopathol. 2012;50:91–109. https://doi.org/10.1146/annurev-phyto-081211-173024.
22. Veselská T, Kolařík M. Application of flow cytometry for exploring the evolution of *Geosmithia* fungi living in association with bark beetles: the role of conidial DNA content. Fungal Ecol. 2015;13:83–92. https://doi.org/10.1016/j.funeco.2014.08.007.

SUPPORTING INFORMATION

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