

Genome size estimates for six rust (*Pucciniales***) species**

Estimativa do tamanho do genoma de seis espécies de ferrugens (*Pucciniales*)

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RESUMO

Os fungos que causam ferrugens caraterizam-se pela especialização relativamente ao hospedeiro, pela biotrofia, por possuírem ciclos de vida complexos e grandes genomas. Neste trabalho a citometria de fluxo foi empregue para determinar o tamanho do genoma de seis espécies de fungos *Pucciniales (Basidiomycota), Melampsora pulcherrima, Puccinia behenis, P. cichorii, P. pimpinellae, P. vincae e Uromyces dianthi,* agentes causais de ferrugem em *Mercurialis annua, Silene latifolia, Cichorium intybus, Pimpinella villosa, Vinca major* e *Dianthus caryophyllus,* respetivamente. Com resultados entre 182,1 e 566,4 Mpb/1C, este estudo contribuiu para o conhecimento do tamanho dos genomas na ordem *Pucciniales,* reforçando a posição deste táxone como o que engloba os fungos com maior tamanho médio de genoma (335,6 Mpb/1C). Este estudo contribui para uma melhor compreensão dos padrões de distribuição de tamanhos de genoma ao longo da filogenia dos fungos, sugerindo uma ligação entre caraterísticas biológicas e o tamanho do genoma. Em particular, os tamanhos dos genomas de fungos *Pucciniales* variam fortemente dentro do género, mas também diferem de forma vincada dos genomas de outras ordens em *Pucciniomycotina* que não *Pucciniales,* sugerindo que a variação do tamanho do genoma possa ser um elemento ativo na evolução dos agentes causais de ferrugens.

Palavras-chave: citogenómica, citometria de fluxo, ferrugem, Fungi, tamanho de genoma

ABSTRACT

Rust fungi (*Basidiomycota, Pucciniales*), one of the largest groups of phytopathogenic organisms, are characterised by host specialisation, biotrophy, complex life cycles and enlarged genomes. In this work we have used flow cytometry to determine the genome size of six rust species, *Melampsora pulcherrima, Puccinia behenis, P. cichorii, P. pimpinellae, P. vincae* and *Uromyces dianthi*, the causal agents of rust on *Mercurialis annua, Silene latifolia, Cichorium intybus, Pimpinella villosa, Vinca major* and *Dianthus caryophyllus,* respectively. With results ranging between 182.1 and 566.4 Mbp/1C, this study has contributed to the knowledge of genome sizes in the order *Pucciniales,* reinforcing this group as the one with the largest average genome size among fungi, with 335.6 Mbp/1C. By comparing genome size and their variability across the fungal kingdom, this study has contributed for understanding the patterns of genome size distribution along the fungal phylogeny, suggesting links between biological specificities and genome sizes. In particular, genome sizes of rust fungi vary greatly within genera, but also differ sharply from other non-*Pucciniales* orders in the *Pucciniomycotina,* suggesting that genome size variation may be an active element shaping the evolution of rust fungi.

Keywords: cytogenomics, flow cytometry, rust, Fungi, genome size

Introduction

Rust fungi (Basidiomycota, Pucciniales) are responsible for very important plant diseases, namely rusts on cereals, soybean, coffee, poplar, pines, legumes, rose and other ornamental plants. They comprise one of the largest groups of phytopathogenic organisms, most of them with very narrow host ranges. Such pathological specialisation into such a wide botanical range of hosts (including mosses, ferns, conifers, monocotyledons and dicotyledons) follows elaborated host-pathogen interaction patterns according to the gene-for-gene theory, which in fact was developed based on the flax-rust pathosystem. Nutrients are obtained by the fungus from living host plant cells (biotrophy) through specialised feeding structures (haustoria). Rust life cycles are among the most complex in fungi, with up to five different spore types and often requiring two botanically distinct hosts to complete the life cycle (as revised by Fernandez et al., 2013). Rust fungi are also characterised by unusually large genomes (average 334 Mbp, while the overall average for Fungi is 44.2 Mbp), a trait that may favour adaptation and pathological specialisation (Tavares et al., 2014).

The genera Puccinia, Uromyces and Melampsora encompass approximately two thirds of all recognised rust species, comprising the most notorious rust pathogens in temperate regions, such as the agents of cereal, legume and poplar rusts. So far, the average genome size for species of the genus Puccinia is 248 Mbp, ranging widely across 17 species from 77 Mbp to 806 Mbp, i.e., spanning nearly the entire range of genome sizes reported so far for rusts. In fact, at 806 Mbp, P. chrysanthemi is one of the largest fungal genomes ever reported, in opposition to the cereal rusts (P. triticina and P. graminis f. sp. tritici, both at 77 Mbp) which fall among the smallest genomes (Tavares et al., 2014), but still twice as large as the average genome size for fungi. Similarly, the genome size of Uromyces species ranges from 277 (U. rumicis) to 712 Mbp (U. vignae), with an average of 467 Mbp obtained over seven species. Genome sizes known for six species in the genus Melampsora range from 118 (M. larici--populina) to 333 Mbp (M. ricini), with an average of 221 Mbp (Tavares et al., 2014).

Such enlarged genomes found in rusts may be important sources of diversity, particularly in the absence of sexual reproduction, due to the activity of transposable elements (abundant in these large genomes), and the events leading to genome size variation (namely polyploidisation and chromosome transfer) are thought to be important factors related with host specialisation and even with specific 'host genotype-pathogen race' interactions (Duplessis et al., 2011; Spanu, 2012; Stuckenbrock & Croll, 2014). The knowledge of rust genome sizes, besides being of interest to the field of Mycology, is therefore of relevance to Plant Pathology in particular and to Agronomy in general. In this work we have determined the genome sizes of six species causing rust diseases in ornamentals and weeds, namely M. pulcherrima (Dog's Mercury rust, on Mercurialis annua), P. behenis (White Campion rust, on Silene latifolia), P. cichorii (Chicory rust, on Cichorium intybus), P. pimpinellae (Wild Anise rust, on Pimpinella villosa), P. vincae (Blue Periwinkle rust, on Vinca major) and U. dianthi (Carnation rust, on Dianthus caryophyllus). For that, flow cytometry, the state of the art method for fungal genome size determination (D'Hondt et al., 2011), coupled with an optimized protocol for the analysis of biotrophic fungi (Loureiro et al., 2007; Tavares et al., 2014), was applied.

Materials and Methods

Plant material exhibiting rust symptoms were collected in the Lisbon area during 2014 and 2015 (Table 1). Rust species were recognised upon identification of host species and complemented by the examination of spore morphology. **Herbarium spe**cimens were deposited at the João de Carvalho e Vasconcellos Herbarium (LISI; Lisbon, Portugal). Three samples were collected at the teliosporic stage, two at the urediniosporic stage and one at the aecidiosporic stage (Table 1).

Bust	Host ¹	Location, date and status	Type of spores ²	Herbarium reference	Genome size (1C)				Reference	
Kust			and life cycle ³	(LISI- FUNGI)	Mean pg	Mean Mbp	SD Mbp	CV (%)	n	standard ⁴
<i>Melampsora pulcherrima</i> Maire	<i>Mercurialis annua</i> (Eu)	Lisbon; 2/2015; spontaneous	I; Ma, He	00027	0.220	215.6	2.78	1.3	4	Rs
<i>Puccinia behenis</i> G.H. Otth	Silene latifolia (Ca)	Oeiras; 2/2015; spontaneous	III, IV; Ma, Ae	00028	0.186	182.1	10.9	6.0	3	Rs
<i>Puccinia cichorii</i> (DC.) Bellynck.	Cichorium intybus (As)	Oeiras; 6/2014; spontaneous	II; Mi	00029	0.342	334.8	38.1	10.5	2	Rs
<i>Puccinia pimpinellae</i> (Str.) Mart.	Pimpinella villosa (Api)	Azenhas do Mar, Sintra; 6/2014; spontaneous	III; Mi	00030	0.329	321.6	1.15	0.36	3	Rs
<i>Puccinia vincae</i> (DC.) Plowr.	Vinca major (Apo)	Oeiras; 2/2015; spontaneous	II; Ma, Ae	00031	0.579	566.4	28.8	5.0	4	SI
Uromyces dianthi (Pers.) Niessl	Dianthus caryophyllus (Ca)	Azenhas do Mar, Sintra; 1/2015; cultivated	III; Ma, He	00032	0.4282	418.8	-	-	1	Rs

 Table 1 - Rust species in this study with reference to the host plant, location, botanical status, collection date, rust life cycle stage and herbarium reference

¹ Acronyms in brackets refer to host family: Api – Apiaceae; Apo – Apocynaceae; As – Asteraceae; Ca – Caryophyllaceae; Eu – Euphorbiaceae;

² Type of spores present in sampled material: I – Aeciospores; II – Urediniospores; III – Teliospores; IV – Basidiospores (Laundon, 1967);

³ Typical life cycle: Ma – macrocyclic; Mi – microcyclic; Ae – autoecious; He – heteroecious;

⁴ Plant reference standards used: Rs – Raphanus sativus (2C = 1.11 pg DNA); Sl – Solanum lycopersicum (2C = 1.96 pg DNA).

Rust genome sizes were estimated by flow cytometry using a Partec CyFlow Space flow cytometer (Partec GmbH, Görlitz, Germany). For such, nuclei were isolated by simultaneously chopping infected leaves of each sample (listed in Table 1) with leaves of the internal reference standard Raphanus sativus 'Saxa' (2C= 1.11 pg or 1.086 Mbp; (Doležel et al., 1992) and Solanum lycopersicum 'Stupické' (2C = 1.96 pg or 1.917 Mbp; Doležel et al. 1992), as previously described (Tavares et al. 2014). The nuclear suspension was then filtered through a 30 µm nylon filter to remove plant and fungal debris, and 50 µg/mL of propidium iodide (Fluka, Buchs, Switzerland) and 50 µg/mL of RNase (Fluka) were added to stain DNA, only. Data were acquired using Partec FloMax software v2.4d (Partec GmbH) as previously described (Tavares et al., 2014). For each sample, fluorescence peaks of fungal nuclei were identified by comparing fluorescence histograms of rust-infected leaves with healthy leaves of the host plant.

Results and Discussion

Flow cytometry analyses enabled the separation and identification of nuclei of six rust species by comparison with their host nuclei (except for *Mercurialis annua, Silene latifolia* and *Pimpinella villosa*, whose nuclei were out of fluorescence range due to a larger genome size) (Fig. 1). The comparison

to the internal reference standard (either R. sativum or S. lycopersicum) permitted the estimation of the genome sizes for the six rust species (Table 1). The genome size of Melampsora pulcherrima (215.6 Mbp), obtained from the Euphorbiaceae Mercurialis annua, is within the range of the genome sizes known for six other Melampsora species (average 221.2 Mbp; Tavares et al., 2014). Among Melampsora spp. infecting Euphorbiaceae, the genome size for M. pulcherrima is lower than that of M. ricini (in Ricinus communis; 332.8 Mbp) and slightly lower than that of M. euphorbiae (in Euphorbia pterococca; 233.8 Mbp). At 418.8 Mbp, the genome size of Uromyces dianthi (from Dianthus caryophyllus) is within the range of the genome size distribution for Uromyces spp. (average 467.5 Mbp; Tavares et al., 2014), and is larger than any of the Uromyces species with non--Fabaceae hosts known so far (as U. transversalis genome size, from *Gladiolus* sp., is 376.7 Mbp and U. rumicis, from Rumex crispus, is 276.8 Mbp). Ranging from 182.1 to 566.4 Mbp (average 359.4 Mbp), the genome sizes for the four Puccinia species determined in this study are within the range of the 17 Puccinia species with known genome size (average 248.3 Mbp; Tavares et al., 2014). At 321.6 Mbp, the genome size of Puccinia pimpinellae (from Pimpinella villosa) is larger than that of P. smyrnii (at 258.9 Mbp), the other rust from an Apiaceae host with known genome size. At 182.1 Mbp, the genome size of P. behenis (from Silene latifolia) is the smallest determined in this study and, along with U.

dianthi, represent the first two genome sizes determined for rusts infecting Caryophyllaceae plants. The genome size of *P. cichorii*, from *Cichorium intybus*, is 334.8 Mbp, a value smaller than that determined in rusts found on other Asteraceae, namely *Coleosporium inulae* (from *Dittrichia viscosa*; 390.3 Mbp) and *P. chrysanthemi* (from *Dendranthema* sp.; 806.5 Mbp). The *Vinca major* rust, *P. vincae*, with an estimated genome size of 566.4 Mbp, represents the largest genome in this study, and the first known genome size for a rust infecting an Apocynaceae plant. As before (Tavares *et al.*, 2014), no relationship could be established between the genome size and the type of life cycle of each rust species.



Figure 1 - Flow cytometric histograms of relative fluorescence intensities of propidium iodide-stained nuclei simultaneously isolated from: A – Melampsora pulcherrima (Mp) and the plant DNA reference standard, Raphanus sativus (Rs, 2C = 1.11 pg DNA); B – Puccinia behenis (Pb) and the plant DNA reference standard, R. sativus (Rs); C – P. cichorii (Pc), its host plant, Cichorium intybus (Ci), and the plant DNA reference standard, R. sativus (Rs); D – Puccinia pimpinellae (Pp), and the plant DNA reference standard, R. sativus (Rs); D – Puccinia pimpinellae (Pp), and the plant DNA reference standard, R. sativus (Rs); E – P. vincae (Pv), its host plant, Vinca major (Vm), and the plant DNA reference standard, Solanum lycopersicum (Sl, 2C = 1.96 pg DNA); and F – Uromyces dianthii (Ud), its host plant, Dianthus caryophyllus (Dc), and the plant DNA reference standard, Raphanus sativus (Rs); the nuclei of Mercurialis annua, Silene latifolia and Pimpinella villosa (panels A, B and D, respectively) are out of range, and therefore not depicted in the histograms.

Spanning from 192.2 to 567.1 Mbp, the results obtained in this study raise the number of organisms in the Pucciniales with known genome size to 45, making this the third better documented order in the Basidiomycota for this trait (Fig. 2). The average genome size for rust fungi is now 335.6 Mbp, making this the fungal order with the largest average genome size, namely larger than the Glomeralles (158.6 Mbp) and the Pezizales (89.3 Mbp). Moreover, the variability in genome size values among the Pucciniales is over ten-fold, from 77 to 893 Mbp. Comparing the genome size standard deviation across fungi, the Pucciniales are again the most variable order (Fig. 2). In further detail, the comparison of genome sizes across fungal genera from which there is information available for three or more species (Table 2) shows that *Uromyces* is the genus with the largest average genome size (461.4 Mbp), followed by the rust genera *Puccinia* (269.5 Mbp) and *Melampsora* (219.6 Mbp), and immediately by the Ascomycota genus *Octospora* (202.7 Mbp), the Basidiomycota genus *Gomphidius* (199.6 Mbp) and the Glomeromycota genus *Glomus* (181.2 Mbp). The genus *Puccinia* is also very variable, with a coefficient of variation across genome size values of 75.7%, second only to the genus *Glomus*. On the contrary, the genera *Melampsora* and *Uromyces* are less variable than many other fungal genera.



Figure 2 - Average genome sizes (Mbp) for every fungal order for which values from at least three different species were available (number of species in brackets), including the results obtained in this study; lines represent standard deviation; fungal orders are arranged phylogenetically (http://tolweb.org/Fungi/).

Table 2 - Average genome sizes (Mbp) for every fungal genus for which values from at least three different species were available, including the results obtained in this study; fungal orders are arranged phylogenetically (http://tolweb.org/Fungi/); c.v. - coefficient of variation; n - number of species; Colour scale - green to red denote lowest to highest values for genome size (Mbp) and for coefficient of variation (%).

Priprint Zurgingtom Cases Order Taminy Outbot In More Control More Control Also A	Phylum	Sub-obvium	Class	Order	Family	Geour		Geno	me size
Period Definitemy cetes Mucosphare file cese Mucosphare file cese Nucosphare file cese	Phylam	Sub-phylam	Class		Family	Genus		Mbp	c.v. (%)
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Persponse Persponse Persponse Persponse Aspragilaci 19 8,6 10.2 Eurotiomycetes Eurotiales Aspragilaci 10						Aureobasidium	4	26,8	7,2
Eurotiomycetes Eurotiales Argergilaceae Argergilaceae Argergilaceae Argergilaceae Argergilaceae Periode 16 31 32 Nelliso 25 Nelliso 25 5 42 27 43 32 Nelliso 25 5 7 43 32 32 Periode 6 6.3 42 27 43 32 Periode 7 43 32 32 33 32 Periode 7 43 34 32 35 36 36 32 35 Periode 7 93 94 35				Pleosporales	Pleosporaceae	Pyrenophora	3	38,6	14,2
Eurobalmycetes Eurobalmycetes Eurobalmycetes Alpergulaciae Percellum 16 13.0 13.0 13.0 Percellum Servellum Percellum 16 0.0 25.0 23.1 Malia S 0.0 20.1 33.0 17.0 <t< td=""><td></td><td></td><td></td><td></td><td></td><td>Aspergillus</td><td>19</td><td>33,6</td><td>10,6</td></t<>						Aspergillus	19	33,6	10,6
Peilomyceta Peilomyceta Hebtales 0 0 3			Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	16	31.3	13.2
 Petromytetia Petro						Dermea	4	37.7	42.3
 						Mollisia	28	66.3	41.2
 Pestomycotia Pesto				Helotiales		Pezicula	9	59.7	33.1
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 						Pyrenoneziza	17	79 1	35.8
Peizomycotia 0 <t< td=""><td></td><td></td><td></td><td>Tapesia</td><td>5</td><td>64.3</td><td>23.7</td></t<>						Tapesia	5	64.3	23.7
Accomycota Petizomycetes Hebitales Petizomycetes Hebitales Circless Circless Hebitales Circless Circless Hebitales 40,0 35,0 7,00 38,0 7,0 28,0 38,0 7,0 28,0 38,0 7,0 28,0 38,0 7,0 28,0 38,0 7,0 28,0 38,0 7,0 28,0						Rispesella		52.0	20.6
Peilomycetia					Helotiaceae	Claussesemuses	5	20,9	12.0
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Ascomyota Rutsomycetes Heibtales Leotionycetes Heibtales Pezizonycotina 5 40, 2 5, 5 5, 2 2, 2 3, 3 5, 2 4, 2 3, 2 2, 2 3, 3 5, 2 3, 3 5, 2 3, 3 5, 2 3, 3 5, 2 3, 3 5, 2 3, 3 5, 2 3, 3 3, 3 </td <td></td> <td></td> <td></td> <td>Crocicreas</td> <td></td> <td>59,7</td> <td>29,1</td>						Crocicreas		59,7	29,1
Pezizomycotia 29 7,3 33,9 Ombroghia 3 9,7 28,1 7,5 12 Calycellina 6 6,5 57.2 57.2 57.2 Calycellina 6 6,5 57.2 <t< td=""><td></td><td></td><td></td><td>Cudoniella</td><td>3</td><td>44,0</td><td>38,4</td></t<>						Cudoniella	3	44,0	38,4
Pezizomycotia isobialies isob						Hymenoscyphus	29	57,3	35,9
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Petionnycetes Hebtales Calycina 4 9,75 11,8 Ascomycota Cistello 4 49,00 15,9 15						Calycellina	6	62,5	57,2
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Peticomycotina Seconda Operational Seconda Peticomycotina Seconda Peticomycotina Seconda Seconda <t< td=""><td></td><td></td><td></td><td></td><td></td><td>Cistella</td><td>4</td><td>49,0</td><td>15,9</td></t<>						Cistella	4	49,0	15,9
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Petizomycotia Hyaloscyphaceae Hyaloscyphaceae Hyaloscyphaceae National 6 8,1 7,2 Ascomycota Petizomycotia 5 6,0 7,2 7,1 1,2 2,1 1,2 2,1 1,2 2,1 1,2 2,1 1,2 2,1 1,2 2,1 1,1 2,1						Hyalopeziza	3	50,0	10,4
Petizomycotia 6 43,8 59, Lachnum 5 43,0 7,2 Ascomycota Feizomycotia 7,2 Molisina 4 4,5 1,5 Ascomycota Rutstroemiaceae Rutstroemiaceae Rutstroemiaceae 4 64,4 2,2 Ascomycota Orbilomycetes Orbilaceae Colorai 3 6,3 2,3 2,3 Orbilomycetes Orbilacea Orbilaceae Orbilaceae Ascobolaceae Ascobolaceae 5 5,3 43,3 4,3 2,3 3,3 Heleacea Helvellacea Ascobolaceae Ascobolaceae 5 5,3 3,3 4,3 2,4 <td></td> <td></td> <td></td> <td></td> <td>Hyaloscyphaceae</td> <td>Hyaloscypha</td> <td>6</td> <td>35,1</td> <td>7,3</td>					Hyaloscyphaceae	Hyaloscypha	6	35,1	7,3
Peizomycotia Lachum 21 4,1 21,1 Ascomycota Hillian 4 5 6,0 7,2 Mallian 4 1,4						Lachnellula	6	43,8	25,9
Pezizomycotina Lasiobelonium 5 36,0 7,2 Accomycota Rutsroemiacea 4 34,5 14,8 Accomycota Rutsroemiacea Rutsroemiacea 4 64,4 27,1 Scierotiniaceae Rutsroemiacea 4 64,4 27,1 Scierotiniaceae Rutsroemia 4 64,2 27,2 Scierotiniaceae Crobila 6 55,5 4,3 Orbiliomycetes Orbilales Orbilaceae Accobolaceae Accobolacea 6,00,5 38,8 Pezizomycetes Pezizomycetes Pezizoacea Peziza 16 60,5 38,8 Pezizonegete Pezizaceae Peziza 16 60,5 38,8 19,8 Pezizonegete Pezizaceae Peziza 16 60,5 38,8 19,8 Pezizonegete Conicohaetales Goimerelales Goimorelaceae Conicohaeta 3 36,5 19,8 Pezizonegete Feziza Goimerelales Goimorelaceae Conicoh						Lachnum	21	41.1	24.1
Pezizomycotia Mollisia 4 35,4 15 Ascomycota Rutsroemia.cea 84,5 14,8 14,5 14,8 14,5 14,8 Ascomycota Rutsroemia.cea Rutsroemia.cea 27,0 10,00 34,6 27,0 Chora 10 53,6 22,8 20,0 20		Pezizomycotina				Lasiobelonium	5	36.0	7.2
Ascomycota Ungukularia 3 4,1 4,2 1,4 Ascomycota Rutsroemiaceae Rutsroemiaceae Rutsroemia 4 64,4 27,1 2,2 2,3 3,0 4,4 2,2 2,2 3,3 3,0 4,2 2,5 5,7 3,3 2,2 2,2 3,3 3,0 1,4 2,2 2,5 5,7 3,3 3,0 3,1 2,2 5,5 3,3 4,0 3,3 4,0 3,3 4,0 3,3 4,0 3,3 4,0 3,2 1,2 2,4 8,4 4,0 3,2 1,2 2,4 8,						Mollisina	4	35.4	1.5
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Noticinal Noticinal <t< td=""><td></td><td rowspan="3"></td><td>Geopora</td><td>3</td><td>74,1</td><td>29,6</td></t<>						Geopora	3	74,1	29,6
Noticitian Noticit						Octospora	4	202,7	44,9
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Glomerellales Glomerellales Glomerellales Glomerellales Colletotrichum 4 52,4 8,4 Bionectriaceae Nectriopsis 3 41,0 38,9 Hypocreales Hypocreaceae Hypomyces 3 37,2 57,5 Nectriaceae Nectriaceae Nectriaceae 3 41,0 38,9 Nectriaceae Hypomyces 3 39,1 8,8 Nectriaceae Nectriaceae Nectriaceae 3 22,3 Ophiostomatales Ophiostomataceae Ophiostoma 3 28,4 19,9 Sordariales Ophiostomataceae Ophiostoma 3 28,4 19,9 Null riales Sordariales Chaetomiaceae Neurospora 3 40,3 1,5 Nylariales Xylariales Xylariaceae Hypoxylon 5 26,2 15,9 Xylariales Saccharomycetales Saccharomycetales Candida 10 13,8 17,8 Saccharomycotina Saccharomycetales Pneumocystidaceae Neumocystidaceae 10 13,8 17,8 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 75 9,9			Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	3	35,8	19,8
Sordariomycetes Hypocreales Bionectriaceae Nectriopsis 3 41,0 38,9 Hypocreales Hypocreales Hypocreaceae Hypocreaceae Hyporeaceae Hyporeaceae 3 47,2 57,5 Nectriaceae Nectriaceae Nectriaceae Nectriaceae S 39,9 13,4 Nectriaceae Nectriaceae Nectriaceae Nectriaceae S 35,5 22,3 Ophiostomatales Ophiostomataceae Ophiostoma 3 28,4 19,9 Sordariales Ophiostomataceae Ophiostoma 5 34,5 12,1 Sordariales Chaetomiaceae Neurospora 3 40,3 1,5 Xylariales Xylariaceae Hypoxylon 5 26,2 15,9 Xylariales Saccharomycetales Saccharomycetales Candida 10 13,8 17,8 Saccharomycotina Saccharomycetse Pneumocystidaceae Pneumocystis 4 7,5 9,9 Taphrinomycotina Pneumocystidales Pneumocystidaceae Pneumocystis 4 13,3 8,9 <td rowspan="2"></td> <td>Glomerellales</td> <td>Glomerellaceae</td> <td>Colletotrichum</td> <td>4</td> <td>52,4</td> <td>8,4</td>				Glomerellales	Glomerellaceae	Colletotrichum	4	52,4	8,4
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Hypocreales Hypocreales Hypocreales Hypocreales Hypocreales Hypocreales Hypocreales Hypocreales Sordariomycets 39,9 13,4 Nectriaceae Nectriaceae Nectria 8 38,5 22,3 Ophiostomatales Ophiostomataceae Ophiostoma 3 28,4 19,9 Sordariales Chaetomiaceae Thielavia 5 34,5 12,1 Sordariales Sordariales Sordariaceae Neurospora 3 40,3 1,5 Xylariales Xylariaceae Neurospora 3 60,5 13,5 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystidaceae Pneumocystidaceae 7 14,5 32,7						Hypocrea	3	47,2	57,5
Sordariomycetes Trichoderma 5 39,1 8,8 Nectriaceae Nectria 8 38,5 22,3 Ophiostomatales Ophiostomataceae Ophiostoma 3 28,4 19,9 Sordariales Ophiostomataceae Ophiostoma 5 34,5 12,1 Sordariales Chaetomiaceae Neurospora 3 40,3 1,5 Xylariales Xylariaceae Neurospora 3 60,5 13,5 Xylariales Xylariaceae Hypoxylon 5 26,2 15,9 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 Schizosaccharomycetales Schizosaccharomycetales Schizosaccharomycetaceae Schizosaccharomyces 7 11,5				Hypocreales	Hypocreaceae	Hypomyces	3	39.9	13.4
Sordariomycetes Nectriaceae Nectria S 38,5 22,3 Ophiostomatales Ophiostomataceae Ophiostoma 3 28,4 19,9 Sordariales Chaetomiaceae Thielavia 5 34,5 12,1 Sordariales Sordariaceae Neurospora 3 40,3 1,5 Xylariales Xylariaceae Neurospora 3 60,5 13,5 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9 Taphrinomycotina Pneumocystidaces Schizosaccharomycetales Schizosaccharomycetaceae Schizosaccharomyces 4 13,3 8,9						Trichoderma	5	39.1	8.8
Ophiostomatales Ophiostomataceae Ophiostomataceae Ophiostomatales Ophiostomataceae Ophiostoma 3 28,4 19,9 Sordariales Chaetomiaceae Thielavia 5 34,5 12,1 Sordariales Chaetomiaceae Neurospora 3 40,3 1,5 Xylariales Xylariaceae Neurospora 3 60,5 13,5 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9					Nectriaceae	Nectria	8	38.5	22.3
Sordariales Opinisormatica Opinisormatica </td <td></td> <td>Ophiostomatales</td> <td>Onbiostomataceae</td> <td>Onhiostoma</td> <td>3</td> <td>28.4</td> <td>19.9</td>				Ophiostomatales	Onbiostomataceae	Onhiostoma	3	28.4	19.9
Sordariales Checkinal case Intervia 3 34,3 12,1 Sordariales Sordariales Sordariaceae Neurospora 3 40,3 1,5 Xylariales Xylariaceae Hypoxylon 5 26,2 15,9 Xylariales Xylariaceae Hypoxylon 5 26,2 13,5 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Saccharomycotina Saccharomycetes Pneumocystidaceae Kluyveromyces 3 11,8 15,9 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9 Taphrinomycotina Schizosaccharomycetales Schizosaccharomycetalees Schizosaccharomycetaceae Schizosaccharomyces 4 13,3 8,9				Sordariales	Chaotomiacaceae	Thiologia		20,4	13,5
Sordariaceae Neurospora 3 40,3 1,5 Xylariales Xylariaceae Hypoxylon 5 26,2 15,9 Xylaria Xylariaceae Xylaria 3 60,5 13,5 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Rluyveromyces 3 11,8 15,9 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9					Condectormaceae	Neurosees		40.7	12,1
Xylariales Xylariaceae Hypoxylon 5 26,2 15,9 Xylariae Xylariaeeae Xylariae 3 60,5 13,5 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Saccharomycotina Saccharomycetes Saccharomycetales Kluyveromyces 3 16,9 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9				Xylariales	Sordariaceae	iveurospora	5	40,3	1,5
Xylaria 3 60,5 13,5 Saccharomycotina Saccharomycetes Saccharomycetales Candida 10 13,8 17,8 Saccharomycetales Saccharomycetales Saccharomycetales Candida 10 13,8 15,9 Saccharomycetales Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 Taphrinomycotina Pneumocystidomycetes Schizosaccharomycetales Schizosaccharomycetaceae Schizosaccharomyces 4 13,3 8,9					Xylariaceae	Hypoxylon	5	26,2	15,9
Saccharomycotina Saccharomycetes Saccharomycetales mitosporic Saccharomycetales Candida 10 13,8 17,8 Saccharomycotina Saccharomycetes Saccharomycetales Saccharomycetales Kluyveromyces 3 11,8 15,9 Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9 Schizosaccharomycetales Schizosaccharomycetales Schizosaccharomycetaceae Schizosaccharomyces 4 13,3 8,9		.				Xylaria	3	60,5	13,5
SaccharomycotinaSaccharomycetesSaccharomycetalesSaccharomycetaceaeKluyveromyces311,815,9SaccharomycotinaPneumocystidomycetesPneumocystidalesPneumocystidaceae714,532,7TaphrinomycotinaPneumocystidomycetesPneumocystidalesPneumocystidaceaePneumocystis47,59,9SchizosaccharomycetesSchizosaccharomycetalesSchizosaccharomycetaceaeSchizosaccharomyces413,38,9		Saccharomycotina	Saccharomycetes	Saccharomycetales	mitos poric Saccharomycetales	Candida	10	13,8	17,8
Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9 Schizosaccharomycetes Schizosaccharomycetales Schizosaccharomycetaceae Schizosaccharomyces 4 13,3 8,9					Saccharomycetaceae	Kluyveromyces	3	11,8	15,9
Taphrinomycotina Pneumocystidomycetes Pneumocystidales Pneumocystidaceae Pneumocystis 4 7,5 9,9 Schizosaccharomycetaes Schizosaccharomycetales Schizosaccharomycetaeeae Schizosaccharomycetaeeaeaeaeaeaeaeaeaeaeaeaeaeaeaeaeaeae					Saccharomycetateae	Saccharomyces	7	14,5	32,7
Schizosaccharomycetae Schizosaccharomycetales Schizosaccharomycetaee Schizosaccharomycetaee 4 13,3 8,9			Pneumocystidomycetes	Pneumocystidales	Pneumocystidaceae	Pneumocystis	4	7,5	9,9
			Schizosaccharomycetes	Schizosaccharomycetales	Schizosaccharomycetaceae	Schizosaccharomyces	4	13,3	8,9

Table 2 - (Continuation) Average genome sizes (Mbp) for every fungal genus for which values from at least three different species were available, including the results obtained in this study; fungal orders are arranged phylogenetically (http://tolweb.org/Fungi/); c.v. – coefficient of variation; n – number of species; Colour scale – green to red denote lowest to highest values for genome size (Mbp) and for coefficient of variation (%).

Phylum	Sub-phylum	Class	Order	Family	Genus		Genome size	
Phylom	Sab priylam			1 dinny	Genus		Mbp	c.v. (%)
	Agaricomycotina	Agaricomycetes		Agaricaceae	Agaricus	3	34,3	14,9
			Agaricales	Againcaceae	Cystoderma	6	36,6	41,0
			Agailcales	Physa la criace ae	Armillaria	7	74,2	28,0
				Pleurotaceae	Pleurotus	8	39,5	39,1
Basidiomycota					Bolete llus	3	71,1	4,6
					Boletus	29	60,1	26,0
				Boletaceae	Leccinum	10	58,6	34,2
					Pulveroboletus	3	52,1	37,0
					Tylopilus	3	69,8	5,5
			Boletales	Boletales incertae sedis	Leucogyrophana	5	38,7	31,4
				Coniophoraceae	Coniophora	4	30,1	18,2
				Gomphidiaceae	Gomphidius	3	199,6	52,3
				Rhizopogonaceae	Rhizopogon	8	56,6	36,1
				Serpulaceae	Austropaxillus	3	110,0	25,0
				Suillaceae	Suillus	21	57,7	36,4
			Polyporales	Polyporaceae	Pycnoporus	3	34,2	5,0
		Tremellomycetes	Tremellales	Treme llaceae Cryptococcus		5	19,8	2,0
	Pucciniomycotina	Pucciniomycetes	Pucciniales	Melampsoraceae	Melampsora	7	219,6	26,7
				Russiningana	Puccinia	21	269,5	75,7
				Fuccinaceae	Uromyces	8	461,4	30,2
Glomeromycota	incertae sedis	Glomeromycetes	Glomerales	Glomeraceae	Glomus	3	181,2	87,5
Microsporidia				Unikaryonidae	Encephalitozoon	4	2,4	14,3
Pezizomycota	Pezizomycotina	Geoglossomycetes	Geoglossales	Geoglossaceae	Geoglossum	3	35,8	2,4
7		incertae sedis	Museralas	Mucoraceae	Mucor	6	39,1	13,2
Zygomycota	willcoromycotina		widcorales	Rhizopodaceae	Rhizopus	3	39,3	29,3

Figure 2 and Table 2 also evidence that while some fungal groups are relatively homogeneous concerning genome sizes of their members (e.g., the Ascomycota orders Eurotiales, Helotiales, Hypocreales, Saccharomycetales and Sordariales and the Basidiomycota families Agaricaceae and Boletaceae), others encompass sharp differences. For instance, in the Ascomycota family Pyronemataceae, the average genome size in the genus Octospora (202.7 Mbp) is approximately 8x larger than that of the genus Cheilymenia (25.5 Mbp), and in the Ascomycota family Xylariaceae, the average genome size in the genus Xylaria (60.5 Mbp) is twice as large as in the genus Hypoxylon (26.2 Mbp). At a higher taxonomic level, mixed scenarios also emerge. In the Ascomycota, a trend for very small genomes in sub-phylla Saccharomycotina and Taphrinomycotina is clear and also below-average values are consistent in the class Eurotiomycetes; still a much higher variability is observed within the classes Pezizomycetes and Sordariomycetes. In the Basidiomycota, sharp differences occur among the Agaricomycotina, with smaller genomes in the orders Agaricales, Polyporales and Tremellales, but also in the family Coniophoraceae of the Boletales, and above-average values in several families of the Boletales. Among the Pucciniomycotina, the large genome size values consistently recorded among the Pucciniales contrast sharply with the low genome size values for non-Pucciniales members of the Pucciniomycotina scattered across different orders (not represented in Figs. 2 nor 3 due to insufficient number of organisms represented in each group): Agaricostilbales (*Agaricostilbum hyphaenes*, 17.9 Mbp); Classiculales (*Naiadella fluitans*, 52.7 Mbp); Microbotryales (*Microbotryum violaceum*, 26.1 Mbp); Mixiomycetales (*Mixia osmundae*, 13.6 Mbp); Sporidiobolales (*Rhodotorula graminis*, 21.0 Mbp, and *Sporobolomyces roseus*, 21.2 Mbp) (as revised by Tavares *et al.*, 2014). The average genome size for these non-Pucciniales members of the Pucciniomycotina is 25.4 Mbp. i.e., over 13x smaller than the average genome size of their sister order Pucciniales (335.6 Mbp after this study).

The mixed scenarios found across fungi regarding genome size variation, in some cases with stable genome size values over large taxonomic groups and in other cases with sharp variations within orders, families and genera (or even intraspecific variation) suggest that genome size is an important player of the speciation processes that shaped the fungal phylogeny, namely through polyploidisation, chromosome transfer and/or the activity of transposable elements (Spanu, 2012; Stuckenbrock & Croll, 2014). Although encompassing over 7000 members, the order Pucciniales is unified by singular biological characteristics, contrasting with other orders in the Pucciniomycotina, a situation that is mimicked by the contrast in genome sizes. The specialization of most rust fungi into single or few host species (frequently requiring two phylogenetically distant host species to complete their life cycles), their strict dependency of life host tissues and their gene-for-gene interaction with their hosts, combined with their large but variable genomes, suggest that genome size variation may be an active element shaping the evolution of rust fungi. Such knowledge is of utmost importance for disease resistance breeding programmes for several crops affected by rust diseases, but also for studies addressing the potential use of rusts as biological control agents of weeds.

Conclusions

In this work we used flow cytometry to determine the genome size of six rust species (Melampsora pulcherrima, Puccinia behenis, P. cichorii, P. pimpinellae, P. vincae and Uromyces dianthi), the causal agents of rust on Mercurialis annua, Silene latifolia, Cichorium intybus, Pimpinella villosa, Vinca major and Dianthus caryophyllus, respectively. Ranging between 192.2 and 567.1 Mbp, the values obtained in this study reinforce the order Pucciniales as the one with the largest average genome size across fungi, at 335.6 Mbp, i.e., nearly 8x larger than the average fungal genome. By comparing the genome sizes (and their variability) across the entire fungal kingdom, this study has provided clues for the importance of genome size variation into the shaping of the fungal phylogeny.

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References

- D'Hondt, L.; Hofte, M.; Van Bockstaele, E. and Leus, L. (2011) - Applications of flow cytometry in plant pathology for genome size determination, detection and physiological status. *Molecular Plant Pathology*, vol. 12, n. 8, p. 815-828.
- Doležel, J.; Sgorbati, S. and Lucretti, S. (1992) Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content

in plants. *Physiologia Plantarum*, vol. 85, n. 4, p. 625-631.

- Duplessis, S.; Cuomo, C. A.; Lin, Y. C.; Aerts, A.; Tisserant, E.; Veneault-Fourrey, C.; Joly, D. L.; Hacquard, S.; Amselem, J.; Cantarel, B. L.; Chiu, R.; Coutinho, P. M.; Feau, N.; Field, M.; Frey, P.; Gelhave, E.; Goldberg, J.; Grabherr, M. G.; Kodira, C. D.; Kohler, A.; Kues, U.; Lindquist, E .A.; Lucas, S. M.; Mago, R.; Mauceli, E.; Morin, E.; Murat, C.; Pangilinan, J. L.; Park, R.; Pearson, M.; Ouesneville, H.; Rouhier, N.; Sakthikumar, S.; Salamov, A. A.; Schmutz, J.; Selles, B.; Shapiro, H.; Tanguay, P.; Tuskan, G. A.; Henrissat, B.; Van De Peer, Y.; Rouze, P.; Ellis, J. G.; Dodds, P. N.; Schein, J. E.; Zhong, S.; Hamelin, R. C.; Grigoriev, I. V.; Szabo, L. J. and Martin, F. (2011) - Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proceedings of the National Academy of Sciences of the USA vol. 108, p. 9166-9171.
- Fernandez, D.; Talhinhas, P. & Duplessis, S. (2013) -Rust fungi: new advances on genomics and host--parasite interactions. In: Kempken, F. (Ed.) - *The Mycota*, vol XI - 2nd ed. Application in Agriculture. Berlin, Springer Verlag, pp. 315-341.
- Laundon, G.F. (1967) Terminology in the rust fungi. *Transactions of the British Mycological Society*, vol. 50, n. 2, p. 189-194.
- Loureiro, J.; Rodriguez, E.; Doležel, J. and Santos, C. (2007) - Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Annals of Botany*, vol. 100, n. 4, p. 875-888.
- Spanu, P. D. (2012) The genomics of obligate (and nonobligate) biotrophs. *Annual Review of Phytopathology*, vol. 50, May 1, p. 91-109.
- Stukenbrock, E. H. and Croll, D. (2014) The evolving fungal genome. *Fungal Biology Reviews*, vol. 28, n. 1, p. 1-12.
- Tavares, S.; Ramos, A. P.; Pires, A. S.; Azinheira, H.
 G.; Caldeirinha, P.; Link, T.; Abranches, R.; Silva,
 M. C.; Voegele, R. T.; Loureiro, J. and Talhinhas,
 P. (2014) Genome size analyses of Pucciniales reveal the largest fungal genomes. *Frontiers in Plant Sciences*, vol. 5, p. 422.