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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 sizes 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 specimens 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).

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

Rust	Host[1]	Location, date and status	Type of spores[2] and life cycle[3]	Herbarium reference (LISI-FUNGI)	Genome size (1C)					Reference standard[4]
					Mean pg	Mean Mbp	SD Mbp	CV (%)	n	
Melampsora pulcherrima Maire	*Mercurialis annua* (Eu)	Lisbon; 2/2015; spontaneous	I; Ma, He	00027	0.220	215.6	2.78	1.3	4	Rs
Puccinia behenis 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
Puccinia cichorii (DC.) Bellynck.	*Cichorium intybus* (As)	Oeiras; 6/2014; spontaneous	II; Mi	00029	0.342	334.8	38.1	10.5	2	Rs
Puccinia pimpinellae (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
Puccinia vincae (DC.) Plowr.	*Vinca major* (Apo)	Oeiras; 2/2015; spontaneous	II; Ma, Ae	00031	0.579	566.4	28.8	5.0	4	Sl
Uromyces dianthi (Pers.) Niessl	*Dianthus caryophyllus* (Ca)	Azenhas do Mar, Sintra; 1/2015; cultivated	III; Ma, He	00032	0.4282	418.8	-	-	1	Rs

[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.

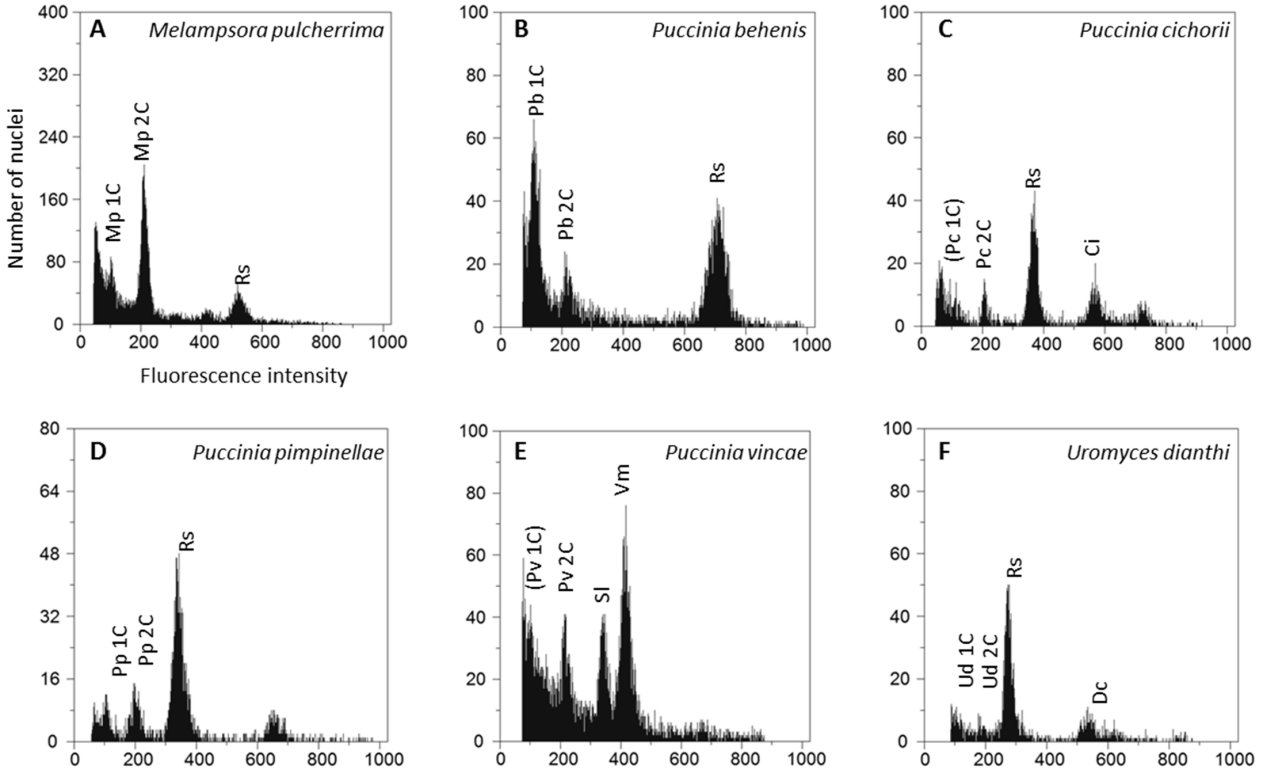

Figure 1 - Flow cytometric histograms of relative fluorescence intensities of propidium iodide-stained nuclei simultaneously isolated from: A – *Melampsora pulcherrima* (Mp) and the plant DNA reference standard, *Raphanus sativus* (Rs, 2C = 1.11 pg DNA); B – *Puccinia behenis* (Pb) and the plant DNA 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); 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.

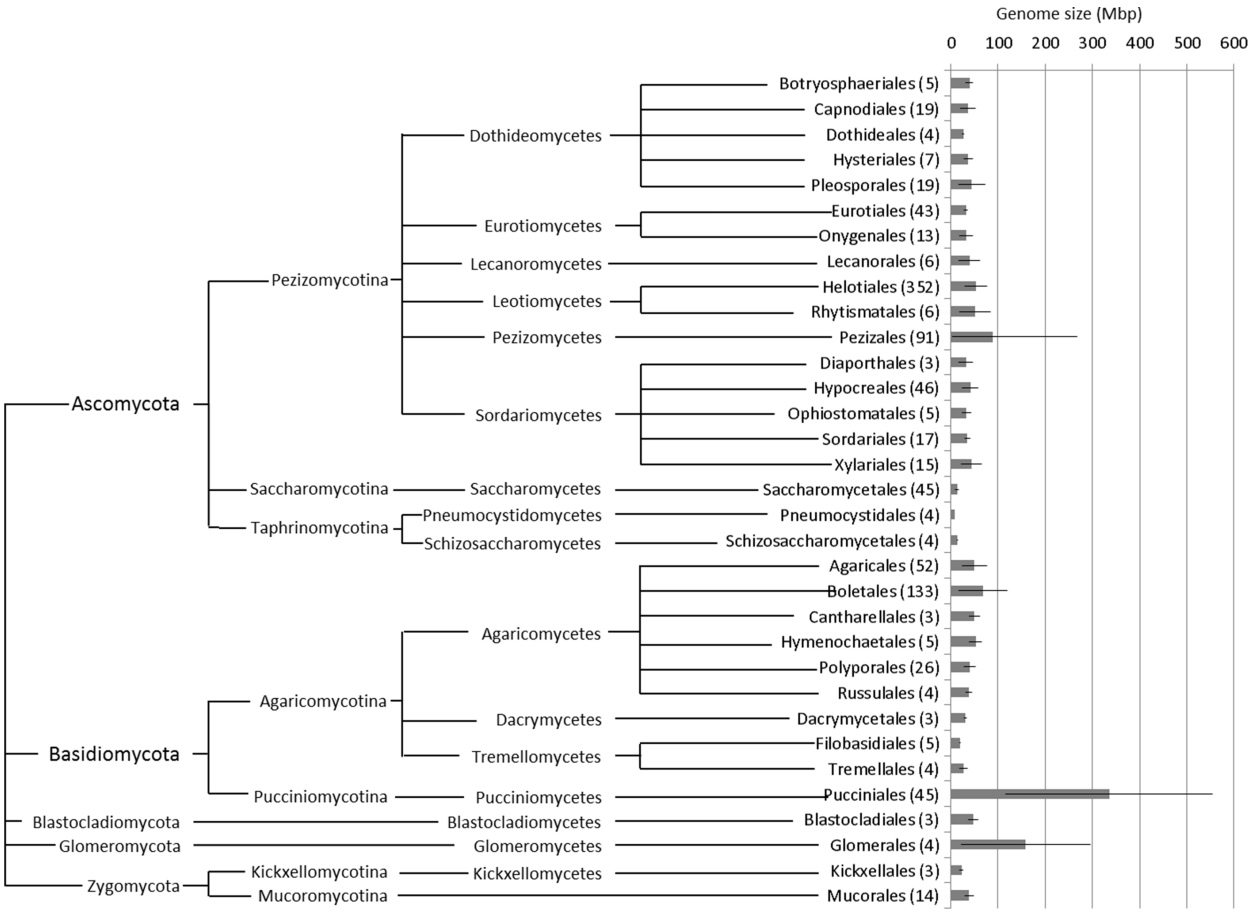

Figure 2 - Average genome sizes (Mbp) for every fungal order for which values from at least three different species were available (number of species in brackets), including the results obtained in this study; lines represent standard deviation; fungal orders are arranged phylogenetically (http://tolweb.org/Fungi/).

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 (%).

Phylum	Sub-phylum	Class	Order	Family	Genus	n	Genome size	
							Mbp	c.v. (%)
Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella	5	50,1	43,6
			Dothideales	Aureobasidiaceae	Aureobasidium	4	26,8	7,2
			Pleosporales	Pleosporaceae	Pyrenophora	3	38,6	14,2
		Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	19	33,6	10,6
					Penicillium	16	31,3	13,2
		Leotiomycetes	Helotiales	Dermateaceae	Dermea	4	37,7	42,3
					Mollisia	28	66,3	41,2
					Pezicula	9	59,7	33,1
					Pirottaea	4	61,9	27,5
					Pyrenopeziza	12	79,1	35,8
					Tapesia	6	64,3	23,7
				Helotiaceae	Bisporella	6	53,9	30,6
					Claussenomyces	5	29,8	13,0
					Crocicreas	7	59,7	29,1
					Cudoniella	3	44,0	38,4
					Hymenoscyphus	29	57,3	35,9
					Ombrophila	3	49,7	28,1
				Hyaloscyphaceae	Calycellina	6	62,5	57,2
					Calycina	4	37,5	1,9
					Cistella	4	49,0	15,9
					Dasyscyphus	3	40,8	27,9
					Hyalopeziza	3	50,0	10,4
					Hyaloscypha	6	35,1	7,3
					Lachnellula	6	43,8	25,9
					Lachnum	21	41,1	24,1
					Lasiobelonium	5	36,0	7,2
					Mollisina	4	35,4	1,5
					Unguicularia	3	41,5	14,8
				Rutstroemiaceae	Rutstroemia	4	64,4	27,1
				Sclerotiniaceae	Ciboria	10	53,6	22,8
					Encoelia	4	34,2	28,7
					Psilachnum	7	45,6	20,9
		Orbiliomycetes	Orbiliales	Orbiliaceae	Orbilia	6	53,9	44,3
		Pezizomycetes	Pezizales	Ascobolaceae	Ascobolus	5	52,3	33,0
				Helvellaceae	Helvella	6	69,5	38,8
				Pezizaceae	Peziza	16	56,0	29,8
				Pyronemataceae	Cheilymenia	4	25,5	5,7
					Geopora	3	74,1	29,6
					Octospora	4	202,7	44,9
					Otidea	3	35,8	4,0
					Scutellinia	7	31,2	12,9
		Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	3	35,8	19,8
			Glomerellales	Glomerellaceae	Colletotrichum	4	52,4	8,4
			Hypocreales	Bionectriaceae	Nectriopsis	3	41,0	38,9
				Hypocreaceae	Hypocrea	3	47,2	57,5
					Hypomyces	3	39,9	13,4
					Trichoderma	5	39,1	8,8
				Nectriaceae	Nectria	8	38,5	22,3
			Ophiostomatales	Ophiostomataceae	Ophiostoma	3	28,4	19,9
			Sordariales	Chaetomiaceae	Thielavia	5	34,5	12,1
				Sordariaceae	Neurospora	3	40,3	1,5
			Xylariales	Xylariaceae	Hypoxylon	5	26,2	15,9
					Xylaria	3	60,5	13,5
	Saccharomycotina	Saccharomycetes	Saccharomycetales	mitosporic Saccharomycetales	Candida	10	13,8	17,8
				Saccharomycetaceae	Kluyveromyces	3	11,8	15,9
					Saccharomyces	7	14,5	32,7
	Taphrinomycotina	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	n	Genome size	
							Mbp	c.v. (%)
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Agaricaceae	Agaricus	3	34,3	14,9
					Cystoderma	6	36,6	41,0
				Physalacriaceae	Armillaria	7	74,2	28,0
				Pleurotaceae	Pleurotus	8	39,5	39,1
			Boletales	Boletaceae	Boletellus	3	71,1	4,6
					Boletus	29	60,1	26,0
					Leccinum	10	58,6	34,2
					Pulveroboletus	3	52,1	37,0
					Tylopilus	3	69,8	5,5
				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	Tremellaceae	Cryptococcus	5	19,8	2,0
	Pucciniomycotina	Pucciniomycetes	Pucciniales	Melampsoraceae	Melampsora	7	219,6	26,7
				Pucciniaceae	Puccinia	21	269,5	75,7
					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
Zygomycota	Mucoromycotina	incertae sedis	Mucorales	Mucoraceae	Mucor	6	39,1	13,2
				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 phylo-

genetically 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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