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Genome Sequence Resources of *Colletotrichum abscissum*, the Causal Agent of Citrus Post-Bloom Fruit Drop, and the Closely Related Species *C. filicis*

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Keywords
genomics, plant-pathogenic fungus, post-bloom fruit drop

Genome Announcement

Colletotrichum is one of the most diverse and destructive plant pathogenic fungi containing genus, responsible for significant losses in agriculture and forest plants. In the present study, we present the draft whole-genome sequence of two closely related species belonging to the *Colletotrichum acutatum* species complex: *C. abscissum*, the causal agent of citrus post-bloom fruit drop and *C. filicis*, a rare species described to accommodate an isolate obtained from an identified fern in Costa Rica. The data resources presented here will provide insights into genetic elements associated with citrus post-bloom fruit drop and into the evolution of *Colletotrichum*.

Colletotrichum species are associated with a broad range of plant diseases, generally referred to anthracnose. Virtually every cultivated plant grown in the world is susceptible to one or more species of *Colletotrichum* and it has been considered scientifically and economically one of the most important groups of plant pathogenic fungi (Dean et al. 2012). *Colletotrichum* comprises more than 257 species listed and grouped into 15 species complexes; among those, the *C. acutatum* species complex, which is composed of a diverse and relatively closely related group of plant pathogenic fungi within the genus, and hence it was suggested as a model system to study evolution and host specialization in plant pathogens (Baroncelli et al. 2017).

C. abscissum is the causal agent of citrus post-bloom fruit drop (Crous et al. 2015). The fungus is restricted to *Citrus* spp. (Rutaceae) and *Psidium* spp. in the American continent (Bragança et al. 2016; Crous et al. 2015; Talhinhas and Baroncelli 2021). *C. abscissum* strain Ca142 (also named LGMF1258) was obtained from blossom blight symptoms of sweet orange (*Citrus sinensis*) petal collected in a commercial orchard in Santa Cruz do Rio Pardo, São Paulo, Brazil. Pathogenicity of *C. abscissum* strain Ca142 was confirmed in vivo and in vitro experiments (Goulin et al. 2019). The strain described here is available at the Plant Pathogenic Fungi Collection of the Department of Phytopathology and Nematology (Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, Brazil).

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Table 1. Summary statistics of *Colletotrichum abscissum* and *C. filicis* genomes

	Statistics	
Variables	***C. abscissum***	***C. filicis***
Culture collection	LGMF1258	CBS101611
Average coverage	210×	115×
Number of scaffolds	423	367
Total assembly length (mb)	54.00	62.97
Scaffold N50 (bp)	321,765	325,017
Scaffold L50 (bp)	46	60
GC (%)	51.10	46.00
BUSCO completeness (%)	98.70	98.90
Number of predicted genes	15,499	17,391
Secreted proteins	2,020	2,096
Biosynthetic gene clusters	59	57
Genome accession	SDAQ00000000.1	MOOC00000000.1

C. filicis is a newly described fungal species to accommodate an isolate (CBS 101611: ex-type culture) obtained from an unidentified fern (Pteridophyta) in Costa Rica (Crous et al. 2021). There are no other reports of this fungus worldwide, and other species of *Colletotrichum* were reported from ferns in Costa Rica, raising serious concern about the conservation status of *C. filicis* (Talhinhas and Baroncelli 2021). The strain was retrieved and is maintained in the culture collection of the Westerdijk Fungal Biodiversity Institute.

Both strains (*C. abscissum* Ca142 and *C. filicis* CBS 101611) were grown in potato dextrose agar medium (Merk), over the cellophane membrane, at 28°C for 3 days. The mycelium was harvested with a sterile spatula, placed in a mortar, and ground with liquid nitrogen and a pestle into fine powders. The genomic DNA was extracted following the Raeder and Broda method (Raeder and Broda 1985), precipitated with 2-propanol, washed in ethanol (70%), dried, and dissolved in Milli-Q water. The treatment with RNase was conducted before the precipitation process. The quality of DNA extracted was evaluated by agarose gel electrophoresis and its concentration was measured with an ND-1000 UV-Vis spectrophotometer (NanoDrop, Thermo Scientific).

Genomic DNA libraries were constructed using the NEB Ultra II DNA Library Prep Kit and sequenced 300PE on an Illumina MiSeq sequencer (Illumina Inc., San Diego, CA). The quality of the sequences was checked on FastQC v0.11.7 (Andrews 2010) and low-quality reads and adaptors were trimmed with Trimmomatic v0.33 (Bolger et al. 2014). De novo assemblies were performed with SPAdes v3.11.1 (Bankevich et al. 2012). Low coverage scaffolds were identified and removed while scaffolds belonging to mtDNA and rRNA clusters were identified and masked. The assembly completeness was assessed based on the sordariomyceta_odb9 lineage dataset using BUSCO v.3 (Simão et al. 2015); for both genomes, the final score value was above 98% (Table 1). The nuclear genome of *C. abscissum* Ca142 consists of 423 scaffolds, with a total assembly length of 54.00 Mbp (N50 = 321,765 and L50 = 46), 51.10% GC-content, and a maximum scaffold size of 2,556,205 bp. The nuclear genome of *C. filicis* CBS 101611 consists of 367 scaffolds, with a total assembly length of 62.97 Mbp (N50 = 325,017 and L50 = 60), 46.00% GC-content, and a maximum scaffold size of 1,547,318 bp (Table 1).

MAKER 3.01.02 pipeline (Holt and Yandell 2011) was used for gene annotation as described by Baroncelli et al. (2016). Available transcriptomic data of *C. abscissum* Ca142 were used to train Augustus v2.5.5 (Stanke et al. 2006) and as biological evidence in MAKER3, while GeneMark-ES v4.48 (Borodovsky and Lomsadze 2011) was used for ab initio gene prediction. Overall, 15,499 and 17,391 protein-coding gene models were predicted in the nuclear genome of *C. abscissum* and C. *filicis*, respectively. SignalP v5.0 (Almagro Armenteros et al. 2019) revealed that 2,020 and 2,096 predicted proteins in *C. abscissum* Ca142 and *C. filicis* CBS 101611, respectively, contain a secretion signal peptide. EffectorP v3.0 (Sperschneider and Dodds 2022) was used to predict secreted effector candidates. Overall, 640 and 711 proteins in *C. abscissum* and C. *filicis*, respectively, might be putatively involved in fungal pathogenicity. Secondary metabolite biosynthetic gene clusters (BGCs) were predicted with antiSMASH v6.1.1 (Blin et al. 2021) using a relaxed detection strictness. In total, 59 and 57 BGCs were identified in the genome of *C. abscissum* and C. *filicis*, respectively, of which 55 syntenic between the two species (Goulin et al. 2022).

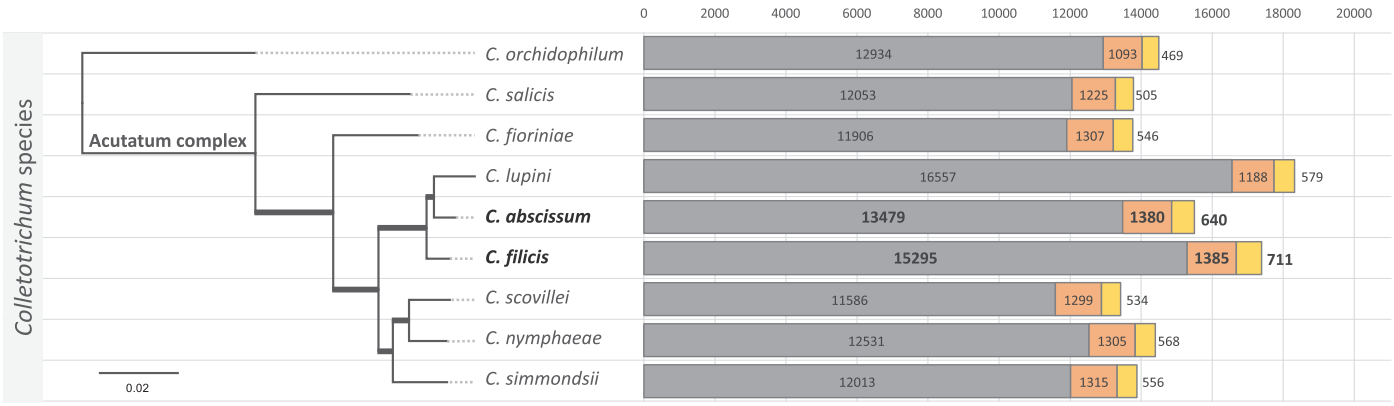

Fig. 1. Comparative genomics of species belonging to the *Colletotrichum acutatum* species complex. On the left, the phylogenomic tree was performed with FastTree v 2.1.11 (Price et al. 2010) based on 8,140 single copy orthologue sequences (5,206,550 characters) identified with Orthofinder v 2.5.4 (Emms and Kelly 2019). Bar chart reports the number of protein-coding genes for each genome (not secreted predicted protein in darkest gray, secreted proteins not predicted to be candidate effectors in orange (medium gray), and candidate effectors in yellow (light gray)).

A comparative analysis of the newly sequenced genomes with those of other members of the acutatum complex, publicly available, revealed that genome features of *C. abscissum* and C*. filicis* are similar to those of closely related species (Fig. 1) (Baroncelli et al. 2014, 2016, 2018, 2021; Huo et al. 2021).

In this study, we provide draft genome sequences of *C. abscissum* and *C. filicis*. To our knowledge, these are the first available genomes for *C. abscissum* and *C. filicis*. These genomes will provide a new useful resource for future research on citrus post-bloom fruit drop and comparative genomic studies of the genus *Colletotrichum*.

Data Availability

The Whole-Genome Shotgun projects have been deposited in GenBank under the accession numbers SDAQ00000000 (*C. abscissum*, BioProject PRJNA516018, BioSample SAMN10780915) and MOOC00000000 (*C. filicis*, BioProject PRJNA350378, BioSample SAMN05938703).

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