

# Conserved Meiotic Machinery in *Glomus* spp., a Putatively Ancient Asexual Fungal Lineage

Sébastien Halary<sup>†,1</sup>, Shehre-Banoo Malik<sup>†,2</sup>, Levannia Lildhar<sup>3</sup>, Claudio H. Slamovits<sup>2</sup>, Mohamed Hijri<sup>1</sup>, and Nicolas Corradi<sup>\*,3</sup>

<sup>1</sup>Département de sciences biologiques, Institut de recherche en biologie végétale, Université de Montréal, Canada

<sup>2</sup>Canadian Institute for Advanced Research, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Canada

<sup>3</sup>Canadian Institute for Advanced Research, Department of Biology, University of Ottawa, Canada

\*Corresponding author: E-mail: ncorradi@uottawa.ca.

<sup>†</sup>These authors contributed equally to this work.

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The accession numbers of all sequences analyzed in this study are provided.

## Abstract

Arbuscular mycorrhizal fungi (AMF) represent an ecologically important and evolutionarily intriguing group of symbionts of land plants, currently thought to have propagated clonally for over 500 Myr. AMF produce multinucleate spores and may exchange nuclei through anastomosis, but meiosis has never been observed in this group. A provocative alternative for their successful and long asexual evolutionary history is that these organisms may have cryptic sex, allowing them to recombine alleles and compensate for deleterious mutations. This is partly supported by reports of recombination among some of their natural populations. We explored this hypothesis by searching for some of the primary tools for a sustainable sexual cycle—the genes whose products are required for proper completion of meiotic recombination in yeast—in the genomes of four AMF and compared them with homologs of representative ascomycete, basidiomycete, chytridiomycete, and zygomycete fungi. Our investigation used molecular and bioinformatic tools to identify homologs of 51 meiotic genes, including seven meiosis-specific genes and other “core meiotic genes” conserved in the genomes of the AMF *Glomus diaphanum* (MUCL 43196), *Glomus irregulare* (DAOM-197198), *Glomus clarum* (DAOM 234281), and *Glomus cerebriforme* (DAOM 227022). Homology of AMF meiosis-specific genes was verified by phylogenetic analyses with representative fungi, animals (*Mus*, *Hydra*), and a choanoflagellate (*Monosiga*). Together, these results indicate that these supposedly ancient asexual fungi may be capable of undergoing a conventional meiosis; a hypothesis that is consistent with previous reports of recombination within and across some of their populations.

**Key words:** comparative genomics, meiosis, fungi, arbuscular mycorrhizal fungi, genome evolution, ancient asexuals.

## Introduction

Meiosis, a hallmark of eukaryotic cells, is necessary for the production of gametes (e.g., spores). It is a major driver of recombination in all eukaryotes, resulting in the shuffling of genomic material between chromosomes. Although predominant throughout eukaryotes (Malik et al. 2008), the advantages versus costs of sexual reproduction and meiosis are still a matter of debate (Ackerman et al. 2010; Archetti 2010). Indeed, although evolutionary theory predicts a rapid extinction of asexual lineages as a consequence of the accumulation of deleterious mutations (Otto and Lenormand 2002; Otto 2009), a number of eukaryotes commonly referred to as “ancient asexuals” (Maynard-Smith 1986) have thrived across diverse

ecosystems for millions of years without sex. These ancient asexuals include evolutionarily distant groups such as the bdelloid rotifers, the arbuscular mycorrhizal fungi (AMF) and a number of protist lineages (Maynard-Smith 1986; Haig 1993; Judson and Normark 1996; Gordo and Charlesworth 2000; Normark 2003; Schurko et al. 2009), which are all derived from sexual ancestors (Ramesh et al. 2005).

In the last decade, research on ancient asexuals has provided new insights into how these organisms coped with the absence of observable sexual cycles. First, the majority of these “asexuals” have been found to exhibit genetic recombination consistent with sexual reproduction (Schurko et al. 2009; Heitman 2010). Second, all former putatively asexual

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taxa whose completely sequenced genomes have been surveyed so far—including a number of medically important pathogens (i.e., *Giardia intestinalis*, *Trichomonas vaginalis*, *Entamoeba histolytica*, several microsporidia, *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., etc.)—have “core meiotic gene” homologs that encode a set of proteins that only function during meiosis in model animals, fungi, and plants studied, and other general DNA repair proteins that are required for proper completion of meiotic recombination in model organisms (Villeneuve and Hillers 2001; Wong et al. 2003; Ramesh et al. 2005; Lee et al. 2008, 2010; Malik et al. 2008), consistent with the hypothesis that the core meiotic gene products would also function in meiotic recombination (or a similar recently derived parasexual process) in these organisms. Orthologs of meiosis-specific genes would be under relaxed functional constraint and accumulate deleterious mutations in asexual organisms and could not be detected by comparative genomic approaches (discussed further by Schurko and Logsdon 2008; Schurko et al. 2009 and references therein), whereas general function DNA repair proteins required for meiotic and mitotic recombination would persist (e.g., *Rad51*, *Mlh1*, *Pms1*, *Msh2*, *Msh6*, *Rad50*, *Mre11*, etc.). Third, sexual reproduction has recently been identified in other organisms long thought to reproduce only clonally, such as the fungus *Aspergillus fumigatus*, (Poggeler 2002; Heitman 2006, 2010; O’Gorman et al. 2009), indicating that asexuality should not be assumed based solely on absence of a recognizable sexual stage.

AMF represent one of those ancient asexual lineages whose genomes have yet to be explored for the evidence of sex. These coenocytic and multinucleate fungi represent a group of plant symbionts that play a dramatic role in terrestrial ecosystems and that are thought to date back 500 My (Humphreys et al. 2010). Attempts to explain their extreme longevity in the absence of sex have generally focused on their atypical cellular content, which includes hundreds of nuclei per spore that may, or may not, be genetically divergent (Kuhn et al. 2001; Pawlowska and Taylor 2004; Hijri and Sanders 2005; Pawlowska 2005; Stukenbrock and Rosendahl 2005). These nuclei are thought to be exchanged at a certain rate among different AMF through anastomosis (fusion between hyphae) (Croll et al. 2008; Angelard et al. 2010) and to regularly recombine.

In this study, we explore the potential for meiotic recombination in AMF by searching for the core meiotic genes across the genomes of four AMF species. This “core” of genes, a term first coined by Villeneuve and Hillers (2001) with reference to meiotic recombination machinery of model animal, fungal, and plant systems, and later expanded by phylogenomic analyses to include diverse protists (Ramesh et al. 2005; Malik et al. 2008), encodes 30 proteins that comprise the conserved meiotic recombination machinery of eukaryotes. The present comparative genomic survey allows

us to form a hypothetical model for meiosis-like recombination in a cryptic sexual (or parasexual) cycle in AMF, which is consistent with previous reports of recombination in these organisms and may be tested further by future functional studies.

## Materials and Methods

### Acquisition of a Low Coverage Survey of *Glomus diaphanum*, *Glomus irregulare*, *Glomus clarum*, and *Glomus cerebriforme*

Low coverage genome surveys of *G. diaphanum* (MUCL 43196), *G. irregulare* (DAOM-197198), *G. cerebriforme* (DAOM 227022), and *G. clarum* (DAOM 234281) were obtained using the 454 pyrosequencing facility at the Génome Québec Innovation Centre (McGill University, Canada). In all cases, an average of 350 Mb of genome data have been generated using an average read length of 336 bp (median length 368 bp). The assemblies resulted in an average of 46,000 contigs for all species with an average length of 1,010 bp. All AMF sequences identified in this study and their relative accession numbers are shown in table 1 and the [supplementary table S1, Supplementary Material](#) online.

### In Silico Identification of AMF Meiosis Genes

A total number of 87 genes known to be required for proper meiotic recombination in *Saccharomyces cerevisiae* have been searched across the genomes of *G. irregulare* (DAOM 181602) and *G. diaphanum* (MUCL 43196) using three independent, yet highly complementary approaches (table 1; [supplementary table S1, Supplementary Material](#) online). This list of genes is based on previous genomic surveys of fungi and other eukaryotes (Malik et al. 2008; Burns et al. 2010; Nowrousian et al. 2010), which we expanded here to include representatives from those extant fungal phyla with publicly available complete or near-completely sequenced genomes in gene depositories, that is, the Basidiomycota *C. neoformans*, *Ustilago maydis*, and *Coprinus cinereus*; the Chytridiomycota *Batrachomyces dendrobatidis* and *Allomyces macrogynus*; and the Zygomycota *Rhizopus oryzae* and *Phycomyces blakesleeanae*.

Putative meiotic genes were initially searched using reciprocal TblastX and TblastN (Altschul et al. 1997) searches of publicly available expressed sequence tags from *G. irregulare* deposited in GenBank (National Center for Biotechnology Information [NCBI]). This preliminary analysis allowed the identification of several AMF transcripts with unambiguous homology to meiosis genes from a number of more distantly related fungal taxa. The remaining genes were from a low coverage genome survey of *G. diaphanum*, *G. clarum*, and *G. cerebriforme* using reciprocal BlastX, TblastX, and TblastN procedures and by using a polymerase chain reaction (PCR) approach based on degenerate primers. Overall, the combination of these bioinformatics and molecular approaches allowed the identification of

**Table 1**

List of 51 AMF Genes Directly or Indirectly Involved in the Process of Meiosis

Gene	Accession Number	Organism	CS-Blast Best Hit			Pfam/NCBI CDD
			Alignment Score	e Value	% Identity (Identities—Positives)	
DMC1 <sup>a</sup>	FR775428	Sc	512	$\times 10^{-146}$	60—74	PF08423.5 (Rad51); PLN03187 (meiotic recombinase Dmc1)
DNL4	GW_113491.1	Nc	340	$5.00 \times 10^{-94}$	53—73	PF01068.1 (DNA_ligase_A_M), PF04679.9 (DNA_ligase_A_C)
EXO1	GW_104190.1	Sm	125	$4.00 \times 10^{-30}$	43—74	—
HOP2 <sup>a</sup>	FR775431	Sc	174	$7.00 \times 10^{-45}$	21—45	PF07106 (TBPIP)
HRR25	HQ262397	Sm	259	$9.00 \times 10^{-70}$	96—97	PF00069.19 (Pkinase)
HTA1	GW_094651.1	Nc	166	$5.00 \times 10^{-42}$	77—83	PF00125.18 (Histone)
HTA2	GW_095958.1	Nc	130	$2.00 \times 10^{-31}$	84—88	PF00125.18 (Histone)
MEC1	FR775435	Sm	260	$6.00 \times 10^{-70}$	46—69	PF00613.14 (Pl3Ka)
MLH1	FR775257	Nc	319	$1.00 \times 10^{-87}$	43—56	—
MLH3	GW_092048.1	Sm	229	$1.00 \times 10^{-60}$	34—53	—
MND1 <sup>a</sup>	GW_112099.1	Sc	212	$3.00 \times 10^{-56}$	27—46	PF03962.9 (Mnd1)
MRE11	GW_118657.1	Sm	454	$\times 10^{-128}$	47—64	PF00149.22 (Metallophos)
MSC1	FR822520	Sm	148	$1.00 \times 10^{-36}$	45—59	—
MSC7	BM027003.1	Sc	290	$6.00 \times 10^{-79}$	39—63	PF00171.1 (Aldedh)
MSH2	HQ262398	Sc	224	$3.00 \times 10^{-59}$	60—70	PF00488.15 (MutS_V)
MSH4	FR877640	Sc	212	$9.00 \times 10^{-56}$	35—62	PF05192.12 (MutS_III), cd03282 (ABC_MSH4_euk)
MSH5 <sup>b</sup>	FR775430	Sc	231	$1.00 \times 10^{-80}$	34—51	PF00488.15 (MutS_V); cd03243 (ABC_MSH5_euk)
MSH6	GW_100645.1	Sm	346	$8.00 \times 10^{-96}$	61—76	PF01624.14 (MutS_I), PF05188.11 (MutS_II)
MUS81	FR775258	Sm	463	$\times 10^{-131}$	38—55	PF02732.9 (ERCC4)
NAM8/MRE2	FR775259	Sm	216	$1.00 \times 10^{-56}$	61—79	PF00076.16 (RRM_1)
PDS5	GW_121462.1	Sm	257	$5.00 \times 10^{-69}$	25—42	—
PMS1	GW_084934.1	Nc	137	$4.00 \times 10^{-33}$	46—72	PF01119.13 (DNA_mis_repair)
RAD1	FR775256	Sm	251	$2.00 \times 10^{-67}$	53—68	PF02732.9 (ERCC4)
RAD17	FR822519	Nc	253	$4.00 \times 10^{-68}$	47—60	PF03215.9 (Rad17)
Rad2	GW_108841.1	Sm	206	$8.00 \times 10^{-54}$	40—57	—
Rad21	FR877641	Nc	198	$2.00 \times 10^{-51}$	39—55	—
RAD23	BM959423.1	Nc	151	$1.00 \times 10^{-37}$	43—63	PF09280.5 (XPC-binding), PF00627.25 (UBA)
RAD50	FR775261	Sm	279	$1.00 \times 10^{-75}$	36—55	PF02463.13 (SMC_N)
RAD51	FR877639	Nc	377	$\times 10^{-105}$	70—83	PF08423.5 (Rad51)
RAD52	GW_121846.1	Sc	130	$5.00 \times 10^{-31}$	51—68	PF04098.9 (Rad52_Rad22)
RAD54 <sup>b</sup>	GW_109886.1	Sm	195	$8.00 \times 10^{-51}$	39—57	PF00271.25 (Helicase_C)
REC8	FR775434	Nc	146	$2.00 \times 10^{-36}$	30—51	PF04825 (Rad21_Rec8)
RFA1	FR838021	Nc	278	$1.00 \times 10^{-75}$	24—43	PF08646.4 (Rep_fac-A_C)
RFA2	FR838020	Nc	198	$2.00 \times 10^{-51}$	20—32	—
SCC3	GW_117977.1	Nc	299	$1.00 \times 10^{-81}$	23—41	—
SGS1	FR775427	Nc	189	$8.00 \times 10^{-49}$	32—50	PF00271.25 (Helicase_C)
SLX1	FR775433	Nc	197	$4.00 \times 10^{-51}$	35—47	PF01541.18 (GIY-YIG)
SMC1	GW_121295.1	Sm	224	$5.00 \times 10^{-59}$	66—79	PF02463.13 (SMC_N)
SMC2	GW_094523.1	Nc	286	$8.00 \times 10^{-78}$	70—83	PF02463.13 (SMC_N)
SMC3	GW_093795.1	Nc	257	$4.00 \times 10^{-69}$	69—84	PF02463.13 (SMC_N)
SMC4	GW_088627.1	Sm	269	$1.00 \times 10^{-72}$	66—82	PF02463.13 (SMC_N)
SMC5	FR775432	Sm	300	$9.00 \times 10^{-82}$	26—50	—
SMC6	FR775262	Sm	293	$6.00 \times 10^{-80}$	37—61	PF02463.13 (SMC_N)
SPO11	FR822517	Nc	167	$2.00 \times 10^{-42}$	38—66	TP6A_N (Type IIB DNA topoisomerase), PLN00060 (meiotic recombination protein SPO11)
SRS2 <sup>a</sup>	FR822518	Sc	127	$3.00 \times 10^{-31}$	51—74	PF00580.15 (UvrD-helicase)
TOP1	FR775263	Sc	491	$\times 10^{-139}$	48—63	PF01028.14 (Topoisom_I)
TOP2	FR775264	Sm	535	$\times 10^{-152}$	47—62	PF00521.1 (DNA_topoisolV)

Table 1 Continued

Gene	Accession Number	Organism	CS-Blast Best Hit			
			Alignment Score	e Value	% Identity (Identities—Positives)	Pfam/NCBI CDD
TOP3	GW_111366.1	Nc	403	$\times 10^{-113}$	57—71	PF01131.14 (Topoisom_bac)
YKU70	FR775265	Nc	276	$7.00 \times 10^{-75}$	27—43	PF03730.8 (Ku_C)
YKU80	GW_117534.1	Nc	304	$3.00 \times 10^{-83}$	37—55	PF02735.10 (Ku)
Zip4/Spo22	FR822521	Nc	151	$2.00 \times 10^{-37}$	31—47	—

NOTE.—Pfam families and domains were retrieved from the Sanger Pfam utility server (2), for e values less than or equal to  $1 \times 10^{-05}$  (Finn et al. 2008).

<sup>a</sup> Best CS-BLAST hit information (source organism, score, e value, and % identity) of each *Glomus* meiotic protein against a database containing the *Saccharomyces cerevisiae* S288c (Sc), *Neurospora crassa* OR74A (Nc), and *Sordaria macrospora* (Sm) genomes or against the Sc genome when no ortholog is present in the two other ascomycetes.

<sup>b</sup> Alignments were performed using CS-BLAST with two iterations. Meiosis-specific genes are highlighted in gray cells. Each *Glomus* spp. protein best hit information corresponds to the expected fungal ortholog, except for Rad54, for which corresponding hits represent the greatest identity percentage and the greatest hit length.

51 meiosis-related genes in AMF (table 1). Only the best hits, exhibiting a cut off e value lower than  $1 \times 10^{-05}$ , were retained in Blast searches. Homology was also asserted using sequence context-specific profiles for homology searches (CS-BLAST; table 1) (Biegert and Soding 2009) for all meiosis-related genes. CS-BLAST searches were performed as follows: *Glomus* spp. proteins were aligned using CS-BLAST with two iterations, against all *S. cerevisiae* S288c, *Neurospora crassa* OR74A, and *Sordaria macrospora* proteins or against *S. cerevisiae* when no ortholog was present in *N. crassa* or *S. macrospora*, *S. cerevisiae*, and *N. crassa* proteins were obtained from the NCBI and *S. macrospora* proteins were downloaded from “The *S. macrospora* genome site” of the Ruhr-University Bochum ([Nowrousian et al. 2010], <http://c4-1-8.serverhosting.rub.de/public/downloads.html>). Pfam family and domain information were also retrieved from the Sanger Pfam server (<http://pfam.sanger.ac.uk/>). HMMER searches failed to identify additional AMF genes that could not be found using traditional Blast procedures. Other publicly accessible genome sequence databases we searched include the Broad Institute’s databases for *A. macrogynus*, *R. oryzae*, and *B. dendrobatidis* (<http://www.broadinstitute.org/scientific-community/data>) and the Joint Genome Institute’s (JGI) databases for *P. blakesleeanus*, *U. maydis*, and *Monosiga brevicollis* (<http://genome.jgi-psf.org/>).

### Polymerase Chain Reaction

The AMF Msh2 and HRR25 homologues were identified by degenerate PCR. Degenerate PCR was performed using combinations of new primers designed on conserved amino acid motifs of proteins encoding Msh2 (Msh2F: 5′-ATH GAR YTI GGN GTI AAR GA-3′; motif IQLGVK and Msh2R: 5′-AA Y ATG GGN GGI AAR AGY ACI TA-3′; motif NMGGKST), and HRR25 (Hrr25F 5′-CCI CAR YTI GAR TAY GAR GC-3′; motif PQLEYE and Hrr25R: 5′-GGN GTI GTC ATY TTY TTY TCC AT-3′; motif MEKKMTT). Briefly, PCR reactions were performed using conditions described previously for other AMF genes (Corradi et al. 2004) and DNA extracted from in vitro cultures of *G. diaphanum*. Successful PCR amplicons were subjected to

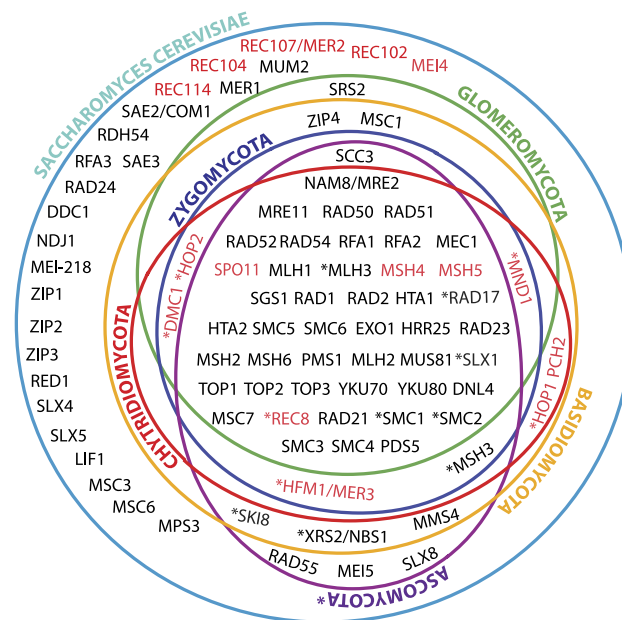
gel extraction, bacterial cloning, and conventional Sanger DNA sequencing.

### Phylogenetic Verification of *Glomus* spp. Meiosis-Specific Protein-Coding Genes in Gene Families

The identification of meiosis-specific gene homologs in *Glomus* spp. was subject to further scrutiny by phylogenetic comparison to other representative eukaryotes, to ensure that orthologs (vs. paralogs) of the seven meiosis-specific genes (*Rec8*, *Spo11-1*, *Dmc1*, *Hop2*, *Mnd1*, *Msh4*, and *Msh5*) were correctly identified. Generally, we compared *Glomus* spp. sequences with data from representative fungi, animals (*Mus musculus*, *Hydra magnipapillata*), and a choanoflagellate (*M. brevicollis*). Sequences obtained from *G. cerebriforme* (Msh4, Rad21, Dmc1) and *G. clarum* (Rad51, Dmc1) were added to the phylogenetic analyses in an effort to improve resolution.

We assembled *Glomus* spp. meiosis-specific genes and annotated putative open reading frames by using Geneious Pro 5.3.6 (Biomatters Ltd.) with reference to pairwise comparisons made by BlastX of GenBank and to multiple sequence alignments of homologous proteins made with MUSCLE v. 3.7 (Edgar 2004). Where applicable, vector or PCR primer sequences were excluded from the assemblies. Besides *Glomus* spp., homologs of meiosis-specific proteins were identified by BlastP searches of the nonredundant NCBI database, JGI, and the Broad Institute (see above). Multiple amino acid sequence alignments (MUSCLE v. 3.7 [Edgar 2004]) were inspected and adjusted manually using MacClade 4.08 (Maddison WP and Maddison DR 1989), and only unambiguously aligned amino acid sites were used for phylogenetic analyses.

We used RAXML v. 7.2.8 (Stamatakis 2006) and MrBayes v. 3.12 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) for phylogenetic analyses. Amino acid sequence phylogenies were computed using RAXML v. 7.2.8 with the LG model of amino acid substitutions (Le and Gascuel 2008) and 25  $\gamma$ -distributed substitution rate categories (LG + 25 $\gamma$ ) for 1,000 bootstrap replicates. Bootstrap support was estimated from 1,000 replicates using PhyML v3.0 (Guindon and Gascuel 2003) with the LG + I



**Fig. 1.**—Expanded catalog of fungal meiotic genes. Venn diagram showing the presence or absence of the genes known to be directly or indirectly involved in meiotic processes in *Saccharomyces cerevisiae* (green circle) (Nowrousian et al. 2010). The presence or absence of these genes have been scored in the genomes of fungal relatives, including representative species belonging to the phylum Ascomycota (Nowrousian et al. 2010), purple circle), Basidiomycota (Donaldson and Saville 2008; Burns et al. 2010), orange circle), Chytridiomycota (red circle), Zygomycota (dark blue circle), and the AMF Glomeromycota (Green circle), inventoried in detail in [supplementary table S1, Supplementary Material](#) online. Meiosis-specific genes are shown in red text. Asterisks represent genes that are sometimes absent in the genome of one or more members of a given phylum. Data included in the purple circle were reported elsewhere (Nowrousian et al. 2010), and we did not repeat the analyses.

+ 8 $\gamma$  model. We ran MrBayes for 10<sup>6</sup> generations hosted by the CIPRES Science Gateway Portal v. 3.1 at the San Diego Supercomputer Center (Miller et al. 2011), with four incrementally heated Markov chains, a sampling frequency of 10<sup>3</sup> generations, temperature set at 0.5 and Whelan and Goldman model (WAG) + I + 8 $\gamma$  (Whelan and Goldman 2001). Only the RAxML topologies are shown.

### Phylogenetic Analyses of Concatenated DNA Repair Proteins

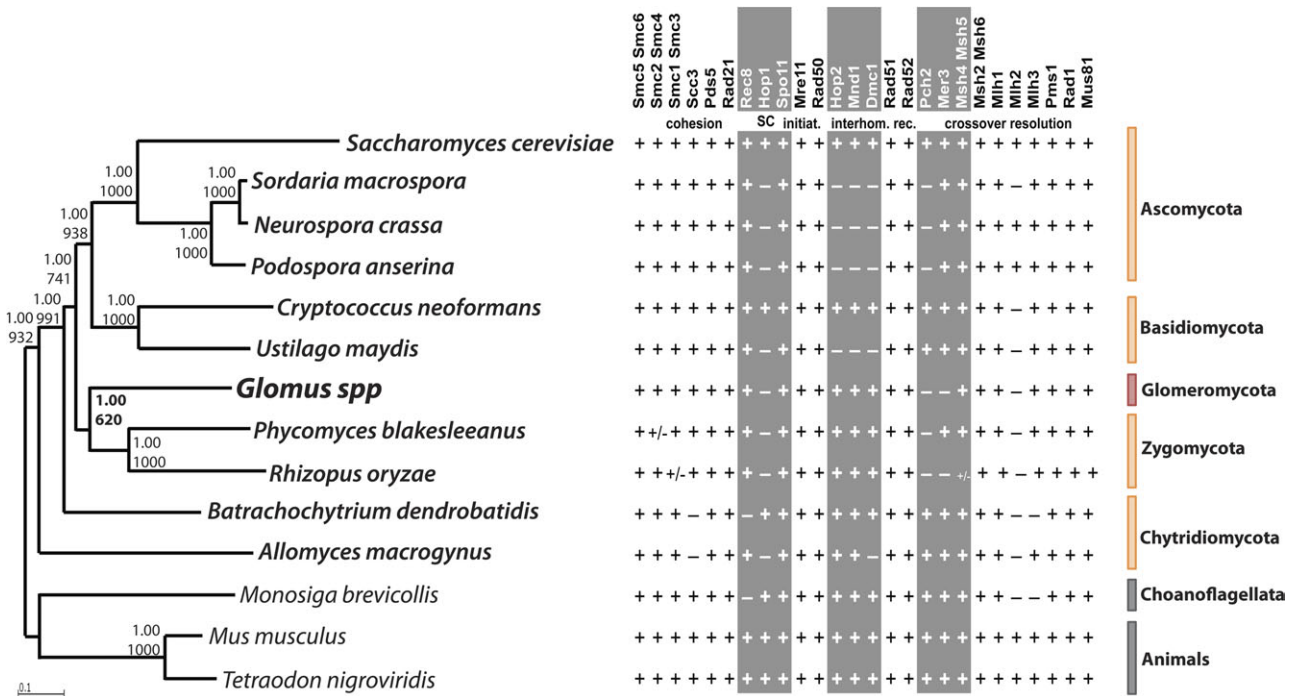
A fungal phylogeny was inferred using 12 orthologous DNA repair proteins among the meiotic proteins retrieved for all surveyed fungal taxa (highlighted as blue cells; [supplementary table S1, Supplementary Material](#) online), as well as in the outgroup species *M. brevicollis* (Choanoflagellata) and the animals *M. musculus* and *Tetraodon nigroviridis*. A multiple sequence alignment was produced using MUSCLE for each protein (Edgar 2004), and divergent or ambiguous positions were removed. Evolutionary models for each protein were determined using ProtTest (Abascal et al. 2005). Several phylogeny inference procedures gave similar trees (data not shown). The alignments were concatenated using Concaterpillar (Leigh et al. 2008). The phylogenetic tree was inferred using PhyML v3.0 (Guindon and Gascuel 2003) and WAG + 4 $\gamma$  with 1,000 bootstrap replicates. A phylogenetic tree was also inferred using MrBayes v3.1.2 (Ronquist

and Huelsenbeck 2003), with 10<sup>7</sup> generations with 4  $\gamma$ -distributed substitution rate categories and separate substitution models for each protein.

## Results and Discussion

In the present study, we identified a total of 51 genes in *Glo-**mus* spp. that are required for the proper completion of meiosis in *S. cerevisiae* (fig. 1 and table 1, [supplementary table S1, Supplementary Material](#) online). Homologues of *S. cerevisiae* genes that could not be identified in *Glo-**mus* spp. were also absent from the representative genomes of other higher fungal groups, including Sordariales among ascomycetes. Overall, among the 87 *S. cerevisiae* genes we searched, none were missing exclusively from our available AMF sequence data set, suggesting that our in silico and molecular approaches have covered most, if not all, of the available AMF predicted meiotic proteome.

Importantly, more than 85% of the core meiotic genes were found to be present in AMF (fig. 2). The only AMF core meiotic genes that could not be detected were homologues of *Pch2*, *Hop1*, *Mei4*, and *Mer3*; all genes whose loss does not affect the successful completion of meiosis in many fungi (Malik et al. 2008; Kumar et al. 2010). In particular, *Pch2*, *Mei4*, *Hop1*, and *Mer3* genes are also absent from the genome of the zygomycete *R. oryzae*, and *Hop1* and *Mer3* are absent from known sexual organisms (i.e., *N. crassa*,



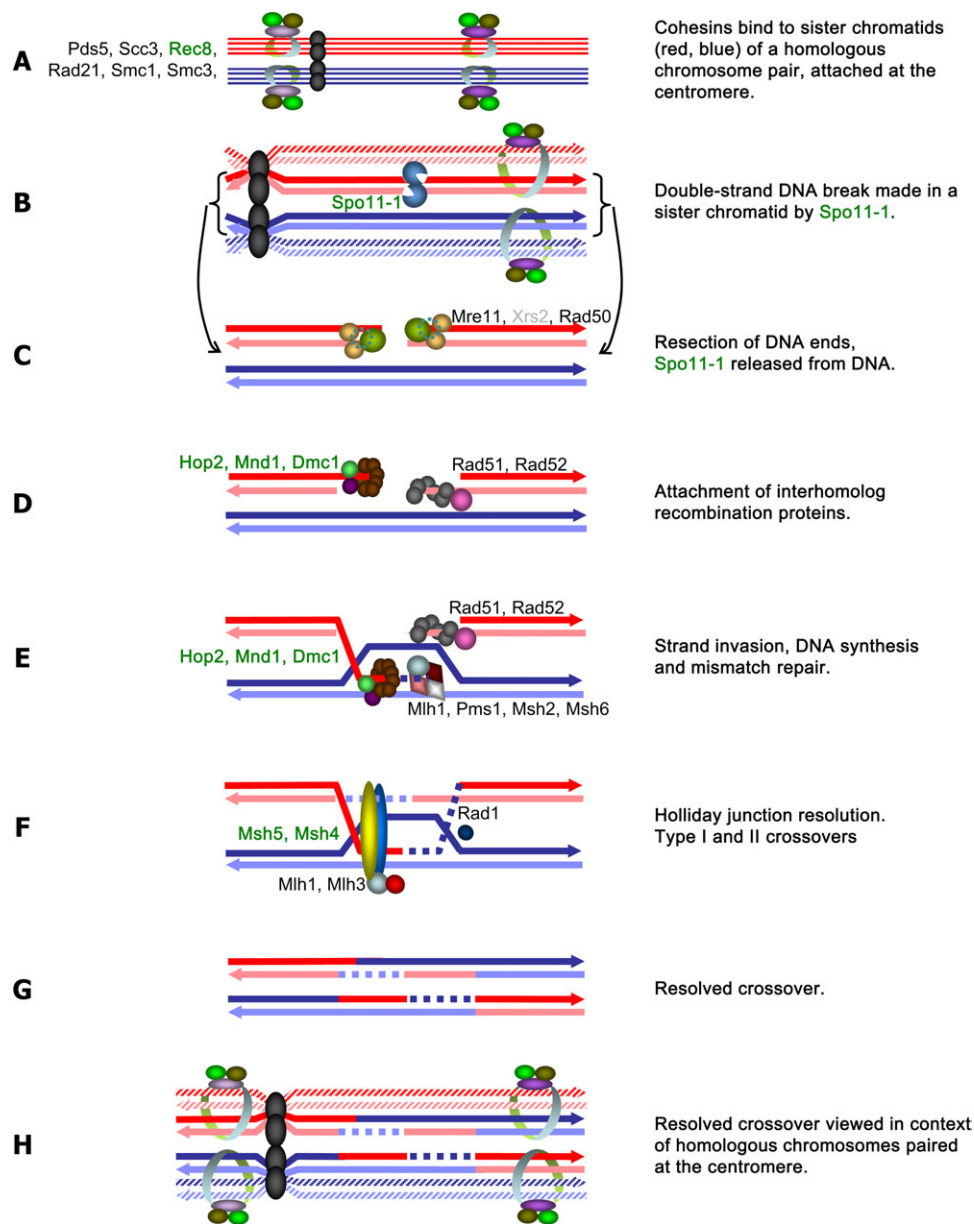
**FIG. 2.**—Phylogenetic tree of 12 concatenated DNA repair protein sequences (*Exo1*, *Rad1*, *Mlh1*, *Mre11*, *Msh6*, *Mus81*, *Smc6*, *Top1*, *Top3*, *Rad23*, *Rad50*, *Rad52*) (left) and list of the core meiotic genes (right). Left side of the figure: *Glomus* spp. (*G. irregulare* and *G. diaphanum*) are highlighted in red, all surveyed fungi (Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota) in orange, and outgroup organisms (one choanoflagellate and two animals) in gray (supplementary table S2, Supplementary Material online). Numbers at nodes correspond to Bayesian posterior probabilities (top) and bootstrap supports from 1,000 replicates of maximum likelihood (PhyML) analysis (bottom). Scale bar represents 0.1 amino acid substitutions per site. Right side of the figure: list of the core meiotic proteins (adapted from references San-Segundo and Roeder 1999; Villeneuve and Hillers 2001; Malik et al. 2008; Joshi et al. 2009 and references therein) and their presence (+) and absence (–, i.e., not detected) in the fungal genomes surveyed in this study. +/– denotes the absence of the given genes in some species belonging to that specific phylum. Meiosis-specific proteins are shown in gray columns. A. Ascomycota; B. Basidiomycota; C. Chytridiomycota; Z. Zygomycota; G. Glomeromycota (i.e., AMF). Orthologs of *Rad21*, *Rad51*, *Pms1*, and *Mlh* and meiosis-specific *Spo11-1*, *Rec8*, *Hop1*, *Hop2*, *Mnd1*, and *Dmc1* genes of basidiomycetes and *B. dendrobatidis* were identified with assistance from Arthur Pightling.

*Gibberella zeae*, and *Drosophila melanogaster*; Malik et al. [2008]).

Our study indicates that AMF genomes contain genes encoding all the tools necessary for meiotic recombination. In particular, they have genes that encode orthologs of seven meiosis-specific proteins involved in sister-chromatid cohesion (*Rec8*), double-strand DNA breaks (*Spo11-1*), interhomolog recombination (*Mnd1*, *Hop2*, and *Dmc1*), and class II crossovers (*Msh4* and *Msh5*) (supplementary figs. S1 and S2, Supplementary Material online). Phylogenetic analyses were used to verify the orthology of these seven meiosis-specific gene homologs in *Glomus* spp. relative to other fungi, with animals and a choanoflagellate as outgroups (supplementary figs. S1 and S2, Supplementary Material online). These proteins were not selected to trace the species genealogy but are sufficient to determine orthology of all *Glomus* spp. meiosis-specific genes we identified and their vertical descent (as opposed to them being specifically related to another organism by lateral gene transfer or contaminants in our cultures). In particular, *Glomus* spp. encode a meiosis-

specific *Rec8* protein that is distinct from the general *Rad21* sister-chromatid cohesion and harbor orthologs of the meiosis-specific transesterase *Spo11-1*. The meiosis-specific *RecA* homolog, *Dmc1*, encoded *Glomus* spp. is also of fungal origin, as is *Rad51*, the general eukaryotic recombinase required for homologous recombination. *Glomus* spp. encode distinct meiosis-specific *Mnd1* and *Hop2* orthologs; these function with *Dmc1* in interhomolog DNA strand exchange during meiosis in model organisms. *Glomus* spp. are also equipped for mismatch repair with *Msh2* and *Msh6* proteins and also for meiosis-specific (class II) crossovers that exhibit interference, with *Msh4* and *Msh5* proteins. Altogether, the presence of these genes in *Glomus* spp. is compelling evidence for an active, hitherto undetected, meiosis-like program in the life cycle of AMF.

The presence of meiotic recombination proteins in AMF is also supported by other independent signatures of sexuality, namely the presence of many retrotransposons (*Ty1-Copia* and *Ty3-Gypsy*; data not shown) (Matic 2001; Wright and Finnegan 2001; Arkhipova 2005; Arkhipova and Meselson



**FIG. 3.**—Hypothetical model of meiotic recombination in AMF *Glomus* spp., depicting likely interactions among proteins identified in this study. The names of meiosis-specific proteins are highlighted in green. Exact stoichiometry is not implied. In meiosis I, cohesins bind to sister chromatids (A), after which double-strand DNA breaks occur, with Spo11 and accessory recombination initiation proteins if present (B). Double-strand break repair is initiated (C). Interhomolog recombination and strand exchange proteins are attracted to the double-strand break (accessory proteins not shown) (D). The resulting heteroduplex (E) may be resolved by class II crossovers, which utilize meiosis-specific proteins (F, G) or by gene conversion (proteins not shown) or Class I crossovers (via Mus81), which do not. This model is derived from the general model that was based on details from *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Arabidopsis thaliana*, and phylogenomic analyses described in references (Malik et al. 2008) and references within.

2005; Gollotte et al. 2006) and recombination within their populations (Vandenkoornhuysse et al. 2001; Croll and Sanders 2009; den Bakker et al. 2010). These evolutionary features, combined with the presence of an expanded suite of conserved meiotic recombination genes, are compelling indicators of sexual reproduction in many eukaryotes (Malik et al. 2008; Schurko et al. 2009). Here, we propose a model

of meiotic recombination in AMF based on the presence of core meiotic genes (fig. 3).

We also identified meiosis-specific gene homologs in *B. dendrobatidis*, a chytridiomycete that lacks any described sexual cycle. Although sex is now known in the other fungi included in our analyses, *B. dendrobatidis*, the fungal pathogen of amphibians, appears to primarily reproduce

asexually (James et al. 2009). Core meiotic genes identified in figure 2 indicate that *B. dendrobatidis* is also capable of undergoing meiotic recombination.

The acquisition of a large sequence data set allowed us to tackle another interesting aspect of AMF evolution, namely their origin from within the fungal kingdom. In particular, we tested the most recent findings suggesting that these ubiquitous organisms may be more closely related to the Zygomycetes than previously thought (Corradi and Sanders 2006; Lee and Young 2009; Liu et al. 2009). We tested this hypothesis by reconstructing a fungal phylogeny of the DNA repair and recombination proteins encoded by all surveyed taxa (fig. 2), as these are fairly well conserved. The resulting phylogenies were all very similar to those identified recently, showing that AMF cluster with relatively strong support as a sister group of Mucorales (phylum Zygomycota). Obviously, the reduced species sampling in our study does not allow any conclusive evidence about the specific evolutionary origin of AMF within the fungal kingdom. However, this relevant phylogenetic signal, together with a virtually identical set of core meiotic genes between those groups (all genes that are absent in AMF are also absent from *R. oryzae*), is a highly intriguing relationship that will hopefully bolster future research in this specific area of comparative genomics upon completion of the first AMF genome sequence (Martin et al. 2008).

Recent advances in the field of population genetics have allowed the identification of several events of recombination both within and across several AMF populations (Vandenkoornhuysen et al. 2001; Croll and Sanders 2009; den Bakker et al. 2010). However, conclusions about the origin of such events (i.e., meiotic vs. mitotic recombination) have been systematically shadowed by a lack of evidence for meiosis in these putative ancient asexuals. By providing the first evidence for an expanded and conserved catalog of AMF meiosis-specific genes, the present study fills an important gap in our understanding of the genetics of these ubiquitous ecologically important organisms. In particular, these conclusions open up the exciting perspective that AMF may not be the evolutionary aberration that they have been long held to be and that they may be able to undergo a cryptic sexual cycle. Future studies such as colocalization or genetic disruption are required to demonstrate the conditions in which the meiosis-specific gene homologs we identified in this study encode products functioning in meiosis in *Glomus* spp. or if they function in a putative parasexual process including interhomolog recombination and crossing over, that is recently derived from a typical meiotic recombination process.

## Supplementary Material

Supplementary figures S1 and S2 and tables S1 and S2 are available at *Genome Biology and Evolution* online (<http://www.gbe.oxfordjournals.org/>).

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