

Comparative Genomics Suggests Primary Homothallism of *Pneumocystis* Species

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ABSTRACT *Pneumocystis* species are fungal parasites of mammal lungs showing host specificity. *Pneumocystis jirovecii* colonizes humans and causes severe pneumonia in immunosuppressed individuals. In the absence of *in vitro* cultures, the life cycle of these fungi remains poorly known. Sexual reproduction probably occurs, but the system of this process and the mating type (*MAT*) genes involved are not characterized. In the present study, we used comparative genomics to investigate the issue in *P. jirovecii* and *Pneumocystis carinii*, the species infecting rats, as well as in their relative *Taphrina deformans*. We searched sex-related genes using 103 sequences from the relative *Schizosaccharomyces pombe* as queries. Genes homologous to several sex-related role categories were identified in all species investigated, further supporting sexuality in these organisms. Extensive *in silico* searches identified only three putative *MAT* genes in each species investigated (*matMc*, *matMi*, and *matPi*). In *P. jirovecii*, these genes clustered on the same contig, proving their contiguity in the genome. This organization seems compatible neither with heterothallism, because two different *MAT* loci on separate DNA molecules would have been detected, nor with secondary homothallism, because the latter involves generally more *MAT* genes. Consistently, we did not detect *cis*-acting sequences for mating type switching in secondary homothallism, and PCR revealed identical *MAT* genes in *P. jirovecii* isolates from six patients. A strong synteny of the genomic region surrounding the putative *MAT* genes exists between the two *Pneumocystis* species. Our results suggest the hypothesis that primary homothallism is the system of reproduction of *Pneumocystis* species and *T. deformans*.

IMPORTANCE Sexual reproduction among fungi can involve a single partner (homothallism) or two compatible partners (heterothallism). We investigated the issue in three pathogenic fungal relatives: *Pneumocystis jirovecii*, which causes severe pneumonia in immunocompromised humans; *Pneumocystis carinii*, which infects rats; and the plant pathogen *Taphrina deformans*. The nature, the number, and the organization within the genome of the genes involved in sexual reproduction were determined. The three species appeared to harbor a single genomic region gathering only three genes involved in sexual differentiation, an organization which is compatible with sexual reproduction involving a single partner. These findings illuminate the strategy adopted by fungal pathogens to infect their hosts.

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Pneumocystis species constitute a group of fungi belonging to the Taphrinomycotina subphylum of the Ascomycota which colonize the lungs of mammals. Genetic and transmission analyses revealed that each *Pneumocystis* species infects specifically a single mammalian host species. *Pneumocystis jirovecii*, the species colonizing humans, can turn into an opportunistic pathogen in immunosuppressed individuals and cause severe, sometimes fatal pneumonia (*Pneumocystis pneumonia* [PCP]). PCP is nowadays the second most frequent life-threatening invasive fungal infection worldwide, with above 400,000 annual cases (1). Comparative genomics suggested the loss of synthesis and assimilation pathways in *Pneumocystis* species and thus that these fungi are obligate parasites without free-living forms (2–4). The character-

istics of these fungi suggest that they are biotrophs of mammals, i.e., parasites retrieving energy and compounds from host cells without killing them (4–7).

The life cycle of *Pneumocystis* species remains poorly known, mostly because of the absence of a method for long-term culture *in vitro*. During the infection, two types of cellular structures are observed: the trophic cells and the asci (also called trophs and cysts, respectively). Microscopic and cytological studies suggested that the trophic cells may undergo binary fission but also diploidization upon conjugation, followed by meiosis and mitosis to produce asci containing eight haploid ascospores (8, 9). The observation of synaptonemal structures within *Pneumocystis* cells (10, 11) and the demonstration of the expression of sex-related

genes (5, 12) further confirmed that the reproduction of these fungi probably includes a sexual phase. Quantitative experiments suggested that the main mode of reproduction of *Pneumocystis* species might be meiotic division, whereas the contribution of mitotic division of trophic cells is still unclear (8, 13, 14). Therefore, the sexual phase might be obligatory for the growth of these fungi. Obligate sexuality is also consistent with the fact that the asci might be the only particles aerially transported and responsible for transmission between hosts (14, 15). Obligate sexuality would imply that trophic cells of compatible mating types must always be present within host lungs in order to allow sexual reproduction.

There are two main systems of sexual reproduction among fungi: heterothallism, involving two compatible mating types, and homothallism, involving a single self-compatible mating type. In heterothallic ascomycetes, the *MAT* loci contain divergent genes (idiomorphs) in opposite mating types (16). In so-called primary homothallic species, the genes of the two idiomorphs are present in the same genome, closely located or not. Another form of homothallism, called secondary, is observed in ascomycetous yeasts such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In this case, the homothallic behavior results from mating type switching and the presence of three *MAT* loci in the same genome: one active and two silent. In *S. pombe*, the *MAT* loci are flanked by *cis*-acting sequences that are involved in a switching mechanism which exchanges the expressed cassette. Primary homothallism is observed in many filamentous ascomycetes (Pezizomycotina or Euascomycetes). As far as *Pneumocystis* species are concerned, their particular system of reproduction is still unknown. The genomic region surrounding the pheromone receptor *ste3* gene has been postulated to constitute a *MAT* locus in these fungi. It would also include genes encoding the protein kinase Ste20 and the homeodomain transcriptional regulator Ste12 and thus, surprisingly, would resemble the *MAT* locus of the basidiomycete *Cryptococcus neoformans* (17).

In the present study, we investigated the system of reproduction of *P. jirovecii* and *Pneumocystis carinii*, the species infecting rats, by the analysis of their genome content and comparative genomics. We also investigated the issue in the Taphrinomycotina relative *Taphrina deformans*, a plant pathogen whose sexual reproduction is also poorly characterized. In order to identify the sex-related genes present in the genomes of these species, genes from the well-annotated Taphrinomycotina member *S. pombe* were used as homology references.

RESULTS

Identification of sex-related genes. Due to the low conservation and great diversity of the sex-related genes among fungi, the identification of homologs of sex-related *S. pombe* proteins within the genomes of *P. jirovecii*, *P. carinii*, and *T. deformans* was done using the bioinformatics strategy shown in Fig. S1 in the supplemental material. The procedure relied on tBLASTn search and recognition by manual inspection of specific domain architecture (see details in Materials and Methods). The 103 *S. pombe* genes used in sequence queries were selected on the basis of their annotation in UniProt and Gene Ontology (GO) terms, as well as of their belonging to relevant protein complexes (see Table S1 in the supplemental material). Genes were classified in one or two of seven role categories: cell fusion, signal transmission, signal transduction, signal regulation, meiosis, mating type locus silencing (RNA in-

terference pathway), and mating type locus switching. Using this strategy, we established the presence or absence of sex-related genes within the genomes of the three fungi (see Table S2 in the supplemental material; the locus reference or genomic location of the genes identified is given in Table S3 in the supplemental material).

Genes homologous to all seven role categories of sex-related *S. pombe* proteins were present in the three genomes investigated (see Table S2 in the supplemental material, green areas). In agreement with their notoriously difficult identification, the presence or absence of the pheromone precursor genes could not be assessed (*map2*, *mfm1*, *mfm2*, and *mfm3*). The genes encoding the two pheromone receptors (*mam2* and *ste3*) and the elements of the pheromone-induced signaling cascade (e.g., *gpa1*, *byr2*, *byr1*, *spk1*, and *ste11*) were present in all three genomes investigated, except that *T. deformans* lacked *mam2*. There was a remarkable similarity of the presence and absence of the genes in the three species investigated, with 51 genes present (see Table S2 in the supplemental material, green areas), and 25 absent (brown areas). Twelve genes were absent only in the two *Pneumocystis* species, suggesting specific features of this genus (signal transmission, *pmd1*; signal transduction, *ste4*; signal regulation, *rst2*, *sxa1*, and *sxa2*; mating type silencing: *ago1*, *arb1*, *arb2*, *chp1*, *dcr1*, *hst2*, *obr1*, and *rdr1*). Thus, more genes were detected in *T. deformans* than in the two *Pneumocystis* species. The Argonaute small interfering RNA chaperone (ARC) complex was absent in the *Pneumocystis* species (mating type silencing: *ago1*, *arb1*, and *arb2*), a feature which we already reported (4). The presence or absence of the gene products of the three species within the reconstructed pathways of *S. pombe* is shown in the supplemental material (see Fig. S2 in the supplemental material).

MAT genes. Genes homologous to the four *S. pombe* *MAT* genes were sought in the three species investigated (see Table S2 in the supplemental material, boxed genes). Extensive *in silico* searches using our bioinformatics strategy (see Fig. S1 in the supplemental material) revealed the presence of only three genes in each *Pneumocystis* species and in *T. deformans*, namely, the homologs of *S. pombe* *matMc*, *matMi*, and *matPi*. The gene *matMc* encodes a transcription factor with the high-mobility-group domain (PTHR10270:SF176 or PTHR10270:SF159), *matMi* encodes a mating type M-specific polypeptide, and *matPi* encodes a transcription factor with a homeobox domain (PTHR11850 or PTHR11850:SF31). The *matMi*-encoded proteins have no known domains and are poorly conserved between species, so that their identification did not rely on domain architecture as for the other genes but on their localization close to *matMc* and opposite orientation relative to *matMc*.

Multiple sequence alignment revealed a fair degree of similarity of the *matMc*-encoded proteins identified, in particular at the high-mobility-group domain, with 27% overall identity with the *S. pombe* protein for all three *P. jirovecii*, *P. carinii*, and *T. deformans* proteins (Fig. 1A). The degree of similarity was lower between the *matPi* proteins and concentrated at the homeobox domain present at the end of the proteins, with 19% overall identity with the *S. pombe* protein for *P. jirovecii* and 18% for both *P. carinii* and *T. deformans* (Fig. 1B). Reasonable alignment of the *matMi* proteins could not be generated because of their low similarity, the identity with the *S. pombe* protein being only 12, 15, and 20% for *P. jirovecii*, *P. carinii*, and *T. deformans* proteins, respectively. Despite this low similarity, the phylogeny of the three putative *MAT*

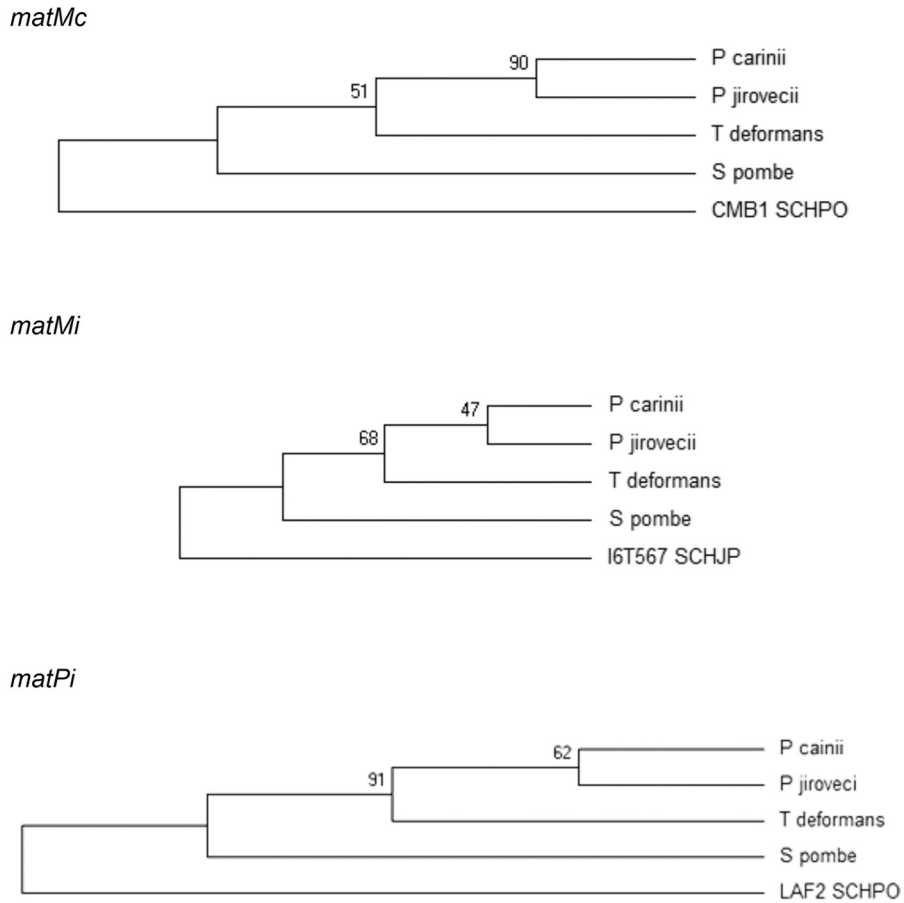


FIG 2 Phylogeny of the *MAT* genes of *S. pombe*, *P. jirovecii*, *P. carinii*, and *T. deformans*. The maximum likelihood method based on the JTT matrix-based model (48) was used to infer the evolutionary history of *matMc*, *matMi*, and *matPi* genes.

a *MAT* locus in *P. carinii* (17). This genomic region was reported to include the protein kinase *Ste20* and the homeodomain transcriptional regulator *Ste12*. In both *P. carinii* and *P. jirovecii*, BLAST analyses using *S. pombe* sequences as queries revealed that the so-called *ste20* gene was in fact an *shk2* homolog with a pleck-

strin homology-like domain (IPR011993) and the so-called *ste12* gene was a truncated version of *ste11*, a signal transduction kinase gene. We identified *ste3* and *shk2* on the same contig in *P. carinii*, whereas *shk2* was on the same contig as the three putative *MAT* genes in *P. jirovecii* but fairly distant (ca. 80 kb). The complete

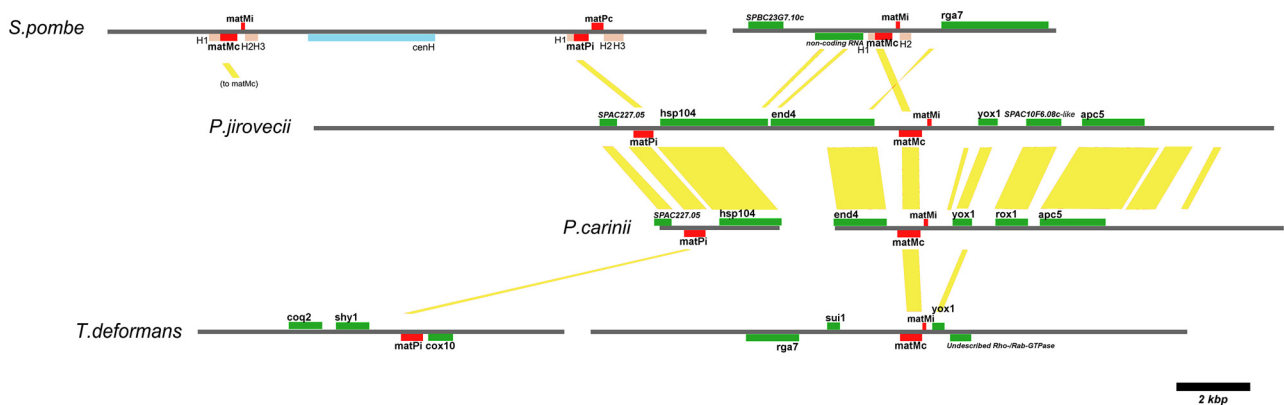


FIG 3 Schematic representation of the *MAT* loci of *S. pombe*, *P. jirovecii*, *P. carinii*, and *T. deformans*. Genes are shown in green, and their synteny with those of the other species is shown by the yellow rectangles. The sequence contigs carrying the genes are shown as gray lines. The centromere-homologous sequence (*cenH*) is shown in blue. The *cis*-acting sequence motifs H1, H2, and H3 involved in mating type switching in *S. pombe* are shown in light brown. The gene name or detected domain name is indicated for each identified open reading frame.

ste11 gene was identified in distinct locations in the two *Pneumocystis* genomes (see Table S3 in the supplemental material).

DISCUSSION

In order to better characterize the life cycle of *Pneumocystis* species and *T. deformans*, we searched for sex-related genes in their genomes. We detected many genes involved in various sex-related processes, such as mating, pheromone signaling, and meiosis. Some of these genes have been previously identified in *P. carinii* (17, 19–23) and *T. deformans* (24), but we report them in *P. jirovecii* for the first time. The expression of several putative mating or meiosis genes in *P. carinii* has been documented elsewhere (5, 12). Together, these observations strongly support the hypothesis that a sexual phase occurs in the life cycle of the *Pneumocystis* species and *T. deformans*.

Our analyses reveal a conserved syntenic genomic conformation of the *MAT* locus between the two *Pneumocystis* species, despite a high level of divergence between the *MAT* genes. This suggests that the conservation of the locus configuration might be more critical for sexual reproduction than that of the mating genes. The most striking feature of our findings was that only three genes homologous to the four *S. pombe* *MAT* genes were identified in each *Pneumocystis* species, as well as in *T. deformans* (*matMc*, *matMi*, and *matPi*). It must be stressed that the *matMi* genes identified remain hypothetical because of their low similarity with that of *S. pombe*. It is likely that no other *MAT* genes were present because (i) the results were similar in the two *Pneumocystis* species and (ii) the genomic and transcriptomic data analyzed are expected to cover most of the genomes. Moreover, we did observe the same synteny and three *MAT* genes also in the *Pneumocystis murina* genome, but our data are unpublished because this genome sequence was released prior to publication under specific terms (see *Pneumocystis murina* Sequencing Project, Broad Institute of Harvard and MIT, <http://www.broadinstitute.org/>). One limitation of our study is that *MAT* genes too divergent from those of *S. pombe* would not have been detected. However, *S. pombe* harbors two different types of DNA binding domains (high-mobility group and homeobox), which increased the probability of detecting such genes. The proximity of three putative *MAT* genes identified in the *Pneumocystis* species and *T. deformans* is atypical and suggests a fusion of two *MAT* loci, one of type minus (M) with *matMc* and *matMi* and one incomplete of type plus (P) with only *matPi*. Consequently, the transcription factors encoded by *matMc* (high-mobility group) and *matPi* (homeobox) would be sufficient to trigger sexual development. Such fusion of two *MAT* loci has been previously observed in other fungi, for example, in the *Stemphylium* genus (25), and the loss of *MAT* transcription factors occurred in *Candida* species other than *C. albicans* (26). A similar fusion of the two *MAT* loci M and P is also present in three *Schizosaccharomyces* species other than *S. pombe*, with only *matMc* in their type M loci (18).

The number and the organization of the putative *MAT* genes in the *Pneumocystis* species and *T. deformans* allow formulation of a hypothesis concerning the system of sexual reproduction of these fungi. First, the presence of only three *MAT* genes seems incompatible with secondary homothallism. Indeed, this system requires generally more genes, i.e., six corresponding to three *MAT* loci in *S. pombe* and *S. cerevisiae* and four corresponding to two *MAT* loci in some methylotrophic yeasts, as recently described (27). This is consistent with the fact that we did not detect any

cis-acting sequences for mating type switching flanking the putative *MAT* genes. Second, the three *MAT* genes in *P. jirovecii* are present on a single DNA molecule (Fig. 3), and this is also the case in *P. murina* (our unpublished findings). The close proximity of these genes on a single DNA molecule is particularly important because it is not compatible with heterothallism. Indeed, if the genome sequences had been derived from a mixture of two compatible heterothallic mating types, two *MAT* loci would have been observed located on two distinct DNA molecules, not on a single one. The alternative possibility, i.e., that the genome sequences correspond to a single heterothallic mating type, is also unlikely. First, the *MAT* locus identified appears to include both types, M and P. Second, asci are present in most *Pneumocystis* infections, if not all, i.e., sexuality appears obligatory. In particular, this was the case in the clinical specimen used for *P. jirovecii* genome sequencing because the diagnosis was made by silver staining of the walls of asci (3).

The loss of the transcription factor gene *matPc* (high-mobility group) in the *Pneumocystis* species and *T. deformans* suggests that the P-specific genes may not be expressed and that P cells may be lacking. Thus, these species may use a single mating type for sexual reproduction, a system previously observed in *C. neoformans* (28) and *Candida albicans* (29). An alternative hypothesis is that expression of the P-specific genes, including that encoding the P-factor, is ensured thanks to a rewiring of the *MAT* pathways, a phenomenon frequently observed in fungi (30, 31). The latter hypothesis would be consistent with the presence of the receptor *mam2* to the P-factor in the *Pneumocystis* species. The presence of *matPi* in all three genomes investigated might reflect that both this gene and the *matMi* gene seem necessary for the expression of *mei3* and thus for entry into meiosis (32). Further experiments are required in order to decipher the system of sexual reproduction in the *Pneumocystis* species and *T. deformans*.

Our results do not allow us to ascertain that the genes identified are *bona fide* *MAT* genes of these species, because we cannot rule out the possibility that there are other transcription factors involved in mating type determination elsewhere in the genomes. Also, one cannot formally exclude secondary homothallism. However, the close vicinity and arrangement of the putative *MAT* genes are consistent with primary homothallism in the *Pneumocystis* species as well as in *T. deformans*. The latter system of reproduction implies the presence of the same *MAT* locus in all isolates of each species that we investigated. This is what we found in the *P. jirovecii* isolates of six patients, further supporting the hypothesis of primary homothallism. This hypothesis is also compatible with the *T. deformans* cell cycle. Indeed, single haploid yeast cells inoculated on peach leaves are able to produce, without cell-cell conjugation, dikaryotic hyphae that give rise to asci containing eight ascospores (33, 34).

Primary homothallism has been hypothesized to be advantageous for pathogens (35), including *Pneumocystis* species (28, 29). Our results are compatible with this hypothesis. Although it involves a single strain, primary homothallism has been shown in *C. neoformans* to avoid accumulation of deleterious mutations, as well as to increase genetic diversity and virulence (36). In the case of *P. jirovecii*, the fact that the majority of infections involve two or more distinct genotypes (37) suggests that the genetic diversity may be further increased by mating among these genetic variants.

The presence of many genes that we classified in the silencing and switching categories in the *Pneumocystis* species and *T. defor-*

mans appears contradictory to the putative absence of secondary homothallism. Nevertheless, their presence could be simply due to their involvement in other cellular or metabolic processes. For example, some *swi*-encoded proteins are known to be necessary for DNA metabolism. On the other hand, the absence of two silencing (*clr1* and *swi6*) and two switching (*sap1* and *swi2*) genes in all three species investigated might be significant because these genes are apparently dedicated to silencing or switching of *MAT* genes (see Table S1 in the supplemental material).

As far as *P. carinii* is concerned, we confirmed the location of *ste3*, the so-called *ste20* gene, and the so-called *ste12* gene within the genomic region which was postulated to be a *MAT* locus resembling those of the basidiomycete *C. neoformans* (17). However, the so-called *ste20* and *ste12* genes turned out to be *shk2* and a truncated version of *ste11*, respectively. These different findings may result from the constant improvement of the databases, the increase in knowledge about these genes since 2001, and the fact that in this study we dealt with more than one genome. The confusion between *ste12* and *ste11* probably results from the fact that *S. cerevisiae ste12* is the ortholog of *S. pombe ste11*. Consequently, the genomic region surrounding *ste3* in the *Pneumocystis* species may constitute a cluster of sex-related genes, rather than a *MAT* locus resembling that of *C. neoformans*.

The computational observation of putative *MAT* genes does not prove conclusively that a species is sexual, because these genes may have been conserved from a sexual ancestor. In conclusion, our analyses suggest the working hypothesis that primary homothallism is the system of reproduction of the *Pneumocystis* species as well as of *T. deformans*.

MATERIALS AND METHODS

Source data. The annotated genome sequences of *P. jirovecii* and *T. deformans* were obtained from the European Nucleotide Archive (38) (CAKM000000000 and CAHR000000000, last accessed on 14 May 2013). The *P. jirovecii* transcriptome assembly (HAAA01000001 to HAAA01003261) was downloaded from EBI (<http://www.ebi.ac.uk>, last accessed 11 November 2013). These genome sequences and transcriptome correspond to the data that we published previously (3, 24). *P. carinii* assembled contigs were downloaded from the project site (<http://pgp.cchmc.org>, last accessed 14 April 2013) and correspond to data published by Slaven et al. (39). The *S. pombe* protein sequences were obtained from the annotated proteome present in UniProt (<http://www.uniprot.org>, last accessed 30 July 2014) and correspond to the data published previously (40).

Search for candidate genes and manual curation. The bioinformatics strategy used to identify homologs of *S. pombe* proteins within *P. jirovecii*, *P. carinii*, and *T. deformans* genomes is shown in Fig. S1 in the supplemental material. Each *S. pombe* sequence used as a query was characterized in order to identify specific domains or domain architecture using the InterProScan4 tool (41), with a particular consideration for the PANTHER subfamilies (42). tBLASTn analyses (Blast+ suite, NCBI, version 64bit) (43) with relaxed parameters (E value from $1E-4$ down to its default value) were used in order to obtain a list of candidate genomic regions within the target genome, which presumably included the genes of interest. The already existing annotation of the genes was considered, and existing collinear coding sequences (CDSs) were retained after assessment using the InterProScan4 tool. When no CDSs were already annotated, a CDS was proposed based on the alignment of the *S. pombe* query protein against the genomic region using GeneWise (44). Only GeneWise global alignments matching most of the query were retained. The start and stop codons of the CDSs were assessed, and the respective exons were corrected manually to obtain the longest CDSs possible. Both existing and newly derived CDSs translated using the EMBOSS_transeq program (45) were

submitted to the InterProScan4 tool. The predicted domains and their architecture were compared to those retained for the corresponding *S. pombe* protein. When strong domain evidence was detected, the CDS was considered the valid homolog. When no or ambiguous domain architecture was detected, the CDSs from all the investigated species were compared in multiple sequence alignments and associated phylogenetic trees. The latter analysis permitted us to validate some homologs whose domain architecture was ambiguous. The overall synteny between the genomic region of interest and that of *S. pombe* was also taken into account. If no CDS could be validated, it was assumed that no homolog existed in the genome investigated. Whenever possible, the original genome annotation was retained, but a complete reappraisal of the CDS definition and of its annotation was required in several cases (see Table S3 in the supplemental material).

Phylogeny analysis. To address specific cases of domain architecture ambiguity, formal phylogeny analysis was carried out using MEGA6 (46) on alignments made by T-Coffee (47). The resulting topologies were chosen on consensus between the results of the different methods and the statistical support for branching. All positions containing gaps and missing data were eliminated. For the inference of the evolutionary history of *matMc*, *matMi*, and *matPi* genes, the maximum likelihood approach was used. Initial trees for the heuristic search were obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model (48). The discrete gamma distribution was used to model the evolutionary rate differences among sites, and the distribution parameter value was determined through maximum likelihood based on the sequence alignment. For every analysis, appropriate out-group sequences were chosen based on length, similar domain architecture, and distinct function.

Synten analysis. The assessment of the degree of synteny between the relevant genomic regions was carried out using the ACT tool (49). Whenever necessary, sequences were trimmed and/or concatenated. BLASTN comparisons were used for less divergent genomes (*Pneumocystis*), whereas tBLASTn was used for comparison of the other genomes.

PCR amplification and sequencing of the putative *MAT* genes from *P. jirovecii* isolates. A genomic region of 1,240 bp encompassing the putative *P. jirovecii* *matMc* and *matMi* genes was amplified using primers 5' ATC CGA TAA ATA CAT ACA CG 3' (positions 56360 to 56379 in sequence CAKM01000262.1 of *P. jirovecii* genome [contig 262]) and 5' GAG GCT GTA AAA AGC ATA AG 3' (positions 57581 to 57600). The template was genomic DNA extracted as previously described (37) from the bronchoalveolar lavage samples of patients with PCP. The PCR began by an initial denaturation step of 3 min at 94°C, which was followed by 35 cycles consisting of 30 s at 94°C, 30 s at the annealing temperature of 56°C, and 90 s at 72°C. The last reaction cycle was terminated with 10 min of extension at 72°C. The final MgCl₂ concentration in the PCR mixtures was 4.5 mM. A region of 889 bp including the putative *P. jirovecii* *matPi* gene was amplified using primers 5' ATG TTA CTA TAT ATA AGA CC 3' (positions 47271 to 47290) and 5' ATG AAT TAG AAG GCA AAA AG 3' (positions 48141 to 48160). The PCR conditions were exactly the same as for amplification of *matMc* and *matMi*, except that the annealing temperature was 54°C. Sequencing of both strands of the PCR products was performed with the two primers used for PCR amplification, as well as the BigDye Terminator DNA sequencing kit and the ABI Prism 3100 automated sequencer (both from PerkinElmer Biosystems, Rotkreuz, Switzerland).

Data accessibility. Curated annotations and scripts are in the folder *Pneumocystis_Mating* available from GitHub (https://github.com/ocisse/Pneumocystis_comparative).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.02250-14/-DCSupplemental>.

Table S1, DOCX file, 0.03 MB.

Table S2, DOCX file, 0.03 MB.

Table S3, DOCX file, 0.02 MB.

Figure S1, PPTX file, 0.1 MB.

Figure S2, TIF file, 1.7 MB.

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