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Review

# Genomics and evolution of *Pneumocystis* species

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ABSTRACT

The genus *Pneumocystis* comprises highly diversified fungal species that cause severe pneumonia in individuals with a deficient immune system. These fungi infect exclusively mammals and present a strict host species specificity. These species have co-diverged with their hosts for long periods of time (> 100 MYA). Details of their biology and evolution are fragmentary mainly because of a lack of an established long-term culture system. Recent genomic advances have unlocked new areas of research and allow new hypotheses to be tested. We review here new findings of the genomic studies in relation with the evolutionary trajectory of these fungi and discuss the impact of genomic data analysis in the context of the population genetics. The combination of slow genome decay and limited expansion of specific gene families and introns reflect intimate interactions of these species with their hosts. The evolutionary adaptation of these organisms is profoundly influenced by their population structure, which in turn is determined by intrinsic features such as their self-fertilizing mating system, high host specificity, long generation times, and transmission mode. Essential key questions concerning their adaptation and speciation remain to be answered. The next cornerstone will consist in the establishment of a long-term culture system and genetic manipulation that should allow unravelling the driving forces of *Pneumocystis* species evolution.

## 1. Background

### 1.1. History

*Pneumocystis* species form a group of opportunistic fungi that cause severe pulmonary infections in mammals with a deficient immune system. These organisms infect exclusively mammals. They were first described by (Chagas, 1909), and wrongly classified as special forms of trypanosomes. They were later identified as a bona fide separate species by the Delanoë couple at the Pasteur Institute in Paris (Delanoë and Delanoë, 1912). Their taxonomic classification remained then elusive because of a phenotypic resemblance with the protists. The issue was resolved using molecular phylogeny based on sequencing ribosomal DNA, which clearly indicated their fungal nature (Edman et al., 1988).

### 1.2. Phylogeny and taxonomy

*Pneumocystis* species belong to the subphylum of Taphrinomycotina within the Ascomycota (Eriksson and Winka, 1997; Sugiyama et al., 2006). The Taphrinomycotina subphylum is monophyletic and encompasses mostly plant-associated or soil-dwelling fungi (Liu et al., 2009). *Pneumocystis* closest relatives are *Schizosaccharomyces pombe* and *Taphrina deformans,* their common ancestor having diverged from the other Taphrinomycota members ca. 467 million years ago (MYA) (Beimforde et al., 2014).

Although all *Pneumocystis* species are ubiquitous, each mammal species can be infected with only one or two of them. Five species have been formally described so far based on the requirements of the International Code of Botanical Nomenclature (ICBN): *Pneumocystis jirovecii* in *Homo sapiens* (Frenkel, 1999), *Pneumocystis carinii* in *Rattus norvegicus* (Frenkel, 1999), *Pneumocystis wakefieldiae* also in *Rattus norvegicus* (Cushion et al., 1993; Cushion et al., 2004), *Pneumocystis murina* in *Mus musculus* (Keely et al., 2004), and *Pneumocystis oryctologi* in Old World rabbits (*Oryctolagus cuniculus*; Dei-cas et al., 2006). Antigenic and DNA based studies suggest the presence of distinct species also in macaques, ferrets, bats, shrews, horses, pigs, and dogs (Banerji et al., 1994; Peters et al., 1994; Christensen et al., 1996; English et al., 2001; Guillot et al., 2004).

*P. jirovecii* is the only species known to infect humans and has never been detected in any other animals. *P. carinii* is the best studied species because of the availability of protocols for experimental or natural infections in laboratory rats. *P. wakefieldiae* was reported either mixed with *P. carinii* (Cushion et al., 1993; Cushion, 1998; Cushion et al., 2004; Chabé et al., 2010), or alone (Palmer et al., 2000). The two latter species are different in terms of electrophoretic karyotypes, gene localization on the chromosomes, sequence identity (4–7% nucleotide divergence in seven orthologs; Cushion, 1998; Cushion et al., 2004), antigenic profiles (Vasquez et al., 1996), and major surface glycoproteins (MSG) expression (Schaffzin and Stringer, 2000). They might be competing against each other for resources when present together within the same rat (Icenhour et al., 2006a).

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### 1.3. Species divergence

According to the evolutionary rates of several genomic loci, the radiation of the *Pneumocystis* genus occurred ca.100 MYA (Keely et al., 2003a; 2004), which roughly overlaps with the radiation of the mammalian species (Holmes, 1991; dos Reis et al., 2015). *P. murina* would have diverged from *P. carinii* between 51 and 71 MYA (Keely et al., 2003a), while *P. carinii* and *P. wakefieldiae* diverged between 15 and 22 MYA (Cushion et al., 2004; Fischer et al., 2006). The neat superposition of multiple *Pneumocystis* species phylogenetic trees with those of their respective hosts supports a co-evolution of these organisms (Guillot et al., 2001). Therefore, a plausible co-speciation scenario is that each species became physically separated from the other species, the hosts acting as barriers that led to the accumulation of genetic differences and the gradual reproductive isolation over time. The absence of gene flow or mating among the different species has been inferred based on linkage disequilibrium analysis consistent with an ancient reproductive isolation (Mazars et al., 1997; Keely et al., 2004; Keely and Stringer, 2009). Furthermore, no evidence of hybridization was detected between *P. carinii* and *P. wakefieldiae,* even during co-infection of the same rat (Cushion, 1998; Cushion et al., 2004). However, caution is warranted because the absence of gene flow was inferred from a small set of conserved markers, which may have not allowed detecting all genetic events. Consequently, whole genome sequencing studies are necessary to validate these findings.

### 1.4. Life cycle

The life cycle of *Pneumocystis* organisms is still hypothetical and mostly derived from microscopic and molecular studies on *P. carinii* (Fig. 1). As fungal organisms with an obligate parasitic behavior, the cycle would occur only inside host's lungs, and begin with the inhalation of infectious *asci*. Once inhaled, each ascus would release first eight ascospores which will evolve to what is known as trophic forms that bind to the type I pneumocytes of the alveolar epithelium. The cycle would then alternate between asexual multiplication of metabolically active trophic cells by binary fission, and sexual reproduction upon mating of two trophic cells that would culminate by the production of *asci* containing eight ascospores (Fig. 2). Trophic cells are amoeboid in shape and represent generally 90–98% of the populations in the infected lungs (Aliouat-Denis et al., 2009). These forms are mononuclear, 2–8 μm in diameter (Dei-Cas et al., 2004), and mostly haploid (Stringer and Cushion, 1998; Wyder et al., 1998; Martinez et al., 2011). Multiploid forms are rare and possibly caused by asymmetrical or post-mating divisions (Martinez et al., 2011). Trophic cell surface is composed of a single layer of electron dense material containing glycoproteins, but possibly no β-glucans. Indeed, the enzymes responsible for

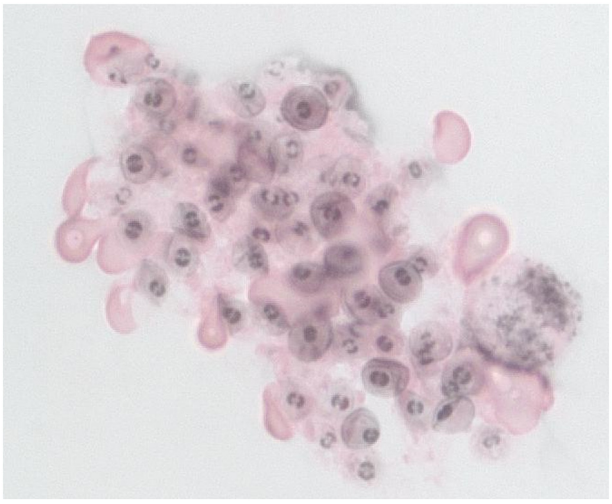

**Fig. 2.** | Cluster of *P. jirovecii asci*.
Cluster of *P. jirovecii asci* stained with Grocott's Methenamine silver (Churukian and Schenk, 1977) within a patient's bronchoalveolar lavage. The structures darker than the rest of the wall on each ascus are the parentheses-like structure (picture from the Institute of Microbiology, Lausanne University Hospital).

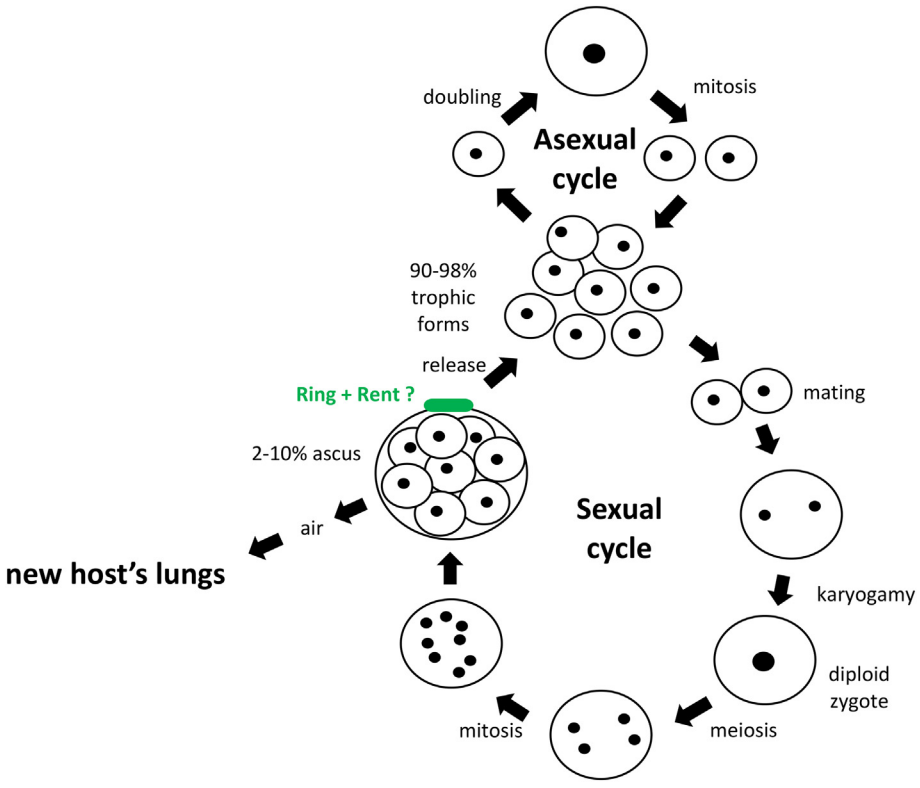


**Fig. 1.** | Cell cycle.
The whole cell cycle of *Pneumocystis* species would take place within the host's lungs, airborne *asci* ensuring transmission to new hosts. The cycle is thought to include two phases: sexual and asexual. The trophic forms tightly adhere to the host's alveolar epithelial pneumocytes type I, whereas *asci* are generally localized within the alveolar lumen. The ring shown in green might allow the formation of a rent upon contact with humidity and so the release of the ascospores. This ring may correspond to the parentheses-like structure visible on Fig. 2. This Figure does not include new features relatively to models previously proposed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)