Co-evolution of the parasitic fungi *Pneumocystis* and their Muridae rodent hosts in Southeast Asia

Alice Latinne^{1,2,3}, François Bezé⁴, Serge Morand^{5,6}, Magali Chabé⁷

¹ Departement of Life Sciences-Conservation Genetics, University of Liège, Liège, Belgium

² Institut des Sciences de l'Evolution (ISEM), Université de Montpellier 2, Montpellier, France

³ Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

⁴ Dunkerque Hospital, Medical laboratory, Dunkerque, France

⁵ CIRAD, UR AGIRs, F-34398 Montpellier, France

⁶ CNRS-CIRAD, Centre d'Infectiologie Christophe Mérieux du Laos, Vientiane, Lao PDR

⁷ Biology and Diversity of Emerging Eukaryotic Pathogens (BDEEP), Center for Infection and Immunity of Lille (CIIL), Univ. Lille Nord de France, Institut Pasteur de Lille, Lille, France

Pneumocystis species are opportunistic and airborne-transmitted fungi that infect the lungs of numerous mammalian species. These highly diversified fungi are characterized by strong host specificity, probably associated with co-speciation. In this study, we investigate the *Pneumocystis* genetic diversity and infection rate in Muridae rodents of Southeast Asia in relation to environmental habitats. A total of 445 wild rodents belonging to 18 Southeast Asian Muridae species were tested for the presence of *Pneumocystis* in their lungs through PCR amplification of two *Pneumocystis* mitochondrial genes (mtLSU rRNA and mtSSU rRNA).

Pneumocystis DNA was detected in 215 (48.3%) out of these 445 rodents. Eight highly divergent *Pneumocystis* lineages were retrieved in our phylogenetic tree. Three of these lineages correspond to the described species *Pneumocystis murina* (infecting *Mus* species), *P. carinii* (infecting *Rattus* species) and *P. wakefieldiae* (also infecting *Rattus* species). Three individuals belonging to *Rattus norvegicus* were found co-infected by both *P. carinii* and *P. wakefieldiae*. The five remaining lineages may correspond to several new undescribed *Pneumocystis* species and infect the lungs of *Cannomys* (lineage 1), *Bandicota* (lineage 2), *Berylmys* (lineage 3), *Rattus* (lineage 4) and *Maxomys*, *Niviventer* and *Leopoldamys* (lineage 5) Muridae genera. The congruence between phylogenies of *Pneumocystis* and their rodent hosts has been tested using co-phylogenetic analyses and the number of inferred co-speciation events is significantly greater than expected by chance. Rodent species, age and sex have no influence on the *Pneumocystis* infection rate among Muridae rodents but individuals trapped close to human settlements in patchy habitat were more likely infected by *Pneumocystis* parasites.