

**OR016***Phylogenetic distribution, stability and function of attine ant gut microbiota***Panagiotis Sapountzis**, Lars H. Hansen, Søren J. Sørensen, David R. Nash, Jacobus J. Boomsma, Morten Schiott

The attine fungus-growing ants are a monophyletic group that switched to an almost exclusive fungal diet ca. 50 MYA. They have become a model of complex symbiosis after several additional fungal and bacterial symbionts were discovered, but their gut bacteria have never been studied. Here we present the first comparative and functional gut-microbiome data for the attine fungus-growing ants based on 454 sequencing and FISH confocal microscopy. We show that gut-microbiomes of nine Panamanian species, representing eight genera, are dominated by few bacterial species belonging mostly to the Alpha-Proteobacteria and Mollicutes and that closely related bacterial species belonging to these clades are often mutually exclusive. The dominant bacterial species are located in specific gut tissues and some of them are clearly intracellular. They appear to mediate the acquisition and/or preservation of nitrogen as prokaryote *nifH* genes were found in almost all ant samples, particularly in Rectal Papillae and Malpighian Tubules where nitrogenous waste-excretion normally takes place. Guts and surrounding organs of higher attine, and particularly leaf-cutting, ants harbored most of these bacteria, consistent with the symbiosis remaining protein-limited in spite of evolutionary transitions in specialization of the crop fungus and later adaptations to using fresh leaves as fungal substrate. The composition of the gut microbiomes was correlated by the presence or absence of a cuticular microbiome of actinomycete bacteria: attine species that have cuticular actinomycetes appear to have more stable gut microbiomes, possibly because cuticular antibiotics protect gut microbiomes against inadvertent invasions by non-symbiotic bacteria. Our results suggest that gut microbiomes and their interactions with external microbiomes may have played important roles in shaping the mutualism between attine ants and their fungal crops over evolutionary time.