Molecular phylogenetic analysis of Spirotrichonymphida protists in the gut of termites

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SUMMARY

The phylum Parabasalia is a group of anaerobic flagellates that possess characteristic parabasal apparatus. The majority of parabasalid species are symbionts of various animal hosts as either parasites or mutualists. Parabasalia represents a complex assemblage of species and its taxonomy recently received extensive reorganization. Presently, Parabasalia is divided into four orders: Spirotrichonymphida, Cristamonadida, Trichonymphida, and Trichomonadida. The former three orders occur exclusively in the guts of termites and related insects. Almost all gut-dwelling members are extremely difficult to cultivate. Their existence in complex microbial communities has prevented molecular phylogenetic studies of them. Particularly, only four species of Spirotrichonymphida have been investigated. In this study, we obtained the sequences of small subunit rRNA genes of spirotrichonymphids from the mixed population of protists in the gut of the termite *Hodotermopsis sjoestedti*. Fluorescent-labeled oligonucleotide probes specific for each sequence were designed and used for the *in situ* identification of spirotrichonymphids species by whole-cell hybridization. Five species of spirotrichonymphids—*Spironympha obtuse*, *S. oblanga*, *Microjoenia minima*, *Spirotrichonympha cincta*, and *Holomastigotes elongatum*—were identified. The phylogeny of parabasalids was inferred using these newly identified sequences to establish more reliable taxonomic classification of spirotrichonymphids.