Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers

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Abstract

Aims: The aims of this study were to design universal markers for different protozoan parasites of *Bombus* spp. based on the phylogenetic position of two important bumblebee parasites *Crithidia bombi* and *Apicystis bombi*.

Methods and results: Standard PCR and extraction techniques were used to amplify and sequence 18S rDNA. Phylogenetic analysis of the rDNA was performed in order to predict the parasite-range of the primers.

Conclusion: *C. bombi* phylogenetically clusters with the trypanosomatids with slowly-evolving SSU-rRNA sequences (SE), while *A. bombi* is the closest sister group of *Mattesia*. A multiplex was designed containing an internal control and two broad-range primer pairs, detecting *C. bombi* and other SE trypanosomatids and also *A. bombi* and other neogregarines.

Significance and impact of study**:** Sequence data generated will further improve the current systematics of insect trypanosomatids and gregarines which remain troublesome. Broad-range markers for bumblebee parasites are necessary tools enabling the screening of commercially imported colonies and thus controlling their worldwide distribution and to discover related emerging parasites.