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.