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论著

基于线粒体基因分析的中华血吸虫分子种系发生研究

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摘要

目的 测定中华血吸虫线粒体细胞色素 C氧化酶亚基 1(CO1)和 NADH脱氢酶亚基 1(ND1)基因序列 ,并根据这些序列构建分子系统发生树 ,探讨中华血吸虫在裂体属内的系统发生位置。 方法 以GNT- K法抽提虫体基因组 DNA,用特异引物 PCR扩增目的基因。PCR扩增产物经纯化后克隆于质粒载体 ,以纯化后的阳性质粒 DNA作为模板 ,M13(F/ R)为引物于 L icor测序仪测序。检索 Gen Bank,查找曼氏血吸虫等相关血吸虫两线粒体基因序列 ,作基因排序及比较分析后 ,用 PHYL IP和 MEGA以邻接法和最大简约法绘制系统发生树。 结果 克隆了中华血吸虫的 CO1和 ND1基因片段 ,并测定了两基因片段的核苷酸序列 ,根据这些序列构建了系统发生树。 结论 中华血吸虫 CO1和ND1基因的系统发生树结果一致。提示中华血吸虫归属于亚洲血吸虫组

关键词 中华血吸虫 CO1 ND1 系统发生分析

分类号

Study on Molecular Phylogeny of Schistosoma sinensium Based on Mitochondrial Genes

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Abstract

Objective To determine the phylogenetic position of Schistosoma sinensium in the genus Schistosoma using mitochondrial cytochrome C oxidase 1 (CO1) and NADH dehydrogenase 1(ND1) as molecular markers. Methods The genomic DNA of adult worms were extracted by the GNTK method. The target regions were amplified by PCR using specific primers. The PCR products were purified before ligation into the plasmid Zero-Blunt. Recombinant plasmids were amplified in E.coli, extracted and purified using routine methods and then sequenced using M13 primers (F/R) on a Licor long read autosequencer. Sequences of related schistosomes were retrieved from GenBank and aligned with our data in the sequence editor ESEE. Gene trees were constructed in PHYLIP and MEGA using both maximum parsimony and neighborjoining methods. For parsimony analysis, all characters were treated as unordered and with equal weights. At least 3 000 cycles of bootstrapping were carried out. For analysis in MEGA, all gap columns were deleted. The third position of codon was included. Results The nucleotide and amino acid sequences of CO1 and ND1 of S.sinensium were obtained. Conclusion The phylogenetic trees from these molecular data suggested that S.sinensium belongs to the Asian schistosome group, and the results coincided with the previous rDNA (ITS2 & LSU) analysis results.

Key words Schistosoma sinensium cytochrome C oxidase 1(CO1) NADH dehydrogenase 1 (ND1) phylogenetic analysis

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