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基于核糖体DNA ITS区和*COX*1基因鉴别华支睾吸虫囊蚴

杨庆利1，2，申继清3，蒋智华2，杨益超2，李红梅1，陈颖丹1，周晓农1 *

1 中国疾病预防控制中心寄生虫病预防控制所，卫生部寄生虫病原与媒介生物学重点实验室，世界卫生组织疟疾、血吸虫病和丝虫病合作中心，上海200025；2 广西壮族自治区疾病预防控制中心，南宁530028；3 广西医科大学寄生虫学教研室，南宁530021

Identification of *Clonorchis sinensis* Metacercariae Based on PCR Targeting Ribosomal DNA ITS Regions and *COX*1 Gene

YANG Qing-li1，2，SHEN Ji-qing3，JIANG Zhi-hua2，YANG Yi-chao2，LI Hong-mei1，CHEN Ying-dan1，ZHOU Xiao-nong1*

1 National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention；Key Laboratory of Parasite and Vector Biology, MOH; WHO Collaborating Centre for Malaria, Schistosomiasis and Filariasis，Shanghai 200025，China；2 Center for Disease Control and Prevention of Guangxi Zhuang Autonomous Region，Nanning 530028，China；3 Department of Parasitology，Guangxi Medical University，Nanning 530021，China

摘要 目的 基于核糖体DNA ITS区和*COX*1基因，PCR鉴别鱼体内华支睾吸虫囊蚴。 方法 于2013年5月底采集广西横县某水库的麦穗鱼，用消化法从鱼肉组织中分离单个华支睾吸虫囊蚴及其他吸虫囊蚴；PCR检测华支睾吸虫核糖体DNA ITS区和*COX*1基因，并分析检测的灵敏性和特异性。 结果 分离到不同发育阶段的华支睾吸虫囊蚴并经PCR检测证实。用针对核糖体DNA ITS区和*COX*1基因的PCR均能检测到单个华支睾吸虫囊蚴DNA，其特异性扩增条带大小分别为437/549、156/249和195/166 bp。用核糖体DNA ITS1和ITS2区的通用引物和特异性引物扩增华支睾吸虫囊蚴DNA的阳性数之比分别为0.905和0.952。针对*COX*1基因的特异性扩增均检测到目标DNA，并且扩增条带较亮。用该方法对其他吸虫囊蚴检测的对比试验显示特异性引物均未出现任何非特异性扩增反应；用ITS区通用引物检测其他吸虫囊蚴则显示出严重的非特异性扩增反应。 结论 通过形态观察结合PCR法识别出了不同发育阶段的华支睾吸虫囊蚴。用华支睾吸虫核糖体DNA ITS区的特异性引物进行PCR检测的灵敏性和特异性均优于相应通用引物。针对*COX*1基因的特异性PCR检测的灵敏性和特异性与核糖体DNA ITS区的检测结果相当。

关键词： 华支睾吸虫 囊蚴 ITS区 COX1基因 PCR

Abstract： Objective To identify *Clonorchis sinensis* metacercariae using PCR targeting ribosomal DNA ITS region and *COX*1 gene. Methods Pseudorasbora parva were collected from Hengxian County of Guangxi at the end of May 2013. Single metacercaria of *C. sinensis* and other trematodes were separated from muscle tissue of *P. parva* by digestion method. Primers targeting ribosomal DNA ITS region and *COX*1 gene of *C. sinensis* were designed for PCR and the universal primers were used as control. The sensitivity and specificity of the PCR detection were analyzed. Results *C. sinensis* metacercariae at different stages were identified by PCR. DNA from single *C. sinensis* metacercaria was detected by PCR targeting ribosomal DNA ITS region and *COX*1 gene. The specific amplicans have sizes of 437/549, 156/249 and 195/166 bp, respectively. The ratio of the two positive numbers in PCR with universal primers and specific primers targeting *C. sinensis* ribosomal DNA ITS1 and ITS2 regions was 0.905 and 0.952, respectively. The target gene fragments were amplified by PCR using *COX*1 gene-specific primers. The PCR with specific primers did not show any non-specific amplification. However, the PCR with universal primers targeting ribosomal DNA ITS regions performed serious non-specific amplification. Conclusion *C. sinensis* metacercariae at different stages are identified by morphological observation and PCR method. Species-specific primers targeting ribosomal DNA ITS region show higher sensitivity and specificity than the universal primers. PCR targeting *COX*1 gene shows similar sensitivity and specificity to PCR with specific primers targeting ribosomal DNA ITS regions.

Keywords： *Clonorchis sinensis* Metacercaria ITS region *COX*1 gene PCR

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