

论著

中国14省不同地区常见嗜尸性苍蝇 mtDNA中 CO I 基因序列检测

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摘要:

目的: 通过检测嗜尸性苍蝇线粒体DNA(mtDNA)上细胞色素氧化酶亚单位 I (CO I)中278 bp基因序列, 鉴定嗜尸性苍蝇种类, 为法医鉴别嗜尸性苍蝇种类提供可靠依据。方法: 随机采集内蒙古包头、赤峰、天津、南宁、福州、山东临沂、石家庄、银川、兰州、北京怀柔、河南新乡及南阳、山西大同、安徽芜湖、浙江衢州、湖南长沙及株洲、永州等14个省不同地区室外多点放置兔、狗、猪尸体上的嗜尸性苍蝇38只。利用改进的小型昆虫DNA匀浆方法提取上述苍蝇mtDNA, 进行PCR扩增; 7%聚丙烯酰胺非变性凝胶连续缓冲体系垂直电泳和银染显色技术进行扩增结果检测; PCR胶回收试剂盒纯化; ABI3730测序仪测序; MEGA4软件包进行序列分析和构建UPGMA系统发育树。结果: 有效鉴定出上述38只嗜尸性苍蝇, 3科(蝇科、丽蝇科、麻蝇科), 10属(家蝇属、齿股蝇属、阿丽蝇属、带绿蝇属、裸金蝇属、原伏蝇属、金蝇属、绿蝇属、黑麻蝇属、别麻蝇属), 12种(家蝇、开普齿股蝇、叉叶绿蝇、亮绿蝇、巨尾阿丽蝇、瘦叶带绿蝇、白头裸金蝇、新陆原伏蝇、大头金蝇、丝光绿蝇、黑尾黑麻蝇、棕尾别麻蝇)。结论: mtDNA上CO I中278 bp基因序列分析能有效地对嗜尸性苍蝇进行种类鉴定。该检测方法快速、简便和精确, 可以作为法医鉴别嗜尸性苍蝇种类的依据。

关键词: 法医昆虫学 嗜尸性苍蝇 种类鉴定 线粒体DNA 细胞色素氧化酶亚单位 I

Sequencing of mitochondrial DNA cytochrome oxidase subunit I gene in sarcosaphagous flies from 14 provinces in China

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Abstract:

ObjectiveTo detect the 278 bp region of gene of the cytochrome oxidase subunit I (CO I) in mitochondrial DNA (mtDNA) of sarcosaphagous flies, identify the species of sarcosaphagous flies, and provide reference for forensic application.MethodsSamples were collected in Baotou and Chifeng of Inner Mongolia, Tianjin, Nanning, Fuzhou, Linyi of Shandong, Shijiazhuang, Yinchuan, Lanzhou, Huairou of Beijing, Xinxiang and Nanyang of Henan, Datong of Shanxi, Wuhu of Anhui, Quzhou of Zhejiang, Changsha, Zhuzhou and Yongzhou of Hunan. A total of 38 flies were randomly collected from rabbits, dogs and pigs which were set outdoors, then the flies' mitochondrial DNA (mtDNA) were extracted by the improved small insects DNA homogenate method. Amplification was conducted by Perkin-Elmer 9600 thermal cycler, then vertical non-denaturing 7% polyacrylamide gelelectrophoresis. PCR products were purified using the nucleic acid purification kit. Sequences of both strands were obtained by direct sequence of the double-stranded PCR product using one of the PCR primers and the ABI PRISM big dye terminator cycle sequencing dit. Sequence reactions were electrophorsed on ABI Model 3730 DNA Sequencers. A UPGMA tree was contrasted using the maximum composite likelihood method in MEGA4.ResultsThe 38 sarcosaphagous flies belonged to 3 families (Muscidae, Calliphoridae, and Sarcophagidae), 10 genres (Musca Linnaeus, Hydrotaea Robineau-Desvoidy, Aldrichina Townsend, Hemipyrellia Townsend, Achoetandrus Bezzi, Protophormia Townsend, Chrysomya Robineau-Desvoidy, Lucilia Robineau-Desvoidy, Helicophagella Enderlein, and Boettcherisca Rohdendorf), and 12 species [Musca domestica (Linnaeus), Hydrotaea(Ophyra)capensis (Wiedemann), Lucilia caesar (Linnaeus), Lucilia illustris (Meigen), Aldrichina graham (Aldrich), Hemipyrellia ligurriens, Achoetandrus (Chrysomya) ruffacies(Macquary), Protophormia terraenovae(Robineau-Desvoidy), Chrysomya megacephala (Fabricius), Lucilia sericata (Meigen), Helicophagella melanura (Meigen), and Boettcherisca peregrine (Robineau-Desvoidy)]. ConclusionThe genus of the sarcosaphagous flies can be identified by 278 bp gene sequence analysis of CO I in mtDNA. This method is rapid, convenient and precise.

Keywords: forensic entomology; sarcosaphagous flies; species identification; mitochondrial deoxyribonucleic acid; cytochrome oxidase subunit I

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