

## 桔小实蝇幼体及成虫残体DNA条形码识别技术的建立与应用

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Establishment and application of DNA barcoding technology for identification of the immatures and adult debris of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

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**摘要** 实蝇类害虫多为国内外检疫对象, 其鉴定识别方法主要依据成虫的外部形态特征, 而传统的形态学识别法对口岸经常截获的幼体及残缺的虫体, 则无能为力。本研究以桔小实蝇*Bactrocera dorsalis*的幼体(卵、幼虫、蛹)以及成虫残体(足、翅、头部、胸部、腹部)为对象, 利用DNA条形码技术, 构建实蝇类害虫快速鉴定技术体系, 并以其他4种常见实蝇(包括番石榴实蝇*B. correcta*、瓜实蝇*B. cucurbitae*、南亚果实蝇*B. tau*、柑桔大实蝇*B. minax*)为对象对该技术体系进行应用验证。结果显示, 桔小实蝇幼体以及成虫残体的碱基序列与数据库中靶标种CO I基因碱基序列的一致性为99.51%~99.84%, 其他4种实蝇相应序列与数据库中靶标种CO I基因序列的一致性分别为100%, 100%, 99.81%~99.83%和100%; 以邻接法(NJ法)构建系统发育树, 靶标种实蝇均与数据库中对应种实蝇聚为一支, 且置信度均为100%。以K2-P模型计算种内及种间遗传距离得出, 5种实蝇的种间遗传距离为0.0597~0.2363, 平均为0.1693; 种内遗传距离为0.0000~0.0041, 平均为0.0019。这些结果表明, 基于DNA条形码的物种识别技术完全可用于口岸截获的实蝇类害虫幼体及残体的准确鉴定。

**关键词:** 桔小实蝇 DNA条形码; 实蝇类; 幼体; 成虫残体; 线粒体DNA 细胞色素C氧化酶亚基I基因; 种类识别

**Abstract:** Many species in the family Tephritidae are quarantine pests worldwide. Usually, identification of the tephritid fruit flies is mainly based on external morphological characteristics of adults. In this research, the immatures (including egg, larva and pupa) and adult debris (including leg, wing, head, thorax and abdomen) of *Bactrocera dorsalis* (Hendel) were used to develop a rapid identification technique for tephritid fruit flies based on DNA barcoding technology. The other four tephritid fruit fly species, i.e., *B. correcta*, *B. cucurbitae*, *B. tau* and *B. minax*, were used to verify the feasibility of the fruit fly identification technique developed. The results showed that the nucleotide sequence identity of the partial CO I gene between the immatures or adult debris of *B. dorsalis* and the target gene from GenBank database is 99.51%-99.84%, while between other four tephritid fruit fly species and the target gene from GenBank database it is 100%, 100%, 99.81%-99.83% and 100%, respectively. Neighbor-joining tree was established based on the analysis of CO I gene sequences. The target species and the corresponding species in the database cluster in the same branches. All bootstrap values of the original divergence within one species are 100%. The intra- and inter-species genetic distances were calculated with MEGA version 5.0 software using the Kimera 2-Parameter model. The intra-species genetic distances are 0.0000-0.0041, with an average of 0.0019. The interspecies genetic distances are 0.0597-0.2363, with an average of 0.1693. There is no overlap between intra- and inter-species genetic distances. The results indicated that the developed DNA barcoding identification techniques based on the partial CO I gene can provide a rapid and accurate method for identification of immatures or adult debris of tephritid fruit fly species.

**Key words:** *Bactrocera dorsalis* DNA barcoding fruit fly immature adult debris mtDNA CO I gene identification

收稿日期: 2011-12-23; 接受日期: 2012-02-12

基金资助:

国家重点基础研究发展计划(“973”计划)项目(2009CB119200); 公益性行业(农业)科研专项(200903047); “十一五”国家科技支撑计划重大项目(2006BAD08A18)

引用本文:

刘慎思, 张桂芬, 武强等. 桔小实蝇幼体及成虫残体DNA条形码识别技术的建立与应用[J]. 昆虫学报, 2012, 55(3): 336-343.

LIU Shen-Si, ZHANG Gui-Fen, WU Qiang et al. Establishment and application of DNA barcoding technology for identification of the immatures and adult debris of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)[J]. ACTA ENTOMOLOGICA SINICA, 2012, 55(3): 336-343.

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