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Phylogenetic Analysis Within Tephritidae of Diptera Based on the Concatenated 13 Mitochondrial Protein Coding Genes of mt Genomes

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ABSTRACT

The mitochondrial genome of insects has been proven to be a useful genetic marker for molecular and evolutionary studies. Tephritidae of Diptera is the most agriculturally important worldwide serious pests. The objective of this study is to infer phylogenetic relationships among 8 sequenced mitochondrial genomes within Tephritidae. According to conserved (C)/variable (V) sites ratio test, all 13 mtDNA Protein Coding Genes (PCGs) were combined into a single data set for use in determining phylogeny. The same gene regions of *Pteronarcys princeps*, *Lucilia sericata* and *Drosophila yakuba* were used as outgroup. When MP and ML analysis were applied to the combined data set, two fairly similar trees were shown. Accorded with all previous morphological and molecular evidence, the monophyly of family Tephritidae was strongly recovered in our result. The data set led to a likelihood tree in which *B. dorsalis* and *B. papayae* was provided relative closely relationship meanwhile *B. carambolae* formed a clade basal to them.

Key words: Mitochondrial genomes, phylogeny, tephritidae

INTRODUCTION

Insects mitochondrial (mt) genomes consists of a typically double-strand, circular molecule of 15-20 kb in length, which encodes 37 genes including 2 ribosomal RNA (rRNA), 22 transfer (tRNA) genes and 13 protein-coding subunits from three of the oxidative phosphorylation complexes (Boore, 1999). The only major noncoding area of the mtDNA is the adenine (A)+thymine (T)-rich region, variable length, which serves as the regulation and initiation of mtDNA replication and transcription (Boore, 1999).

The mitochondrial genome of insects is maternal inheritance, relatively small size, generally nonrecombining, orthologous genes and constant gene content. At present, this closed circular molecule has been completely sequenced in over more than 170 species from a variety of insects, thus confirming the versatility of this genetic marker for molecular and evolutionary studies (Li *et al*., 2009; Nardi *et al*., 2010). The Diptera (flies) is the most species in terms of their mt genomic coverage. Within the Diptera, Tephritidae (fruit flies) has 8 sequenced mitochondrial genomes (Table 1).

Tephritidae is the most agriculturally important pests and has been listed as a kind of serious economically pests by 64 countries and areas (White and Elson-Harris, 1992; Aluja and Norrbom, 2000). There are about 5,000 known species in almost 500 genera of the family Tephritidae (White and Elson-Harris, 1992; Aluja and Norrbom, 2000). Many governments adopt a series of serious quarantine methods to avoid the introduction of fruit flies and pay more attention to their classification and identification (Hui, 2001; Liu *et al*., 2007; Chen and Ye, 2008).

Within the family Tephritidae, phylogenetic studies had been conducted by Korneyev (1999) based on morphology; by Han (2000) and Han *et al*. (2006) based on mitochondrial 16S rDNA gene; by Smith *et al*. (2003) based on 16S rDNA and COII genes and by Zhang *et al*. (2010) based on 16S rDNA and CO I sequence genes. In the tree based on mtDNA sequences, the bootstrap of a few clades had been limited to low bootstrap value (<50%) and their relationships were still unclear and rarely been discussed. A better understanding of the phylogenetic relationship of Tephritidae required an expansion of gene numbers. Therefore, the objective of this study was to infer phylogenetic relationships among 8 sequenced mitochondrial genome within Tephritidae. The inferred relationships based on this study were further compared to those proposed by morphological analysis (Korneyev, 1999) and molecular sequence analysis (Han, 2000; Smith *et al*., 2003; Han *et al*., 2006; Nardi *et al*., 2010; Zhang *et al*., 2010).

MATERIALS AND METHODS

Conserved (C)/variable (V) sites ratio test: Conserved (C) / variable (V) sites ratio (Lee *et al*., 2006) were performed to test the estimation of reliability of alignment. According to Lee *et al*. (2006), 13 protein coding genes (PCGs) all show >0.5 of C/V ratio (Fig. 1) and were further used for the phylogenetic analysis.

The concatenation of 13 protein coding gene sequences: All 13 PCGs were aligned using DNAStar (DNAStar Inc.) and manually corrected. Subsequently a set of protein-coding genes were concatenated. Sequences of *Pteronarcys princes* (Plecoptera: Pteronarcyidae, NC-006133), *Lucilia sericata* (Diptera, NC_009733) and *Drosophila yakuba* (Diptera, NC_001322) were used as outgroup in present study. All 13 single gene alignments were concatenated to give a final dataset of 11 sequences by aligned 11300 positions.

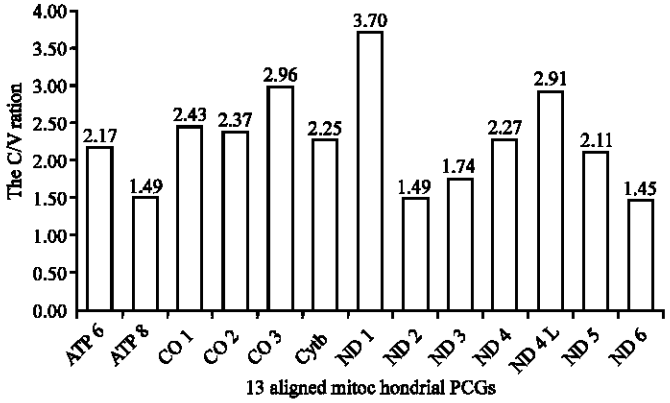

Fig. 1: C/V ratio of aligned 13 mitochondrial PCGs within tephritidae of diptera

Phylogenetic analysis: Phylogenetic reconstructions of the aligned combined data set was performed with PAUP 4.0b10 (Swofford, 2002) for Maximum likelihood (ML) and maximum-Parsimony (MP) analysis independently.

ML analysis was done with a heuristic search algorithm using 100 random-addition sequences and performing TBR branch swapping. The confidence values of the ML tree were evaluated via the bootstrap test with 100 iterations.

Before undertaking likelihood-model-based phylogenetic searches, a preferred model of the aligned combined data set for ML analysis were chosen using Akaike Information Criterion (AIC) as implemented in JModeltest 0.1.1 (Posada, 2008). The GTR+I+G was inevitably identified as the best-fit model for ML tree with the following parameter values: nucleotide frequencies were estimated from the data (A: 0.3829, C: 0.1576, G: 0.1019 and T: 0.3577); substitution rates: R(a) [A-C] = 12.3056; R(b) [A-G] = 44.2922; R(c) [A-T] = 24.0826; R(d) [C-G] = 23.8279; R(e) [C-T] = 141.6046; and R(f) [G-T] = 1.0000. The proportion of invariable sites was 0.3860 and the estimated gamma distribution shape parameter was 1.1290.

For MP analysis, nucleotides were treated as unordered and equally weighted characters; the gaps were considered as missing data in all analysis. MP analysis was done with a heuristic search algorithm using 100 random-addition sequences and performing TBR branch swapping. Bootstrap analysis with 1000 replicated times was used to measure support of the resulting topologies.

RESULTS AND DISCUSSION

All 13 mtDNA Protein Coding Genes (PCGs) were combined into a single data set for use in determining a Tephritidae molecular phylogeny (Fig. 2). When MP and ML analysis were applied to the combined data set, two fairly similar trees were shown (Fig. 2; MP tree was not shown. MP analysis showed the same topology as the ML tree though its support values were slight lower). The trees obtained using the combined data were consistently more stable (i.e., less variable when the tree reconstruction methods were changed), more resolved (i.e., fewer nodes with support values below 50%, irrespective of the method used) and more robust (i.e., the resolved nodes were supported by higher support values) than the trees constructed using 16S rDNA, CO_2 and CO_1 genes before (Han, 2000; Smith *et al*., 2003; Han *et al*., 2006; Zhang *et al*., 2010) . The use of PCG sequences of the mitochondrial genomes as a reliable molecular marker were suitable to resolve the phylogenetic relationships, as previously reported by Cameron *et al*. (2007) and Boore *et al*. (2005).

The family Tephritidae was clearly as monophyly by the shape of the subcosta (bent sharply forward subapically and usually weaker or fold like beyond the bend) for adult fruit flies (Aluja and Norrbom, 2000). This was also well supported as monophyletic in terms of our result and other previous molecular data (Han, 2000; Smith *et al*., 2003; Han *et al*., 2006; Nardi *et al*., 2010; Zhang *et al*., 2010).

The genus *Bactrocera* in present study included seven well-known species (Table 1). Because of their scientifically significance and economic importance, many species (i.e., *B. dorsalis* and *B. oleae*) had been well-studied and served as prominent model organisms for ecological, physiological and genetic studies (Nardi *et al*., 2003; Chen and Ye, 2008; Shi *et al*., 2010). Recently, relationships within *Bactrocera* were discussed by Zhang *et al*. (2010). Fifty-five Bactrocera species were defined by CO_1 and 16S rDNA gene fragments, totalling approximately 1034 bp, including *C. capitata* used as out-group for the phylogenetic analysis. An identical topology within each cluster of *Bactrocera* was obtained from this study and the result of Zhang *et al*. (2010), though their support values were very low (<50%).

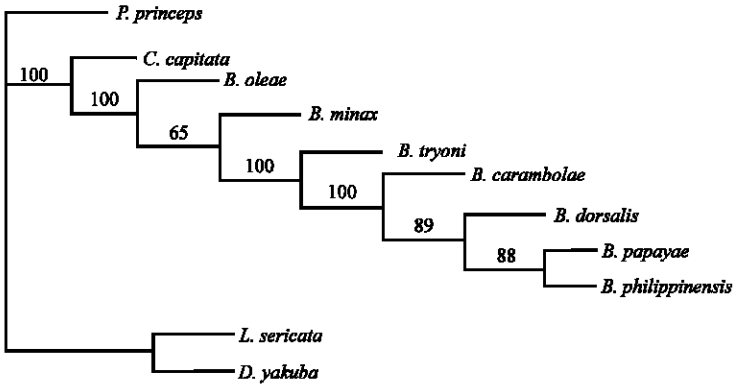

Fig. 2: Maximum likelihood trees derived from analysis of 13 mitochondrial PCGs data set, Numbers above nodes indicated bootstrap values

Table 1: The complete Mitochondrial genome sequences within tephritidae of diptera

Genus	Species	Length	GenBank ID
Bactrocera	*B. tryoni* (Nardi *et al*., 2010)	15925	NC_014611
Bactrocera	*B. minax*	16043	NC_014402
Bactrocera	*B. oleae* (Nardi *et al*., 2003)	15815	NC_005333
Bactrocera	*B. philippinensis*	15915	NC_009771
Bactrocera	*B. carambolae*	15915	NC_009772
Bactrocera	*B. papayae*	15915	NC_009770
Bactrocera	*B. dorsalis*	15915	NC_008748
Ceratitis	*C. capitata* (Spanos *et al*., 2000)	15980	NC_000857

Smith *et al*. (2003) suggested the close relationship between *B. dorsalis* and *B. carambolae* and then *B. papayae* included to group one clade in their strict consensus parsimonious trees. In the study on *Bactrocera* phylogeny inferred from CO_2 and 16S rDNA gene, Smith *et al*. (2003) displayed *B. papayae* at the base of three species. Another extensive study of the phylogeny of the Diptera had been performed by Nardi *et al*. (2010). Phylogenetic tree had been yielded by the concatenated 37 dipteran mitochondrial genes. *B. carambolae* was placed as the basal clade to *B. dorsalis* and *B. papayae*.

In present study, the position of three species reached a similar conclusion with Zhang *et al*. (2010) and Nardi *et al*. (2010). Our data set led to a likelihood tree in which *B. dorsalis* and *B. papayae* was provided relative closely relationship with high bootstrap support (89%) meanwhile *carambolae* formed a clade basal to them with wholly bootstrap value (100%) (Fig. 2).

CONCLUSION

Accorded with all previous morphological and molecular evidence, the monophyly of family Tephritidae was strongly recovered in our result. Our data set led to a likelihood tree in which *B. dorsalis* and *B. papayae* was provided relative closely relationship meanwhile *B. carambolae* formed a clade basal to them.

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