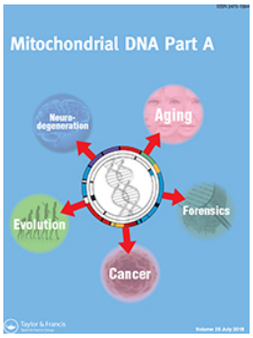

Taylor & Francis
Taylor & Francis Group

Mitochondrial DNA Part A

DNA Mapping, Sequencing, and Analysis

ISSN: 2470-1394 (Print) 2470-1408 (Online) Journal homepage: http://www.tandfonline.com/loi/imdn21

Genetic analysis of *Bactrocera zonata* (Diptera: Tephritidae) populations from India based on *cox1* and *nad1* gene sequences

Jaipal S. Choudhary, Naiyar Naaz, Moanaro Lemtur, Bikash Das, Arun Kumar Singh, Bhagwati P. Bhatt & Chandra S. Prabhakar

To cite this article: Jaipal S. Choudhary, Naiyar Naaz, Moanaro Lemtur, Bikash Das, Arun Kumar Singh, Bhagwati P. Bhatt & Chandra S. Prabhakar (2018) Genetic analysis of *Bactrocera zonata* (Diptera: Tephritidae) populations from India based on *cox1* and *nad1* gene sequences, Mitochondrial DNA Part A, 29:5, 727-736, DOI: 10.1080/24701394.2017.1350952

To link to this article: https://doi.org/10.1080/24701394.2017.1350952

MITOCHONDRIAL DNA PART A
2018, VOL. 29, NO. 5, 727–736
https://doi.org/10.1080/24701394.2017.1350952

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Genetic analysis of *Bactrocera zonata* (Diptera: Tephritidae) populations from India based on *cox1* and *nad1* gene sequences

Jaipal S. Choudhary[a], Naiyar Naaz[a], Moanaro Lemtur[a], Bikash Das[a], Arun Kumar Singh[a], Bhagwati P. Bhatt[b] and Chandra S. Prabhakar[a,c]

[a]ICAR Research Complex for Eastern Region, Research Centre, Ranchi, India; [b]ICAR Research Complex for Eastern Region, ICAR Parisar, P. O. Bihar Veterinary College, Patna, India; [c]Department of Entomology, Veer Kunwar Singh College of Agriculture (Bihar Agricultural University, Sabour), Dumraon, India

ABSTRACT

The peach fruit fly, *Bactrocera zonata*, is among the most serious and polyphagous insect pest of fruit crops in many parts of the world under genus *Bactrocera*. In the present study, the genetic structure, diversity and demographic history of *B. zonata* in India were inferred from mitochondrial *cytochrome oxidase 1* (*cox1*) and *NADH dehydrogenase 1* (*nad1*) sequences. The efficiency of DNA barcodes for identification of *B. zonata* was also tested. Genetic diversity indices [number of haplotypes (*H*), haplotype diversity (*Hd*), nucleotide diversity (π) and average number of nucleotide differences (*k*)] of *B. zonata* populations across India maintain high level of genetic diversity without isolation by distance among the geographic regions. Non-significant negative correlation between pairwise *F*st and geographic distance suggests a high level of gene flow among studied populations of *B. zonata*. The possibility of sudden expansion of *B. zonata* revealed through mismatch distribution analysis as well as negative Tajima's *D* and Fu's *Fs* values further supported by star-like network of haplotypes. DNA barcoding analysis suggests that *B. zonata* specimens can be clearly differentiated from other species with 100% accuracy of identification. Therefore, *cytochrome oxidase 1* (*cox1*) barcode sequences generated in the present study could be a valuable source for the rapid identification and global population genetic study of *B. zonata*.

ARTICLE HISTORY
Received 26 April 2017
Accepted 2 July 2017

KEYWORDS
Bactrocera; *cox1* gene; *nad1* gene; genetic diversity; DNA barcode

Introduction

The peach fruit fly, *Bactrocera zonata* (Saunders), is a serious pest of horticultural crops mainly tropical fruits in many parts of the world particularly in South Asia (India, Bhutan, Bangladesh, Nepal and Pakistan), Southeast Asia (Laos, Myanmar, Thailand and Vietnam) and some parts of Africa (Egypt, Libya and Mauritius) (White and Elson-Harris 1992; El-Samea and Fetoh 2006; CABI/EPPO 2013; EPPO 2014). *B. zonata* was first recorded from Bengal (India) by Saunders during 1842 (Kapoor 1993) and is considered as one of the most destructive and widely distributed species of fruit fly in India. Crop losses of 25–100% in peach (*Prunus persica*), apricot (*Prunus armeniaca*), guava (*Psidium guajava*) and figs (*Ficus carica*) (Gupta et al. 1990; Rana et al. 1990; Rai et al. 2008; Choudhary et al. 2012; Prabhakar et al. 2012a; EPPO 2014). In certain areas of north India and Pakistan, it has been more notorious than *Bactrocera dorsalis* (Qureshi et al. 1991; Kapoor 1993). Being multivoltine and highly polyphagous, *B. zonata* can infest fleshy fruits of more than 50 species of cultivated and wild plants (Duyck et al. 2004).

Information regarding rapid identification and characterization of different species, genetic diversity, genetic structure, gene flow and factors facilitates population expansion in the past and is helpful in the planning of appropriate management strategies (Roderick and Navajas 2003; Wan et al. 2012). For example, in sterile insect technique (SIT), sterile males fight with the normal males for mating chance with normal females in the field; nevertheless, this method requires millions of sterile insects with same genetic makeup (Itô et al. 2003; Prabhakar et al. 2012 b; Kunprom et al. 2015). Information about genetic makeup, effective population size and gene flow, which can be assumed from genetic studies of target insect populations (Aketarawong et al. 2011), is important in the planning for release of sterile insects in the development of area-wide management program. The genetic studies with molecular markers could also add to our understanding on the impact of past climatic conditions (e.g. Pleistocene glaciations), domestication and distribution of host crops on current insect populations. Previous studies on insects, such as mosquitoes (O'Loughlin et al. 2008), black flies (Pramual et al. 2005, 2011) and other species of fruit fly, viz. *Bactrocera cucurbitae* (Coquillett), *Bactrocera tau* (Walker), *Bactrocera latifrons* (Hendel) and *Bactrocera correcta* (Bezzi) (Prabhakar et al. 2012 b; Prabhakar et al. 2013; Meeyen et al. 2014; Kunprom et al. 2015), highlighted the importance of

CONTACT Chandra S. Prabhakar csprabhakar.ento@gmail.com ICAR Research Complex for Eastern Region, Research Centre, Plandu, Ranchi 834 010, Jharkhand, India

Supplemental data for this article can be accessed here.

historical climatic changes and host range in determining the genetic structure and diversity of South and Southeast Asian faunas.

Mitochondrial DNA (mtDNA) is one of the most used and universally accepted molecular markers, in particular for the phylogeographical studies of insect species (Wan et al. 2011). Features such as maternal inheritance, non-recombinant, multiple copy and high rate of evolution make mtDNA as a strong molecular marker for study intra- and interspecific relationships when compared with nuclear markers (Simon et al. 1994; Roderick 1996; Mun et al. 2003; Nardi et al. 2005; Wan et al. 2012; Prabhakar et al. 2012 b, 2013; Meeyen et al. 2014; Choudhary et al. 2016). Mitochondrial DNA genes, i.e., *cytochrome oxidase I* (*cox1*) and *NADH dehydrogenase* gene (*nad1*), are very useful tools for genetic studies which showed greater nucleotide substitution rates and more variation than other mitochondrial genes (Shi and Ye 2007; Wan et al. 2011, 2012; Wu et al. 2014). *nad1* with *cox1* genes could be better in understanding the genetic structure, recognition of cryptic species, new invasion and evolution of insect species (Wu et al. 2014; Choudhary et al. 2016).

In spite of being an important pest species which causes significant economic losses, documented information on genetic structure and geographical variability of *B. zonata* based on molecular/genetic markers is presently not available. Most of the studies on molecular systematics of tephritid fruit flies included *B. zonata* for phylogenetic analysis with different *Bactrocera* species (Muraji and Nakahara 2001; Nakahara and Muraji 2008; Zhang et al. 2010; Asokan et al. 2011; Prabhakar 2011). The detailed genetic structure of *B. zonata* has not been studied yet from any region of the world.

Keeping all these facts in view during the present study, both *cox1* and *nad1* gene sequences were used to investigate genetic variation, population genetic structure and population history of *B. zonata* from India, considered as the native region of the species. We also tested the efficiency of *cox1* gene sequences as barcode for the identification of *B. zonata*. In addition to the knowledge of genetic structure and diversity of this pest gathered from this study, the *cox1* barcoding sequences generated in this study would strengthen further identification of *B. zonata* from closely related species and immature stages, whose morphological identification is difficult.

Materials and methods

Sample collection and species identification

Adults of *B. zonata* (male) were collected from the seven locations in India that covers different climatic zones, during 2012–2014. Flies were collected with the help of parapheromone (methyl eugenol) traps placed in mango orchards that cover the major endemic areas of *B. zonata* in India. Details of the *B. zonata* collection sites are presented in Table 1. The collected samples were identified on the basis of available morphological literature of Drew and Raghu (2002), Prabhakar et al. (2012c) and Choudhary et al. (2014). Collected and identified samples were stored in 95% ethanol at −20 °C until DNA extraction from the specimens.

Table 1. Details of sampling locations and diversity indices of *B. zonata* populations collected from India.

							cox1 (N)			*nad1* (N)			*cox1* and *nad1* (N)		
Location, State	ID code	Coordinates	Collection year	*cox1* (N)	*nad1* (N)	*cox1* and *nad1* (N)	*H*	*Hd*	$\pi \pm SD$	*H*	*Hd*	$\pi \pm SD$	*H*	*Hd*	$\pi \pm SD$
Bengaluru, Karnataka	BGR	12°96′N, 77°56′E	2012	14	10	10	11	0.956	0.014 ± 0.007	7	0.933	0.005 ± 0.003	9	0.978	0.007 ± 0.004
Paria, Gujarat	PAR	20°45′N, 72°96′E	2012	16	9	9	9	0.908	0.009 ± 0.005	7	0.944	0.005 ± 0.003	9	1.0	0.008 ± 0.004
Ranchi, Jharkhand	RNC	23°35′N, 85°33′E	2012	5	10	5	5	1.000	0.024 ± 0.015	10	1.0	0.006 ± 0.004	5	1.0	0.016 ± 0.010
Patna, Bihar	PTA	25°61′N, 85°14′E	2013	14	6	6	13	0.989	0.024 ± 0.013	5	0.933	0.005 ± 0.004	6	1.0	0.013 ± 0.007
Vengurla, Maharashtra	VGR	15°85′N, 73°63′E	2012	8	4	4	7	0.964	0.007 ± 0.005	4	1.000	0.006 ± 0.004	4	1.0	0.004 ± 0.003
Indore, Madhya Pradesh	IDR	22°70′N, 75°90′E	2013	/	27	/	/	/	/	16	0.963	0.007 ± 0.004	/	/	/
Mumbai, Maharashtra	MBI	18°97′N, 72°82′E	2014	/	21	/	/	/	/	15	0.967	0.007 ± 0.004	/	/	/

H: number of Haplotypes; *Hd*: haplotype diversity; π: nucleotide diversity; *SD*: standard deviation.

DNA extraction, amplification and sequencing

Total DNA was extracted from individuals of *B. zonata* following the protocol described by Prabhakar et al. (2009) with minor modifications. Two fragments (~680 bp of *cox1* and ~600 bp of *nad1*) of the mitochondrial genome were amplified from each individuals using primer pairs *cox1*, UEA7 (5′-TACAGTTGGAATAGACGTTGATAC-3′)/UEA10 (5′-TCCAATGCACTAATCTGCCATATTA-3′) (Lunt et al. 1996) and *nad*1-F (5′-TTTAGTTGCTTGGTTGTGTATTCC-3′)/*nad*1-R (5′-GAAAAAGGTAAAAAACTCTTTCAAGC-3′) (Nardi et al. 2005). PCR amplifications of *cox1* gene were performed with 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C, with an initial denaturation step of 3 min at 94 °C and a final elongation at 72 °C for 30 min. The first subunit of the *NADH dehydrogenase* gene was amplified with an initial denaturation step of 5 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C, 1 min 30 sec at 72 °C and a final extension of 10 min at 72 °C. The PCR products were separated in 2% (w/v) agarose gel using TAE buffer (40 mM Tris acetate, 1 mM EDTA). Amplified PCR products were freeze-dried and sent for custom sequencing using *cox1* and *nad1* primers (Xcelris Labs Limited, Ahmedabad, India). As nuclear copies of mitochondrial genes (numts) can generate doubtful results (Hazkani-Covo et al. 2010), we systematically double-checked the obtained chromatograms to ensure that double peaks did not occur and that the haplotypes were functional coding genes (absence of indels or stop codons). After manual correction and assembly, unique sequences (haplotypes) were deposited in GenBank under accession numbers KY367445–KY367487 for *cox1* and KY367398–KY367444 for *nad1* gene sequences.

Data analysis

DNA sequences were aligned using ClustalW program implemented in MEGA ver. 6.0. (Tamura et al. 2013). Unique haplotypes were identified in Arlequin ver. 3.5 (Excoffier and Lischer 2010). Descriptive statistics, i.e., number of haplotypes (*H*), haplotype diversity (*Hd*) and nucleotide diversity (π), was calculated with DnaSP ver. 5.0 software (Librado and Rozas 2009). Statistical significance of nucleotide diversity (π) was assayed through a non-parametric permutation method (5000 permutations) in Arlequin ver. 3.5.

Analysis of molecular variance (AMOVA) was performed to assess the genetic structure and genetic variability within and between the populations of *B. zonata*. Population pairwise *F*st and AMOVA analyses worked by partitioning the total variations between and within populations of *B. zonata* and were run using Kimura 2-parameter model (K2P) in Arlequin ver. 3.5. Genetic distances among *B. zonata* population pairs were also calculated using Kimura 2-parameter methods implemented in MEGA 6.0. To depict the evolutionary and geographical relationships among haplotypes, a median joining of haplotype genealogical relationship network of both the gene sequences was constructed with Network ver. 4.6 (Bandelt et al. 1999). The major clusters of *B. zonata* haplotypes were coloured according to the sampling locations of *B. zonata* populations to draw migration and invasion pattern of the species across India. Mantel test (Mantel 1967) was also used to determine the relationship between genetic distance (*F*st) and geographic distance matrices (km in log scale) to test an isolation-by-distance (IBD) model. The Mantel test was performed through IBD web service ver. 3.23 using 10,000 randomizations (Jensen et al. 2005). The geographical distance between each pair of populations was estimated by Google Maps Distance Calculator (http://www.daftlogic.com/projects-google-maps-distance-calculator.htm).

Mismatch distribution was used to test the signature of population expansion (Rogers and Harpending 1992). The demographic history was studied using mismatch distributions in DnaSP ver. 5.0 (Librado and Rozas 2009). Tajima's *D* and Fu's F_S values were also calculated to test for neutrality (Tajima 1989; Fu 1997). Population size before expansion (θ_o), population size after expansion (θ_1), raggedness index and sum of squared deviations (SSD) between observed and expected mismatch distributions were similarly calculated. All parameters were tested against the expected values under the hypothesis of a recent population expansion based on 1000 bootstrap replicates.

For *B. zonata* species identification through *cox1* barcoding sequences, 108 *cox1* gene sequences (51 sequences were obtained from previous publications and 57 from the present study) representing 29 fruit fly species of the genus *Bactrocera* were included in the analysis (Supplementary Table S1). The sequences were further analyzed using MEGA 6.0 software to obtain intra- and interspecific distances, while maximum-parsimony (MP) trees were constructed to determine evolutionary relationships between *B. zonata* and other *Bactrocera* species using the Tamura 3-parameter + I distance model. The 'Best Close Match' and 'All Species Barcodes' methods in the program TaxonDNA (Meier et al. 2006) were performed to test the frequency of successful identification. This method assigns sequence into species based on the level of sequence similarity below the threshold value, which is below 95% of all intraspecific distances (Meier et al. 2006).

Results

Mitochondrial DNA sequence variation

No insertions, deletions or stop codons were present in the alignment. All sequences were truncated to the same length to eliminate missing data. Most of the individuals were sequenced for both *cox1* and *nad1* gene regions whereas Indore and Mumbai populations were sequenced only with *nad1*. The final sequences of length 549 and 555 bp of *cox1* and *nad1*, respectively, were used for analyses. Collapsing of individual sequences led to the identification of 43 haplotypes from 57 individuals and 47 haplotypes from 87 individuals for *cox1* and *nad1* genes, respectively (Supplementary Table S2 and Supplementary Table S3), whereas 33 haplotypes were detected from 34 individual sequences concatenated for both the markers for each individual (Table 1). Among 43 haplotypes detected with *cox1* gene, only two haplotypes were shared by at least two populations and the remaining 41 were exclusive haplotypes not shared by any other populations (Supplementary Table S2, Figure 1). Among 47 haplotypes detected with *nad1* gene, 12 and 35

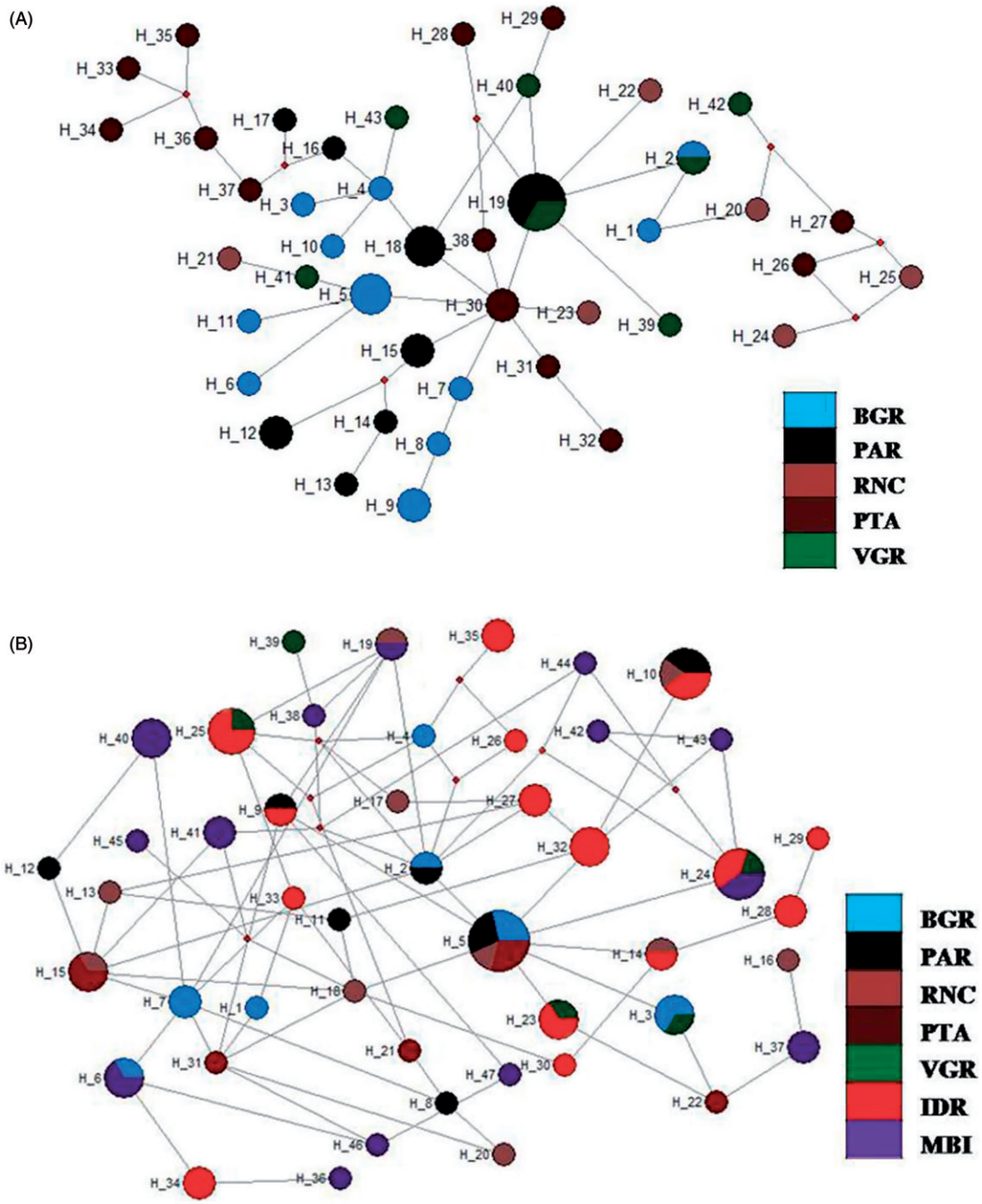

Figure 1. Median joining networks of mtDNA haplotypes *B. zonata.* (**A**) MJ network of *cox1* haplotypes and (**B**) MJ network of *nad1* haplotypes. Each circle represents a haplotype, and circle diameter is relative to haplotype frequency. Colours represent the geographic origin of specimens. Smallest red squares represent median vectors.

haplotypes were detected as shared and unique haplotypes, respectively (Supplementary Table S3). The most common haplotype was H5 in *nad1* sequences, comprised of four individuals from different populations of peach fruit fly.

Results obtained from basic descriptive genetic diversity analysis based on concatenated sequences as well as both genes are summarized in Table 1. Haplotype diversity (*Hd*) ranged from 0.908 to 1.0, 0.933 to 1.0 and 0.978 to 1.0 and nucleotide diversity (π) ranged from 0.007 ± 0.005 to 0.024 ± 0.013, 0.005 ± 0.003 to 0.007 ± 0.004 and 0.004 ± 0.003 to 0.013 ± 0.007 were observed in *cox1*, *nad1* and concatenated sequences, respectively, whereas the haplotype diversity, nucleotide diversity and average number of nucleotide differences were 0.996, 0.010 and 11.351, respectively, for the entire *B. zonata* population used in this study (Table 2). Values observed from genetic diversity indices showed that all the populations retain fairly high levels of genetic diversity.

Mitochondrial genealogy

Median joining (MJ) networks of *cox1* and *nad1* haplotypes were generated using Network ver. 4.6 and are depicted in Figure 1. Networks are normally star-like with limited

Table 2. Genetic diversity and demographic history parameters of *B. zonata* populations.

Gene	*H*	*Hd*	*k*	π	θ_o	θ_1	Tajima's *D*	Fu's *Fs*	SSD	Raggedness index
cox1	43	0.984	9.227	0.017	4.850	20025.021	−0.50557	−27.814	0.029	0.063
nad1	47	0.980	3.538	0.006	0.250	85721.034	−0.32424	−51.137	0.030	0.097
Concatenated	33	0.996	11.351	0.010	6.07563	80030.56375	−0.41107	−24.966	0.058	0.125

H: number of haplotypes; *Hd:* haplotype diversity; *k*: average number of nucleotide differences; π; nucleotide diversity; θ_o: effective population size before expansion; θ_1: effective population size after expansion; SSD: sum of squared deviations between observed and expected mismatch distributions under a sudden expansion model.

Table 3. Summary of AMOVA analysis of different populations of *B. zonata*.

Gene analyzed	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation index
cox1	Among populations	4	46.540	0.68634Va	14.42	*Fst* 0.14420*
	Within populations	52	211.811	4.07328Vb	85.58	
	Total	56	258.351	4.75962		
nad1	Among populations	6	13.055	0.03766Va	2.12	*Fst* 0.02121*
	Within populations	80	139.060	1.73824Vb	97.88	
	Total	86	152.115	1.77591		
Concatenated	Among populations	4	35.081	0.57634Va	10.40	*Fst* 0.10401*
	Within populations	29	143.978	4.96475Vb	89.60	
	Total	33	179.059	5.54109		

*$p < .05$.

Table 4. Population-specific *Fst* and pairwise *Fst* among different populations of *B. zonata* based on *cox1* and *nad1* gene.

Populations	BGR (0.04062)	PAR (0.04168)	RNC (0.02007)	PTA (0.04103)	VGR (0.04286)	IDR (0.01219)	MBI (0.00554)
BGR (0.14861)		−0.00206*	−0.03486*	−0.06031*	−0.14728*	0.01237*	0.01166*
PAR (0.17305)	0.08892		−0.07886*	−0.02965*	−0.05993	0.00105*	0.08150
RNC (0.11151)	0.19200	0.27747		−0.08282*	−0.10039*	−0.03222*	0.06655
PTA (0.09539)	0.14386	0.19278*	0.05244*		−0.14061*	−0.01290*	0.04851*
VGR (0.18464)	0.07833	0.02702	0.23240	0.16051*		−0.06684*	−0.04637
IDR	/	/	/	/	/		0.10281
MBI	/	/	/	/	/	/	

Distance values below diagonal are based on *cox1* sequences and above diagonal are *nad1* sequences obtained by a bootstrap procedure (1000 replicates).
Values given in parenthesis are population-specific *Fst* based on respective sequences.
*Values (distances) shown are significantly differed at *$p < .05$.

substructure. Some haplotypes positioned in the centre of the networks were found at higher frequency in all population (i.e. H19 for *cox1* and H5 for *nad1*), with most remaining haplotypes that were found in one single population, generally at low frequency and connecting to central haplotypes through few number of mutations.

Population genetic structure

The analysis of molecular variance (AMOVA) showed that most of the genetic variations were present inside the populations (89.60% based on concatenated sequences, 85.58% from *cox1* and 97.88% from *nad1* gene sequences) than among the populations (14.42%, 2.12% and 10.40%) (Table 3). The overall fixation index (*Fst*) calculated for all populations with concatenated and both genes was statistically significant (0.14420, 0.02121 and 0.10401 at $p < .05$ level) (Table 4). Population specific fixation index (*Fst*) was found highest for VGR (Vengurla, Maharashtra) population (0.18464) with *cox1* gene while PTA (Patna, Bihar) population (0.04103) with *nad1* gene. Population pairwise *Fst* values revealed that most populations were not genetically different in *cox1* gene analysis in comparison with *nad1* gene analysis (Table 4). Relatively, high *Fst* values were found between Patna and the other regions while considering both the genes (Table 4).

Isolation by distance analyses were only performed with *nad1* data. Analysis showed an extremely weak, negative correlation between pairwise *Fst* values and pairwise geographic distances but was statistically non-significant ($r^2 = -0.2596$, $p = .1550$).

Demographic history and neutrality test

The sequence variations of *cox1*, *nad1* gene and concatenated sequences were used to perform Tajima's *D* and Fu's *Fs* test to determine deviations from neutrality. Tajima's *D* ($p > .10$) and Fu's *Fs* statistic ($p > .10$) neutrality tests with all were non-significant and indicates compatible with neutrality. As a test of recent population expansion, applied mismatch distribution analysis indicated non-significant multimodal (concatenated sequences)/bimodal (*cox1*) and unimodal (*nad1*) shape distribution (Figure 2). However, sudden population expansion model was in accord with concatenated and both gene sequences (Table 2).

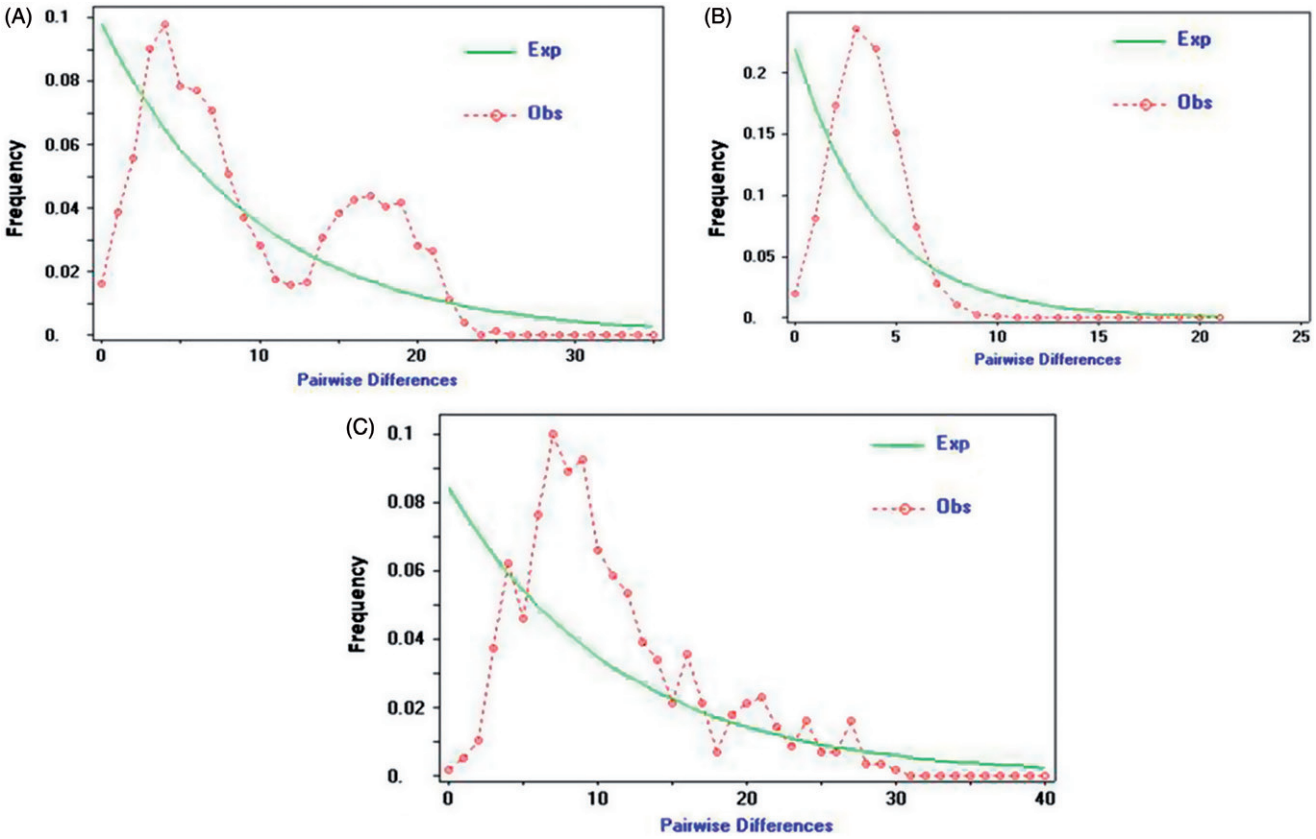

Figure 2. Observed and simulated mismatch distribution of populations of *B. zonata* collected from India. (A) Mismatch distribution graph from *cox1* gene sequences, (B) mismatch distribution graph from *nad1* gene sequences and (C) mismatch distribution graph from concatenated sequences of *cox1* and *nad1* gene.

The ratio between estimated effective population size after expansion (θ_1) and before expansion (θ_o), an estimate of the extent of population growth, was 13172.389X for concatenated sequences, 2128.870 for *cox1* and 342884.136 for *nad1* gene sequences implied for population growth. Analysis of sequences showed population expansion time (s) varied for both genes from 3.538 to 3.707 million years.

DNA barcode

Intraspecific genetic divergence of 57 *cox1* sequences of *B. zonata* ranged between 0% and 4.70% with an average of 1.70% and maximum sequences fall within a range of 0.5–1.0% genetic divergence. Nucleotide frequencies were 31.2% (A), 35.7% (T), 19.0% (C) and 14.1% (G). The base composition of *cox1* gene fragment was found to be biased towards adenine and thymine, which together constituted 66.9%. The overall transition (ti)/transversion (tv) bias of nucleotide sequence was R = 1.20. Interspecific genetic divergence ranged between 8.84% (compared with *B. nigrotibialis*) and 26.30% (compared with *B. tau*) with an average of 16.4%. The best close match methods identified the 95% intraspecific genetic divergence threshold value of *cox1* sequences of *B. zonata* included in the analysis was 1.78%. Based on the best close match method, all of *B. zonata* specimens were correctly identified. Phylogenetic analysis using the best model of nucleotide substitution according to the lowest Akaike information criterion (AIC) was Tamura 3-parameter + I in maximum-likelihood statistical method (Figure 3) that found a strong bootstrap support for the monophyly of *B. zonata* with other closer species.

Discussion

Population genetic analysis

The genetic variation in natural populations of any organism is the outcome of a balance between evolutionary and demographic processes and thus provides tools to interpret the evolutionary potential of a species (Li et al. 2013). In the present study, genetic distance of *B. zonata* (ranges between 0.000% to 4.70% for *cox1* and 0.00% to 2.02% for *nad1* gene sequences) demonstrated high level of genetic variation in India which is similar in the pattern of closely related fruit fly species viz. *B. dorsalis* (0.7%–2.0%) (Shi et al. 2012; Choudhary et al. 2016), *B. correcta* (0.760% and 3.250%) (Kunprom et al. 2015) and *Bactrocera tryoni* (Froggatt) (0.5%–1.8%) (Blacket et al. 2012). However, low level of genetic variation has also been observed for other species of fruit fly, namely *B. cucurbitae*, population collected from India, China as well as Southeast Asia by Prabhakar et al. (2012b) and Hu et al. (2008); *B. latifrons* (0.09–0.86%) (Meeyen et al. 2014) from Thailand and *Bactrocera oleae* (Rossi) (0.09%–0.48%) (Dogac et al. 2013) from Turkey. High genetic divergence in fruit flies may result in the presence of different forms of the species in different populations. It can be demonstrated by investigating morphological, cytological, molecular ground and *Wolbachia* infection in the populations of target species (Jamnongluk et al. 2003; Kunprom et al. 2015). In this study,

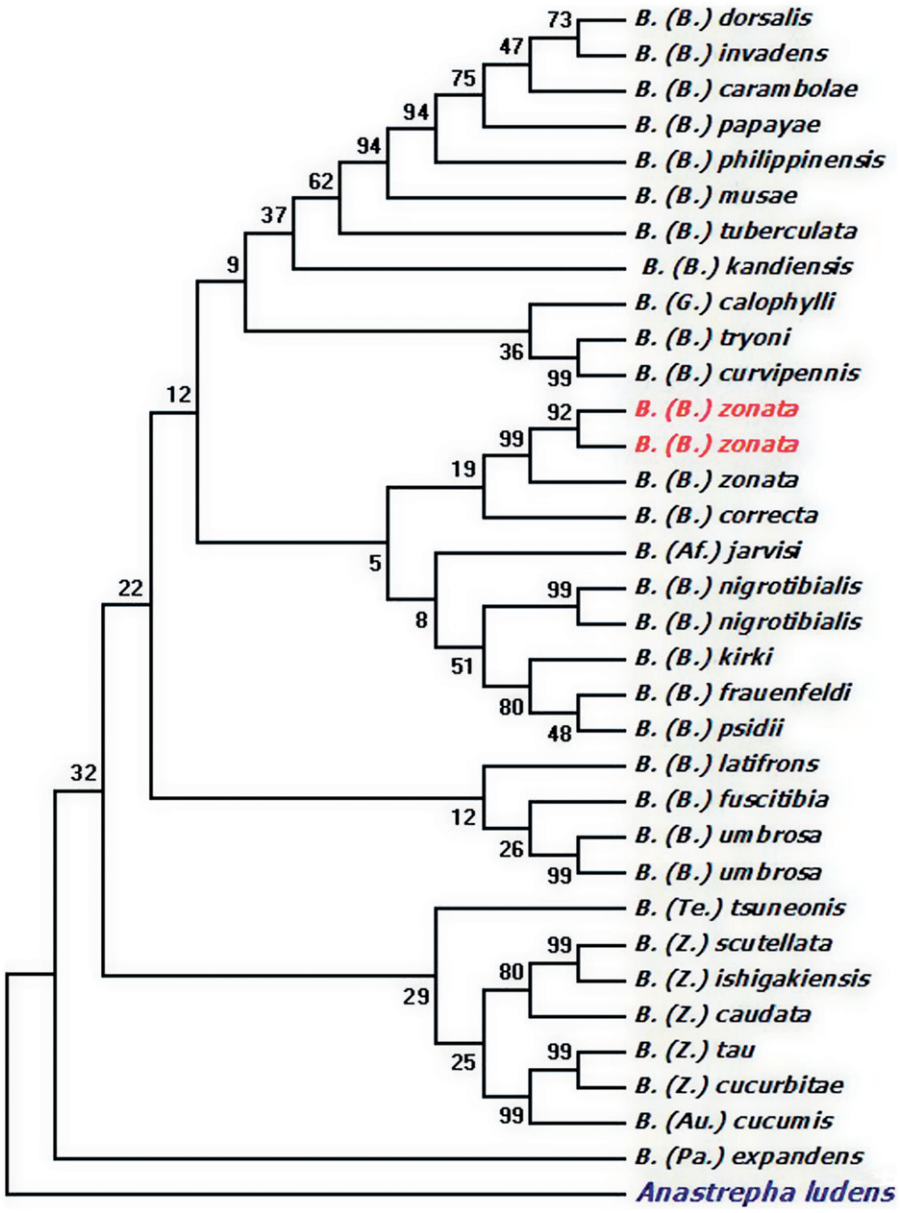

Figure 3. Maximum-likelihood estimation of the phylogenetic relationship among 29 species of the genus *Bactrocera* including present study species sequences (red colour) with bootstrap support (1000 replicates) value near the branch. *Anastrepha ludens* (HM538319) (blue colour) was used as outgroup species.

high level of genetic diversity might have maintained due to ecological and behavioural characteristics of the *B. zonata*, i.e., high reproductive potential (Shehata et al. 2008) and high dispersal capabilities (Peterson and Denno 1998) along with stability and high adaptability of the fly in tropical and subtropical climatic conditions of India. Additionally, the abundance of suitable host plants, such as guava, mango, sapota, peach, plum, banana, in India might have exerted little novel selective pressure in the past for the establishment and dispersal of population. Furthermore, high level of genetic diversity is observed when species is considered as ancient existence because ancestors show significantly more genetic diversity than derived population due to founder effect (Grant and Bowen 1998). Generally, invasive species are low in genetic diversity due to selection pressure (Suarez and Tsutsui 2008) and genetic drift which may temporarily reduce population size in founder colonies (Grapputo et al. 2006), whereas *B. zonata* considered as a species originated in India. This may be one of the reason that *B. zonata* retain fairly high level of genetic diversity in Indian populations. Similar trends of high genetic diversity were also observed in *B. dorsalis* populations collected from Southeast Asia (Wu et al. 2014), Yunnan Province of China (Shi et al. 2010; Wan et al. 2011) and India (Choudhary et al. 2016). It has been proven fact that when the values of haplotype ($Hd > 0.5$) and nucleotide ($\pi > 0.005$) diversity are high, the analyzed population is stable with long evolutionary history (Rosetti and Remis 2012). *B. zonata* populations studied here fall within these two conditions of high haplotype and nucleotide diversity suggests apparent population stability with long evolutionary history.

Mismatch distribution analysis for testing demographic expansion of the species with expected unimodal shape of graph over observed unimodal/bimodal shape for both genes and in concatenated dataset revealed population expansion of *B. zonata* to a demographic equilibrium or a stable

population. In general, a multimodal (including bimodal) mismatch distribution indicates diminishing population sizes or structured size; and unimodal distribution suggests that population underwent expansion (Excoffier et al. 1992; Rogers and Harpending 1992). The MJ networks showed randomly distributed haplotypes of *B. zonata* populations collected from India, in line with population that underwent expansion. MJ networks did not display any fundamental structure which could not be allowed to identify a phylogenetic haplogroup in the population of *B. zonata*. This is generally interpreted as indicative of a population that has recently expanded in size from a small number of founders following a population bottleneck (Slatkin and Hudson 1991). The notion that the species underwent a significant population expansion is further in line with the observation that MJ networks for both the genes have a distinctly star-like structure, typical of expansion demographic processes.

The test of neutrality generated negative Tajima's *D* value for *cox1* and concatenated *cox1* and *nad1* genes suggest the possibility of recent *B. zonata* population expansion (Campbell et al. 2004), whereas the negative Fu's *Fs* statistical value observed in *B. zonata* populations for both the genes and their concatenated analyses provide strong evidence for population expansion (Fu and Li 1993). The hypothesis that the observed data fit the sudden expansion model was tested using the sum of square deviations (SSD) (Schneider and Excoffier 1999) and the raggedness index (Harpending 1994). Here, our observations on non-significant values for SSD and raggedness index suggest that population have expanded suddenly in past (Rogers 1995). This notion is also supported by observations from analysis of molecular variance and showed nearly 90% of variability was observed within the population, and a non-significant correlation between genetic distances and geographic distances suggests no isolation by distance among populations. This may be due to the accumulation of beneficial genetic variation and pass on to the next generation in a rapidly growing population (Su et al. 2001). Diverse climatic conditions and ecological suitability of the region and species capabilities may be the reason for high genetic diversity and gene flow in *B. zonata*. Natural barriers like big mountains and rivers are present in the sampling areas which may limit the gene flow in *B. zonata*. However, these natural barriers exert insignificant pressure to lower the gene flow in the present study. Mountain ranges are also important geographic barriers to gene flow in many species (Meeyen et al. 2014; Kunprom et al. 2015).

Identification of species using DNA barcode

In this study, we used DNA barcoding technique and our results indicate that DNA barcodes based on *cox1* sequences are highly useful for *B. zonata* identification. Species identifications based on DNA barcode are considered as reliable and accurate, if large gaps exist between intraspecific and interspecific genetic divergence (Hebert et al. 2004; Meyer and Paulay 2005). Barcode gap can be detected by recording the overlap between the highest intraspecific and the lowest interspecific genetic distances (Meier et al. 2008). Our results showed large gap between intra- and interspecific genetic distances for all *Bactrocera* species used in this study, suggesting the reliability of results and consistent with previous reports on DNA barcoding of fruit flies. Analysis of base composition of the *cox1* gene fragment showed that A + T content in *B. zonata* population was 66.90%. These data are in agreement with A + T content of *cox1* region gene sequences in species of genus *Bactrocera* (63–68%) (Jamnongluk et al. 2003; Choudhary et al. 2015). Efficiency of *cox1* barcoding sequences for species identification has tested earlier and suggest the DNA barcode is effectively successful in demarcating morphological fruit fly species although the bias between members of the same species complex was limited (Armstrong and Ball 2005; Meeyen et al. 2014). There has been no previous morphological indication of the *B. zonata* as a species complex, which is consistent with present findings. Therefore, identification of this species based on *cox1* barcoding sequences is simple and accurate. Morphologically, *B. zonata* resembles with *B. correcta* based on wing pattern, i.e., the discontinuous or an extremely narrow costal band with section distal to apex R_{2+3} before expanding into a spot in wing. *B. zonata* is different in having red-brown scutum (Drew and Raghu 2002; Prabhakar et al. 2012c; Choudhary et al. 2014). In this context, our study also showed that *B. zonata* and *B. correcta* form a closely related clade with a very strong support by ML analysis, supporting recent *cox1* barcoding and phylogenetic studies (Muraji and Nakahara, 2001; Nakahara and Muraji 2008; Zhang et al. 2010; Asokan et al. 2011). Accurate and successful DNA barcoding depends on the availability of the sequences of particular species in the data library (Virgilio et al. 2010; Jinbo et al. 2011). A factor that could lead to incorrect species identification using the DNA barcode is insufficient geographic sampling of the taxa (Jinbo et al. 2011). In this context for *B. zonata*, present study significantly contributes to the database of GenBank *cox1* barcoding sequences, which will foster the molecular identification of this important pest species.

Conclusions

Collection and genetic analysis of the peach fruit fly, *B. zonata*, in our study suggest widely distribution of the species in India with high genetic diversity. High gene flow among *B. zonata* populations present in different areas of India due to round the year availability and continuous geographical presence of wide range of host. The present investigation also suggests that there is no cryptic species present in *B. zonata*, although high genetic variability is present in the fly populations across India. Therefore, the management of *B. zonata* could be possible with the genetic control methods like release of sterile insects or genetically engineered insect population. *cox1* gene sequences developed during the study could be helpful in the rapid identification for quarantine the species at the earliest, where *B. zonata* is not present.

Acknowledgments

This research work was funded by Ministry of Agriculture, Government of India through National Initiative on Climate Resilient Agriculture (NICRA)

project under Indian Council of Agricultural Research (ICAR) (ICAR-RCER/RC R/E.F./2011/29). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Authors thank two anonymous reviewers for their critical comments on the earlier version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research work was funded by Ministry of Agriculture, Government of India through National Initiative on Climate Resilient Agriculture (NICRA) project under Indian Council of Agricultural Research (ICAR) (ICAR-RCER/RC R/E.F./2011/29).

References

Aketarawong N, Chinvinijkul S, Orankanok W, Guglielmino CR, Franz G, Malacrida AR, Thanaphum S. 2011. The utility of microsatellite DNA markers for the evaluation of area-wide integrated pest management using SIT for the fruit fly, *Bactrocera dorsalis* (Hendel), control programs in Thailand. Genetica. 139:129–140.

Armstrong KF, Ball SL. 2005. DNA barcodes for biosecurity: invasive species identification. Philos Trans R Soc Lond B Biol Sci. 360:1813–1823.

Asokan R, Rebijith KB, Singh SK, Sidhu AS, Siddharthan S, Karanthi PK, Ellango R, Ramamurthy VV. 2011. Molecular identification and phylogeny of *Bactrocera* species (Diptera: Tephritidae). Florida Entomol. 94:1026–1035.

Bandelt H, Forster P, Roehl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 16:37–48.

Blacket MJ, Semeraro L, Malipatil MB. 2012. Barcoding Queensland fruit flies (*Bactrocera tryoni*): impediments and improvements. Mol Ecol Resour. 12:428–436.

CABI/EPPO. 2013. *Bactrocera zonata*. [Distribution map]. Distribution Maps of Plant Pests. Wallingford (UK).

Campbell P, Schneider CJ, Adnan AM, Zubaid A, Kunz TH. 2004. Phylogeny and phylogeography of old world fruit bats in the *Cynopterus brachyotis* complex. Mol Phylogenet Evol. 33:764–781.

Choudhary JS, Kumari A, Das B, Maurya S, Kumar S. 2012. Diversity and population dynamic of fruit flies species in methyl eugenol based parapheromone traps in Jharkhand region of India. The Ecoscan. 1:57–60.

Choudhary JS, Naaz N, Prabhakar CS, Das B, Maurya S, Kumar S. 2014. Field guide for identification of fruit fly species of Genus *Bactrocera* prevalent in and around mango orchards. Technical booklets No. R-43/Ranchi-16. Ranchi: ICAR Research Complex for Eastern Region, Research Centre, pp 1–16.

Choudhary JS, Naaz N, Prabhakar CS. Moanaro 2016. Genetic analysis of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) populations based on mitochondrial *cox1* and *nad1* gene sequences from India and other Asian countries. Genetica. 144:611–623.

Choudhary JS, Naaz N, Prabhakar CS, Srinivasa Rao M, Das B. 2015. The mitochondrial genome of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae): complete DNA sequence, genome organization, and phylogenetic analysis with other tephritids using next generation DNA sequencing. Gene. 569:191–202.

Dogac E, Kande Mir I, Taskin V. 2013. The genetic polymorphisms and colonization process of olive fly populations in Turkey. PLoS One. 8:e56067.

Drew RAI, Raghu S. 2002. The fruit fly fauna (Diptera: Tephritidae: Dacinae) of the rainforest habitat of the Western Ghats, India. Raffles Bull Zool. 50:327–352.

Duyck PF, Sterlin JF, Quilici S. 2004. Survival and development of different life stages of *Bactrocera zonata* (Diptera: Tephritidae) reared at five constant temperatures compared to other fruit fly species. Bull Entomol Res. 94:89–93.

El-Samea SAA, Fetoh BEA. 2006. New record of *Bactrocera zonata* (Saundera) (Diptera: Tephritidae) on potatoes in Egypt. Egyptian J Agric Res. 84:61–63.

EPPO. 2014. PQR database. Paris (France): European and Mediterranean Plant Protection Organization. [accessed 2016 Dec 15]. http://www.eppo.int/DATABASES/pqr/pqr.htm

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 10:564–567.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131:479–491.

Fu YX, Li WH. 1993. Statistical tests of neutrality of mutations. Genetics. 133:693–709.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 147:915–925.

Grant WS, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J Hered. 89:415–426.

Grapputo A, Bisazza A, Pilastro A. 2006. Invasion success despite reduction of genetic diversity in the European populations of eastern mosquito fish (*Gambusia holbrooki*). Italian J Zool. 73:67–73.

Gupta D, Verma AK, Bhalla OP. 1990. Population of fruit-flies (*Dacus zonatus* and *D. dorsalis*) infesting fruit crops in north-western Himalayan region. Indian J Agric Sci. 60:471–474.

Harpending HC. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human Biol. 66:591–600.

Hazkani-Covo E, Zeller RM, Martin W. 2010. Molecular poltergeists: mitochondrial DNA copies (numts) in sequenced nuclear genomes. PLoS Genet. 6:e1000834.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of birds through DNA barcodes. PLoS Biol. 2:1657–1663.

Hu J, Zhang JL, Nardi F, Zhang RJ. 2008. Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae), from China and Southeast Asia. Genetica. 134:319–324.

Itô Y, Kakinohana H, Yamagishi M, Kohama T. 2003. Eradication of the melon fly, *Bactrocera cucurbitae*, from Okinawa, Japan, by means of the sterile insect technique, with special emphasis on the role of basic studies. J Asia Pac Entomol. 6:119–129.

Jamnongluk W, Baimai V, Kittayapong P. 2003. Molecular phylogeny of tephritid fruit flies in the *Bactrocera tau* complex using the mitochondrial COI sequences. Genome. 46:112–118.

Jensen JL, Bohonak AJ, Kelley ST. 2005. Isolation by distance, web service. BMC Genetics. 6:13–23.

Jinbo U, Kato T, Ito M. 2011. Current progress in DNA barcoding and future implications for entomology. Entomol Sci. 14:107–124.

Kapoor VC. 1993. Indian fruit flies (Insecta: Diptera: Tephritidae). New York (NY): International Sciences Publisher.

Kunprom C, Sopaladawan PN, Pramual P. 2015. Population genetics and demographic history of guava fruit fly *Bactrocera correcta* (Diptera: Tephritidae) in northeastern Thailand. European J Entomol. 112:227–234.

Li J, Ye Y, Wu C, Qi P, Guo B, Chen Y. 2013. Genetic variation of *Mytilus coruscus* Gould (Bivalvia: Mytilidae) populations in the East China Sea inferred from mtDNA gene sequence. Biochem Syst Ecol. 50:30–38.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25:1451–1452.

Lunt DH, Zhang DX, Szymura JM, Hewitt GM. 1996. The insect *cytochrome oxidase I* gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol Biol. 5:153–165.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.

Meeyen K, Nanorksopaldawan P, Pramual P. 2014. Population structure, population history and DNA barcoding of fruit fly *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae). Entomol Sci. 17:219–230.

Meier R, Shiyang K, Vaidya G, Ng PKL. 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. Syst Biol. 55:715–728.

Meier R, Zhang G, Ali F. 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the 'barcoding gap' and leads to misidentification. Syst Biol. 57:809–813.

Meyer CP, Paulay G. 2005. DNA barcoding: error rate based on comprehensive sampling. PLoS Biol. 3:422.

Mun J, Bohonak AJ, Roderickm GK. 2003. Population structure of the pumpkin fruit fly *Bactrocera depressa* (Tephritidae) in Korea and Japan: Pliocene allopatry or recent invasion? Mol Ecol. 12:2941–2951.

Muraji M, Nakahara S. 2001. Phylogenetic relationships among fruit flies, *Bactrocera* (Diptera, Tephritidae), based on the mitochondrial rDNA sequences. Insect Mol Biol. 10:549–559.

Nakahara S, Muraji M. 2008. Phylogenetic analyses of *Bactrocera* fruit flies (Diptera: Tephritidae) based on nucleotide sequences of the mitochondrial *COI* and *COII* Genes. Res Bull Plant Prot Ser Japan. 44:1–12.

Nardi F, Carapelli A, Dallai R, Roderick GK, Frati F. 2005. Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). Mol Ecol. 14:2729–2738.

O'Loughlin SM, Okabayashi T, Honda M, Kitazoe Y, Kishino H, Somboon P, Sochanta T, Nambanya S, Saikia PK, Dev V, et al. 2008. Complex population history of two *Anopheles dirus* mosquito species in south Asia suggests the influence of Pleistocene climate change rather than human-mediated effects. Evol Biol. 21:1555–1569.

Peterson MA, Denno RF. 1998. The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. Amer Nat. 152:428–446.

Prabhakar CS, Sood P, Kapoor V, Kanwar SS, Mehta PK, Sharma PN. 2009. Molecular and biochemical characterization of three bacterial symbionts of fruit fly, *Bactrocera tau* (Tephritidae: Diptera). J Gen Appl Microbiol. 55:213–220.

Prabhakar CS, Sood P, Mehta PK, Sharma PN. 2013. Population genetic structure of the pumpkin fruit fly, *Bactrocera tau* (Walker) (Diptera: Tephritidae) in Himachal Pradesh, India. Biochem Syst Ecol. 51:291–296.

Prabhakar CS, Sood P, Mehta PK. 2012a. Fruit fly (Diptera: Tephritidae) diversity in cucurbit fields and surrounding forest areas of Himachal Pradesh, a North-Western Himalayan state of India. Arch Phytopathol Plant Prot. 45:1210–1217.

Prabhakar CS, Mehta PK, Sood P, Singh SK, Sharma P, Sharma PN. 2012b. Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) based on mitochondrial *cytochrome oxidase* (*COI*) gene sequences. Genetica. 140:83–91.

Prabhakar CS, Sood P, Mehta PK. 2012c. Pictorial keys for predominant *Bactrocera* and *Dacus* fruit flies (Diptera: Tephritidae) of north western Himalaya. Arthropods. 1:101–111.

Prabhakar CS. 2011. Biodiversity of fruit flies (Tephritidae: Diptera) and utilization of gut bacteria in their management [Ph.D. Thesis]. Palampur (Himachal Pradesh, India): Department of Entomology, CSK Himachal Pradesh Agricultural University.

Pramual P, Kongim B, Nanork Sopaladawan P. 2011. Phylogeography of *Simulium siamense* Takaoka and Suzuki complex (Diptera: Simuliidae) in Thailand. Entomol Sci. 14:428–436.

Pramual P, Kuvangkadilok C, Baimai V, Walton C. 2005. Phylogeography of the black fly *Simulium tani* (Diptera: Simuliidae) from Thailand as inferred from mtDNA sequences. Mol Ecol. 14:3989–4001.

Qureshi ZA, Hussain T, Siddiqui QH. 1991. Relative preference of mango varieties by *Dacus zonatus* (Saunders) and *D. dorsalis* Hendel. Pakistan J Zool. 23:85–87.

Rai S, Uma S, Bhagat RM, Gupta SP. 2008. Population dynamics and succession of fruit fly on sub-tropical fruits under rainfed condition in Jammu Region. Indian J Entomol. 70:12–15.

Rana JS, Parkash OM, Verma SK. 1990. A note on relative susceptibility of some guava cultivars to fruit fly, *Dacus zonatus* (Saunders). Haryana J Hortic Sci. 19:131–133.

Roderick GK, Navajas M. 2003. Genes in new environments: genetics and evolution in biological control. Nat Rev Genet. 4:889–899.

Roderick GK. 1996. Geographic structure of insect populations: gene flow, phylogeography, and their uses. Ann Rev Entomol. 41:263–290.

Rogers A, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol. 9:552–569.

Rogers AR. 1995. Genetic evidence for a Pleistocene population explosion. Evolution. 49:608–615.

Rosetti N, Remis MI. 2012. Spatial genetic structure and mitochondrial DNA phylogeography of Argentinean populations of the Grasshopper *Dichroplus elongatus*. PLoS One. 7:e40807.

Schneider S, Excoffier L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics. 152:1079–1089.

Shehata NF, Younes MWF, Mahmoud YA. 2008. Biological studies on the peach fruit fly, *Bactrocera Zonata* (Saunders) in Egypt. J Appl Sci Res. 4:1103–1106.

Shi W, Kerdelhue C, Ye H. 2010. Population genetic structure of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) from Yunnan province (China) and nearby sites across the border. Genetica. 138:377–385.

Shi W, Kerdelhue C, Ye H. 2012. Genetic structure and inferences on potential source areas for *Bactrocera dorsalis* (Hendel) based on mitochondrial and microsatellite markers. PLoS One. 7:e37083.

Shi W, Ye H. 2007. Genetic structure in four geographic populations of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) located in the seasonal occurrence zone in Yunnan Province. Acta Ecol Sin. 6:2477–2482.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am. 87:651–701.

Slatkin M, Hudson RR. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics. 129:555–562.

Su B, Fu Y, Wang Y, Jin L, Chakraborty R. 2001. Genetic diversity and population history of the red panda (*Ailurus fulgens*) as inferred from mitochondrial DNA sequence variations. Mol Biol Evol. 18:1070–1076.

Suarez AV, Tsutsui ND. 2008. The evolutionary consequences of biological invasions. Mol Ecol. 17:351–360.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 3:585–595.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 30:2725–2729.

Virgilio M, Backeljau T, Nevado B, Meyer MD. 2010. Comparative performances of DNA barcoding across insect orders. BMC Bioinformatics. 11:206.

Wan X, Liu Y, Zhang B. 2012. Invasion history of the oriental fruit fly, *Bactrocera dorsalis*, in the Pacific-Asia Region: two main invasion routes. PLoS One. 7:e36176.

Wan X, Nardi F, Zhang B, Liu Y. 2011. The oriental fruit fly, *Bactrocera dorsalis*, in China: origin and gradual inland range expansion associated with population growth. PLoS One. 6:e25238.

White IM, Elson-Harris M. 1992. Fruit flies of economic significance: their identification and bionomics. Wallingford, (UK): International Institute of Entomology; CAB International.

Wu ZZ, Li HM, Bin SY, Ma J, He HL, Li XF, Gong FL, Lin JT. 2014. Sequence analysis of mitochondrial *ND1* gene can reveal the genetic structure and origin of *Bactrocera dorsalis* s.s. BMC Evol Biol. 14:55.

Zhang B, Liu YH, Wu WX, Wang ZL. 2010. Molecular phylogeny of *Bactrocera* species (Diptera: Tephritidae: Dacini) inferred from mitochondrial sequences of *16S rDNA* and *COI* sequences. Florida Entomol. 93:369–377.