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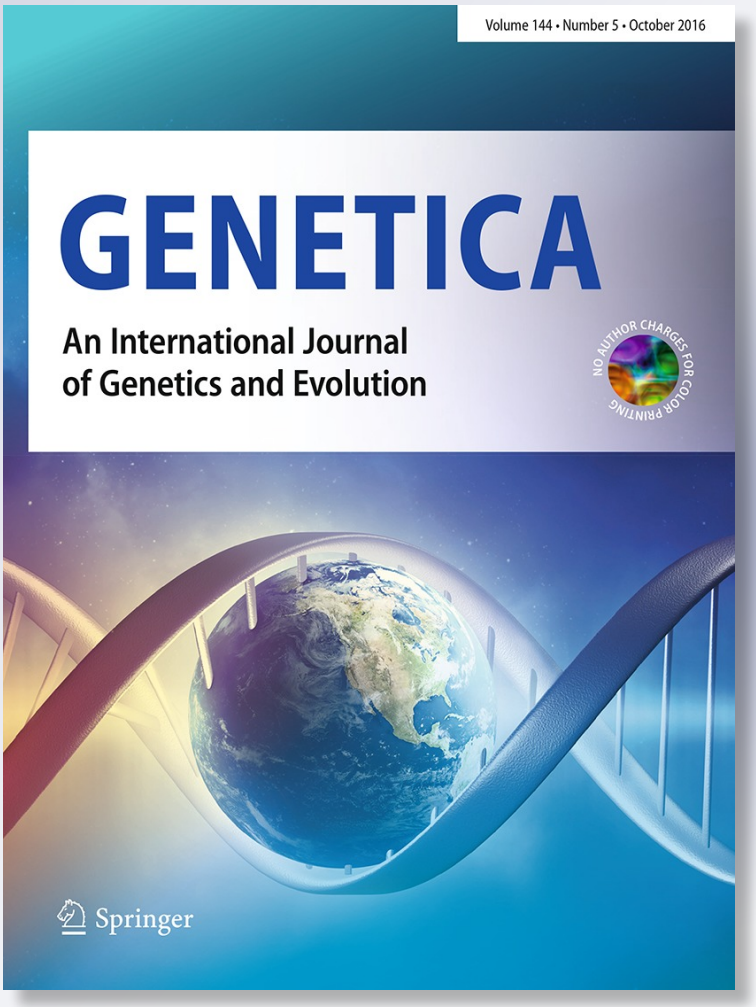

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Genetic analysis of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) populations based on mitochondrial *cox1* and *nad1* gene sequences from India and other Asian countries

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Abstract The study examined the genetic diversity and demographic history of *Bactrocera dorsalis*, a destructive and polyphagous insect pest of fruit crops in diverse geographic regions of India. 19 widely dispersed populations of the fly from India and other Asian countries were analysed using partial sequences of mitochondrial *cytochrome oxidase I* (*cox1*) and *NADH dehydrogenase* 1 (*nad1*) genes to investigate genetic diversity, genetic structure, and demographic history in the region. Genetic diversity indices [number of haplotypes (*H*), haloptype diversity (*Hd*), nucleotide diversity (π) and average number of nucleotide difference (*k*)] of populations revealed that *B. dorsalis* maintains fairly high level of genetic diversity without isolation by distance among the geographic regions. Demographic analysis showed significant (negative) Tajimas' *D* and Fu's F_S with non significant sum of squared deviations (*SSD*) values, which indicate the possibility of recent sudden expansion of species and is further supported through distinctively star-like distribution structure of haplotypes among populations. Thus, the results indicate that both ongoing and historical factors have played important role in determining the genetic structure and diversity of the species in India. Consequently, sterile insect technique (SIT) could be a possible management strategy of species in the regions.

Keywords *Bactrocera dorsalis* · *cox1* gene · *nad1* gene · Genetic diversity

Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) is a polyphagous and destructive insect pest of many tropical and subtropical fruits. *B. dorsalis* was first reported from Taiwan Island, during 1912 (Hardy 1973) and it was first reported in India by Bezzi between 1913 and 1916. Presently, it has been recorded throughout South, South East, Far East Asia and some pacific islands including Hawaii and recently its widespread presence was reported from many countries of African continent (Kapoor 1993; Lux et al. 2003; Prabhakar et al. 2012b; Schutze et al. 2014; Shi et al. 2012; Stephens et al. 2007; Wan et al. 2012; Yu et al. 2007). In spite of *B. dorsalis* being a well established pestiferous fruit fly species in India (Choudhary et al. 2012; Kapoor 1993) and economic and ecological threats associated with its infestation in new areas, data on its phylogeography has not been studied in India and not included in any studies so far. It causes losses up to 80 % in mango crop (Verghese and Jayanthi 2001), besides huge losses in many other economically important fruit crops viz. guava, cherry, plum, pear, peaches, loquat, apple, banana, fig citrus and mandarin (Kapoor 1993). *B. dorsalis* is a serious concern in terms of its possible introduction to the other important fruit growing regions of the world and listed as one of the most important quarantine pest because of its high invasiveness and adaptability to new environment (CABI 2016). The annual economic losses in India due to

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this pest in different fruit crops have been estimated to the tune of $ 450 million (Stonehouse 2001).

With increasing inter-continental agricultural commodity trade and in the era of globalization, species are repeatedly transported outside their natural boundaries (Wilson et al. 2009). A number of barriers need to be broken, to become established and ultimately invade new environments for an introduced species (Blackburn et al. 2011). The impact of alien species invasion can be diverse, from direct impact on local (native) biodiversity and natural resources to affecting human well-being as well as agriculture. Reconstructing colonization history of invasive species, i.e. revealing their origin and genetic composition, understanding the invasion, spread pathways, and factors facilitating population expansion in the past are helpful for planning quarantine and timely implementation of management strategies in case of invasion (Wan et al. 2012; Aketarawong et al. 2014).

Molecular genetics provides powerful tools for invasion patterns of introduced species and inferring their potential sources (Estoup and Guillemaud 2010; Lombaert et al. 2010; Aketarawong et al. 2014). Mitochondrial DNA (mtDNA) is a very popular molecular marker in particular for phylogeography (Wan et al. 2011) and a robust evolutionary markers for determining intra- and inter-specific relationships, owing to features such as non recombinant, high copy numbers, accelerated rate of evolution, simple maternal inheritance and a lower effective population size (Mun et al. 2003; Nardi et al. 2005; Prabhakar et al. 2012a, 2013; Roderick 1996; Simon et al. 1994; Wan et al. 2012). The mitochondrial DNA is particularly informative to reconstruct historical processes, such as the identification of the area of origin of a species, the pathways of invasion, spread and historical demography of alien species (Bryja et al. 2010; Finn et al. 2006; Miller et al. 2009; Rollins et al. 2011; Siedchlag et al. 2010; Suare et al. 2009; Venkatesan et al. 2007). The information about patterns of genetic variation, population structure and gene flow is consequently helpful in large-scale efforts to control insect pests with Area Wide Integrated Pest Management (AW-IPM) and Sterile Insect Technique (SIT) programme (Roderick and Navajas 2003; Krafsur 2005).

In recent past, population genetic structure and demographic history of *B. dorsalis* from Chinese and Southeast Asian populations were studied using both microsatellite mitochondrial DNA markers (Aketarawong et al. 2007; Li et al. 2007, 2009, 2012; Wan et al. 2011; Wu et al. 2014). Recently among *B. dorsalis* species complex, *B. dorsalis* has acquired the status of a senior synonym of *B. papaya*, *B. philippinensis* and *B. invadens* and these species are now known as *Bactrocera dorsalis* (Schutze et al. 2015).

Therefore, we analyse two mitochondrial genes (*cox1* - and *nad1*) sequences of adult *B. dorsalis* collected from wide geographical locations of India to infer the genetic diversity of *B. dorsalis* and their historic and present relationship with other Asian population. An attempt was made to assess both the distribution and genetic diversity of the fly within and between populations and to understand the pattern of gene flow of *B. dorsalis* within India and other Asian countries to devise an area wide management programme. Also mitochondrial DNA sequence data generated in the present study will enrich the *B. dorsalis* mitochondrial gene database and would be a valuable source of Indian population of *B. dorsalis* for global phylogeographic studies.

Materials and methods

Sample collection

Adult male flies of *B. dorsalis* were collected from 12 locations in India during 2012–2014 represent different climatic zones. Flies were captured using traps baited with parapheromone (methyl eugenol) placed in mango orchards. Sampling locations covered the major areas with prominent *B. dorsalis* infestation (Table 1; Fig. 1). The collected samples were identified on the basis of morphological descriptions suggested by Drew and Raghu (2002) and Choudhary et al. (2014). Identified specimens were stored in 95 % ethanol at −20 °C until use for DNA extraction.

DNA extraction

Total DNA was extracted from individuals of *B. dorsalis* following the protocol described by Prabhakar et al. (2009) with minor modifications. The preserved samples of oriental fly were transferred to 1.5 ml microtubes and was grinded in 700 µL of CTAB-based buffer (2 % w/v cetyltrimethyl-ammonium-bromide) using sterile micro pestle and incubated in water bath at 65 °C for 1 h. Same volume of chloroform : isoamyl alcohol (24:1 v/v) was added and mixed gently. Tubes were centrifuged at 10,000 rpm for 12 min and aqueous phase was transferred to new tubes. The DNA was precipitated by adding 450 µl pre-chilled isopropanol and kept at −20 °C for 20–30 min and subsequently centrifuged for 14 min at 12,000 rpm at 4 °C. Then, DNA pellet was washed thrice with cold 70 % ethanol at 10,000–12,000 rpm for 5–10 min at 4 °C, dried and re-suspended in 100 µl of Tris–EDTA (ethylene diamine tetra acetic acid) buffer.

PCR amplification of mitochondrial *cox1* and *nad1* genes

Two fragments of the mitochondrial genome were amplified from each individual using primer pairs *cox1*, UEA7 (5′-TACAGTTGGAATAGACGTTGATAC-3′)/UEA10 (5′-TCCAATGCACTAATCTGCCATATTA-3′) (Lunt et al. 1996)

Table 1 Sampling locations information of *B. dorsalis* populations

Sr. no	Location, state/province (country)	ID code	Number (*n*)	Latitude (N)	Longitude (E)	Elevationm (amsl)	Collection year
1.	Patna, Bihar (India)	PT	11	25°61′	85°14′	58	2013
2.	Bangalore, Karnataka (India)	BG	8	12°96′	77°56′	965	2012
3.	Paria, Gujarat (India)	PA	7	20°45′	72°96′	18	2012
4.	Ranchi, Jharkhand (India)	RC	5	23°35′	85°33′	651	2012
5.	Lucknow, Uttar Pradesh (India)	LUK	7	26°84′	80°94′	126	2013
6.	Vengurla, Maharashtra (India)	VGR	7	15°85′	73°63′	19	2012
7.	Sangareddy, Telangana (India)	SR	5	17°62′	78°09′	524	2013
8.	Udaipur, Rajasthan (India)	UD	4	24°58′	73°68′	598	2012
9.	Basar, Arunachal Pradesh (India)	BS	4	27°98′	94°67′	580	2013
10.	Dindigul, Tamil Nadu (India)	DG	6	10°35′	75°95′	268	2013
11.	Bhubaneswar, Odisha (India)	BBN	4	20°27′	85°84′	45	2013
12.	Mumbai, Maharashtra (India)	MB	5	18°97′	72°82′	17	2014
13.	Bangkok (Thailand)*	BGK	7	16°97′	101°09′	2	–
14.	Guangzhou, Guangdong (China)*	GZ	8	23°18′	113°28′	21	2009
15.	Hekou, Yunnan (China)*	HK	8	22°59′	103°88′	87	2009
16.	Fuzhou, Fujian (China)*	FZ	5	26°15′	119°28′	67	2009
17.	Nanning, Guangxi (China)*	NN	9	22°78′	108°46′	50	2010
18.	Taibei, Taiwan (China)*	TW	10	24°95′	121°55′	9	–
19.	Laos*	LOU	5	20°06′	102°35′	300	2008

* Populations are shown with geographical coordinates and sample collection year that have been approximated to our best knowledge using the information provided in the GenBank record/original publication

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 s 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 *nad1* and *cox1* primers (Xcelris Labs Limited, India). After manual correction and assembly, unique sequences were deposited in Genbank under accession numbers KT119929-KT119946, KM359573-KM359624 for *cox1* and KT119947-KT120001 for *nad1* gene sequences.

Data analysis

DNA sequences were aligned using ClustalW programme implemented in MEGA ver. 6.0. software (Tamura et al. 2013). Unique haplotypes were identified in ARLEQUIN ver. 3.5 (Excoffier and Lischer, 2010) and Dnasp ver. 5.0 software (Librado and Rozas 2009). Descriptive statistics [number of haplotypes (*H*), haloptype diversity (*Hd*), nucleotide diversity (π), average number of nucleotide difference (*k*)] were calculated with Dnasp ver. 5.0 software. Statistical significance of derived indices was assayed through a non-parametric permutation method (5000 permutations) in ARLEQUIN ver. 3.5 software.

An AMOVA hierarchical analysis of variance was performed using ARLEQUIN ver. 3.5 software to partition total variance in its components within and among populations. To depict the evolutionary and geographical relationships among haplotypes, a median joining haplotype network of both the gene sequences was constructed with NETWORK ver. 4.6 (Bandelt et al. 1999). The major clusters of the *B. dorsalis* haplotypes were coloured according to the sampling locations of *B. dorsalis* populations to draw migration and invasion pattern of the species across India.

Mantel test (Mantel 1967) was 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).

The demographic history was studied using mismatch distributions in Dnasp ver. 5.0. Mismatch distributions were calculated to test sudden population expansion model for *B.*

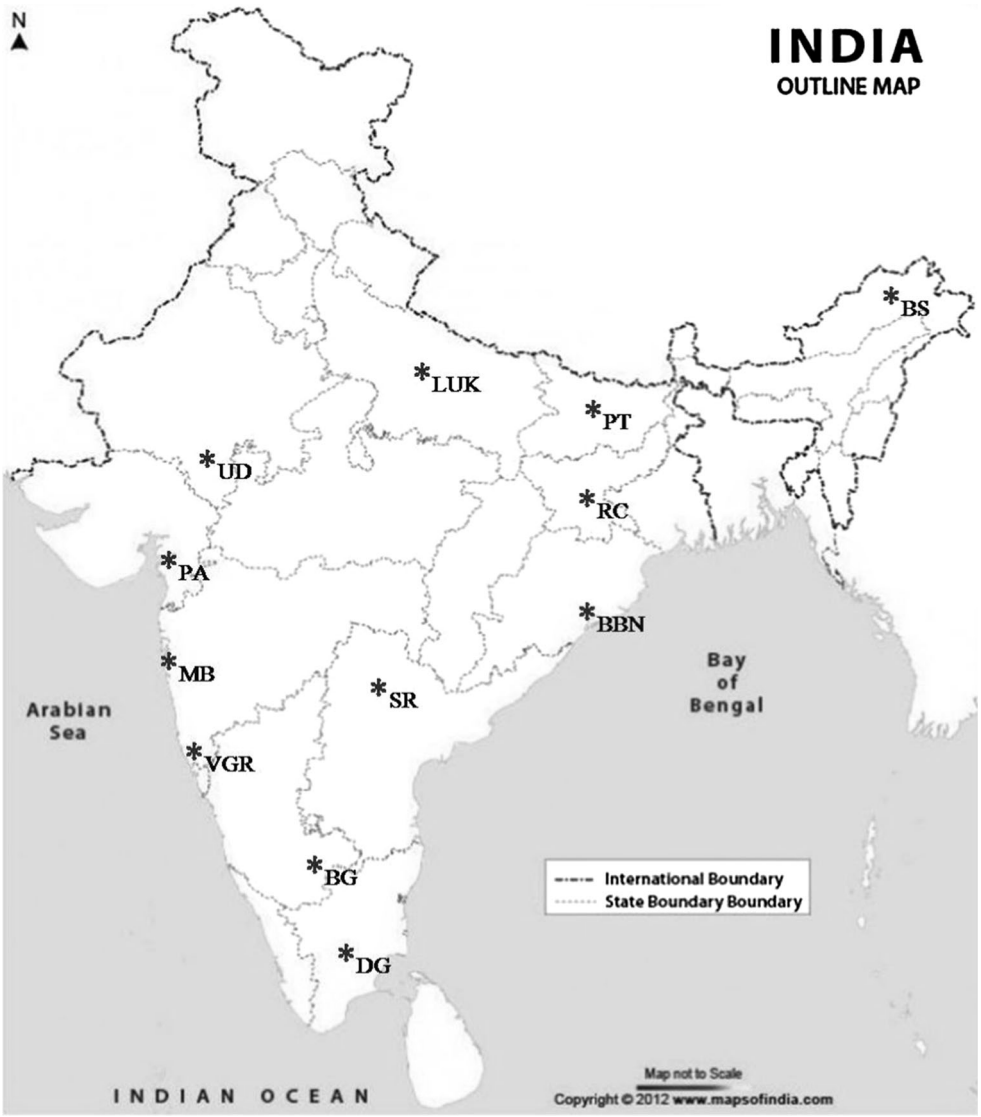

Fig. 1 Map of *B. dorsalis* collection sites from different parts of India

dorsalis (Rogers and Harpending 1992). Tajimas' *D* and Fu's F_S were also calculated to test for neutrality (Fu 1997; Tajima 1989). All parameters were tested against the expected values under the hypothesis of a recent population expansion based on 1000 bootstrap replicates. Genetic distances among *B. dorsalis* population pairs were calculated using Kimura two parameter methods implemented in MEGA 6.0 software.

Results

Nucleotide information

In the present study, we merged the sequences obtained from 12 locations of Indian region (accession number of *cox1*: KT119929-KT119946, KM359573-KM359624 and *nad1*: KT119947-KT120001). A total of 73 sequences each of *cox1* and *nad1* gene sequenced in the present study and 52 sequences each of *cox1* and *nad1* gene sequences collected from NCBI Genbank from other countries were aligned together and used for population genetic study of Indian populations of *B. dorsalis* in relation to other Asian countries (Table 1). The final sequences length of 503 and 504 bp for *cox1* and *nad1* genes respectively, were used for analyses. Overall 87 variable sites, including 29 parsimony informative sites and 58 singleton sites in *cox1* gene and 110 variable sites, including 40 parsimony informative sites and 70 singleton sites in *nad1*gene were observed after nucleotide sequence alignment.

Intra-population diversity

Analysis of sequences led to identification of 84 haplotypes in *cox1* gene and 86 haplotypes in *nad1* gene from all the populations of *B. dorsalis* (Tables 2 and 3). Among 84

Table 2 Genetic diversity indices among *cox1*and *nad1* sequences

Sr. no.	Locations	*cox1*				*nad1*			
		H	Hd	K ± SD	π ± SD	H	Hd	K ± SD	π ± SD
1	Patna	10	0.981	4.25454 ± 2.283848	0.008458 ± 0.005122	10	0.981	3.745455 ± 2.045296	0.007417 ± 0.004569
2	Bangalore	6	0.928	7.4285 ± 3.887194	0.01476 ± 0.0088	8	1.000	4.178571 ± 2.320511	0.008274 ± 0.005234
3	Paria	7	1.000	10.571429 ± 5.492499	0.02101 ± 0.01250	7	1.000	7.523810 ± 4.001943	0.014899 ± 0.009078
4	Ranchi	5	1.000	10.600000 ± 5.832959	0.021074 ± 0.013557	5	1.000	2.400000 ± 1.556732	0.004752 ± 0.003604
5	Lucknow	7	1.000	8.190476 ± 4.328241	0.016283 ± 0.009857	6	0.952	4.476190 ± 2.506556	0.008864 ± 0.005686
6	Vengurle	7	1.000	2.761905 ± 1.658384	0.005491 ± 0.003777	7	1.000	4.476190 ± 2.506556	0.008864 ± 0.005686
7	Sangareddy	5	1.000	5.00000 ± 2.92174	0.00994 ± 0.006791	5	1.000	2.200000 ± 1.450315	0.004356 ± 0.003357
8	Udaipur	4	1.000	11.66667 ± 6.7232	0.02319 ± 0.015961	4	1.000	4.166667 ± 2.609437	0.008251 ± 0.006170
9	Basar	3	0.833	2.33333 ± 1.5922	0.004639 ± 0.003780	4	1.000	2.833333 ± 1.871311	0.005611 ± 0.004425
10	Dindigul	4	0.866	4.20000 ± 2.424871	0.00835 ± 0.005567	5	0.933	1.600000 ± 1.095445	0.003168 ± 0.002505
11	Bhubaneswar	4	1.000	3.166667 ± 2.0564	0.006296 ± 0.004882	3	0.833	2.833333 ± 1.871311	0.005611 ± 0.004425
12	Mumbai	4	0.900	5.200000 ± 3.02606	0.010338 ± 0.007033	5	1.000	5.800000 ± 3.338760	0.011485 ± 0.007729
13	Bangkok	7	1.000	3.619048 ± 2.083686	0.007195 ± 0.004745	8	1.000	21.238095 ± 10.700532	0.042056 ± 0.024272
14	Guangzhou	5	0.785	2.285714 ± 1.397567	0.004544 ± 0.003165	8	1.000	6.000000 ± 3.199771	0.011881 ± 0.007218
15	Hekou	8	1.000	5.619048 ± 3.068340	0.011171 ± 0.006988	6	0.952	3.714286 ± 2.130765	0.007355 ± 0.004833
16	Fuzhou	2	0.600	3.60000 ± 2.18955	0.007157 ± 0.005089	5	1.000	4.200000 ± 2.503851	0.008317 ± 0.005796
17	Nanning	8	0.972	3.61111 ± 2.01923	0.007179 ± 0.004554	9	1.000	5.277778 ± 2.814407	0.010451 ± 0.006323
18	Taibei	5	0.800	3.955556 ± 2.161756	0.007864 ± 0.004860	10	1.000	4.644444 ± 2.486970	0.009197 ± 0.005570
19	Laos	5	1.000	5.20000 ± 3.026066	0.010338 ± 0.007033	5	1.000	4.200000 ± 2.503851	0.008317 ± 0.005796

H number of Haplotypes, *Hd* Haplotypes diversity, *K* average number of nucleotide differences, π nucleotide diversity, *SD* standard deviation

Table 3 Genetic diversity and demographic history parameters of *B. dorsalis* populations

Gene	*H*	*Hd*	*K*	π	θ_o	θ_1	τ	Tajima's *D*	Fu's *Fs*	SSD
cox1	84	0.984	6.576	0.0130	2.08704	68431	4.41530	−1.89603*	−101.726**	0.09337
nad1	86	0.990	5.65626	0.01122	1.02622	84214	3.75915	−2.41782**	−117.518**	0.04900

H number of haplotypes, *Hd* haplotypes diversity, K, average number of nucleotide differences; π, nucleotide diversity; θ_o, effective population size before expansion; θ_1, effective population size after expansion; τ, population expansion time (million years), *SSD* sum of squared deviations between observed and expected mismatch distribution under a sudden expansion model

** $P < 0.01$; * $P < 0.05$

haplotypes detected within *cox1* gene, only 16 haplotypes were shared by at least two populations and the remaining 68 haplotypes were exclusive haplotypes not shared by any other populations (Supplementary Table 1). Likewise only 18 out of 86 haplotypes detected in *nad1* gene were shared haplotypes (Supplementary Table 2). The most common haplotypes were H7 in *cox1* gene sequences and H17 in *nad1* gene sequences, comprised of 11 and 6 individuals from different populations of *B. dorsalis*, respectively.

Results obtained from descriptive genetic diversity analysis of 19 populations based on both gene sequences are summarized in Table 2. Haplotype diversity (*Hd*), average number of nucleotide differences (*k*), nucleotide diversity (π) varied from 0.6000 to 1.000, 2.285714 ± 1.397567 to 11.66667 ± 6.7232 and 0.004544 ± 0.003165 to 0.02319 ± 0.015961, respectively in *cox1* gene sequences, while from 0.833 to 1.000; 1.600000 ± 1.095445 to 21.238095 ± 10.70 0532 and 0.003168 ± 0.002505 to 0.014899 ± 0.009078, respectively in *nad1* gene sequences. Results of genetic diversity indices indicate that almost all the populations retain fairly high levels of genetic diversity.

Median Joining networks among haplotypes

Median Joining (MJ) networks of *cox1* and *nad1* haplotypes were generated from 125 individuals of *B. dorsalis* using NETWORK ver. 4.6 (Fig. 2), which suggested H7 in *cox1* and H17 in *nad1* as most common and ancestral/original haplotypes present in *B. dorsalis* population from India and other Asian countries. MJ networks reconstructed for evolutionary relationships among haplotypes for both genes used in this study showed a star-like shape, characteristic of population expansion (Slatkin and Hudson 1991). Other major haplotypes (i.e. H8 and H65 in *cox1*, H8, H11, H12, H22, H31, H33, and H37 in *nad1*) connected to widely distributed haplotypes through several mutational steps suggest diverse population present in *B. dorsalis*.

Genetic segregation among populations

The analysis of molecular variance (AMOVA) showed that most of genetic variation was present within populations (78.93 % from *cox1* and 92.90 % from *nad1* gene sequences, $P < 0.001$) than among the populations (21.07 % from *cox1* and 7.10 % from *nad1* gene sequences, $P < 0.001$) (Table 4). The overall fixation index (F*st*) calculated for all populations with both genes were statistically significant (0.21074 at $P < 0.1$ and 0.07096 at $P < 0.05$ levels) (Table 4). Population pairwise F_{st} of *B. dorsalis* was highest for BS (Basar, Arunachal Pradesh, India) population (0.284) with *cox1* gene while DG (Dindigul, Tamil Nadu) population (0.171) with *nad1* gene. The F*st* statistics values were lower, explaining the importance of haplotype divergence in the population structure pattern. Pairwise F*st* test among 19 populations showed significant differences in 119 and 82 out of 171 population pair comparisons with *cox1* and *nad1* genes respectively, after Bonferroni corrections. The highest differentiation was found between GZ (Guangzhou, China) and LUK (Lucknow, UP, India) populations for *cox1* gene (Table 5).

Mantel tests were used to predict pattern of isolation by distance, revealed a non-significant negative relationship for *cox1* and non-significant positive relationship for *nad1* between pairwise genetic and geographic distances for entire region of *B. dorsalis* ($r = -0.0430$ and $r = 0.0246$ in *cox1* and *nad1* genes, respectively at $P > 0.001$). These results suggest that genetic level of differentiation among the populations was not strongly associated with geographical distance.

Demographic history and neutrality test

The sequence variations of *cox1* and *nad1* gene sequences were used to perform Tajima's *D* and Fu's *Fs* test to determine deviations from neutrality. Significant negative Tajima's *D* and Fu's *Fs* values were found among both gene sequences (Table 3), suggesting that the *B. dorsalis* populations of studied region did not conform to the theory of neutral evolution.

The mismatch distribution plots of both genes were smooth and clear unimodal revealed that these populations were undergoing population expansion (Fig. 3). Even though sudden expansion model was in turn to be rejected for study area in accord with both gene sequences (SSD = 0.09337 in *cox1* and 0.04900 in *nad1* gene).

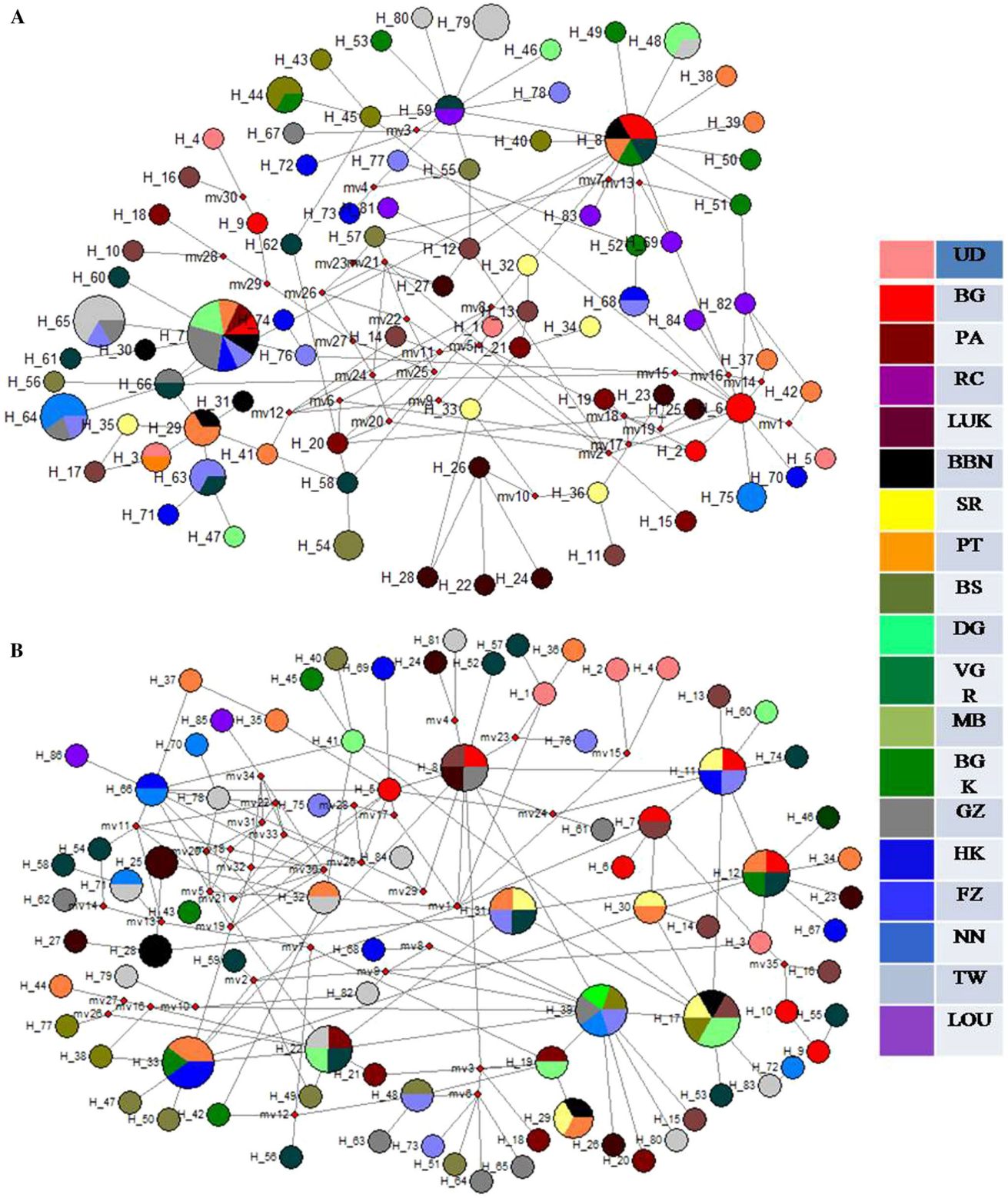

Fig. 2 Median joining networks of mtDNA haplotypes *B. dorsalis* **a** network of *cox1* haplotypes, **b** network of *nad1* haplotypes. *Each circle* represents a haplotype, and circle diameter is relative to haplotype frequency. *Colours* represent the geographic origin of specimens and size also indicates the proportion of individuals sampled in different populations within the study area. *Smallest red squares* represent median vectors

Table 4 Summary of AMOVA analysis of different populations of *B. dorsalis*

Gene analysed	Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	Fixation index
cox1	Among populations	18	128.989	0.69994Va	21.07	F_{st} 0.21074*
	Within populations	105	275.253	2.62146 Vb	78.93	
	Total	123	404.242	3.32140		
nad1	Among populations	18	71.033	0.20148 Va	7.10	F_{st} 0.07096**
	Within populations	105	276.999	2.63809 Vb	92.90	
	Total	123	348.032	2.83957		

** $P < 0.05$

* $P < 0.1$

Analysis of sequences showed population expansion time (τ) varied for both genes from 3.75915 to 4.41530 million years.

Discussion

The analysis of mtDNA sequences demonstrated high level of genetic diversity as exemplified by high values of *Hd, k* and π from both *cox1 and nad1* gene analyses (Table 2). The three genetic diversity parameters (*Hd, k* and π) were not influenced by the sample size indicating that they were appropriate indices of genetic diversity for *B. dorsalis* populations. It is evident that when value 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). Most of the studied populations fall within these conditions of *Hd* and π except BS (Basar, Arunachal Pradesh, India) and GZ (Guangzhou, China) in *cox1* and DG (Dindigul, Tamil Nadu, India), SR (Sangareddy, Telangana, India), RC (Ranchi, Jharkhand, India) and BGK (Bangkok, Thailand populations with -*nad1* gene analysis. These populations of *B. dorsalis* showed low value of π and high value of *Hd* suggesting that the existence of small portion of populations might have been suffered recent population growth. High level of genetic diversity of *B. dorsalis* in Indian populations suggested ancient existence of species because ancestors show significantly more genetic diversity than derivative population due to founder effect (Grant and Bowen 1998; Nei and Li 1979). In this investigation, some populations of *B. dorsalis* showed high value of *k* comparable with South-East Asian populations of the fly and, also considered as origin region of the species (Wu et al. 2014). High genetic diversity among *B. dorsalis* populations was also observed in South-East Asia (Aketarawong et al. 2007) and Yunnan Province of China (Shi et al. 2005, 2010; Wan et al. 2011) in earlier studies. Generally invasive species are low in genetic diversity due to selection pressure (Suarez and Tsutsui 2008) and genetic drift which may be due to temporarily reduced population size in founder colonies (Grapputo et al. 2006). High level of genetic diversity in India might be maintained due to both ecological suitability of the region and excellent biological traits of the species (i.e. high reproductive potential, short life cycle, a rapid and long dispersal ability) of *B. dorsalis* (Chen et al. 2006; Liang et al. 2001; Sridhar et al. 2014). Moreover, free trade of fruits which may carry eggs or maggots from one part to another part of the region may facilitate multiple introductions of the species, can counteract the drop in genetic variability associated with colonization or rescue a species from an actual loss in genetic diversity (Shi et al. 2012; Suarez and Tsutsui 2008). Multiple introductions of source population and frequent crossing among distantly related populations may also help to retain high level of genetic diversity in the species.

According to analysis of molecular variance, high level of genetic differentiation was observed between the populations even though it was less as compared to differences observed within populations. These results are in contrast to Chinese populations of *B. dorsalis* (Wu et al. 2014). High level of variability inside the populations was found in species those have not expanded in large areas in recent times (Prabhakar et al. 2012a).

The MJ networks showed randomly distributed haplotypes of *B. dorsalis* populations sampled from India and other Asian countries. MJ networks did not display any fundamental structure which could not be allowed to identify a phylogenetic haplogroup. This notion is also supported by a non-significant correlation between genetic distances and geographic distances suggesting no isolation by distance among populations (Mantel test). Diverse climatic conditions and ecological suitability of the region and species capabilities may be the reason for high genetic diversity and gene flow. Nevertheless, natural barriers like big mountains and rivers are present in the sampling region which may limit the gene flow in region. A similar situation has also been described in China where weak genetic

Table 5 Population specific F*st* and pair wise differences among different populations of *B. dorsalis* based on *cox1* and *nad1* gene

Populations	UD (0.113)	BG (0.096)	PA (0.007)	RC (0.152)	LUK (0.091)	BBN (0.145)	SR (0.157)	PT (0.104)	BS (0.145)	DG (0.171)
UD (0.092)		0.110*	0.071	0.390*	0.205*	0.282*	0.287*	0.170*	0.317*	0.457*
BG (0.154)	−0.062		0.034	0.300*	0.151*	0.203*	0.116*	0.057	0.159*	0.275*
PA (0.084)	−0.076	0.007		0.102*	0.067	−0.030	−0.080	0.041	−0.023	0.030
RC (0.100)	−0.048	0.041	−0.086		0.171*	0.174*	0.212*	0.109*	0.147*	0.126
LUK (0.140)	0.234*	0.352*	0.149*	0.098		−0.058	0.092*	0.025	0.017	0.100*
BBN (0.267)	0.101	0.035	0.075	0.124*	0.440*		−0.064	0.054	0.056	0.042
SR (0.222)	0.119*	0.210*	−0.049	−0.048	0.099	0.383*		0.014	0.020	−0.012
PT (0.226)	0.137*	0.045	0.123*	0.173*	0.459*	−0.050	0.342*		−0.008	0.065
BS (0.284)	0.200*	0.177*	0.202*	0.284*	0.503*	0.522*	0.519*	0.349*		−0.038
DG (0.236)	0.168*	0.080*	0.173*	0.223*	0.489*	−0.017	0.426*	0.041*	0.361*	
VGR (0.267)	0.187*	0.058*	0.173*	0.266*	0.513*	0.203*	0.464*	0.068*	0.316*	0.075
MB (0.218)	0.126*	0.070	0.106*	0.177*	0.441*	0.067	0.363*	0.098*	0.382*	0.106
BGK (0.247)	0.143*	0.055	0.115*	0.173*	0.469*	−0.051	0.367*	0.005	0.312*	−0.044
GZ (0.277)	0.322*	0.203*	0.300*	0.341*	0.583*	0.076	0.572*	0.135*	0.581*	0.054
HK (0.200)	0.101*	−0.001	0.105*	0.162*	0.437*	−0.001	0.337*	0.007	0.187*	0.000
FZ (0.253)	0.175*	0.108*	0.184*	0.235*	0.492*	0.181	0.457*	0.132*	0.450*	0.167*
NN (0.244)	0.214*	0.099*	0.207*	0.248*	0.519*	−0.002	0.444*	0.051	0.336*	−0.024
TW (0.234)	0.287*	0.177*	0.278*	0.327*	0.552*	0.112	0.494*	0.148*	0.385*	0.031
LOU (0.218)	0.097*	0.014	0.093*	0.152*	0.424*	0.123*	0.346*	0.039	0.268*	0.095*

Populations	VGR (0.091)	MB (0.065)	BGK (−0.368)	GZ (0.045)	HK (0.112)	FZ (0.106)	NN (0.063)	TW (0.080)	LOU (0.106)
UD (0.092)	0.304*	0.180*	0.054*	0.199*	0.208*	0.361*	0.151*	0.274*	0.285*
BG (0.154)	0.151*	0.181*	0.090*	0.138*	0.089*	0.287*	0.085*	0.210*	0.150*
PA (0.084)	0.104*	0.098*	0.035	0.079*	0.060	0.100*	0.023	0.083*	0.069
RC (0.100)	0.149*	0.024*	0.012	0.001	0.122*	0.122	0.015	0.024	0.132
LUK (0.140)	0.030	0.136*	0.067*	0.113*	0.031	0.150*	0.063*	0.079*	−0.001
BBN (0.267)	0.067	0.142*	−0.004	0.109*	0.157*	0.161*	0.048	0.070	0.075
SR (0.222)	0.118*	0.167*	0.022	0.079	0.127*	0.158*	0.000	0.055	0.130*
PT (0.226)	0.006	0.081*	0.102*	0.070*	−0.045	0.141*	0.002	0.054	−0.029
BS (0.284)	−0.001	0.108*	−0.031	−0.002	−0.001	0.025	−0.035	−0.101	−0.002
DG (0.236)	0.124*	0.196*	0.031	0.069	0.116*	0.029	0.011	−0.038	0.112*
VGR (0.267)		0.091*	0.060*	0.091*	0.031	0.159*	0.057	0.079	−0.071*
MB (0.218)	0.140*		0.027	0.007	0.052	0.172*	−0.013	0.104*	0.067
BGK (0.247)	0.047	0.047		0.035	0.045	−0.024	0.042	0.061*	−0.012
GZ (0.277)	0.303*	0.299*	0.116*		0.020	0.058	−0.027	0.036	0.066*
HK (0.200)	−0.017	0.041	−0.038	0.145*		0.116*	−0.047	0.020	−0.022
FZ (0.253)	0.237*	0.236*	0.190*	0.258*	0.127		0.057	−0.053	0.087
NN (0.244)	0.110*	0.163*	−0.050	0.032	−0.01*	0.150*		0.002	0.024
TW (0.234)	0.150*	0.207*	0.071	0.093	0.089	0.238*	0.048		0.012
LOU (0.218)	0.060	0.103	0.088*	0.268*	−0.01*	0.160*	0.127*	0.176*	

Distances 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 F*st* based on respective sequences

* Values (distances) shown are significantly differed

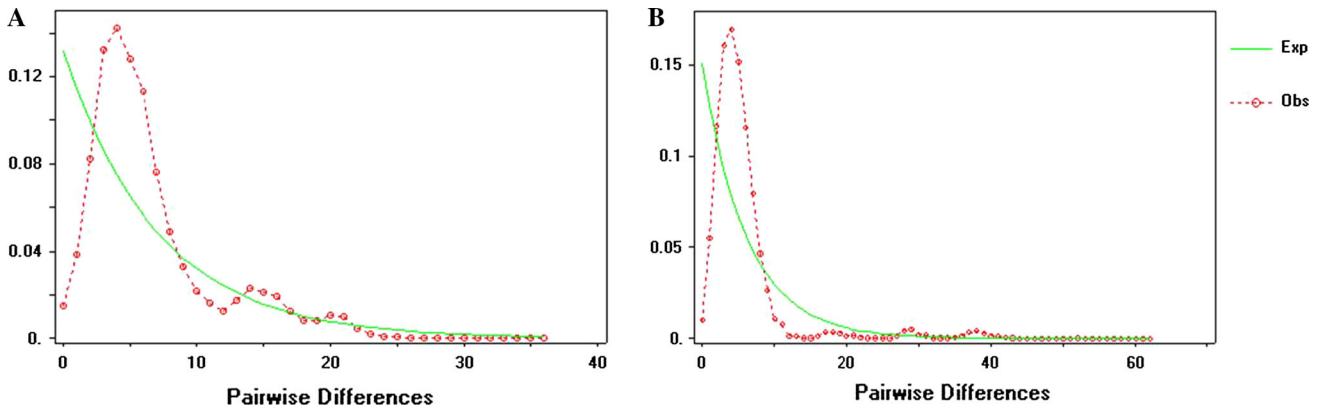

Fig. 3 Observed and simulated mismatch distribution of Indian populations of *B. dorsalis*. **a** Mismatch distribution graph from *cox1* gene sequences, **b** mismatch distribution graph from *nad1* gene sequences

structure was observed among 14 populations of Yunnan region (Shi et al. 2010) as well as in entire distribution range of *B. dorsalis* (Wan et al. 2011) despite presence of natural barriers i.e. big rivers and mountain ranges.

Results of demographic analyses obtained from both genes, showed significant (negative) Tajima's *D* and Fu'*Fs* values of studied populations which do not fit a simple model of selective neutrality and bottleneck analysis (Harpending et al. 1998). A negative Tajima's *D* value is produced when more than expected numbers of polymorphic sites have low frequencies within a sample. This pattern can be explained by either a recent increase in size of the population or recent selection (Slatkin 1985). The data suggested possibility of recent expansion of *B. dorsalis* populations in India in terms of new geographic areas and in the intensity of populations. Unimodal mismatch distribution with non significant *SSD* values for both the gene analyses suggested *B. dorsalis* population experiencing recent population expansion. The notion that the species underwent a significant population expansion is further in line with the observations from MJ networks, have a distinctively star-like structure, typical of expansion demographic processes.

Based on high genetic diversity in the region and weak genetic structure of populations, we hypothesize that this process of colonization may be interpreted as a gradual to fast, range expansion associated with high population numbers and population growth as observed in Chinese population of *B. dorsalis* (Wan et al. 2011). A CLIMEX software based prediction model showed that *B. dorsalis* incidence may increase in north and northeastern parts of India in next few decades and central and western part of India will be unsuitable for the species growth (Sridhar et al. 2014). Whereas, high genetic diversity, ineffectiveness of natural barriers, ecological suitability, crop diversification and increased domestic travelers and trade in the region suggested *B. dorsalis* might be the major insect pest of fruit crops in India. Our results also suggest that *B. dorsalis* populations from central and western part of India i.e. PA (Paria, Gujarat), VGR (Vengurla, Maharashtra), UD (Udaipur, Rajasthan) and MB (Mumbai, Maharashtra) have sufficient genetic variation which could be enough for evolutionary and ecological adaptation in response to rescue the negative impact of climate change scenarios in coming decades as detected by Sridhar et al. (2014).

Population genetic data generated during this study suggested low nucleotide diversity and high level of gene flow among Indian population of *B. dorsalis*. Torres et al. (2007) studied seven population of New World screwworm *Cochliomyia hominivorax* (Coquerel) from Uruguay with mtDNA analysis and suggested that the seven populations from Uruguay are very similar sharing homogenous haplotype. Analyses indicate that this species forms a single panmitic population in Uruguay (Lyra et al. 2005; Torres et al. 2007). Results of the present study also suggest any one Indian population of *B. dorsalis* can be used for the mass rearing, gamma irradiation and release of sterile insects for an AW-IPM programme. The unique haplotype detected in the present study will also be helpful in identification of inter population movement of the species in future.

Conclusion

Genetic diversity, expansion history of *B. dorsalis* in Indian subcontinent with other Asian countries was investigated using two mitochondrial DNA markers (*cox1* and *nad1*). High levels of genetic diversity with weak genetic structure have been observed among all the populations. Earlier studied ecological prediction model

based on climatic suitability also suggested that in future pest intensity may increase in north and northeastern region of subcontinent which is also supported with sufficient genetic variation in existing population, suitable for evolutionary stability in response to environmental changes. Present study suggests that the distant population of *B. dorsalis* may be mating compatible and suitable for the management through the area wide management program with proper inclusion of Sterile Insect Technique (SIT).

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