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GENETIC EVIDENCE FOR DIVERSITY OF SPIRALLING WHITEFLY, *ALEURODICUS DISPERSUS* (HEMIPTERA: ALEYRODIDAE) POPULATIONS IN INDIA

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Abstract

The spiralling whitefly *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), a highly polyphagous pest, has since about 2000 been an important pest of agricultural and horticultural crops in India. Despite its economic importance, little is known about the level and patterns of genetic variability in populations of the *A. dispersus*. In this study, we used the simple sequence repeats - polymerase chain reaction (SSR-PCR) technique was used to study the genetic diversity of *A. dispersus* in India. The present study is the first of its kind in India to utilize SSR markers for characterizing *A. dispersus* populations. A total of 356 different bands were scored using 18 primers for amplification. About 66.0% of alleles were polymorphic in *A. dispersus* populations. The SSR survey clearly detected moderate levels of polymorphism among the whitefly populations; multiple alleles were identified in many markers. The polymorphism information content (PIC) for SSR primers ranged from 0.0000 to 0.9541. Average genetic distances were estimated in our effort to investigate the level of DNA variation among the 7 geographic populations of *A. dispersus* from southern and northeastern India. The highest average genetic distance (0. 8182) was found between populations from Karnataka and Andhra Pradesh and Meghalaya and Mizoram. An UPGMA dendrogram based on similarity coefficients was constructed for the 7 populations analyzed. The *A. dispersus* population from Maharashtra was separated from those in the other 6 states viz., Tamil Nadu, Kerala, Andhra Pradesh, Karnataka, Mizoram and Meghalaya.

Key Words: *Aleurodicus dispersus*, populations, SSR primers, genetic diversity, dendrogram

Resumen

La mosca blanca espiral, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), es una plaga altamente polífaga, la cual desde acerca del año 2000 ha sido una plaga importante de cultivos agrícolas y hortícolas en la India. A pesar de su importancia económica, poco se sabe sobre el nivel y el patrón de variabilidad genética en las poblaciones de *A. dispersus*. En este estudio, utilizamos la técnica de una secuencia simple que se repite - reacción en cadena de la polimerasa (SSR-PCR) para estudiar la diversidad genética de *A. dispersus* en la India. El presente estudio es el primero de su clase en la India que utiliza marcadores de SSR para caracterizar las poblaciones de *A. dispersus*. Se obtuvo un total de 356 bandas diferentes utilizando 18 iniciadores (primers) para la amplificación. Acerca del 66.0% de los alelos fueron polimórficos en poblaciones de *A. dispersus*. El sondeo de SSR claramente detectó niveles moderados de polimorfismo entre las poblaciones de mosca blanca; se identificaron múltiples alelos en muchos de los marcadores. El contenido de información de polimorfismo (CIP) para los iniciadores de SSR varía desde 0.0000 hasta 0.9541. Se estimaron el promedio de las distancias genéticas en nuestro esfuerzo para investigar el nivel de variación del ADN entre

las 7 poblaciones geográficas de *A. dispersus* del sur y del noreste de la India. Se encontró el promedio de la distancia genética más alto (0.8182) entre las poblaciones de Karnataka y Andhra Pradesh y Meghalaya y Mizoram. Se construyó un dendrograma UPGMA basado en los coeficientes de similitud de las 7 poblaciones analizadas. La población de *A. dispersus* de Maharashtra fue separada de los otros 6 estados: Tamil Nadu, Kerala, Andhra Pradesh, Karnataka, Mizoram y Meghalaya.

Palabras Clave: *Aleurodicus dispersus*, poblaciones, iniciadores SSR, diversidad genética, dendrograma

The spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), is an insect pest of many tropical and sub-tropical crops. *Aleurodicus dispersus*, native to the Caribbean region of Central America (Russell 1965), is a highly polyphagous pest, which has an extensive host range covering 481 plant species belonging to 295 genera from 90 families of vegetables, fruits and ornamentals trees (Srinivasa 2000). In India, it was first recorded in 1993 at Thiruvananthapuram, Kerala on cassava (Palaniswami et al. 1995). Later it was reported from several parts of Kerala (David & Regu 1995; Ranjith et al. 1996), Tamil Nadu (David & Regu 1995), Karnataka (Mani et al. 2000), Andra Pradesh, Maharashtra (Charati et al. 2003), Orissa (CABI/EPPO 2006) and Mizoram (Boopathi 2008). The whitefly might have been introduced into India from neighboring countries like the Maldives (Muniappan 1996), Sri Lanka (Ranjith et al. 1996) and Myanmar (Boopathi 2008). Since 1993 the species has spread throughout the southern and northeastern India, thereafter it has become an important pest of agricultural and horticultural crops (Boopathi & Karuppuchamy 2013; Boopathi et al. 2013a). Direct feeding damage was caused by the piercing and sucking of sap from foliage by immature and adult stages of whiteflies (Boopathi et al. 2013b). When feeding, dense populations of this polyphagous pest caused premature leaf drops and produced ample honeydew which serves as a substrate for sooty mold growth, which led to the abandonment of cassava fields (Akinlosotu et al. 1993). Sooty mold blackens the leaf, decreases photosynthetic activity and decreases vigor often causing disfigurement of the host (Kumashiro et al. 1983; John et al. 2007). However, the colonization process and geographic source of the original population remain unknown. A molecular technique based on the polymerase chain reaction (PCR), the simple sequence repeats - polymerase chain reaction SSR-PCR, is being widely used in applied entomology (Harry et al. 1998). Many works have been done on the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), including genetic studies (Callejas et al. 2005), but very little on *A. dispersus* especially in India. Despite the economic importance of *A. dispersus*, little is known about the level and patterns of genetic variability in *A. dispersus* populations. Thus, in this project the SSR-PCR technique was used to obtain information on genetic variability of *A. dispersus* populations of India.

Materials and Methods

Insect Collections

Aleurodicus dispersus population was collected from 7 geographic regions of southern and northeastern States of India (Table 1). *Aleurodicus dispersus* adults were preserved in 70% ethanol until DNA extraction. Five adults from each population were analyzed to determine the genetic diversity of *A. dispersus* populations in India.

Isolation of DNA

The DNA was isolated by the CTAB (cetyl trimethylammonium bromide) method adopted from Gawel & Jarrett (1991) with necessary modifications. Prior to extraction, 1 adult female was taken into an autoclaved mortar containing 50 µL of pre-heated CTAB extraction buffer and the sample was ground using pestle. The samples were incubated at 65 °C for 45 min with CTAB extraction buffer. Equal volume of chloroform: isoamyl alcohol mixture (24: 1) was added and mixed by inversion for 15 min. The tubes were centrifuged at 12,000 rpm for 15 min. at 4 °C. The clear aqueous phase was transferred to a new sterile tube. One-third volume of ice cold isopropanol was added and mixed gently by inverting the tubes until DNA was precipitated out by incubating the tubes at -20 °C overnight. Then, it was centrifuged at 12,000 rpm for 20 min. in 4 °C to pellet out the DNA. The DNA pellet was washed with 70% ethanol and dissolved in 20 µL of 1X TE buffer (pH 8.0). To eliminate the RNA and protein contamination from DNA, one-tenth volume of RNase (10 mg mL^{-1}) and 1 µL of proteinase K (25 mg mL^{-1}) was added to DNA sample and incubated at 37 °C for 30 min.

SSR - PCR (Polymerase Chain Reaction) Analysis

DNA samples from *A. dispersus* populations collected from 7 states of India were amplified using a set of 18 SSR primer pairs which included 7 SSR primers of *A. dispersus* (Ma et al. 2011)

TABLE 1. *ALEURODICUS DISPERSUS* DNA SAMPLES COLLECTED FROM 7 GEOGRAPHIC REGIONS OF SOUTHERN AND NORTHEASTERN STATES OF INDIA.

Code	Locations	Hosts	Collected	Latitude	Longitude	Altitude (meter)
TN	Coimbatore, Tamil Nadu	*Manihot esculenta* Crantz.	28.12.2012	11° 0'47.39"N	76°56'14.19"E	434.0
KL	Trissur, Kerala	*Carica papaya* L.	06.11.2012	10°31'34.94"N	76°12'53.79"E	26.0
AP	Nandyal, Andhra Pradesh	*Psidium guajava* L.	13.12.2012	15°28'59.88"N	78°28'59.88"E	211.0
KA	Bengaluru, Karnataka	*Acalypha hispida* Burm. f.	24.04.2012	13° 1'36.90"N	77°35'5.07"E	928.0
MH	Pune, Mahashtra	*Psidium guajava* L.	08.01.2013	18°29'31.69"N	73°59'12.96"E	556.0
MZ	Kolasib, Mizoram	*Psidium guajava* L.	12.01.2013	24°12'39.57"N	92°40'35.18"E	635.0
ML	Umiam, Meghalaya	*Rosa* spp.	02.02.2013	25°41'24.46"N	91°55'20.47"E	965.0

and 11 SSR primers of *B. tabaci* (De Barro et al. 2003; Tsagkarakou & Roditakis 2003; Gauthier et al. 2008) as detailed in Table 2. Preliminary studies were done using SSR primers of *B. tabaci* to know the genetic variation among the *A. dispersus* populations.

Polymerase chain reactions were performed in a thermocycler (BIORAD DNA engine, PTC-0200, Mexico). Eighteen different primers were used for amplification. Reactions were performed in a volume of 25.0 µL containing 16.5 µL of double distilled water, 2.5 µL of Tag buffer, 1.0 µL of dNTPS, 0.5 µL of $MgCl_2$, 1.0 µL of forward primer, 1.0 µL of reverse primer, 0.5 µL of Tag DNA polymerase and 2.0 µL of the extracted Genomic DNA.

The reaction cycle comprised an initial 5.0 min of pre-denaturation at 95 °C, 40 cycles of amplification (1.0 min of denaturation at 95 °C; 1.0 min of annealing temperature ranging from 50.0 to 60.0 °C for different SSR primers as in Table 2; 1.0 min of extension at 72 °C), and 5.0 min of final extension at 72 °C. Followed the amplification, the samples were subjected to denaturing polyacrylamide gel electrophoresis as detailed below.

Polyacrylamide Gel Electrophoresis

PCR products were run on 6% polyacrylamide gels, using a gel apparatus. The gel was pre-run for about 20 min. before loading the sample and maintained at 70 W for 2 h. After electrophoresis, the gel was separated from the plates and treated for 15 min in fixation solution (double distilled water and 0.5% v/v acetic acid) with gentle shaking, the gel was washed 2-times with distilled water for 2 min. The gel was stained in staining solution (0.1% w/v silver nitrate), the gel was washed 2 times with distilled water for 2 min, and then the gel was transferred to developing solution (1.5% w/v sodium hydroxide, 0.3% formaldehyde). The reactions were stopped by using stopper solution (double distilled water and 0.5% v/v acetic acid). The bands were then visualized by silver staining (Bassam et al. 1991; Chalhoub et al. 1997), dried overnight and photographed.

Statistical Analysis

Images were used to score the data for SSR analysis. Clearly resolved, unambiguous polymorphic bands were scored with the help of AlphaEase® FC Software (Version 6.0). The scores were obtained in the form of a matrix with '1' and '0', which indicated the presence and absence of bands in each location, respectively. Allele scoring was done using the 100 bp marker lane as a reference. The sizes of the alleles are approximated by comparing with the band migration distance of the standard 100 bp ladder. The polymorphism information content (PIC) values are calculated for each SSR marker using the following algorithm.

TABLE 2. SSR PRIMERS USED FOR CHARACTERIZATION OF *ALEURODICUS DISPERSUS* POPULATIONS COLLECTED FROM 7 GEOGRAPHIC REGIONS OF SOUTHERN AND NORTH-EASTERN STATES OF INDIA.

Primer name	Sequences (5' - 3')		AT (°C)	Amplification size (bp)
	Forward	Reverse		
Btls1-9	GTG TTT GAG GAG GTG GG	CTA TTT ATC TAT TTG GGT CA	55.0	262-282
Btls1-14	CTC GAA CTC GAT CAT CC	ATT GGA AGC CTC GAA TAC	55.0	130-150
Btls1-13	CTA AGA CCG ATT CCT CC	CAA TAC TAC ACC TTC AAT TAC C	55.0	113-121
AY145456	AGC AGC ATC AAC AGG CTC	CTA GAT TCT GCT TGA AAG G	50.0	142-262
AY145458	GTC TTT GGG AGA GCC AGA AT	AAC AAG ACG GTG GCA GCG A	56.0	74-119
AY145464	TGA CGA CCT GAG GCT GAG AG	TGC AAC GGC AAC AGC AAG CAA	59.8	72-87
AY183673	GAG ATC ATA TCC CCA TTG TTT C	ATC ACG GGT CAT AGA TCA CG	50.2	279-305
AY183675	AAA TTA ACT GCC GCT CAA CG	ATA TCG ATA CAA TCT TAC CCG	50.2	290-302
AY183678	ATT CGG TTC GTC TTA GGG AC	ACG ATG TTT CCA AAC TGA GC	50.2	152-186
AY183679	GCT CAA CCG AAT ACA TCC AC	AAG TCT AAA GGA AGC GTG GAA	50.2	140-142
AY183682	ACG ACA CAA ATT GGC ATT ACA T	ACA AGT CAA CAT CCT CTA GGT A	50.2	200-222
AD2	CTC CAT GCT GTT CTT GAT	CAG GCA CCT ATA AAC CG	56.7	251-275
AD3	CGA CGA TTT ATA CGA ACG CA	ACA CGA ATT GAA GTT GAG GG	53.0	241-261
AD12	TCA CCA GAC CCC ACC CAC CGA C	CAC AAA TGC TCC CAA TAC C	55.3	242-246
AD13	CGA CAA CAG GAA ACA ACG GT	AAA CTG GCA AAG GCG GAC	53.0	308-320
AD15	CAT TGA GTG GGT CCA TTG TT	CGG GAA ATG ATG TCA GGA GG	53.0	278-290
AD20	TGC GGG CTC CAA CTA TGT	TGT GGT CGG CAG GAT TTA	53.0	174-194
AD26	TTA AAT TGC TCG CAT GGC	TAA AAT AGG CTT CAG ACC C	50.2	191-194

AT: Annealing Temperatures

PIC = 1-Σ f_i^2 i = 1 where f_i^2 is the frequency of ith allele (Hildebrand et al. 1992).

The data matrix was used to calculate Jaccard's similarity coefficient (Sneath & Sokal 1973) which does not consider the joint absence of a marker as an indication of similarity. A dendrogram was constructed by the Unweighted Pair-Group Method (UPGMA) (Sneath & Sokal 1973). The resulting matrix was used to calculate Manhattan distances between all pairs of populations. These analyses were performed using NTSYS-pc software, version 2.0 (Rohlf 1998).

Results

In the SSR analysis, all amplification products obtained were reproducible and consistent. The genetic variation among the seven *A. dispersus* populations collected from various geographic regions of India was investigated using SSR markers. Eighteen of the 45 primers screened produced clear bands on the SSR amplifications and were used for analysis. These primers amplified a total of 356 alleles (Table 3). The total number of alleles obtained from each primer ranged from 4 (*AD26*) to 79 (*AY145458*) with an average of 19.78 alleles per primer. Two hundred and thirty five (66.0%) out of 356 alleles were polymorphic for *A. dispersus* populations (13.06 polymorphic alleles per primer). Among 18 primers, 16 primers were polymorphic and the polymorphism ranged from 21.4 to 100%. The primers, Btls1-9, AY145464, AY183673, AY183675, AY183678, AY183679, AY183682, AD2, AD13, AD15, and AD26 were produced 100 % polymorphic patterns, while Btls1-14 and Btls1-13 produced monomorphic pattern. The polymorphism information content (PIC) for SSR primers ranged from 0.0000 to 0.9541. The PIC value was the highest in AD12 (0.9541), AY145458 (0.9492), and AD3 (0.9339). The size of amplification products from different primers ranged from 72 to 320 bp. An example of SSR amplification patterns of primer, AD3 is shown in Fig. 1 for different geographical regions of India.

Genetic relationships between populations are shown in Table 4. The average genetic distances were estimated to investigate the level of DNA variation among the 7 geographic populations of *A. dispersus*. The genetic distance varied from 0.6364 to 0.8182 among the 7 geographic populations of *A. dispersus*. The lowest average genetic distance was found between populations from Maharashtra and Karnataka (0.6364). The highest average genetic distance (0. 8182) was found between populations from Karnataka and Andhra Pradesh and Meghalaya and Mizoram.

An UPGMA dendrogram based on similarity coefficient was constructed for the 7 populations analyzed (Fig. 2). Three major clusters were evident. The first cluster contained *A. dispersus* populations from Tamil Nadu, Kerala, Andhra Pradesh and Karnataka. In the first cluster,

Table 3. Polymorphism information content (PIC) of SSR markers in *Aleurodicus dispersus* populations of 7 geographic regions of southern and northeastern states of India.

S. No	Primer code	Number of alleles	Number of polymorphic alleles	% polymorphism	PIC value
1	Btls1-9	34	34	100.0	0.7457
2	Btls1-14	11	0	Monomorphic	0.0000
3	Btls1-13	11	0	Monomorphic	0.0000
4	AY145456	44	33	75.0	0.8704
5	AY145458	79	35	44.3	0.9492
6	AY145464	11	11	100.0	0.3967
7	AY183673	12	12	100.0	0.5972
8	AY183675	11	11	100.0	0.4959
9	AY183678	7	7	100.0	0.4898
10	AY183679	8	8	100.0	0.7188
11	AY183682	5	5	100.0	0.6400
12	AD2	12	12	100.0	0.4444
13	AD3	59	37	62.7	0.9339
14	AD12	14	3	21.4	0.9541
15	AD13	10	10	100.0	0.0000
16	AD15	5	5	100.0	0.0000
17	AD20	19	8	42.1	0.8227
18	AD26	4	4	100.0	0.3750
	Total	356	235		
	Mean	19.78	13.06		

PIC : Polymorphism Information Content

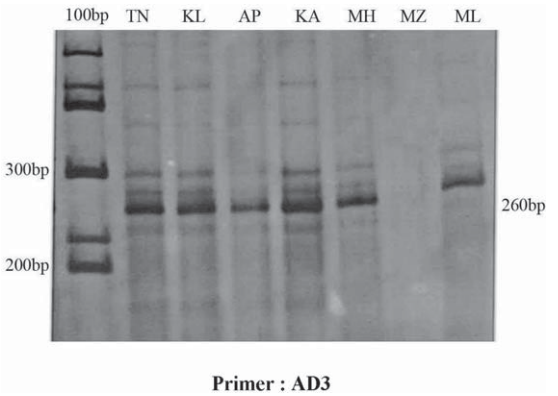

Fig. 1. SSR profiles of the 7 *Aleurodicus dispersus* populations collected from southern and northeastern States of India. Abbreviations: TN: Tamil Nadu, AP: Andhra Pradesh, KA: Karnataka, MH: Mahashtra, MZ: Mizoram and ML: Meghalaya.

Tamil Nadu population was separated from the other 3 states. The second cluster had populations from Mizoram and Meghalaya. The third cluster contained *A. dispersus* populations from Maharashtra which was separated from other 6 states of India. From cluster analysis is evident that Maharashtra and Tamil Nadu populations were distinct from each other.

Discussion

Microsatellites (SSR markers) are short DNA fragments located throughout the genome, which contain tandemly repeated patterns of 2 to 6 basepairs. Because of their high polymorphism and co-dominance, these markers provide an effective tool of population genetic studies. SSR markers were isolated and utilized for studying genetic diversity in *B. tabaci* (De Barro et al. 2003; Tsagkarakou et al. 2007; Gauthier et al. 2008). SSR markers have been used to differentiate *B. tabaci* variants and to estimate the biotypes and genetic relationships of closely related populations from the same geographical location (De Barro et al. 2003; Tsagkarakou et al. 2007; Gauthier et al. 2008). The primer sequences of around 10 SSR markers are publicly available for studying the *A. dispersus* genome (Ma et al. 2011).

The present study is the first of its kind in India to utilize SSR markers for characterizing *A. dispersus* populations. Eighteen SSR primers were amplified in *A. dispersus* and they produced 356 alleles. The number of alleles produced by different primers ranged from 4 (*AD26*) to 79 (*AY145458*) with an average of 19.78 alleles per primer. Our SSR survey clearly detected moderate levels of polymorphism among the *A. dispersus* populations; multiple alleles were identified in many markers. These SSR allele numbers are higher than those reported from *B. tabaci,* i.e., up to 13 (Tsagkarakou & Roditakis 2003), 16 (De Barro 2005) and 24 (Gauthier et al. 2008) alleles. This might be due to smaller number of populations collected from geographically closer distances in the present study, whereas the populations from different countries across continents were compared in other studies. The polymorphism information content (PIC) value of each SSR marker is a measure of marker's diversity. PIC provides an estimate of discriminatory power of a locus by taking into account not only the number of alleles expressed, but also the relative frequency of those alleles.

In the present study, SSR markers showed 66.0% polymorphic alleles from 7 populations collected from different geographical regions of India. Similar results were reported by Ma et al. (2011) who observed that microsatellite markers had heterozygosity from Hainan and Canary Island, which ranged from 0.100 to 0.933. Several studies have been carried out to characterize the extent of genetic diversity and differentiation of whitefly populations (Rekha et al. 2005; De Barro 2005; Chu et al. 2007; Tsagkarakou et al. 2007). However, earlier Callejas et al. (2005) had reported that there were no differences in RAPD patterns among *A. dispersus* populations from different islands of the Canaries.

Cluster analysis showed that, in general, *A. dispersus* populations are scattered independently in the localities where the samples were

Table 4. Average genetic distances among 7 *Aleurodicus dispersus* populations collected from southern and northeastern states of India.

	Tamil Nadu	Kerala	Andhra Pradesh	Karnataka	Maharashtra	Mizoram	Meghalaya
Tamil Nadu	1.0000						
Kerala	0.7273	1.0000					
Andhra Pradesh	0.7636	0.7818	1.0000				
Karnataka	0.7273	0.7818	0.8182	1.0000			
Maharashtra	0.7273	0.7091	0.7455	0.6364	1.0000		
Mizoram	0.6909	0.7091	0.7455	0.7091	0.6727	1.0000	
Meghalaya	0.7636	0.6727	0.7818	0.6727	0.6364	0.8182	1.0000

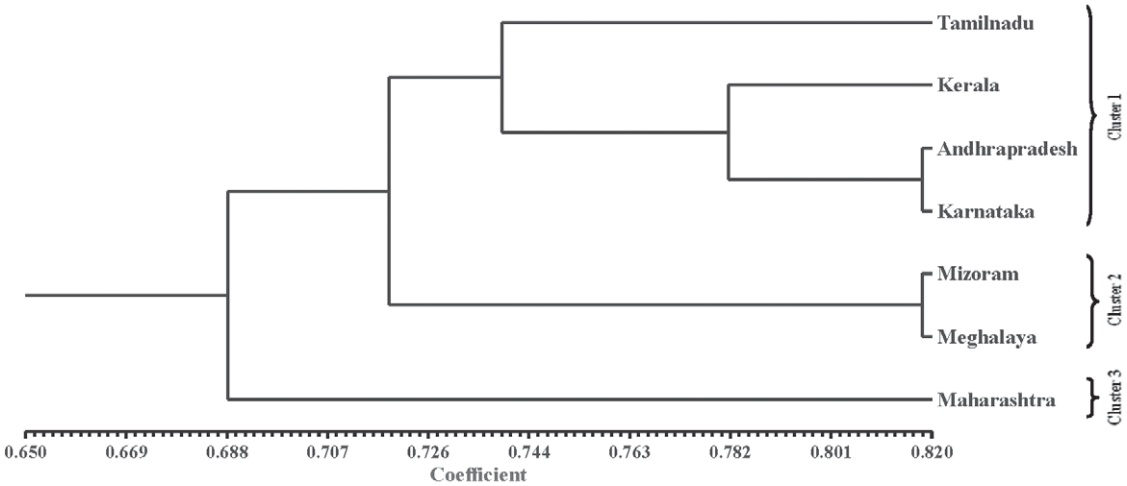

Fig. 2. Dendrogram showing the UPGMA clustering of *Aleurodicus dispersus* populations collected from southern and northeastern States of India based on SSR markers.

collected, especially samples from the Maharashtra and Tamil Nadu populations and theses populations were distinct from each other. Nevertheless, some clusters were evident, joining populations according to the regions. This result suggests that a differentiation of populations has already occurred, mainly according to the geographical regions where populations are localized. The cluster analyses of the *A. dispersus* populations showed the heterogeneous set of groups and sub-groups, probably due to differences between several nutritional and behavioral factors. The differentiation observed could also be explained by the introduction of *A. dispersus* populations in different geographical regions of India from several rather than a single founder population, by the massive spread of this populations from other countries like Maldives (Muniappan 1996), Sri Lanka (Ranjith et al. 1996) and Myanmar (Boopathi 2008) in these populations that could have affected the allele frequencies in different geographical regions.

Genetic variation was detected in all the populations analyzed. Considering only *A. dispersus* populations, an average Jaccard similarity of 0.7273 was observed between all populations analyzed. Moreover, UPGMA showed the clustering of individuals by geographical regions. More studies are necessary concerning molecular sequences (mitochondrial and nuclear genomes), host range phenotypes and mating compatibility to elucidate this controversy.

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