Genetic structures of sand fly (Diptera: Psychodidae) populations in a leishmaniasis endemic region of Turkey

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ABSTRACT: The object of this study was to determine the genetic structures of three vector species, *Phlebotomus tobbi, Phlebotomus papatasi,* and *Phlebotomus sergenti,* in the Cukurova Region of Turkey, an endemic focus of cutaneous leishmaniasis. The genetic diversity indices, neutrality tests and hierarchical analysis of molecular variance (AMOVA) were performed using partial sequences of ITS2 and cytochrome b gene regions. In all species, within population genetic variation was higher than between population variation for ITS2 gene region. Fst values were low and non-significant for *P. sergenti*, and were higher for *P. papatasi* and *P. tobbi* indicating a weak structuring between populations. AMOVA tests suggest any substantial isolation between populations within species. AMOVA analysis of cyt b gene region revealed significant genetic structuring between populations for *P. papatasi* and *P. sergenti*. Fst values were relatively high and significant for these species indicating a certain degree of isolation between populations. However, in *P. tobbi,* any significant population genetic structuring was detected. Tajima's D and Fu's Fs values were negative and significant in all three species might be indicating a demographic expansion. ***Journal of Vector Ecology* 36 (Supplement 1): 32-48. 2011.**

Keyword Index: Phlebotomus papatasi, P. tobbi, P. sergenti, genetic structure, nucleotide diversity, population extension, leishmaniasis, Turkey.

INTRODUCTION

Leishmaniasis is transmitted by several different species of phlebotomine sand fly vectors (Volf et al. 2002). The disease is endemic in all the countries of the Mediterranean basin and its epidemiology is rapidly evolving (Schönian et al. 2008). As a Mediterranean country, Turkey represents a crossroad between the three continents of Africa, Europe and Asia, with ecological and climatic features that are important in the epidemiology of leishmaniasis. Two clinical types of leishmaniasis exist in Turkey. Human cutaneous leishmaniasis (CL) caused by *Leishmania tropica* Wright, 1903 (Alptekin et al. 1999, Akman et al. 2000, Volf et al. 2002) and *L. infantum* Nicolle 1908 (Serin et al. 2005, Svobodova et al. 2009), which is highly endemic in south and southeast Anatolia, and human visceral leishmaniasis (VL), caused by *L. infantum,* which is endemic along the Aegean and Mediterranean costs and occurs sporadically in other regions (Ozbel et al. 1995, Ok et al. 2002, Volf et al. 2002, Yaman and Ozbel 2004).

The Cukurova Basin has become a second endemic focus of CL within Turkey. Because it is an ecotone between western and eastern Anatolia, is one of the crossroads between Asia and Europe in terms of animal and plant dispersion, and almost 70% of the region is covered by agricultural area, the plain is appealing to a large human population. Accordingly, there is a significant increase of CL cases over the past 10 years. Most of the species found in this region are known vectors of CL (Simsek et al. 2007, Svobodova et al. 2009). According to recent studies, the etiological agent of CL in the region was identified as *L. infantum,* and *Phlebotomus (Larroussius) tobbi* Adler, Theodor et Lourie 1930 was shown to be the vector of CL in the Cukurova focus (Svobodova et al. 2009).

Populations are usually found in nature as separated subunits as a result of ecological, behavioral, or genetic diversification. These subpopulations may exhibit different phenotypic and genotypic features from each depending on such factors as gene flow, geographic distance and diversifying selection (Hendrick 2005). Strong genetic differentiation between phlebotomine sand fly populations are expected since they deposit their larvae into the soil and are weak fliers as adults, making them highly habitat specific (Depaquit et al. 2002). This genetic differential and subsequent adaptation to the current environment can cause differences in vectorial capacities of sand flies. Previous studies showed that *P. tobbi* is prevalent throughout Turkey and constitutes the most abundant species in the Cukurova region (Simsek et al. 2007, Erisoz Kasap et al. 2009). Therefore, it is necessary to determine the genetic structures of *P. tobbi* populations in order to improve our knowledge of leishmaniasis transmission in this area. Together with *P. tobbi,* the genetic structures of *P. (Paraphlebotomus) sergenti* Parrot 1917 and *P. (Phlebotomus) papatasi* Scopoli 1786 populations were examined because the former is the proven vector of *L. tropica* in the southeastern part of the Turkey (Alptekin et al. 1999, Akman et al. 2000, Volf et al. 2002) while the latter is another vector of *L. major* (Killick-Kendrick 1990, Parvizi et al. 2005), which was found in several places (Akman et al. 2000) and is largely distributed

all over the country.

The aim of this study was to determine the genetic structure of *P. tobbi*, *P. papatasi* and *P. sergenti* populations within the Cukurova region. Determining the genetic structures of these populations will increase our knowledge of leishmaniasis transmission within this area. Population genetic structure was assessed using two genes; one nuclear, the internal transcribed spacer 2 (ITS2) gene region, and one mitochondrion, cytochrome b (cyt b) gene region. These two gene regions are known for their relative high mutation rates making them highly useful for studies at the intra population level (Esseghir et al. 1997, Mukabayire et al. 1999, Depaquit et al. 2000, 2002, Di Muccio et al. 2000, Yahia et al. 2004, Moin-Vaziri et al. 2007, Hamarsheh et al. 2007).

MATERIALS AND METHODS

Study area

The Cukurova Basin is approximately 28,000 km^2 in area surrounded by the West Taurus Mountains on the west, Taurus Mountains on the north, Amanos Mountains on the east, and the Mediterranean Sea on the south. Seyhan (on the west) and Ceyhan (on the east) rivers nourish the plain. The study was carried out at Tarsus village, Adana and Osmaniye provinces, and their environment (Figure 1, Table 1).

The plain has a temperate climate with long summers and short and rainy winters. This climate enables the cultivation of three or more agricultural products per year. It is also preferable by sand flies and especially the warm season coincides with their reproductive cycles (Lewis 1982, Alptekin et al. 1999, Volf et al. 2002). Most of the area is fertile (mollisol and alluvial soil) and used for agricultural activities but *Pinus* and *Abies* forests are also cultivated. The villages are usually surrounded by citrus orchards and cotton fields, most commonly in the eastern part. Residents live in single-family houses built from briquette, adobe, stone and cement, surrounded by gardens with henhouses and sheep or cattle sheds.

Sand fly collection and identification

Seventy-one of the thousands of specimens collected from 32 villages of the study area were selected for determining the genetic structures of the populations (Table 2). Indoor and outdoor sand fly collections used CDC light traps (John W. Hock, U.S.A.), sticky papers and mouth aspirators (Alexander 2000). Specimens collected from each trap were stored in alcohol for morphological identification. Identifications were based on the morphology of male genitalia and female spermathecae and pharynges using the keys of Theodor (1948), Lewis (1982), Killick-Kendrick, (1991). Detailed information on the distribution, abundance and seasonal dynamics of species within the study area and within villages are given in Belen and Alten (2011).

DNA extraction, amplification and sequencing

DNA was isolated from thoraces following the procedures for the Qiagen DNeasy Blood and Tissue Isolation kit. The samples were dried before DNA extraction. PCR was performed in a 50 µl volume using 1.5 µl extracted DNA solution and 100 pmol of each of the two primers for ITS2. Primer sequences are: C1a: 5'–CCT GGT TAG TTT CTT TTC CTC CGC T-3' and JTS3: 5' –CGC AGC TAA CTG TGT GAA ATC-3' (Depaquit at al. 2000). The PCR mix contained 42.25 µl distillated water, 5 µl 10Xbuffer, 0.15 µl 25 mM dNTP, 1 µl 1.5 mM $MgCl_2$ and 0.25 µl (1.25 unit) *Taq* polymerase (Bioron). Initial denaturation at 93° C for 1 min was followed by 35 cycles of denaturation at 94° C for 45 s, annealing at 56° C for 45 s, extension at 72° C for 45 s, and a final elongation time of 2 min at 72° C.

Similarly for the cyt b gene region, PCR was performed in a 50 µl volume using 1.5 µl extracted DNA solution and 2 µl (10 pmol=1/10 diluted) of each of the two primers were added. Primer sequences for cyt b are: CB1-SE: 5'-TAT GTA CTA CCC TGA GGA CAA ATA TC-3' and CB-R06: 5'-TAT CTA ATG GTT TCA AAA CAA TTG C-3' (Parvizi and Ready 2006). PCR mix was prepared using 35.75 µl distillated water, 5 µl 10Xbuffer, 3 µl 2.5 mM dNTP, 2 µl 1.5 mM $MgCl_2$ and 0.25 µl (1.25 unit) *Taq* polymerase (Bioron). Initial denaturation at 94° C for 2 min was fallowed by 10 cycles of denaturation at 94° C for 30 s, annealing at 40° C for 30 s and extension at 72° C for 30 s. These cycles were followed by another 25 cycles of 94° C for 30 s, annealing at 48° C for 30 s and extension at 72° C for 30 s with a final elongation time 3 min at 72° C. Amplicons were analyzed by electrophoresis in 80 ml, 1.5% agarose gel containing 10 µl etidium bromide.

All PCR products were purified with the Invisiorb PCR Purification kit before sequencing. Purification products were both direction sequenced directly with the ABI Big Dye Terminator Cycle Sequencing kit with the primers used for DNA amplification reactions.

Sequence analysis

Sequences were aligned and edited using Clustal W2 software. Nucleotide diversity between and within populations were estimated using the Φ (Watterson, 1975) and π (Tajima, 1983) statistics. We also calculated neutrality indices Tajima's D and Fu's Fs using the mtDNA cyt b gene region in order to determine whether there was any deviation from the assumption of neutrality. Tajima's D and Fu's Fs indices were not calculated for the ITS2 gene region since multi-copy genes violate the assumptions of the tests. A molecular analysis of variance (AMOVA) was conducted in order to determine the genetic structure of populations. (Cockerham 1969, 1973, Weir and Cockerham 1984). Statistical significance of F_{st} values was determined using a non-parametric permutation approach (Excoffier et al. 1992). All analyses were performed using Arlequin v.3.1.1 (Excoffier et al. 2005) software.

Table 1. Sampling stations in the study area.

Villages	Coordinates		Altitude	Villages	Coordinates		Altitude
	North	East			North	East	
Kulak (KUL)	36 47 37.7	34 52 01.8	1 m	Döşeme (DOS)	37 25 28.1	35 51 59.2	175 m
Dedepınarı (DDP)	36 55 36.6	35 28 56.4	4 m	Camili (CAM)	37 20 19.5	35 36 38.5	181 m
Adalı (ADA)	36 38 04.8	35 32 33.0	7 m	Tepeçaylak (TCK)	37 03 33.6	35 03 34.7	188 m
Yemişli (YEM)	36 39 18.6	35 21 31.0	9 m	Tepecikören (TEP)	37 21 50.8	35 37 38.9	189 m
Eğriağaç (ERA)	36 46 40.8	35 26 50.4	9 m	Tehçi	37 09 14.8	36 20 01.9	192 m
Ziyalı (ZIY)	36 49 35.4	35 34 22.4	18 m	Karakütük (KKK)	37 24 40.1	36 07 15.8	217 m
Doğankent (DK)	36 50 51.9	35 20 19.0	20 m	Sarımazı (SMZ)	36 58 21.5	35 58 32.4	222 m
Yenice (YEN)	36 58 39.2	35 02 57.4	42 m	Dutlupınar (DUT)	37 01 39.7	36 01 03.7	222 m
Hamamköy (HAM)	37 19 08.7	35 49 02.8	45 m	Otluk (OT)	37 18 05.2	35 31 05.3	237 m
Narlık (NAR)	36 55 03.6	35 51 14.3	48 m	Gökbüket (GOK)	37 07 39.1	35 32 48.6	244 m
İsalı (ISA)	36 55 11.9	35 43 04.9	62 m	Aydın (AY)	37 24 35.6	35 35 43.8	278 m
Bucak (BUC)	37 26 54.4	35 54 19.5	66 m	Zerdali (ZER)	37 24 18.2	35 37 51.2	292 m
Tumlu (TUM)	37 08 49.9	35 42 26.3	66 m	Sofular (SOF)	37 22 53.9	36 14 20.0	316 m
Küçük Tüysüz (KTUY)	37 02 57.0	36 05 31.4	78 m	Kızyusuflu (KIZ)	37 19 54.8	36 12 36.7	373 m
Baklalı (BAK)	37 02 11.2	35 38 16.6	124 m	Akçakoyunlu (AKC)	37 11 15.4	36 25 13.3	373 m
Koyunevi (KOY)	37 17 21.3	35 39 23.6	146 m	Gedikli (GED)	37 30 10.1	35 51 40.5	381 m

RESULTS

Analysis of ITS2 sequences

Summary of genetic diversity indices, neutrality tests and results of hierarchical analysis of molecular variance (AMOVA) for all species using ITS2 gene sequences is shown in Table 3.

The total length of sequences after alignment and pruning were 490, 480 and 400 bp for *P. papatasi*, *P. sergenti* and *P. tobbi* respectively. Sequences were A-T rich in all species (71.33% for *P. papatasi*; 71.27% for *P. sergenti*; 68.15% for *P. tobbi*). Appendix 1 contains the unique haplotypes obtained from ITS2 sequences for all species.

Within *P. papatasi,* we determined seven unique haplotypes containing 10 polymorphic sites with no indels. Total number of substitutions was 10 (5 transitions and 5 transversions). Nucleotide diversity was generally low (Θs=3.075 ± 1.437; $\Theta \pi$=2.057 ± 1.368) however total haplotype diversity was high 0.781 ± 0.102. The distribution of *P. papatasi* haplotypes within the Cukurova region is shown in Figure 1A. Haplotype 5 (H5) was the most widespread haplotype fallowed by H3 while H2 and H6 were only found in one village.

Within *P. sergenti* we determined eight haplotypes containing 6 variable sites with no indels. Total number of substitutions was 6 with a transition-transversion ratio of 5:1. Similar to *P. papatasi,* nucleotide diversity was low (Θs=1.887 ± 0.996; $\Theta \pi$=1.725 ± 1.199). Haplotype distributions are shown in Figure 1C. H3 was the most common haplotype while other haplotypes were mostly unique to one sampling location. Haplotype diversity was highest in the Kızyusuflu (KIZ) village (4 haplotypes).

Within *P. tobbi* we determined 14 unique haplotypes containing 22 polymorphic sites with five indels. A total of 17 substitutions was observed with a transition to transversion ratio of 13:4. Nucleotide diversity was relatively high (Θs=5.028 ± 2.093; $\Theta \pi$=4.044 ± 2.375) and haplotype diversity was 0.926 ± 0.058. The distribution of *P. tobbi* haplotypes is given in Figure 1E, with nearly all haplotypes unique to sampling locations. The most widespread haplotype was H5 that was sampled from three locations.

Separate AMOVA analyses of *P. papatasi*, *P. sergenti* and *P. tobbi* populations, revealed no genetic structuring between populations within the Cukurova region (Table 3). In all species, within population genetic variation was higher than between population variation and variance components for between population variation was non significant (*P. papatasi*, 31.95%, Va=0.347, p=0.122; *P. sergenti,* -12.46%, Va=-0.106; p=0.653; *P. tobbi*, 40.27%, Va=0.824, p=0.073). In *P. sergenti* Fst values were extremely low and non-significant (Fst = -0.126, P = 0.105). Fst values for *P. papatasi* and *P. tobbi* were higher indicating a weak structuring between populations ($Fst_{P. papatasi}$=0.320; p=0.014; $Fst_{P. tobbi}$=0.402; p=0.008). Overall AMOVA tests conducted separately for all three species suggests that there is no substantial isolation between populations within species.

Analysis of cyt b sequences

Summary of genetic diversity indices, neutrality tests and results of hierarchical analysis of molecular variance (AMOVA) for all species using cyt b gene sequences is shown in Table 3.

Table 2. Haplotype distributions of ITS2 and cyt b gene regions for the three species. H: ITS2 haplotypes; h: cyt b haplotypes. Frequencies of haplotypes are in parentheses.

Villages	Altitude	*P. papatasi*		*P. sergenti*		*P. tobbi*	
		ITS2	cyt b	ITS2	cyt b	ITS2	cyt b
Kulak (KUL)	1 m		h3				
Dedepınarı (DDP)	4 m	H2, H3	h1, h3				
Adalı (ADA)	7 m					H7	h7
Yemişli (YEM)	9 m					H1, H2, H3	h1, h2, h3,h4
Eğriağaç (ERA)	9 m					H6	h6
Ziyalı (ZIY)	18 m	H3					
Doğankent (DK)	20 m	H4(4), H5, H6	h3(6)				
Yenice (YEN)	42 m	H1	h3				
Hamamköy (HAM)	45 m						h14, h15
Narlık (NAR)	48 m					H5	h5(2)
İsalı (ISA)	62 m					H5	h8
Bucak (BUC)	66 m						h13
Tumlu (TUM)	66 m					H10	h11
Küçük Tüysüz (KTUY)	78 m						h5
Baklalı (BAK)	124 m					H13	h5
Koyunevi (KOY)	146 m		h3				
Döşeme (DOS)	175 m						h17
Camili (CAM)	181 m	H3, H4	h4		h5	H9	h9, h10
Tepeçaylak (TCK)	188 m	H4	h1, h2				
Tepecikören (TEP)	189 m		h3, h6	H2, H8	h5		h2(2), h12
Tehçi	192 m					H11	h16
Karakütük (KKK)	217 m						h2
Sarımazı (SMZ)	222 m			H1, H2	h1, h2		
Dutlupınar (DUT)	222 m			H3, H4	h1, h2		
Otluk (OT)	237 m	H6	h5				
Gökbüket (GOK)	244 m					H8	h2
Aydın (AY)	278 m	H4	h7	H3	h2(2)	H12	h2
Zerdali (ZER)	292 m			H3	h7		h18
Sofular (SOF)	316 m				h6	H10	h5
Kızyusuflu (KIZ)	373 m			H3, H5, H6, H7	h6(3)	H4, H5	h2, h5
Akçakoyunlu (AKC)	373 m			H3, H4	h2		
Gedikli (GED)	381 m			H8	h3, h4	H14	h13
Total number of haplotypes		6	7	8	7	14	18
Total number of specimens		15	20	14	16	17	31

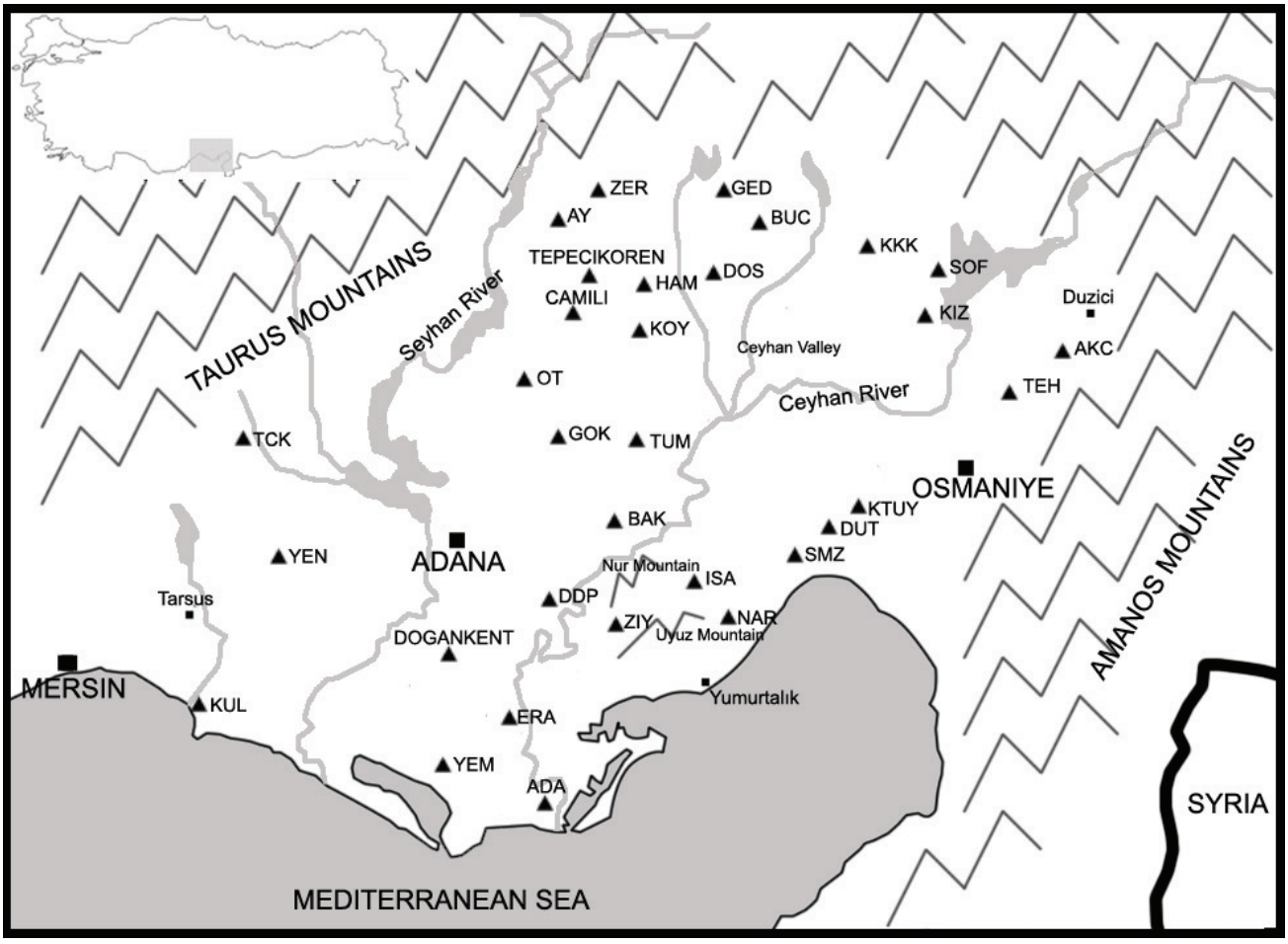

Figure 1. Map of the study area showing the main sites sampled, the main geographical barriers between the sites, cities. Triangles indicate the sampling sites.

The total length of sequences after alignment and pruning were 639, 625 and 653 bp for *P. papatasi*, *P. sergenti* and *P. tobbi,* respectively. Similar to ITS2, the sequenced region was A-T rich for all species (70.23% for *P. papatasi*; 69.84% for *P. sergenti*; 73.85% for *P. tobbi*). Appendix 2 contains unique haplotypes obtained from cyt b sequences for all species.

Within *P. papatasi,* we determined seven unique haplotypes containing seven polymorphic sites with no indels. Total number of substitutions was five (one transition and one transversion). Nucleotide diversity was generally low (Θs = 1.409 ± 0.758; $\Theta \pi$ = 0.936 ± 0.749) and haplotype diversity was moderate compared to ITS2 sequences (0.516±0.132). The distribution of *P. papatasi* haplotypes within the Cukurova region is shown in Figure 1B. Haplotype 3 (h3) was the most widespread haplotype within the region followed by Haplotype 1 (h1) sampled from 2 locations. All other haplotypes were unique to their respective sampling locations.

Within *P. sergenti,* we determined seven unique haplotypes containing six polymorphic sites with one indel. Total number of substitutions was six (four transitions and two transversions). Nucleotide diversity was generally low (Θs=1.887 ± 0.996; $\Theta \pi$=1.725 ± 1.199) however haplotype diversity was high 0.850 ± 0.060. The distribution of *P. sergenti* haplotypes within the Cukurova Region is shown in Figure 1D. Haplotype 2 (h2) was the most widespread haplotype within the region sampled from 4 of the 10 locations.

Among all three species, *P. tobbi* had the highest number of unique haplotypes (18) with a total of 68 polymorphic sites with no indels. The total number of substitutions was 68 (46 transitions and 23 transversions). Nucleotide diversity was substantially higher than the other two species (Θs=17.021 ± 5.518; $\Theta \pi$=9.815 ± 5.137) and haplotype diversity was 0.905 ± 0.038). Figure 1F shows the distribution of *P. tobbi* haplotypes in the study area. h2 and h5 were the most common and widespread haplotypes while the remaining haplotypes were mostly sampled from one location. Haplotype diversity was highest in the Yemisli (YEM) village with four haplotypes.

Results of separate AMOVA analyses of *P. papatasi*, *P. sergenti* and *P. tobbi* populations are given in Table 3. AMOVA analysis revealed significant genetic structuring between populations for *P. papatasi* and *P. sergenti*. In these species, between population genetic variation was higher than within population genetic variation and the associated variance components were significant (*P. papatasi*, 67.06%,

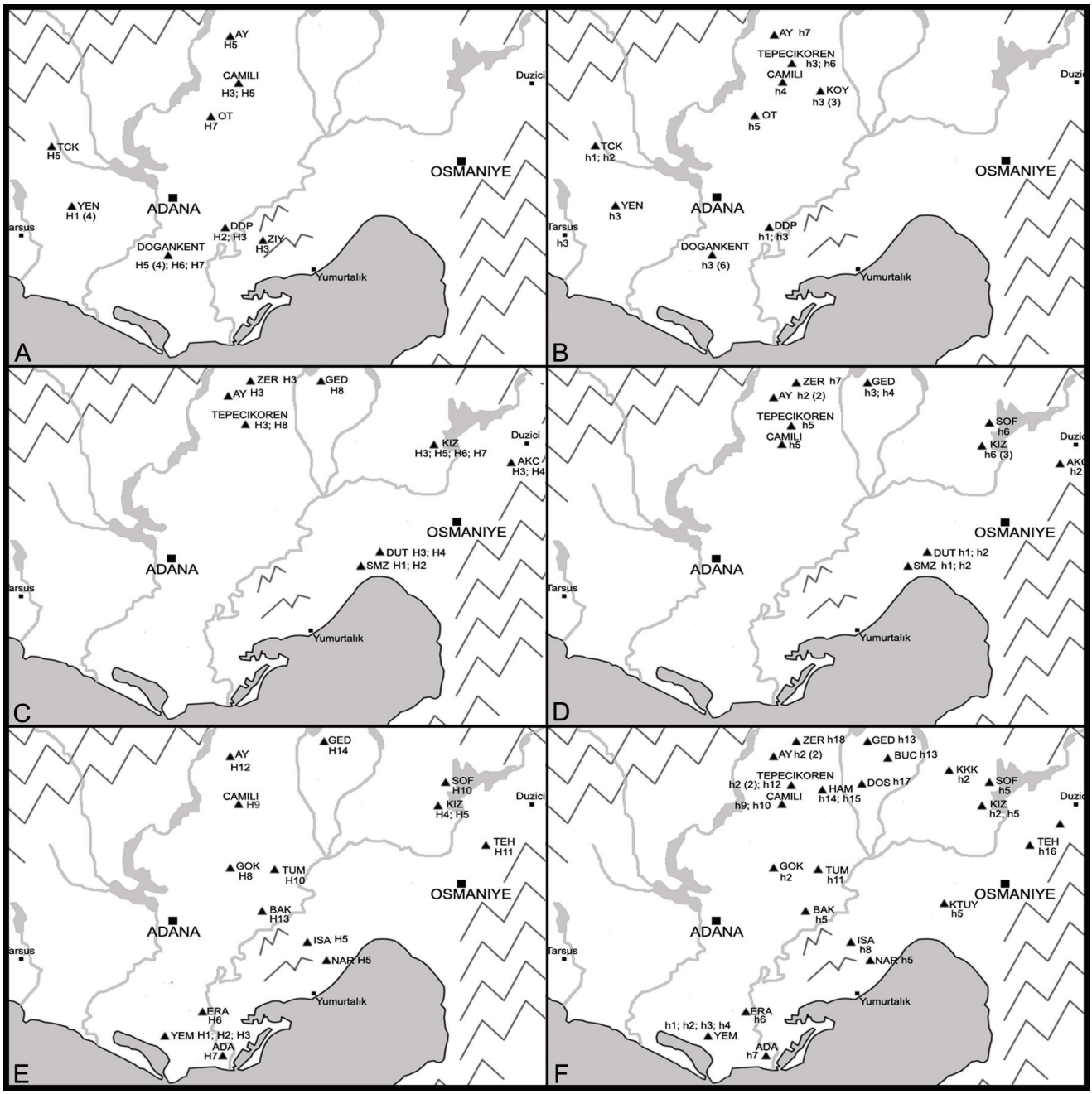

Figure 2. Haplotype distributions of ITS2 and cyt b gene regions for the three species. H: ITS2 haplotypes; h: cyt b haplotypes. **A**: *P. papatasi*-ITS2; **B**: *P. papatasi*-cyt b; **C**: *P. sergenti*-ITS2; **D**: *P. sergenti*-cyt b; **E**: *P. tobbi*-ITS2; **F**: *P. tobbi*-cyt b. H: ITS2 haplotypes; h:cyt b haplotypes. Frequencies of haplotypes are given in parenthesis.

Va = 0.339, p = <0.001; *P. sergenti*, 66.67%, Va = 0.500, p = <0.001) variation and variance components for between population variation was not significant (*P. papatasi*, 31.95%, Va=0.347, p=0.122; *P. sergenti,* -12.46%, Va=-0.106; p=0.653; *P. tobbi*, 40.27%, Va=0.824, p=0.073). In both *P. papatasi* and *P. sergenti*, Fst values were relatively high and significant (*P. papatasi*, Fst = 0.671; p = 0.003; *P. sergenti*, Fst = 0.666; p < 0.001) indicating a certain degree of isolation between populations. In *P. tobbi* however, we did not detect any significant population genetic structuring, as most of the variation was attributed to within population variation (82%). Genetic variation between populations was low (17%) and the related variance component was not significant (Va = 0.875, p = 0.277). Fst values were also low for populations of *P. tobbi* (Fst = 0.177; p <0.001) indicating little population isolation.

Results of neutrality tests designed to assess whether nucleotide polymorphism deviated from expectation under neutrality for all species are shown in Table 3. Tajima's D and Fu's Fs values were negative and significant in all three species. The significant negative values of these test statistics might indicate that the Cukurova populations of *P. papatasi*, *P. sergenti* and *P. tobbi* are undergoing processes of demographic expansion.

DISCUSSION

This study is the first concerning the genetic structure of sand fly populations within Turkey. ITS2 and cyt b gene sequences that are useful markers for intra population level studies were used in order to determine the genetic diversity and structures of *P. papatasi, P. sergenti,* and *P. tobbi* populations. In this study, sequences were A-T rich, as expected, which is a common observation for both gene regions (Simmons and Weller 2001, Parvizi and Ready 2006, Depaquit et al. 2002, 2008).

Analyses conducted using the ITS2 gene region revealed no genetic structuring between populations of *P. papatasi, P. sergenti and P. tobbi* in the Cukurova Region. In contrast, analyses conducted using the cyt b gene region determined the presence of genetic structuring in *P. papatasi* and *P. sergenti* populations. However, even though AMOVA analyses returned positive results for *P. papatasi* and *P. sergenti* populations, haplotype distributions did not support this conclusion since haplotype distribution was uniform with no apparent geographic clustering. Therefore, we can conclude that even if there is some genetic structuring in *P. papatasi* and *P. sergenti* populations according to the cyt b gene region, this is weak at best.

P. papatasi has a widespread distribution between the 20th and 45th latitudes and can be found throughout much of Europe, Asia, and Africa. One would expect such a widespread taxa to have some sort of genetic structuring within its distributional range, but studies dealing with

Table 3. Summary of molecular results for ITS2 and cyt b gene regions of the three species.

Species	*P. papatasi*		*P. sergenti*		*P. tobbi*	
Gene region	ITS2	cyt b	ITS2	cyt b	ITS2	cyt b
Number of individuals	15	20	14	16	17	31
Fragment length (bp)	490	639	480	625	400	653
A-T ratio (%)	71,33	70,23	71,27	69,84	68,15	73,85
G-C ratio (%)	28. 67	29,77	28,73	30,16	31,85	26,15
Number of haplotypes	7	7	8	7	14	18
Haplotype diversity	0.781±0.102	0.516±0.132	0.890±0.060	0.850±0.060	0.926±0.058	0.905±0.038
Number of polymorphic sites	10	7	6	6	22	68
Substitutions	10	5	6	6	17	68
Transition	5	4	5	4	13	46
Transversion	5	1	1	2	4	23
Nucleotide diversity	High	Low	Low	Low	High	High
θS	3.075±1.437	1.409±0.758	1.887±0.996	1.808±0.941	5.028±2.093	17.021±5.518
θπ	2.057±0.102	0.936±0.749	1.725±1.199	1.441±1.036	4.044±2.375	9.815±5.137
Source of variation						
Among populations (%)	31,95	67,06	-12,46	66,67	40,27	17,75
Within populations (%)	68,05	32,94	112,46	33,33	59,73	82,25
Fst	0.320; p=0.014	0.670; p=0.002	-0.126; p=0.105	0.666; p=0.000	0.402; p=0.008	0.177; p=0.000
Isolation	Low	High	Not significant	High	Low	Low
Neutrality analyses						
Tajima - D	-	-1,460	-	-0,694	-	-1,590
Fu FS	-	-3,242*	-	-26,060*	-	-24,550*
Expansion	-	Significant	-	Significant	-	Significant

the genetic diversity of *P. papatasi* have revealed little to no genetic variation within this species distributional range (Esseghir et al. 1997, Parvizi and Ready 2006, Depaquit et al. 2008). Thus, it was not surprising that we found a similar result with *P. papatasi* populations showing little to no genetic structuring within the Cukurova basin, a small and closed area. Esseghir et al. (1997) determined the presence of 16 haplotypes in 27 *P. papatasi* specimens collected from 12 countries including India, Iraq, Syria, Cyprus, Spain, Italy, Morocco and Egypt. The papa1 haplotype from the Mediterranean basin was determined to be the ancestral sequence, but nucleotide diversity between haplotypes were low as they were separated from each other by only one to four nucleotide substitutions Researchers explained the lack of genetic structure within the distributional range of *P. papatasi* by the absence of vicariance in Mediterranean basin and they concluded that the Mediterranean Sea is not an effective barrier to prevent the dispersal of this species (Esseghir et al. 1997). Congruently, Parvizi and Ready (2006) tested for the presence of isolation by distance within *P. papatasi* populations in Iran. However, they found no support for isolation by distance and gene flow seemed to continue between geographically separated populations. In their study concerning the molecular homogeneity of different geographical populations of *P. papatasi*, Depaquit et al. (2008) collected *P. papatasi* specimens from 18 countries and revealed a few number of ITS2 and ND4 (mtDNA) haplotypes shared between populations with no geographic connections. One study where the presence of some genetic diversity was found within *P. papatasi* was that of Hamarsheh et al. (2007) in which researchers found significant genetic differentiation between distantly separated populations of *P. papatasi* in relation to mtDNA cyt b sequences. Hamarsheh et al. (2007) stated that the main factor determining the degree of genetic diversity was latitude and not climatic conditions. Researchers also concluded that the determined level of genetic differentiation was not enough to support the existence of a species complex.

The known vector of *L. tropica* in Southeastern Anatolia, Turkey, *P. sergenti*, showed no geographical structuring within the Cukurova basin in terms of the distribution of haplotypes obtained from both ITS2 and cyt b gene regions. As one of the most widely distributed sand fly species, *P. sergenti* has a patchy distribution within its range and sub-populations are significantly different in vector capacities (Depaquit et al. 1998). It has also been pointed out that the distribution of the species is wider than epidemics of leishmaniasis and that this taxon should be treated as a species complex. Depaquit et al. (2000, 2002) revealed *P. sergenti* populations to be separated in two major lineages pertaining to northeast and south and west populations according to ITS2 sequence variation. They also suggested that the northeast branch is the ancestral clade showing no or very little between population variations, although there are marked differences between populations in terms of ecology, host preference and potential vector capacity. In addition, ITS2 sequences of three populations sampled from Turkey (one population from Adana and two populations from Şanlıurfa) were totally identical. Dvorak et al. (2006) using laboratory colonies (Turkey and Israel) of *P. sergenti* investigated the two clades in the above study using molecular (RAPD and ITS2 sequences), morphometric (wing shape and size) and cross-mating methods. They found variation between sub groups of Israeli colonies and significant wing deformations between Israeli and Turkish colonies. Also, F1 progeny obtained from the cross-mating experiments formed a distinct group with an intermediate position between the Turkish and Israeli groups, indicating that these two populations, although geographically separated, have not reached reproductive isolation. Yahia et al. (2004) collected populations from different altitudes in Morocco but could not find any significant difference between haplotype frequencies in relation to altitude. However, Baron et al. (2008) detected high diversity within Spanish populations of *P. sergenti.* In a similar study, Moin-Vaziri et al. (2007) showed that the three different monotypes of *P. sergenti* within Iran showed no significant genetic differentiation.

Among the three species evaluated in this study, *P. tobbi* showed the highest number of polymorphic sites and nucleotide diversity. Although haplotype diversity was high in both gene regions, haplotypes showed no geographical structuring within the Cukurova basin. The findings showing *P. tobbi* to be the vector of *L. infantum* in the study area have increased the importance of this species (Svobodova et al. 2009). The antropophilic property of the species may stem from the fact that due to the socio-economical conditions prevalent within the region, humans live in close proximity to animals and animal shelters.

Genetic researches conducted on the *Larroussius* subgenus have usually concentrated on phylogenetic, identification of species complexes or phylogeographic assessments of taxa. Esseghir et al. (1997, 2000) recorded low between-population variation for two *Larroussius* species, *P. perniciosus* and *P. perfiliewi*. West Mediterranean populations of *P. perniciosus* and *P. tobbi* were similar to each other both morphologically and genetically, and their populations sampled from Tunisia, Malta, and Italy also showed low haplotype diversity. On the other hand, Parvizi and Assmar (2007) used the nuclear elongation factor -1α" (EF-1α) and detected seven haplotypes from eight specimens of *P. tobbi* from Iran, thereby indicating that the diversity was quite high despite the narrow distribution of the species. This study supports our findings of high haplotype diversity of *P. tobbi* in The Cukurova region.

Statistically significant and negative Tajima's D and Fu's Fs values suggest an excess of rare alleles, population expansion (demographic instability), or purifying selection at the sequenced loci (Nei and Kumar 2000, Paupy 2008). In this study, statistics showed negative values for cyt b gene loci but only Fu's Fs statistics were significant indicating that population expansion more than selection might be responsible for deviations from neutrality, since Fu's Fs value is highly sensitive to range expansions, which lead to high negative values (Fu 1997, Ramos-Onsins and Rozas 2002). Simsek et al. (2007) has claimed that the Taurus and

Amanos mountains surrounding the Cukurova basin do not act as barriers limiting the dispersal of sand fly populations. Therefore, our results suggest that vector species within the Cukurova basin are increasing their distributional range. This will undoubtably have important consequences for the spread of vector borne diseases to neighboring areas. Accordingly future studies will be conducted using a larger number of loci and individuals and will focus on the whole of Turkey together with neighboring biogeographic regions. This will enable us to extract not only more detailed and reliable results but will also help us frame our results on a more global scale.

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Appendix 1

Haplotypes obtained from ITS2 gene region.

A: *Ph. papatasi*

```
          10        20        30        40        50        60        70        80        90       100
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H1  AGCTAACTGTGTGAAATCGTGTGAACTGCAGGACACATGAACATCGACATTTTGAACGCATATTGCGGTCCATGCAAAAGTTTAAATTTTTGTTTAA
P_H2  ....................................................................................................
P_H3  ....................................................................................................
P_H4  ....................................................................................................
P_H5  ....................................................................................................
P_H6  ....................................................................................................
P_H7  ....................................................................................................

         110       120       130       140       150       160       170       180       190       200
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H1  ACTGCATGGACCACGTATGGTTGAGTGTTGTAAATATTAAGCAATTGAAATTGTTTTTATACATTTGTATAAAAACATTGGAGGTATGGAATTATATTTC
P_H2  ..........................C.....................................................C.................
P_H3  ............C.............C.....................................................G.C.................
P_H4  ..........................C.....................................................C.................
P_H5  ..........................C.....................................................C.................
P_H6  ..........................C.....................................................C.................
P_H7  ..........................C.....................................................C.................

         210       220       230       240       250       260       270       280       290       300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H1  ATGCTCTTAATATATATTTATTTTT?AAAGCACATTTGAATGTACCCAATGTATAATATTAAAAGTTGAAATATATATATAAGGGGTATATCATAGTCAT
P_H2  ................??.....T.....................................................................
P_H3  ...............A.?.....T.....................................T.....................................
P_H4  ................??.....T.....................C...............................................
P_H5  ................???....T.....................................................................
P_H6  ................????...C.....................................T.....................................
P_H7  ...............A.?.....T.....................................................................

         310       320       330       340       350       360       370       380       390       400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H1  TGGATAATTTATTTTAAAGACATATTGTTATACAGAAAAACTTATTATATTAAAAAACTTTTAATAAAATGGGATTATTCAATATAATAATGTGCTACAA
P_H2  C...........C.....................................................................................
P_H3  ............C.....................................................................................
P_H4  ............C.........................C.....................................................................
P_H5  ............C.....................................................................???.............
P_H6  ............C.....................................................................................
P_H7  ............C.....................................................................................

         410       420       430       440       450       460       470       480       490
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H1  TAATTTAATATTATTGCGATCTCAACTCATACGTGACTACCCCCTGAATTTAAGCATATTAATAAGCGGAGGAAAAGAAAC?TAACCAGG
P_H2  ..........................................................................?.............
P_H3  ..........................................................................A.............
P_H4  ..........................................................................?.............
P_H5  ..........................................................................?.............
P_H6  ..........................................................................?.............
P_H7  ..........................................................................?.............
```

B: *Ph. sergenti*

```
          10        20        30        40        50        60        70        80        90       100
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1  CGCAGCTAACTGTGTGAAATCGTGTGAACTGCAGGACACATGAACATCGACATTTTGAACGCATATTGCGGTCCATGCAAAAGTTTAAACTTGTTTAAAC
S_H2  ..............................................................................?.....................
S_H3  ....................................................................................................
S_H4  ....................................................................................................
S_H5  ....................................................................................................
S_H6  ....................................................................................................
S_H7  ....................................................................................................
S_H8  ....................................................................................................
```

```
           110       120       130       140       150       160       170       180       190       200
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 TGCATGGACCACGTATGGTTGAGTATCGTAAATATTAAGCAATTGAAATTGTTTTTTTTCTTCTATTTCTCTATAGAAAAAGAAAACATTGGAGTTATGA
S_H2 ....................................................................T.....................................
S_H3 ....................................................................T.....................................
S_H4 ....................................................................T.....................................
S_H5 ....................................................................T.....................................
S_H6 ....................................................................T.....................................
S_H7 ....................................................................T.....................................
S_H8 ....................................................................T.....................................

           210       220       230       240       250       260       270       280       290       300
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 AATTTTTTTTCATGCTCTTAATATGTATTAAAGTATATTTGAATGTACCCAATATATATATATATTAAATTAAAAAGAATATATGTGGTATATCATAGTC
S_H2 ....................................................C.....................................C.....
S_H3 ....................................................T.....................................T.....
S_H4 ....................................................C.....................................C.....
S_H5 ....................................................T.....................................T.....
S_H6 ....................................................T.....................................T.....
S_H7 ....................................................T.....................................T.....
S_H8 ....................................................C.................G.....................C.....

           310       320       330       340       350       360       370       380       390       400
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 ATTGAATTATATCTTGCCATTGTTATACAAAACGTATATATATTTTATATTTAATATAGGGATTATTCATATAAGAAAATGTGCAAAATAAAAAATTATT
S_H2 ..........................G.....................................................................G.....
S_H3 ..........................G.....................................................................G.....
S_H4 ....................................................................T.....................G.....
S_H5 ....................................................................T.....................A.G.....
S_H6 ....................................................................T.....................G.....
S_H7 ....................................................................T.....................G.....
S_H8 ....................................................................T.....................G.....

           410       420       430       440       450       460       470       480
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 TTAATTGCGATCTCAACTCATACGTGACTACCCCCTGAATTTAAGCATATTAATAAGCGGAGGAAAAGAAACTAACCAGG
S_H2 ....................................................................T.....
S_H3 ....................................................................T.....
S_H4 ....................................................................T.....
S_H5 ....................................................................T.....
S_H6 ....................................................................T.....
S_H7 ....................................................................T.....
S_H8 ....................................................................T.....
```

C: *Ph. tobbi*

```
             10        20        30        40        50        60        70        80        90        10
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1   TGTGAACTGCAGGACACATGAACATCGACATTTTGAACGCACATTGCGGTCCATGCTAATGTTTAACTTGTTAAACTGCATGGACCACGTATGGTTGAGT
T_H2   ....................................................................T.....................................
T_H3   ..........................................................-.........T.....................................
T_H4   ....................................................................T.....................................
T_H6   ....................................................................T.....................................
T_H7   ..............................................-.....................T.....................................
T_H8   ....................................................................T.....................................
T_H5   ....................................................................T.....................................
T_H9   ....................................................................T.....................................
T_H10  ....................................................................T.....................................
T_H11  ..............................................-.....................T.....................................
T_H12  ....................................................................T.....................................
T_H13  ....................................................................T.....................................
T_H14  ..............................................-.....................T.....G...............

             110       120       130       140       150       160       170       180       190       200
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1   GTCGTAAATAATAAGCAGAACAAATTGTTTTTATTCTCTATATGGGATTAGAAA-CATTGGAGCTATGTAAGTGTTATACTTTCATGCTCTTAAAGTTTT
T_H2   ................A.....................................-.....................T.....................
T_H3   ................A.....................................-.....................T.....................
T_H4   ................A.....................................-.....................T.....................
T_H6   ................A.....................................-.....................T.....................
T_H7   ....................................................-.....................T.....................
T_H8   ................A.....................................-.....................T.....C...............
T_H5   ................A.....................................-.....................T.....................
T_H9   ................A.....................................-.....................T.....................
T_H10  ................A.....................................-.....................T.....................
T_H11  ................A.....................................-.....................T.....................
T_H12  ................A.....................................-.....................T.....................
T_H13  ................A.....................................A.....................C.....................
T_H14  ..........C.....A.....................A.........A.....................T.....................
```

```
                 210       220       230       240       250       260       270       280       290       300
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1        TTTTTGACAACGCATTTGAATGTACTCAATGAAAAATGTAATAAAACACACATTGAGTGTAACATCAAAATCATTGCATAAATGTAAAGATCTTTGTTAC
T_H2        ..........................................................................................................
T_H3        ..........................................................................................................
T_H4        ..........................................................................................................
T_H6        ........AC.......................G..........................................................................
T_H7        ........AC..........................................................................................
T_H8        ..........................................................................................................
T_H5        ..........................................................................................................
T_H9        ..........................................................................................................
T_H10       ..........................................................................................................
T_H11       ........................................-.................................................................
T_H12       ..........................................................................................................
T_H13       ..................................................................T.....................A...........
T_H14       ..........................................................................................................

                 310       320       330       340       350       360       370       380       390       400
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1        ATTCAGAGTTTTATTACTATGAGATTATTCATAAGAATTGTGTATCTATATATATATTAAAGCGACCTCAACTCATGCGTGACTACCCCCTAAATTTAAG
T_H2        .....A.....................................................................A.....................
T_H3        ..........................................................................A.....................
T_H4        ..........................................................................................G.....
T_H6        ......................................-...................................A.....................
T_H7        .....................................-....................................A.....................
T_H8        ..........................................................................A.....................
T_H5        ......................................-...................................A.....................
T_H9        ............G.............................................................A.....................
T_H10       ..........................................................................A.....................
T_H11       ........................................A.G...............................A.....................
T_H12       ..........C.................................................................A.....................
T_H13       ..........................................................................A.....................
T_H14       ......................................-.....................................
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Appendix 2

Haplotypes obtained from cyt b gene region.

A: *Ph. papatasi*

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                10        20        30        40        50        60        70        80        90       100
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H2  CCCTTATCTAGGAACAATATTAGTTCAATGAATCTGAGGAGGATTTGCTGTAGATAATGCAACTTTAACACGATTTTTTACATTCCACTTTTTATTCCCA
P_H3  ....................................................................................................
P_H6  ....................................................................................................T...
P_H1  ....................................................................................................
P_H7  ....................................................................................................
P_H5  ....................................................................................................
P_H4  ....................................................................................................

               110       120       130       140       150       160       170       180       190       200
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H2  TTTATTATTGCTGCTATAACTATAATTCATTTATTATTCCTCCATCAAACAGGTTCTAATAACCCCTTAGGATTAAATAGAGATTCAGATAAAATCCCCT
P_H3  ....................................................................................................
P_H6  ....................................................................................................
P_H1  ....................................................................................................
P_H7  ....................................................................................................
P_H5  ....................................................................................................
P_H4  ....................................................................................................

               210       220       230       240       250       260       270       280       290       300
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H2  TTCATCCTTATTTCTCTTTTAAGGATTTAATTGGATTTATTGTTATAATTATAATATTAAGAATTCTAACAATCACAGCCCCTTATTTTCTTGGAGATCC
P_H3  ....................................................................................................
P_H6  ....................................................................................................
P_H1  ....................................................................................................
P_H7  ..............................G.....................................................................
P_H5  ....................................................................................................
P_H4  ....................................................................................................

               310       320       330       340       350       360       370       380       390       400
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H2  AGATAATTTTATTCCAGCAAATCCTCTTGTAACCCCTCCTCATATTCAACCAGAATGATACTTCCTATTTGCTTATGCAATTTTACGTTCAATTCCTAAT
P_H3  ....................................................................................................
P_H6  ....................................................................................................
P_H1  ....................................................................................................
P_H7  ....................................................................................................
P_H5  ....................................................................................................
P_H4  ....................................................................................................

               410       420       430       440       450       460       470       480       490       500
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H2  AAATTAGGAGGAGTAATTGCCCTTGTTATATCAATTGCTATCCTTTTCCTTATACCTTTACTCCATACAAACCAATCACAAGGACTTCAATTTTACCCGT
P_H3  ..........................................................T.........................A.
P_H6  ..........................................................T.........................A.
P_H1  ..........................................................T..........................
P_H7  ..........................................................T..........................
P_H5  ..........................................................T.........................A.
P_H4  ....................................................T...T.........................A.

               510       520       530       540       550       560       570       580       590       600
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P_H2  TAAATCAAATCCTATTCTGATATATAGTAATTACTATTATTCTATTAACATGAATCGGAGCTCGTCCTGTTGAAACTCCTTATATTTTAACAGGACAAAT
P_H3  ....................................................................................................
P_H6  ....................................................................................................
P_H1  ....................................................................................................
P_H7  ....................................................................................................
P_H5  ....................................................................................................
P_H4  ....................................................................................................

               610       620       630
      ....|....|....|....|....|....|....|....
P_H2  TTTAACTGTTCTTTACTTCTCTTATTATATTTTAAATCC
P_H3  ....................................
P_H6  ....................................
P_H1  ....................................
P_H7  ....................................
P_H5  ....................................
P_H4  ....................................
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B: *Ph. sergenti*

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           10        20        30        40        50        60        70        80        90       100
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 CCCTATATTAGTTCAATGAATTTGAGGAGGATTTGCAGTAGATAATGCAACCCTTACCCGATTTTTTACTTTTCATTTTCTGTTTCCCTTCATTATCGCA
S_H2 ....................................................................................................
S_H3 .A..................................................................................................
S_H4 .A..................................................................................................
S_H5 ....................................................................................................
S_H6 ..........................................................C.........................................
S_H7 ....................................................................................................

           110       120       130       140       150       160       170       180       190       200
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 GCAATAACTATAATTCACTTATTATTTTTACATCAAACAGGCTCTAATAACCCCCTAGGATTAAATAGAAACTCAGATAAAATCCCGTTTCATCCTTATT
S_H2 ....................................................................................................
S_H3 ....................................................................................................
S_H4 ....................................................................................................
S_H5 ....................................................................................................
S_H6 ....................................................................................................
S_H7 ............C.......................................................................................

           210       220       230       240       250       260       270       280       290       300
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 TTTCTTTCAAAGACTTAATTGGATTCATTATTATAATTATAATATTAAGAATTCTAACAATTATAGCTCCTTACTATTTAGGAGATCCAGATAATTTTAT
S_H2 ....................................................................................................
S_H3 ....................................................................................................
S_H4 ....................................................................................................
S_H5 ....................................................................................................
S_H6 ....................................................................................................
S_H7 ....................................................................................................

           310       320       330       340       350       360       370       380       390       400
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 TCCAGCAAATCCTTTAGTTACCCCTCCTCATATTCAACCTGAATGATATTTTTTATTT-GCTTATGCAATTTTACGGTCAATTCCTAATAAACTTGGAGG
S_H2 ....................................................-.....................................
S_H3 ....................................................-.....................................
S_H4 ....................................................-.....................................
S_H5 ....................................G...............-.....................................
S_H6 ....................................................-.....................................
S_H7 ....................................................-.....................................G..

           410       420       430       440       450       460       470       480       490       500
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 AGTAATTGCTCTTGTCATATCTATTGCAATTCTATTTTTAATACCCCTTTTACACACTAACAAAACCCAGGGTCTTCAATTTTACCCACTAAATCAAATT
S_H2 ....................................................G.....................................
S_H3 ....................................................................................................
S_H4 ....................................................G.....................................
S_H5 ....................................................G.....................................
S_H6 ....................................................G.....................................
S_H7 ....................................................G.....................................

           510       520       530       540       550       560       570       580       590       600
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S_H1 TTATTCTGATATATAGTAATTATAATTCTTCTATTGACTTGAATTGGGGCCCGTCCAGTAGAAGCTCCCTATATCATTACTGGTCAAATTTTAACTATTC
S_H2 ....................................................................................................
S_H3 ....................................................................................................
S_H4 ....................................................................................................
S_H5 ....................................................................................................
S_H6 ....................................................................................................
S_H7 ....................................................................................................

           610       620
     ....|....|....|....|....|
S_H1 TTTATTTCTCCTACTATATTATTAA
S_H2 .........................
S_H3 .........................
S_H4 .........................
S_H5 .........................
S_H6 .........................
S_H7 .........................
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C: *Ph. tobbi*

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                10        20        30        40        50        60        70        80        90       100
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1       CCCTTATTTAGGAAATATATTAGTGCAATGAATTTGAGGAGGATTTGCTGTAGATAATGCTACTCTTACACGATTTTTTACTTTTCATTTTCTATTCCCT
T_H2       ....................................................................................................
T_H3       ....................................................................................................
T_H4       ....................................................................................................
T_H5       ....................................................................................................
T_H6       ....................................................................................................
T_H7       ....................................................................................................
T_H8       ....................................................................................................
T_H9       .......C.............T.............T..G.............A.................C.........T....T...
T_H10      ....................................................................................................
T_H11      ....................................................................................................
T_H12      ....................................................................................................
T_H13      ....................................................................................................
T_H14      ....................................................................................................
T_H15      ......................A.............................................................................
T_H16      ....................................................................................................
T_H17      ....................................................................................................
T_H18      .......C.............T.............G.............A.................C.........T....T...

               110       120       130       140       150       160       170       180       190       200
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1       TTTATTATTGCTGCAATAACTATAATTCATTTATTATTTTTACATCAAACAGGATCAAATAATCCTCTAGGATTAAATAGAAATTCAGATAAAATTCCTT
T_H2       ....................................................................................................
T_H3       ....................................................................................................
T_H4       ....................................C...............................................................
T_H5       ....................................................................................................
T_H6       ....................................................................................................
T_H7       ....................................................................................................
T_H8       ....................................................................................................
T_H9       ............T..............C..................T.....C..AT............T.....T............
T_H10      ....................................................................................................
T_H11      ....................................................................................................
T_H12      ....................................................................................................C....
T_H13      ........G...........................................................................................
T_H14      ....................................................................................................
T_H15      ....................................................................................................
T_H16      ....................................................................................................
T_H17      ........G...........................................................................................
T_H18      ............T..............C................G..T.....C..AT............T.....T............

               210       220       230       240       250       260       270       280       290       300
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1       TTCACCCTTATTTTTCATTTAAAGATATTATTGGATTTATTATTATAATTATAATTTTATCTATTCTATCAATTATAGCTCCTTATTATTTAGGAGATCC
T_H2       ....................................................................C...............................
T_H3       ....................................................................C...............................
T_H4       ....................................................................C...............................
T_H5       ....................................................................................................
T_H6       ....................................................................................................
T_H7       ....................................................................C...............................
T_H8       ..........................G.........................................C...............................
T_H9       .C..T.....................................G.....................T....T....C...C...............................
T_H10      ....................................................T.............C...............................
T_H11      ....................................................................C...............................
T_H12      ....................................................T.............C...............................
T_H13      ....................................................................................................
T_H14      ....................................................................C...............................
T_H15      ....................................................................C...............................
T_H16      ....................................................................C...............................
T_H17      ....................................................................C...............................
T_H18      .C..T.....................................G.....................T....T....C...C...............................
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           310       320       330       340       350       360       370       380       390       400
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1  AGATAATTTTATTCCTGCTAATCCATTAGTAACCCCTCCCCATATCCAACCAGAATGATATTTTTTATTTGCCTATGCTATTCTCCGTTCTATTCCCAAT
T_H2  ..................................T.....................................................................C
T_H3  ..................................T.....................................................................C
T_H4  ..................................T.....................................................................C
T_H5  ..................................T.....................................................................
T_H6  ..................................T.....................................................................
T_H7  ..................................T.....................................................................C.....C
T_H8  ..................................T.....................................................................C
T_H9  G.....................T.....T..T.....T.....T.........................T.....A.....T...........T...
T_H10 ..................................T.....................G...............................................
T_H11 ..................................T.....................................................................C
T_H12 ..................................T.....................................................................
T_H13 ..................................T.....................................................................
T_H14 ..................................T.....................................................................
T_H15 ..................................T.....................................................................C
T_H16 ..................................T.....................................................................C
T_H17 ..................................T.....................................................................C
T_H18 G.....................T.....T..T.....T.....T.............C...........T.....A.....T...........T...

           410       420       430       440       450       460       470       480       490       500
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1  AAGTTAGGGGGAGTAATTGCCCTAGTTATATCAATTGCTATTTTATTTATTTTACCAATTTTACATACTAATAAATCACAAGGATTACAATTTTATCCTA
T_H2  ..A.....A.....................................................................................
T_H3  ..A.....A.....................................................................................
T_H4  ..A.....A.....................................................................................
T_H5  ..............................................................................................
T_H6  ..............................................................................................
T_H7  ..A...........................................................................................
T_H8  ..A.....A.....................................................................................
T_H9  ..A.....A...........T..T..A.....................................GT..G....A........C.............
T_H10 ..............................................................................................
T_H11 ..A...........................................................................................A.
T_H12 ..............................................................................................
T_H13 ..............................................................................................
T_H14 ..............................................................................................
T_H15 ..A.....A.....................................................................................
T_H16 ..A.....A.....................................................................................
T_H17 ..A.....A.....................................................................................
T_H18 ..A.....A...........T..T..A.....................................GT..G....A........C.............

           510       520       530       540       550       560       570       580       590       600
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
T_H1  TCAATCAAATTTTATTTTGATATATAGTTATTATTATTGTTTTATTAACATGAATTGGAGCCCGCCCTGTTGAAGCTCCTTTTATTTTAACAGGACAAAT
T_H2  ..............................................................................C.....................
T_H3  ..............................................................................C.....................
T_H4  ..............................................................................C.....................
T_H5  ..............................................................................................
T_H6  ..............................................................................................
T_H7  ...................................A..........................................C.....................
T_H8  ..............................................................................C.....................
T_H9  .T..................................A.........................T.....A.....C..A.............T.....
T_H10 ..............................................................................C.....................
T_H11 ..............................................................................C.....................
T_H12 ..............................................................................C.....................
T_H13 ..............................................................................................
T_H14 ..............................................................................................
T_H15 ..............................................................................C.....................
T_H16 ..............................................................................C.....................
T_H17 ..............................................................................C.....................
T_H18 .T..................................A.........................T.....A.....C..A.............T.....

           610       620       630       640       650
      ....|....|....|....|....|....|....|....|....|....|...
T_H1  TTTAACTGTTCTTTACTTCTCTTATTATATTCTAAATCCTATAATTTCGAAAA
T_H2  ..............................................A....
T_H3  .....................................C........A....
T_H4  ..............................................A....
T_H5  ..............................................A....
T_H6  ..............................................
T_H7  ..............................................A....
T_H8  .....................................C........A....
T_H9  .C....A........T..............T.................A....
T_H10 ..............................................?.A....
T_H11 ..............................................A....
T_H12 ..............................................A....
T_H13 ..............................................A....
T_H14 ..............................................A....
T_H15 ..............................................A....
T_H16 .......A......................................A....
T_H17 ..............................................A....
T_H18 .C....A........T..............T.................A....
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