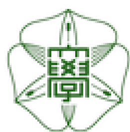
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Genetic divergence in populations of *Lutzomyia ayacuchensis,* a vector of Andean-type cutaneous leishmaniasis, in Ecuador and Peru

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Abstract

Haplotype and gene network analyses were performed on mitochondrial cytochrome oxidase I and cytochrome *b* gene sequences of *Lutzomyia (Lu.) ayacuchensis* populations from Andean areas of Ecuador and southern Peru where the sand fly species transmit *Leishmania (Leishmania) mexicana* and *Leishmania (Viannia) peruviana*, respectively, and populations from the northern Peruvian Andes, for which transmission of *Leishmania* by *Lu. ayacuchensis* has not been reported. The haplotype analyses showed higher intrapopulation genetic divergence in northern Peruvian Andes populations and less divergence in the southern Peru and Ecuador populations, suggesting that a population bottleneck occurred in the latter populations, but not in former ones. Importantly, both haplotype and phylogenetic analyses showed that populations from Ecuador consisted of clearly distinct clusters from southern Peru, and the two populations were separated from those of northern Peru.

Keywords: Phlebotomine sand fly; *Lutzomyia ayacuchensis*; cytochrome oxidase I; cytochrome *b*

1. Introduction

Phlebotomine sand flies are insects of the family Psychodidae in the order Diptera. Approximately 800 sand fly species have been recorded; of these, fewer than 10% have been confirmed as vector species of leishmaniasis. These are in two currently defined genera, *Phlebotomus* in the Old World and the genus *Lutzomyia* in the New World (Munstermann, 2004; Bates, 2007; Kato *et al*., 2010; Alvar *et al*., 2012). Only a restricted number of species support the development of specific *Leishmania* species and consequently transmit the parasites (Kato *et al*., 2010; Ready, 2013). Therefore, surveillance of circulating sand flies is important for predicting the risk and expansion of the disease in endemic and surrounding areas. Sand flies are generally identified by morphologic characteristics; mainly internal structures such as the spermatheca, cibarium and pharynx in females, and terminal genitalia in males (Young and Duncan, 1994). Genetic information on sand flies is accumulating, and several genetic markers have been used to examine the systematics, relationships and evolution among sand fly species (Kato *et al*., 2010). The molecular taxonomy of sand flies mostly supports the traditional morphological classification, and can be applied to the surveillance of circulating species as well as identification of the species responsible for the transmission of *Leishmania* parasites in given endemic areas (Aransay *et al*., 1999; Beati *et al*., 2004; Kato *et al*., 2005, 2007, 2008; Terayama *et al*., 2008; Kuwahara *et al*., 2009; Fujita *et al*., 2012).

In addition to the species differences, intraspecific population divergence caused by multiple environmental factors such as climate, distance, altitude, and geographic barriers is suggested to influence vector competence (Lanzaro *et al*., 1993; Hamarsheh *et al.*, 2009; Ready 2013). Since the maternally inherited mitochondrial genes reflect the

evolutionary history more accurately because of their clonal inheritance, lack of recombination and higher mutation rate compared with nuclear DNA (Avise, 1994; Rokas *et al*., 2003), these genes have been used to estimate the population structure of arthropod vectors, and their geographical variation among populations has been reported in sand flies (Esseghir *et al*., 1997; Ishikawa *et al*., 1999; Hodgkinson *et al*., 2003; Hamarsheh *et al*., 2007; Belen *et al*., 2011; Florin *et al*., 2011; Rocha *et al*., 2011; Cohnstaedt *et al*., 2012; Yamamoto *et al*., 2013; Pech-May *et al*., 2013).

Lutzomyia (Lu.) ayacuchensis is a unique sand fly species distributing mainly in the Andean highlands of Ecuador and Peru (Takaoka *et al*., 1990; Caceres *et al*., 2004; Kato *et al*., 2005, 2008; Gomez *et al*., 2014a,b). The species is a proven vector of *Leishmania (Leishmania) mexicana* in the Ecuadorian Andes (Takaoka *et al*., 1990; Hashiguchi *et al*., 1991; Kato *et al*., 2005, 2008; Gomez *et al*., 2014a,b), whereas the same species transmits *Leishmania (Viannia) peruviana* in Andean areas of southern Peru (Caceres *et al*., 2004). The sand fly populations from Ecuador and Peru were indistinguishable by morphological observation and genomic analysis of the 18S rRNA genes and rRNA internal transcribed spacer (ITS) sequences (Kuwahara *et al*., 2009). *Lutzomyia ayacuchensis* is also distributed in the northern Peruvian Andes where cutaneous leishmaniasis is endemic; however, no transmission of *Leishmania* parasites by sand fly species has been reported in these areas. In the present study, mitochondrial cytochrome oxidase I (COI) and cytochrome *b* (*cyt* b) genes were compared in *Lu. ayacuchensis* populations from Andean areas of Ecuador and northern and southern Peru to assess genetic divergence among populations with different vector competence.

2. Materials and methods

2.1.Sand fly collection

Sand flies were collected in 4 Andean areas of Ecuador; Huigra (1,200 m above sea level), Chanchan (1,500 m a.s.l.), and Alausi (2,300 m a.s.l.), the Province of Chimborazo; and Paute (2,750 m a.s.l.), Province of Azuay (Kato et al., 2008; Kuwahara et al., 2009); and 4 Andean areas of Peru; Higosniyocc, Province of Lucanas, and Saquihuacca (2,250 m a.s.l), Province of Parinacochas, Department of Ayacucho; Zapote (340 m a.s.l.), Province of Lambayeque, Department of Lambayeque; Viza (1,750 m) and El Paraiso (1,400 m a.s.l.), Province of Cutervo, Department of Cajamarca; and La Perla (1,930 m a.s.l.), Province of Huancabamba, Department of Piura (Kato et al., 2008; Kuwahara et al., 2009; Fujita et al., 2012) (Fig.1). All flies were captured between 18:30 and 21:00 by protected human bait, between 18:00 and 22:00 with Shannon traps, and between 19:00 and the next morning at 6:00 by CDC light traps. The sand flies were identified based on the morphology of their spermathecae, measurements of wing veins, the ratio of the palpus length to antenna and the thorax color (Young and Duncan, 1994). These morphologically identified specimens were fixed in absolute ethanol and stored at room temperature for further molecular analysis.

2.2.DNA extraction

Ethanol-fixed sand flies were individually lysed in 50 µl of DNA extraction buffer [150 mM NaCl, 10 mM Tris-HCl (pH 8.0), 10 mM EDTA and 0.1 % sodium dodecyl sulfate (SDS)] with 100 µg/ml of proteinase K. The samples were incubated at 37˚C overnight, heated at 95˚C for 5 min, and then 0.5 µl portions were directly used as the

templates for PCR amplification.

PCR amplification and sequence analysis of Lutzomyia ayacuchensis *cytochrome oxidase I* and *cytochrome* b *genes*

The *Lu. ayacuchensis* COI gene fragment was amplified with universal COI primers (LCO1490: GGTCAACAAATCATAAAGATATTGG and HCO2198: TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al., 1994), and the *cyt* b gene fragment was amplified with primers prepared based on the *Lu. ayacuchensis cyt* b gene sequences (Lay cyt S: TGTCGAGATGTTAACTATGG and Lay cyt R: TGCTATTTAAGCTTATTAAC) (Yamamoto et al., 2013). PCR amplification was carried out in a volume of 15 μl with the primers (0.4 μM each), Ampdirect Plus (Shimadzu Biotech, Tsukuba, Japan), and high fidelity DNA polymerase (KOD-Plus-ver.2; TOYOBO, Tokyo, Japan). After an initial denaturation at 95˚C for 5 min, amplification was performed with 35 cycles of denaturation (95˚C, 1 min), annealing (55˚C, 1 min) and polymerization (72˚C, 1 min), followed by a final extension at 72˚C for 10 min. The PCR products were purified using a FastGene Gel/PCR Extraction kit (NIPPON Genetics, Tokyo, Japan) to remove excessive primers, and the sequences were directly determined with a forward primer by the dideoxy chain termination method using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

2.3.Data analysis

The sequences were aligned with CLUSTAL W software (Thompson et al., 1994) and examined using the MEGA program (Molecular Evolutionary Genetics Analysis)

version 5.2 (Tamura et al., 2011). The pairwise genetic distances between groups were analyzed by MEGA 5.2 using the Kimura two-parameter (Tamura et al., 2011). Phylogenetic analyses were performed by the Maximum Likelihood (ML) method with the distance algorithms available in the MEGA package (Tamura et al., 2011). The number of segregating sites, number of haplotypes, haplotype diversity, average number of differences, and nucleotide diversity for each population were calculated using DnaSP 5.0 (Rozas et al., 2003), and genetic diversity between populations was determined using MEGA 5.2 (Tamura et al., 2011). A haplotype network was constructed using the median-joining methods as implemented in the program NETWORK 4.6.1.2 (http://www.fluxus-engineering.com/sharenet.htm) (Bandelt et al., 1999).

3. Results

3.1.Haplotype analysis of the Lutzomyia ayacuchensis *COI gene*

COI gene sequences of 215 *Lu. ayacuchensis* collected from 4 Ecuadorian Andes sites (Huigra, Chanchan, Alausi, and Paute) and 4 Peruvian Andes sites (Ayacucho, Lambayeque, Cajamarca, and Piura) were determined by direct sequencing, and the sequences of 628bp-fragments were aligned. The sequence analysis identified the presence of 103 haplotypes with 92 segregating sites (Fig. 2). No more than two different nucleotides were identified at each segregating site except for three (nucleotide positions at 310, 349 and 607), and neither nonsense mutations nor insertions/deletions were observed (Fig. 2). The number of segregating sites (S), number of haplotypes (H), haplotype diversity (Hd), average number of differences (K), and nucleotide diversity (Pi) were calculated for each population (Table 1). Haplotype analysis showed that 48 flies from Huigra, 23 from Chanchan, and 21 from Alausi, Province of Chimborazo, belonged to 14, 7, and 7 haplotypes, respectively (Fig. 2, Table 1). On the other hand, 14 flies from Paute, Province of Azuay, consisted of 6 haplotypes (Fig. 2, Table 1). Interestingly, dominant haplotypes, Hap1 and Hap2, were noted in all these areas of Ecuador where *Lu. ayacuchensis* transmits *L. (L.) mexicana* (Fig. 2 and 3). In Ayacucho of Peru, where *Lu. ayacuchensis* is a proven vector of *L. (V.) peruviana*, 45 sand flies composed 28 haplotypes, of which a haplotype, Hap27, was relatively dominant, showed distinct haplotypes from those of endemic areas in Ecuador (Fig. 2 and 3). In other Peruvian areas where *Leishmania* species transmitted by *Lu. ayacuchensis* have not been reported, 16 sand flies from Lambayeque, 16 flies from Cajamarca, and 32 flies from Piura belonged to 16, 12, and 24 haplotypes, respectively, each including less than 4 individuals in the absence of an apparently dominant haplotype (Fig. 2 and 3).

All these haplotypes of *Lu. ayacuchensis* were distinct from those of the *L. (L.) mexicana*-prevalent areas of Ecuador (Huigra, Chanchan, Alausi, and Paute) and *L. (V.) peruviana*-prevalent areas of Ayacucho (Fig. 2 and 3). Statistical analyses supported a higher genetic diversity in populations of Lambayeque (K=12.758, Pi=0.020), Cajamarca (K=3.308, Pi=0.005) and Piura (K=11.921, Pi=0.019), when compared to populations of Ecuador (K=0.909-1.202, Pi=0.001-0.002) and Ayacucho of Peru (K=1.538, Pi=0.002) (Table 1). Intrapopulation genetic divergence was lower in the areas of Ecuador (Huigra, Chanchan, Alausi, and Paute: 0.1-0.2%) and Ayacucho of Peru (0.4%), when compared to the other Peruvian areas, Lambayeque, Cajamarca, and Piura (0.5-2.1%) (Table 2). The genetic divergence between populations was lower between Ecuadorian populations (Huigra, Chanchan, Alausi, and Paute) (0.2-0.3%), and the highest between Ayacucho and Lambayeque (5.3%) (Table 3). The genetic divergence between *Lu. ayacuchensis* populations and *Lu. hartmanni* from Huigra, both of which belong to the subgenus *Helcocyrtomyia*, was 19.0-20.4%. Phylogenetic analysis showed that *Lu. ayacuchensis* from the Ecuadorian Andes (Huigra, Chanchan, Alausi, and Paute) and from Ayacucho of Peru where the species transmits *L. (L.) mexicana* and *L. (V.) peruviana*, respectively, consisted of clearly distinct clusters, which were separated from those of Lambayeque, Cajamarca, and Piura of Peru, where transmission of *Leishmania* by this species has not been reported (Fig. 4).

3.2.Haplotype analysis of the Lutzomyia ayacuchensis cyt *b gene*

The *cyt* b gene sequences of 216 *Lu. ayacuchensis* collected from 4 Ecuadorian sites and 4 Peruvian Andes sites were determined by direct sequencing, and the sequences of 596bp-fragments were aligned. The sequence analysis identified the presence of 97

haplotypes with 136 segregating sites, and neither nonsense mutations nor insertions/deletions were observed (Fig. 5). Haplotype analysis showed that 48 flies from Huigra, 23 from Chanchan, and 24 from Alausi, Province of Chimborazo, belonged to 13, 4, and 7 haplotypes, respectively, whereas 13 flies from Paute, Province of Azuay, consisted of 3 haplotypes (Fig. 5, Table 4). A dominant haplotype, Hap1, was noted in all these areas of Ecuador (Fig. 5). In Ayacucho in Peru, 46 sand flies had 20 haplotypes, of which a haplotype Hap23 was dominant, and showed distinct haplotypes from those of endemic areas in Ecuador (Fig. 5 and 6). In other Peruvian areas (Lambayeque, Cajamarca, and Piura), 16, 12, and 34 sand flies belonged to 15, 9, and 33 haplotypes, respectively, each including less than 3 individuals in the absence of an apparently dominant haplotype (Fig. 5 and 6). All these haplotypes were distinct from those of Ecuador (Huigra, Chanchan, Alausi, and Paute) and Ayacucho of Peru where *Lu. ayacuchensis* transmits *Leishmania* species (Fig. 5 and 6). Statistical analyses supported a higher genetic diversity in populations of Lambayeque (K=9.650, Pi=0.016), Cajamarca (K=5.379, Pi=0.009) and Piura (K=13.865, Pi=0.023), when compared to populations of Ecuador (K=0.498-1.051, Pi=0.001-0.002) and Ayacucho of Peru (K=1.569, Pi=0.003) (Table 4). Intrapopulation genetic divergence was lower in the areas of Ecuador (Huigra, Chanchan, Alausi, and Paute: 0.1%) and Ayacucho of Peru (0.2%), when compared to other areas, Lambayeque, Cajamarca, and Piura (0.7-1.9%) (Table 2). The genetic divergence between populations was lower between Ecuadorian populations (Huigra, Chanchan, Alausi, and Paute) (0.1%), and the highest between Paute and Piura (4.5%) (Table 3). Similar to the results obtained from phylogenetic analysis of the COI gene, *Lu. ayacuchensis* from the Ecuadorian Andes (Huigra, Chanchan, Alausi, and Paute) and from Ayacucho of Peru made up distinct clusters,

which were isolated from those of Lambayeque, Cajamarca, and Piura of Peru (Fig. 7).

4. Discussion

It has been suggested that genetic divergence caused by genetic drift and/or selection may affect the vectorial capacity of sand flies, as well as other arthropods (Lanzaro *et al*., 1993). In the present study, mitochondrial COI and *cyt* b genes were comparatively analyzed in *Lu. ayacuchensis* populations from Andean areas of Ecuador (Huigra, Chanchan, Alausi, and Paute) and southern Peru (Ayacucho), where sand fly species transmit *L. (L.) mexicana* and *L. (V.) peruviana*, respectively, and those from the northern Peruvian Andes (Lambayeque, Cajamarca, and Piura), in which transmission of *Leishmania* by *Lu. ayacuchensis* has not been reported. The results showed apparent genetic divergence among populations with different vector competence.

Lutzomyia ayacuchensis is a rare species with regard to vector competence; this species is a proven vector of *L. (L.) mexicana* in the Ecuadorian Andes whereas it transmits another subgenus species, *L. (V.) peruviana* in southern Peru (Takaoka *et al*., 1990; Hashiguchi *et al*., 1991; Caceres *et al*., 2004; Kato *et al*., 2005, 2008; Gomez *et al*., 2014a,b). Initially, we suspected that sand flies from Ecuador and Peru may be closely related, but not identical species; however, they were indistinguishable after comparative morphologic classification and genomic analysis (Caceres, unpublished; Kuwahara *et al*., 2009). These observations, along with a restricted range of sand fly activity through their development stages (Alexander, 1987; Alexander and Young, 1992; Morrison *et al*., 1993) encouraged the analysis of the genetic divergence among populations. Haplotype analyses targeting the COI and *cyt* b genes revealed higher intrapopulation genetic divergence in Lambayeque, Cajamarca and Piura of Peru, when compared to populations of Ecuador and Ayacucho of Peru. Particularly, 4 Ecuadorian populations (Huigra, Chanchan, Alausi, and Paute) showed extremely low genetic

divergence despite their geographical isolation (e.g. more than 60 km between Alausi and Paute). In general, higher genetic diversity within a geographical location is found in populations that have not undergone drastic size reductions over evolutionary time, whereas the effect of genetic drift is reduced when the population size is expanding. Therefore, the data suggest that a population bottleneck may have occurred in Ayacucho in Peru and especially, in Ecuador (Huigra, Chanchan, Alausi, and Paute), but not in Lambayeque, Cajamarca and Piura. Since sand flies are typically active at night, they may need to adapt to the severe climate in the Andean highlands where active time is limited due to a temperature drop after dark. Less genetic divergence may have some relation to the higher ratio of *L. (L.) mexicana* infection in Ecuador populations.

In the phylogenetic and haplotype analyses of Peruvian specimens, the Ayacucho population was clearly separated from the populations of Lambayeque, Cajamarca and Piura. This may simply reflect the geographic distance between Ayacucho and other Peruvian areas studied; however, it may not be so because the genetic divergence in Ecuadorian populations was comparable in Peruvian populations regardless of the geographic distance. The sample collection sites of Ayacucho and Lambayeque are located on the western Andean slopes at altitudes of 2,250 m a.s.l. and 340 m a.s.l., respectively, and those of Piura and Cajamarca are located on eastern Andean slopes at altitudes of 1,930 m a.s.l. and 1,400-1,750 m a.s.l., respectively. The climate is different between the slopes; it is very dry at the western Andean slope sites regardless of the altitude and becomes cold after dark at highland areas of Ayacucho, whereas eastern Andean slope areas have a subtropical climate and are humid. In a recent study, *Lu. verrucarum* from each valley of Peru was reported to have unique genotypes with genetic divergence of 0.2-1.0% that was not shared with sand flies from other valleys or

from more distant regions (genetic divergence of 1.6-3.1%) (Cohnstaedt *et al*., 2012). The genetic divergence rates of the *Lu. ayacuchensis cyt* b gene between western slope sites, Ayacucho and Lambayeque, and between eastern slope areas, Cajamarca and Piura, were 3.8% and 2.2%, respectively, which were comparable between western and eastern slope areas (1.6-4.4%). The results indicated that slope-specific variation was not observed in *Lu. ayacuchensis* of Peru, which differed from *Lu. verrucarum*. This may partly reflect higher intrapopulation genetic variation in *Lu. ayacuchensis* of Peru, especially in Lambayeque and Piura (1.3-1.9%).

The present study clearly demonstrated apparent genetic divergence among populations of *Lu. ayacuchensis* with different vector competence. Although laboratory studies do not always reflect the natural conditions, experimental infection of *Lu. ayacuchensis* from Ecuador populations by *L. (V.) peruviana* or those from Ayacucho population by *L. (L.) mexicana* may help to understand the vectorial capacity of these sand flies.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Figure Legends

Fig.1. (A) Map of Ecuador and Peru showing the geographic locations where *Lutzomyia ayacuchensis* were collected. HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; PI, Piura; CA, Cajamarca; LA, Lambayeque; AY, Ayacucho.

Fig.2. Variable nucleotides found in the alignment of the *Lutzomyia ayacuchensis* cytochrome oxidase I gene. The cytochrome oxidase I (COI) gene sequence of a 628bp-fragment was analyzed in 215 *Lutzomyia (Lu.) ayacuchensis* collected from 4 Ecuadorian (Huigra, Chanchan, Alausi, and Paute) and 4 Peruvian Andes regions (Ayacucho, Lambayeque, Cajamarca, and Piura). Dots denote identical sequences and numbers show their corresponding positions from the *Lu. ayacuchensis* COI gene fragment obtained in this study. HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; PI, Piura; CA, Cajamarca; LA, Lambayeque; AY, Ayacucho.

Fig.3. Haplotype network of the cytochrome oxidase I sequences of *Lutzomyia ayacuchensis* collected from 4 Ecuadorian (Huigra, Chanchan, Alausi, and Paute) and 4 Peruvian Andes regions (Ayacucho, Lambayeque, Cajamarca, and Piura). Each haplotype is represented by a circle sized in proportion to the frequency of the haplotypes. Each crossbar represents one nucleotide substitution. Small black circles indicate one nucleotide substitution between haplotype. HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; PI, Piura; CA, Cajamarca; LA, Lambayeque; AY, Ayacucho.

Fig.4. Phylogenetic tree of the cytochrome oxidase I sequences among *Lutzomyia ayacuchensis* populations. The scale bar represents 0.005% divergence. HU, Huigra;

CH, Chanchan; AL, Alausi; PA, Paute; PI, Piura; CA, Cajamarca; LA, Lambayeque; AY, Ayacucho.

Fig.5. Variable nucleotides found in the alignment of the *Lutzomyia ayacuchensis* cytochrome *b* gene. The cytochrome *b* (*cyt* b) gene sequence of a 596bp-fragment was analyzed in 216 *Lutzomyia (Lu.) ayacuchensis* collected from 4 Ecuadorian (Huigra, Chanchan, Alausi, and Paute) and 4 Peruvian Andes regions (Ayacucho, Lambayeque, Cajamarca, and Piura). Dots denote identical sequences and numbers show their corresponding positions from the *Lu. ayacuchensis cyt* b gene fragment obtained in this study. HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; PI, Piura; CA, Cajamarca; LA, Lambayeque; AY, Ayacucho.

Fig.6. Haplotype network of the cytochrome *b* sequences of *Lutzomyia ayacuchensis* collected from 4 Ecuadorian (Huigra, Chanchan, Alausi, and Paute) and 4 Peruvian Andes regions (Ayacucho, Lambayeque, Cajamarca, and Piura). Each haplotype is represented by a circle sized in proportion to the frequency of the haplotypes. Each crossbar represents one nucleotide substitution. Small black circles indicate one nucleotide substitution between haplotype. HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; PI, Piura; CA, Cajamarca; LA, Lambayeque; AY, Ayacucho.

Fig.7. Phylogenetic tree of the cytochrome *b* sequences among *Lutzomyia ayacuchensis* populations. The scale bar represents 0.005% divergence. HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; PI, Piura; CA, Cajamarca; LA, Lambayeque; AY, Ayacucho.

Table 1.
Summary statistics for the COI gene from *Lutzomyia ayacuchensis* populations

population[a]	N	S	H	Hd	K	Pi
HU	48	11	14	0.778	1.202	0.002
CH	23	7	7	0.522	0.909	0.001
AL	21	7	7	0.767	1.200	0.002
PA	14	6	6	0.791	1.538	0.002
AY	45	28	30	0.956	2.644	0.004
LA	16	42	16	1.000	12.758	0.020
CA	16	12	12	0.958	3.308	0.005
PI	32	56	24	0.974	11.921	0.019
Total	215	92	103	0.933	18.511	0.029

[a]population: HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; AY, Ayacucho; LA, Lambayeque; CA, Cajamarca; PI, Piura

N, number of sequences; S, number of segregating sites; H, number of haplotypes; Hd, haplotype diversity; K, average number of differences; Pi, nucleotide diversity

The number of segregating sites, number of haplotypes, haplotype diversity, average number of differences, and nucleotide diversity for each population were calculated using DnaSP 5.0 (Rozas et al., 2003).

Table 2.

Genetic divergence of *Lutzomyia ayacuchensis* within populations

	HU[a]	CH	AL	PA	AY	LA	CA	PI
COI	0.002	0.001	0.002	0.002	0.004	0.021	0.005	0.019
cyt b	0.001	0.001	0.001	0.001	0.002	0.013	0.007	0.019

[a]population: HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; AY, Ayacucho; LA, Lambayeque; CA, Cajamarca; PI, Piura

Genetic divergence was determined using MEGA 5.2 (Tamura et al., 2011).

Table 3.

Genetic divergence of *Lutzomyia ayacuchensis* between populations

		cyt b							
		HU[a]	CH	AL	PA	AY	LA	CA	PI
COI	HU		0.001	0.001	0.001	0.044	0.040	0.037	0.044
	CH	0.002		0.001	0.001	0.044	0.040	0.037	0.044
	AL	0.002	0.002		0.001	0.044	0.040	0.037	0.044
	PA	0.002	0.002	0.003		0.044	0.040	0.036	0.045
	AY	0.035	0.036	0.035	0.036		0.038	0.037	0.044
	LA	0.049	0.049	0.049	0.049	0.053		0.019	0.016
	CA	0.050	0.051	0.050	0.050	0.048	0.024		0.022
	PI	0.049	0.049	0.049	0.049	0.052	0.020	0.024	

[a]population: HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute;
AY, Ayacucho; LA, Lambayeque; CA, Cajamarca; PI, Piura

Genetic divergence was determined using MEGA 5.2 (Tamura et al., 2011).

Table 4.
Summary statistics for the *cyt* b gene from the *Lutzomyia ayacuchensis* populations

population[a]	N	S	H	Hd	K	Pi
HU	48	14	13	0.501	0.741	0.001
CH	23	4	4	0.383	0.498	0.001
AL	24	6	7	0.558	0.652	0.001
PA	13	3	3	0.590	1.051	0.002
AY	46	17	20	0.824	1.569	0.003
LA	16	34	15	0.992	9.650	0.016
CA	12	20	9	0.939	5.379	0.009
PI	34	86	33	0.998	13.865	0.023
Total	216	136	97	0.868	20.300	0.034

[a]population: HU, Huigra; CH, Chanchan; AL, Alausi; PA, Paute; AY, Ayacucho; LA, Lambayeque; CA, Cajamarca; PI, Piura
N, number of sequences; S, number of segregating sites; H, number of haplotypes; Hd, haplotype diversity; K, average number of differences; Pi, nucleotide diversity
The number of segregating sites, number of haplotypes, haplotype diversity, average number of differences, and nucleotide diversity for each population were calculated using DnaSP 5.0 (Rozas et al., 2003).

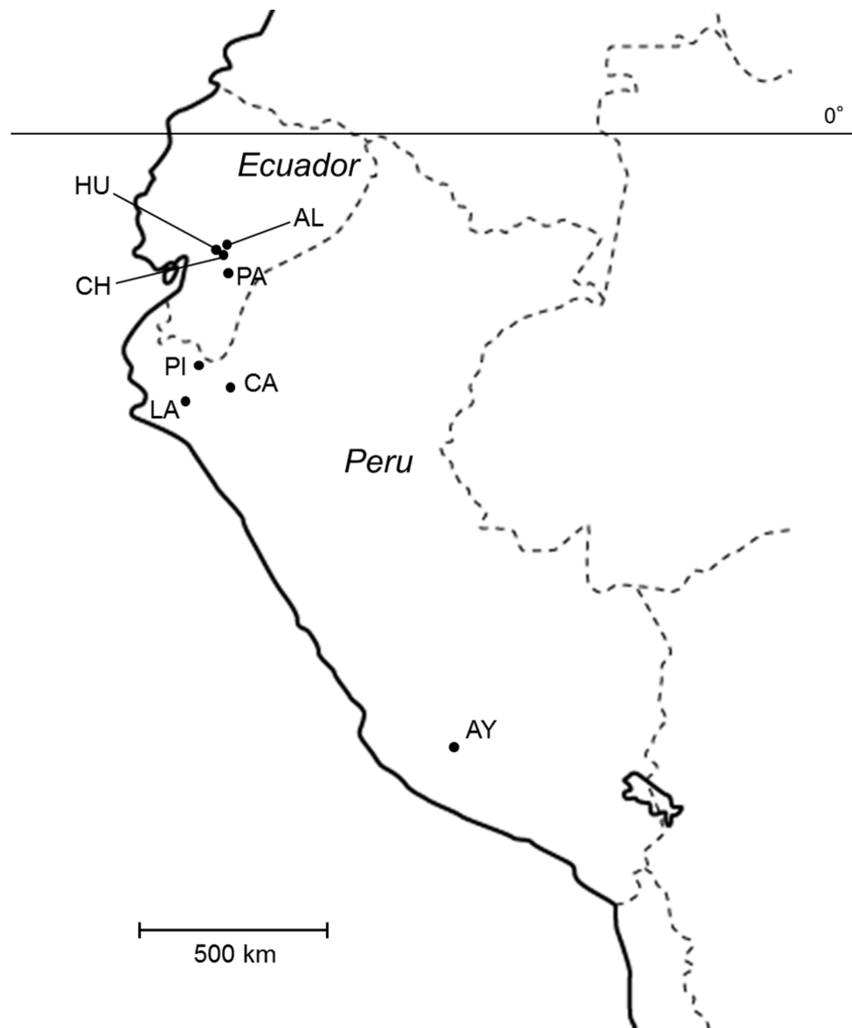

Fig. 1

		No. in population							
	Segregating sites	Ecuador				Peru			
Haplo-type	111111112222222222222223333333333333333444444444444444555555555555556666666 11112223456677890014669900013445567790011224444567789914555677788899011222244556780001112 79346904853847038739826903025457836814017035836798106515821478003815739257467917063461473672	HU	CH	AL	PA	AY	LA	CA	PI
Hap1	GCAGTTATATTGATAATTTTCTAATCGATATATTTCAAGGGTGCCTCGCATTTCTGATGATAATGAACATATATCACCTAATCTCTATAAGT	6	2	5	1				
Hap2	•••••••••••••••G••	21	16	9	6				
Hap3	••T•••	1		1					
Hap4	•••••••••••••••G•••••••••••••••••••••••••••••••••G••	7		3					
Hap5	•••T••••T•••••••••			1					
Hap6	•••••••••••••••G••••••••••••••••••••••••••••••••••G•••			1					
Hap7	•••••••••••••••G••C•••••••••••••		1	1					
Hap8	A•••••••••••G•••		1						
Hap9	•••••••••••••••G•••••••••••••••••••••••A••		1						
Hap10	•••••••••••••••G•••••••••••••••••••••••AA•••		1						
Hap11	•••••••••••A•••G•••••••••••••••••••••••A••	1	1		1				
Hap12	•••••••••••••••G••••••••••••••••••••••••••A•••	1							
Hap13	•••••••••••••••G••••••••••••••••••••••••••••T•••	3			2				
Hap14	•••••••••••••••G••••••••••••••••••••••••••••••••T•••	1							
Hap15	•••••••••••A•••G••	2							
Hap16	•••••••••••••••G••T•••••••	1							
Hap17	•••••••••••••••G••C•••••••••••••••••••••••	1							
Hap18	•••••••••••••••••••••••••••••••••C••	1							
Hap19	•••••••••••••••G•••••••G•••••••••••••••••••••••••G••	1							
Hap20	••T•••	1							
Hap21	•••••••••••••••G••••••••••••••••••••••••••••T•••••••••••••A•••••••••••••••••••••••••••••••••				3				
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Hap31	••••C•••••CA••G•••••T•••••••C•C••••••G••A•AT•••••G••C•••••A•A•G••G•T••••••T••••G••••••T•G•••					1			
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Hap46	••••C•••••CA••G•••••T•••••••••C••••••G••A•AT•••••G••C•••••A•A•G••G•T••••••T••••G•••••CT•••••					2			
Hap47	••••C•••••CA••G•••••T•••••••••C••••••G••A••T•••A•G••C•••••A•A•G••G•T••••••T••••G••••••T•G•••					1			
Hap48	••••C•••••CA••G•••••T••••••••••••••••G••A••TT•••••••C•••••A•A•G••G•T••••••T••••G••••••T•G•••					1			
Hap49	••••C•••••CA••G•••••T•••••••••C••••••G••A••T••••••••C•••••A•A•G••G•T••••••T••••G••••••T•G•••					1			
Hap50	••••C•••••CAG•G•••••T•••••••••C••••••G••A••T•••••G••CT••••A•A•G••G•T••••••T••••G••••••T•G•••					1			
Hap51	••••C•••••CA••G•••••T•••••••C•C••••••G••A••T•••••G••C•••••A•A•G••G•T••••••T••T•G••••••T•G•••					1			
Hap52	••••C•••••CA••G•••••T••••••G••C••••••G••A••T•••••G••C•••••A•A•G••G•T••••••T••••G•••••CT•G•••					1			
Hap53	••••C•••••CA••G••••C•••••••G•G••CCCT••••A•A•••T•••••••••••A•A•••••G•••C•G•T••A••GC••TC•G•G••						1		
Hap54	••••CG•••CCA••GG•••C•••••••G•G••C•CT••••A•••••••••••••••••A•A•••••G•••C•G•T••A••GC••TC•A•G••						1		
Hap55	••••C•••GCCAT••••••C•••••••G•G••C•CTG••AA•A••CT•TG••C•••G•A•A••••G••••C•G•T••A••GC••T••G••••						1		
Hap56	A••••••••CCAG•G••••C•••••T•G•G••C•CTC•••AG••••T••G••••••••A•A••••G••••C•G•TG•A•CG•••TC•G••••						1		
Hap57	••••CG•••CCA•••••••C•••••••••G••CCCT••••A•A•••••••••••••••A•A•••••G••CC•G•T••A••GC••TC•A•G••						1		1
Hap58	••••CG•••CCA••GG•••C•••••••G•G••C•CT••••A•A•••••T•••••••••A•A•••••G•••C•G•T••A••GC••TC•A•G••						1		
Hap59	••••C••••CCAG•GG•••C•••••T•GCG••C•CT••••AG••••••••••••••••A•A••••G••••C•G•TG•A•CG•••TC•G••••						1		
Hap60	••••C••••CCAT••••••C•••••T•G•G••C•CT••••A•••••T•AG••••••G•A•A•••••••••C•G•T••A••GC••T••GG•••						1		
Hap61	••••C•••GCCAT••••••C•••••••••G••C•CTG•••A••••CT•TG••C•••G•A•A••••G••••C•G•T••A••GC••T••G••••						1		
Hap62	••G•C••••CCA••GG•••C•••••T•G•G••C•CT••••A•••••T••G••••••••A•A••••G••••C•G•TG•A•CGC••TC•G••••						1		
Hap63	••••C••••CCA••G••••C•••••••••G••CCCT••••A•A•T•T•T•••••••••A•A••••G•T••C•G•T••A•••C••TC•G•G••						1		
Hap64	••••C•••••CA••G••C•C••G••••G•G••CCCT••••A•A•••T•••••••••••A•A•••••G•••C•G•T••A••GC••TC•G•G••						1		
Hap65	••••C••••CCAT••••••C••••C••G•G••C•CT••••A•••••T•••••C•••G•A•A••••G••••C•G•T••A••GC••TC•C•••A						1		
Hap66	••••CG•••CCA••GG•••C•••••••G•G••C•CT••••A•A•••••••••••••••A•A•••••G•••C•G•T••A••GC••TC•A•G••						1		
Hap67	••••C••••CCAT••G•••C•••••••G•G••C•CT••••A•••••••••••••••G•A•A••••G••••C•G•T••A••GC••T••G••••						1		
Hap68	••••C••••CCA••G••••C•••••••G••••CCCT••••A•A•T•T•T•••••••••A•A••••G••••C•G•T••A•••C••TC•G•G••						1		
Hap69	••••C••••CCAT•G••••C••G••••••G••C•CT••••••ATT•T••G•••••T••A•A••••G••••C•C•T••A••GC••TC•A••••							1	
Hap70	••••C••••CCAT•G••••C••G••••••G••C•CT••••A•ATT•T•••••C•••••A•A••••G••••C•G•T••A••GC••TC•A••••							3	
Hap71	••••C••••CCAT•G••••C••G••••••G••C•CT••••••AT••T••G••••••••A•A••••G••••C•G•T••A••GC••TC•A••••							2	
Hap72	A•••C••••CCAT•G••••C••G••••••G••C•CT••••••ATT•T••G••••••••A•A••••G••••C•G•T••A••GC••TC•A••••							1	
Hap73	••••C••••CCAT•G••••C••G••••••G••C•CT••••••ATT•T••G••C•••••A•A••••G••••C•G•T••A••GC••TC•A••••							2	
Hap74	••••C••••CCAT•G••••CT••••••••G••C•CT••••••AT••T••G••••••••A•A••••G••••C•G•T••A••GC••TC•A••••							1	
Hap75	A•••C••••CCAT•G••••C••G••••••G••C•CT•••A••ATT•T••G••••••••A•A•••CG••••C•G•T••A••GC••TC•A••••							1	
Hap76	••••C•••••CAT•G••••C••G••••••G••C•CT•••A••ATT•T••G••••••••A•A••••G••••C•G•T••A••GC••TC•A••••							1	
Hap77	••••C•••••CAT•G••••C••G•••••••••C•CT•••A••ATT•T••G••••••••A•A••••G••••C•G•T••A••GC••TC•A••••							1	
Hap78	A•••C••••CCAT•G••••C••G••••••G••C•CT••••••ATT•T••G••••••••A•A••••G••••C•••T••A••GC••TC•A••••							1	
Hap79	••••C••••CCAT•G••••C••G••••••G••C•CT••••••ATT•T••G••••••••A•A••••G••••C•G•T••A••GC••TC•A••••							1	
Hap80	••••C••••CCAT•G••••C••G••••••G••C•CT••A•••ATT•T••G••••••••A•A••••G••••C•G•T••A••GC••TC•A••••							1	
Hap81	••••CG•••CCA•••••••C•••••••••G••CCCT••••A•A•••••••••••••••A•A•••••G••CC•G•T••A•CGC••TC•A•G••								1
Hap82	••••C••••CCAC•G•••CC••••C••G••••C•CT••••A•A•••T••G••••••G•A•A••••G••••C•G•T•TA•••C••TC•G•G••								1
Hap83	••••C•••GCCAT••••••C•••••••••G••C•CTG•••A••••CT•T•••C•••G•A•A••••G••••CGG•T••A••GC••T••G••••								1
Hap84	••••CG•••CCA••G••••C•••••••G•G••C•CT••••A•A•••••••••••••••A•A•••••G•••C•G•T••A••GC••TC•A•G••								2
Hap85	•••••••••CCA••••C••C••G••••GC•••C•CT••••A•••••••G•••••••G•AGA••••G••••C•G•T••A•G•C••TCCG••••								1
Hap86	••••C••••CCA••G••••C•••••••G•G••CCCT••••A•A•••T••G••••••G•A•A•••GG••••C•G•T••A••GC•••C•G••••								1
Hap87	••••C••••CCAT•G•••CC••••C••G••••C•CT••••A•A•••T••G••••••G•A•A••••G••••C•G•T•TA•••C••TC•G•G••								3
Hap88	••••C••••CCA••G••••C•••••••G••••CCCT••••A•A•••T•T•••••••••A•A••••G••••C•G•T••A•••C••TC•G•G••								4
Hap89	••••C•••••CA•C•••••C•••••••G•G••C•CT•••••••T••T•••••••••••A•A•••••••••C•G•T•TA•••C••TC•G••••								1
Hap90	••••CG•••CCA••GG•••C•••••••G•G••C•CT••••A•A•••••••••••••••A•A•••••G•••C•G•T••A••GC••TC•A•GA•								1
Hap91	••••C••••CCAT••G••CC••••C••G•••GC•CT••••A•A•••T••G••••••G•A•A••••G••••C•G•T••A•••C••TC•G•G••								1
Hap92	••G•C••••CCA••GG•••C•••••T•G•G••C•CT••••A•••••T•••••••••••A•A••••G••••C•G•TG•A•CGC••TC•G••••								1
Hap93	•••••••••CCA••••C••C•••••••G•G••C•CT••••A•••••••AG•••••••••GA••••G••••C•G•T••A•••C••TCTG••••								1
Hap94	••••C••••CCAT•G••••C••••C••G••••C•CT••••A•A•••T••G••••••••A•A••••G••••C•G•T••A•••C••TC•G•G••								1
Hap95	••••C••••CCAG•G••••C•••••T•G•G••C•C•C•••AA••••T••G••••••••A•A••••G••••C•G•TG•A•CG•••TC•G••••								1
Hap96	••••CG•••CCA••G••••C•••••••G•G••C•CT••••A•A•••••••••••••••A•A•••••G•••C•G•T••A••GC•CTC•A•G••								3
Hap97	••G•C••••CCA••GG•••C•••C•T•G•G••C•CT••••A•••••T••G••••••••A•A••••G••••C•G•TG•A•CGC••TC•G••••								1
Hap98	••••C••••CCA••G••••C•••••••G•G••CCCT••••A•••••T•••••••••••A•A•••••G•••C•GCT••A••GC••TCTG•G••								1
Hap99	•••TCG•••CCA••G••••C•••••••G•G••C•CT••••A•A•••••••••••••••A•A•••••G•••C•G•T••A••GC•CTC•A•G••								1
Hap100	••G•C•GC•CCA••GG•••C•••C•T•G•G••C•CT••••A•••••T•••••••••••A•A••••G••••C•G•TG•A•CGC••TC•G••••								1
Hap101	••••CG•••CCA••G••••C•••••••••G••C•CT••••A•A•••••••••••••••A•A•••••G•••C•G•T••A••GC••TC•A•G••								1
Hap102	••••CG•••CCA••G••••C•••••••G•G••C•CT•••AA•A•••••••••••••••A•A•••••G•••C•G•T•TA••GC••TC•A•G••								1
Hap103	•••••••••CCAG••••••CT••••••G••••C•CT••••A•••••••A•••••••G•A•A••••G••••C•G•T••A•••C••TCCG••••								1
Total		48	23	21	14	45	16	16	32

Fig. 2

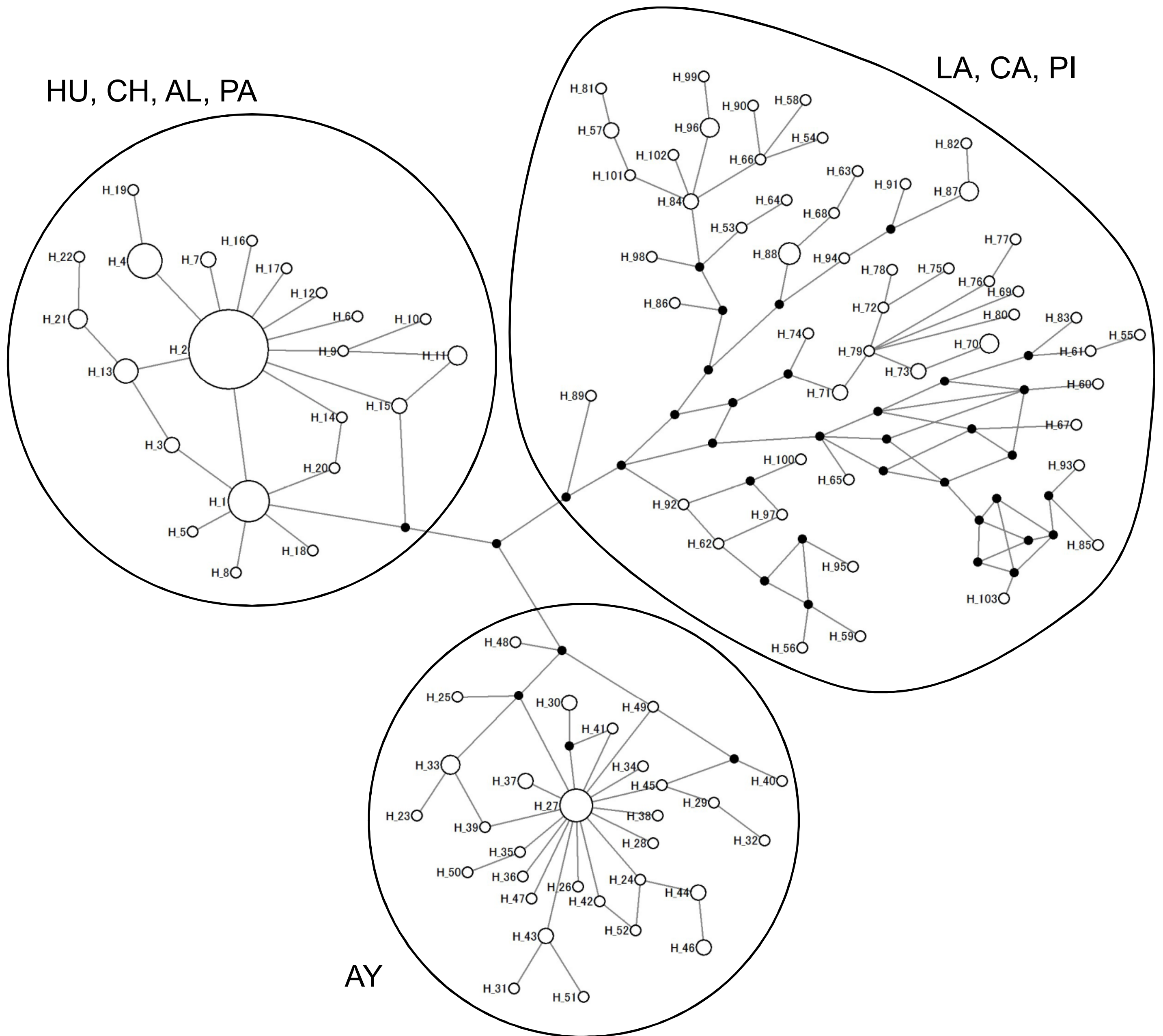

Fig. 3

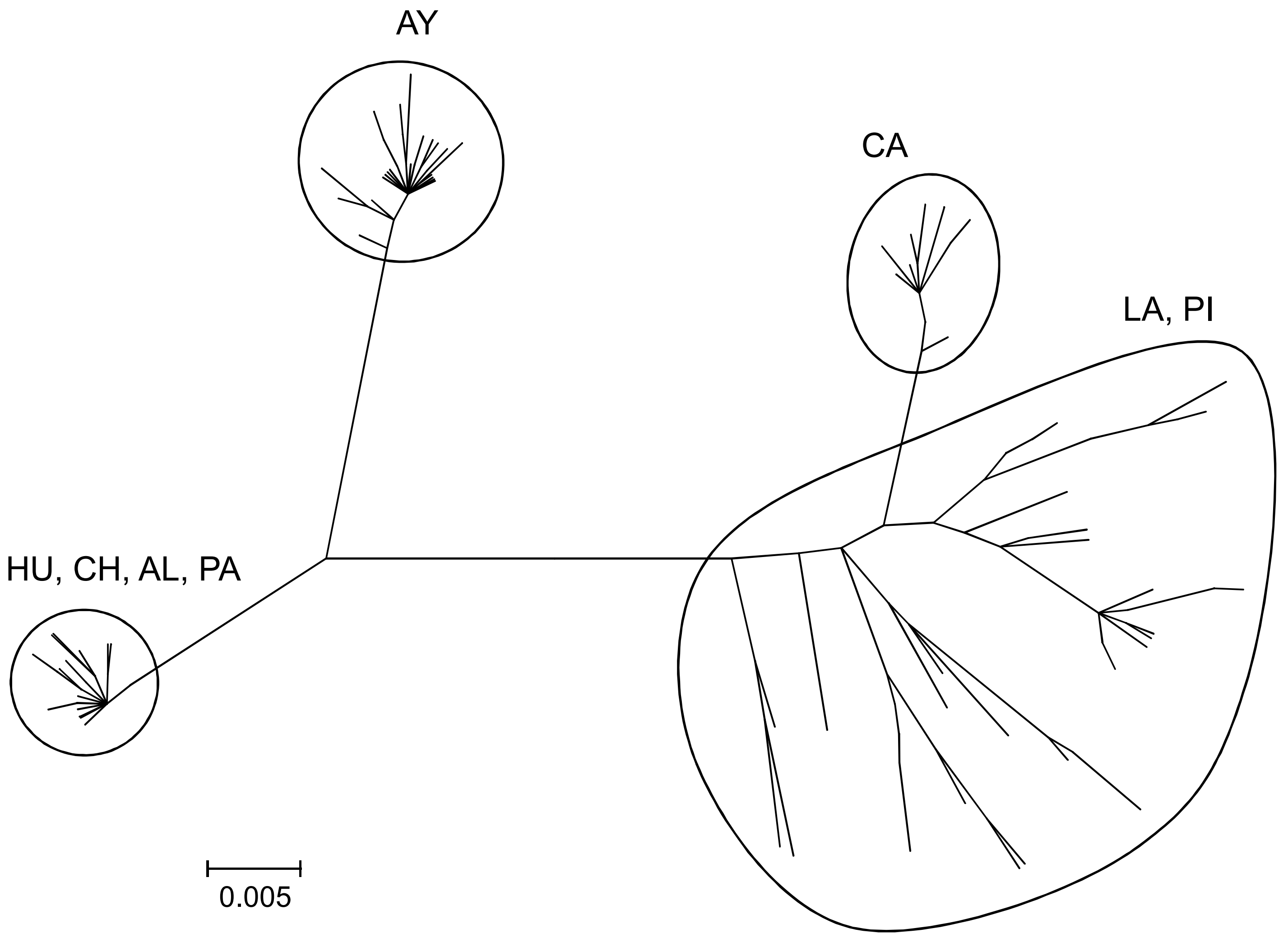

Fig. 4

Haplotype	Segregating sites	HU	CH	AL	PA	AY	LA	CA	PI
		No. in population							
		Ecuador				Peru			
	1111111111111111222222222222222222233333333333333333333444444444444444444444444444444445555555555555555555555555555555555 2334455566666788990112233456777891111233444555567890012225666778888900012223444455556677788899999000122222333444455556677777788888899 25695462845701378578463476958705917380379247069027849277824171036580156732569039815890249684671290135891432356804503590256353456790123561 4	HU	CH	AL	PA	AY	LA	CA	PI
Hap1	CTCTGGAACCTTTCAATTTGATGATGAAAAGAATCTATTATAAATAGTCAACCCGCTTCACCATTACGACTTTTAGTTTTGATATTTAAACCTAAGGGCCGTCTTTAGGCCCCTAAGAGTTTTGCCATTTACGTCT	34	18	16	8				
Hap2	......................................C...			1					
Hap3	....T..			1					
Hap4	................C...			1					
Hap5	.........................A..		3	2					
Hap6	...........................G..	2	1	2					
Hap7	...............................G..			1					
Hap8	...................A..A...........................		1						
Hap9	.................................C..	2							
Hap10	......................A..A..	1							
Hap11	....G...	1							
Hap12	.....A..	1							
Hap13	....G..	1							
Hap14	....C................................	1							
Hap15	....G...	1							
Hap16	.........................A..........G.C...	1							
Hap17	.........................AG...	1							
Hap18	....T....................................	1							
Hap19	....T...	1							
Hap20	....T.....................................				2				
Hap21	......G......................................G..				3				
Hap22	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A.T.T....G.......T......A.TC					2			
Hap23	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T......A.TC					19			
Hap24	...C..G.TT...........CA..A....A.C..C..C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T......A.TC					4			
Hap25	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T............T......A.TC					1			
Hap26	..AC..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TCA...A...T....G.......T......A.TC					1			
Hap27	...C..G.TT...........CAG.A....A.C..C..C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T......A.TC					2			
Hap28	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC..G......TCC...A...............T.......T..TC....A.A.T....G.......T......A.TC					1			
Hap29	...C....TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T......A.TC					2			
Hap30	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.T.......TCC...A...............T.......T..TC....A...T............T......A.TC					1			
Hap31	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....GA......T......A.TC					1			
Hap32	...C..G.TT...........CAG.A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T......A.TC					2			
Hap33	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T........TC					2			
Hap34	...C..G.TT...........CA..A.G..A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T......A.TC					1			
Hap35	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC..G......TCC...A...............T.......T..T.....A.A.T....G.......T......A.TC					1			
Hap36	...C..G.TT...........CAG.A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T............T......A.TC					1			
Hap37	...C..G.TT...........CA..A....A.C...G.C........C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T......A.TC					1			
Hap38	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..T.....A...T....G.......T......A.TC					1			
Hap39	...C..G.TT...........CA..A....A.C.....C........C.....T.TC.TC.........TCC...A...............T.......T..TC...AA...T....G.......T......A.TC					1			
Hap40	...C..G.TT...........CA..A....A.C.....C....G...C.....T.TC.TC.........TCC...A...............T.......T..TC....A...T....G.......T........TC					1			
Hap41	...C..G.TT...........CA..A....A.C.....C........C.....T.TCCTC.........TCC...A...............T.......T..TC....A.G.T............T......A.TC					1			
Hap42	..T.....TT...T...C....A.CA....AGT.T..CC......G......TT..C.T....C.....T......C..............T..........TCCC..A................T..C......C						1		
Hap43	..T.....TT.......C......CAG..GAGT.....C.............TT..C.T....C.....T......C..............T..........TCCC..A.............C..T..C......C						1		
Hap44	........TT.......C......CAGGG.A.C.....C..G.....C....TT..C.T....C.....T.....................T..........TCCC..A.T..............T..C......C						1		
Hap45	........TT.......C......CAG...AGT.....C..G.....C....TT..C.T....C.....T.....................T..........T..C..A................T..C......C						3		
Hap46	..T.....TT.......C......CAG...AGT.....C.............TT..C.T..T.C...A.T......C..............T..........TCCC..A.............C..T..C......C						1		
Hap47	..T.....TT.......C......CAG...AGT.....C.............TT..C.T....C.....T......C..............T..........TCCC..A.............C..T..C......C						1		
Hap48	........TT.......C......CAG...A.C.....C...GG...C....TT..C.T....C.....T..........A..........T..........TCCCG.A.T..............T..C......C						1		
Hap49	........TT.......C......CAG.G.A.C.....C...G....C....TT..C.T....C.....T.....................T..........TCCCG.A................T..C......C						1		
Hap50	........TT.......C......C.G...AGT.....C..G.....C....TT..C.T....C.....T.....................T..........T..C..A................T..C......C						1		
Hap51	..T.....TT..C....C......CAG...AGT.T...C.............TT..C.T........A.TC.....C..............T..........TCCC..A................T..C......C						1		
Hap52	..T.....TT...T...C......CAG...AGT.T..CC......G......TT..C.T....C.....T......C..............T.......T..TCCC..A................T..C......C						1		
Hap53	........TT.......C......CAG.G.A.C...G.C...G....C....TT..C.TG...C.....T.....................T..........TACCG.A.T..............T..C......C						1		
Hap54	..T.....TT.......C......CAG...AGT.....C.............TT....TG...C.....T......C..............T..........TCCC..A.............C..T..C......C						1		
Hap55	........TT.......C......CA....A.C...G.C...G....C....TT..C.TG...C.....T.....................T..........TCCCGAA.T..............T..C......C						1		
Hap56	..T.....TT..C....C......CAG...AGT.T...C.............TT..C.T..........TC.....C..............T..........TCCC..A................T..C......C						1		
Hap57	......G.TA.A.....C......CAG...A.C...G.C..G...........T..C.T..........T.....................T......T...T.CC..A................T..C......C							1	
Hap58	........TT.......C......CAG...A.C...G.C..G..C........T..C.T..........T.....................T......T...T.CC..A................T..C......C							1	
Hap59	......G.TT.......C......CAG...A.C...G.C.............TT..C.T..........T.....................T......T...T.CC..A.T..............T..C......C							1	
Hap60	......G.TAA......C......CAG...A.C...G.C..G...........T..C.T..........T.....................T......T...T.CC..A................T..C......C							1	
Hap61	......G.TT.......C......CAG...A.C...G.C..G...........T..C.T..........T.....................T......T...T.CC..A................T..C......C							3	
Hap62	........TGC......C..C...CAG...A.C...G.C..G..C........T..C.T........C.T....T............C.....C....T...T.CC..A.....C.A........T..CC.....C							1	
Hap63	......G.T.G......C......CAG...A.C...G.C.............TT..C.T..........T.....................T......T...T.CC..A.T....CC........T..C......C							1	
Hap64	......G.TT.......C......CAG...A.C...G.C..G...........TA.C.T..........T.....................T......T...T.CC..A................T..C......C							2	
Hap65	......G.TAA......C......CAG...A.C...G.C..............T..C.T..........T.......C.............T......T...T.CC..A.T..............T..C......C							1	
Hap66	.......GTT.......C......CAG...AGC...G.C...G..T...G..TT..C.T....C.....T.....................T..........T.CC..A.T..............T..C......C								1
Hap67	........TT.......C......CAG.G.A.C.....C...G....C....TT..C.T....C.....T.....................T..........TCCCG.A.T..............T..C......C								1
Hap68	.......GTT.......C......CAG...AGC...G.C...G..T.C....TT..C.T....C.....T.....................T..........T.CC.AA................T..C......C								1
Hap69	..T.....TT.......C......CAG...AGT.....C.......A.....TT..C.T....C...A.T......C..............T..........TCCC..A.............C..T..C......C								2
Hap70	..T.....TT.......C......CAG...AGT.....C.......A.....TT..C.T..T.C...A.T......C..............T..........TCCC..A.....C.......C..T..C......C								1
Hap71	..T.....TT.......C......CAG...AGT.T...C...........G.TT..C.T....C...A.T......C.A...........TTA...A.T...TCCC..A..........G.....T..C......C								1
Hap72	....A..GTT.......C......CAG...AGC...G.C...G..T.C....TT..C.T....C.....T.....................T..........T.CC..A................T..C......C								1
Hap73	.......GTT.......C......CAG...AGC...G.C...G..T.C....TT..C.T....C.....T.....................T..........T.CC..A.T..............T..C......C								1
Hap74	..T.....TT..C....C......CAG...AGT.T...C.......A.....TT..C.T........A.TC..A..C.......A.A.........A.....TCCC..A..........AA....T..C..G...C								1
Hap75	..T.....T........C......CAG.G.AGT.....C.............TT..C.T....C...A.T..C...C..............T..........TCCC.AA.............C..T..C......C								1
Hap76	..T.....TT..C....C......CAG...AGT.T...C.......A.....TT..C.T........A.TC.....C..............T..........TCCC..A.............C..T..C......C								1
Hap77	..T.....TT.......C......CAG...AGT.....C.......A.....TT..C.T....C.....T......C.....AT.......T..........TCCC..A.............C..T..C......C								1
Hap78	.......GTT.....G.C......CAG...A.C...G.C...G..T.C....TT..C.T....C.....T.....................T..G.A.....TCCC.AA..T.C.....A.....T..CC..G..C								1
Hap79	........TT.......CC.....CAG...AGC...G.C..G.....C....TT..C.TGT..C.....T.....................T..........T.CC..A................T..C.T....C								1
Hap80	..T.....TT.......C......CAG...AGT.....C.......A.....TT..C.T....C...A.T......C..............T.....A...ATCCC..A.............C..T..C......C								1
Hap81	........TT.......C......CAG...A.C.....C...GG...C...GTT..C.T....C...AGT........AAT....A.....T...A.A....TCCCGAA.......A........T..C......C								1
Hap82	........TT.......C......CAG...AGC...G.C.C.GG.T.C....TT..C.TG...C.....T................................TCCCG.A................T..C......C								1
Hap83	TC......TT.......C......CAG...AGT.....C..G.....C....TT..C.T....C.....T.....................T..........T..C..A................T..C......C								1
Hap84	..T.....TT.......C......CAG...AGT.....C.............TT..C.T....C.....T......C..............T....A.....TCCC..A.............C..TT.C......C								1
Hap85	..T.....TT..C....C......CAG...AGT.T...C.............TT..C.T........A.TC.....C..............TA....A....TCCC..A..........A.G...T..C......C								1
Hap86	TC....G.TT.......C......CAG...A.C...G.C..G...........T..C.T..........T.....................T..........TCCC..A................T..C......C								1
Hap87	..T.....TT.......C......CAG...AGT.....C.......A.....TT..C.T....C...A.T......C..............T..G.......T.CC..A.....C......AC..T..C......C								1
Hap88	.......GTT.......C......CAG...AGC...G.C...G..T.C....TT..C.T....C.....T.....................T..........T.CC..A................T..C......C								1
Hap89	........TT.......C......CAG...AGT.....C..G.....C....TT..C.T....C.CT..T...........T.........T..........A..C..A................T..C......C								1
Hap90	..T.....TT.......C......CAG...AGT.....C.......A.....TT..C.T....C...A.T......C..............T..........T.CC..A.............C..T..C......C								1
Hap91	..T...G.TT...T...C......CAG...AGT.T...C......G......TT..C.T....C...A.T......C..............T....A.....TCCCAAA................T..C......C								1
Hap92	..T.....TT..C....C......CAG...AGT.T...C.............TT..C.T..........TC.....C..............T..........TCCC..A................T.CC....C.C								1
Hap93	..T.....TT.......C......CAG...AGT.....C.............TT..C.T....CA..A.TC.....C.....................A..AAT.CC.AA.....C....A..CCAT..C......C								1
Hap94	........TT.......C......CAG...AGT.....CG.G.....C....TT..C.T....C.....T.....................T..........T..C..A................T..C......C								1
Hap95	..T.....TT....G..C......CAG...AGT.....C.......A.....TT..C.T....C...A.T......C..............T..........TCCC..A.............C..T.CC......C								1
Hap96	..T.....TT.......C......CAG...AGT.....C.......A.....TT..C.T....C...A.T......C..............T....A.....TCCC.AA.........A...C..T..CC.....C								1
Hap97	........TT.......C......CAG...AGC.....C..G.....C....TT..C.TG...C.....T...............A...T.T...A.A...AA.CC.AA....C....A...C..T..CCT....C								1
Total		48	23	24	13	46	17	12	33

Segregating sites

Fig. 5

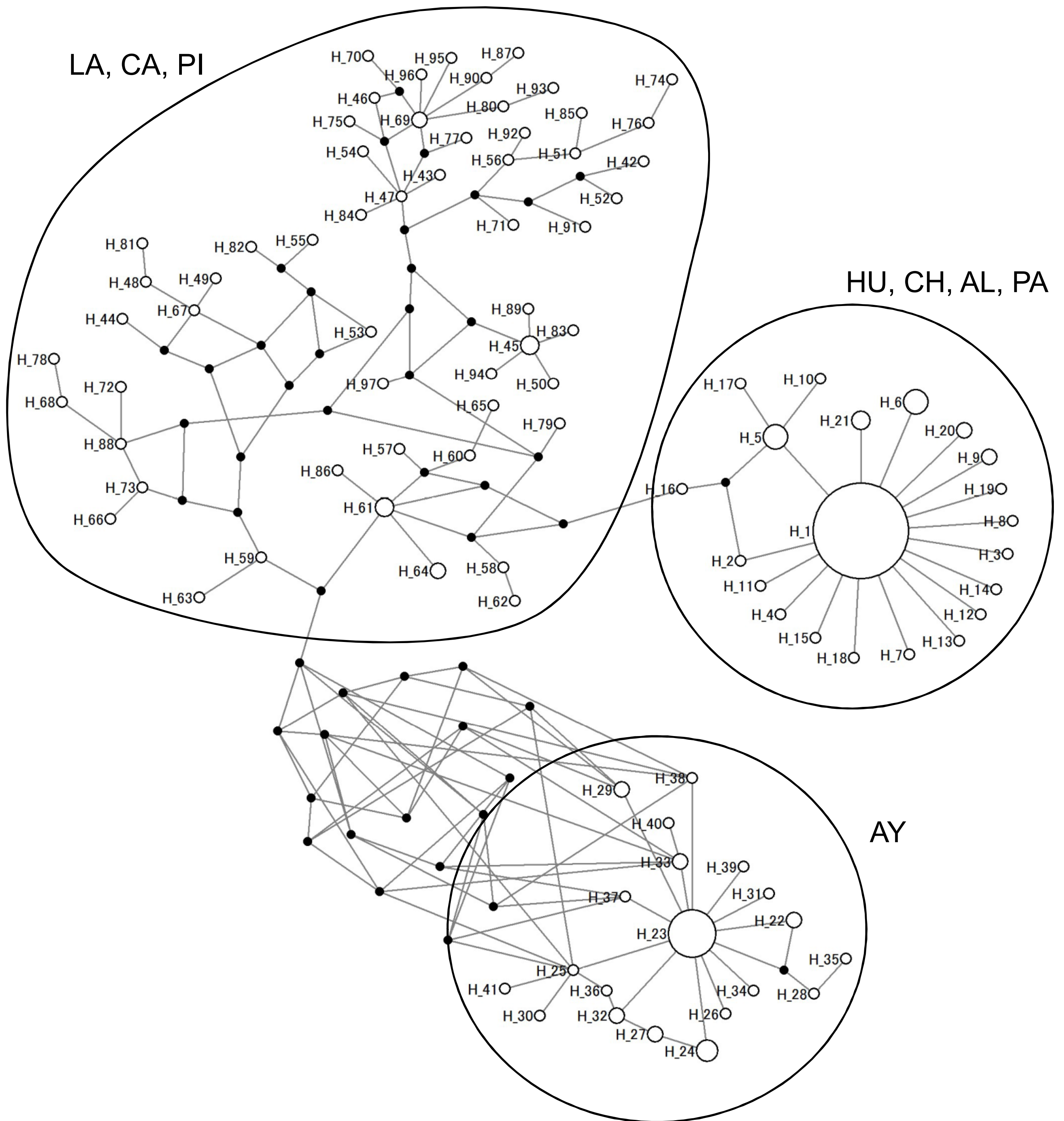

Fig. 6

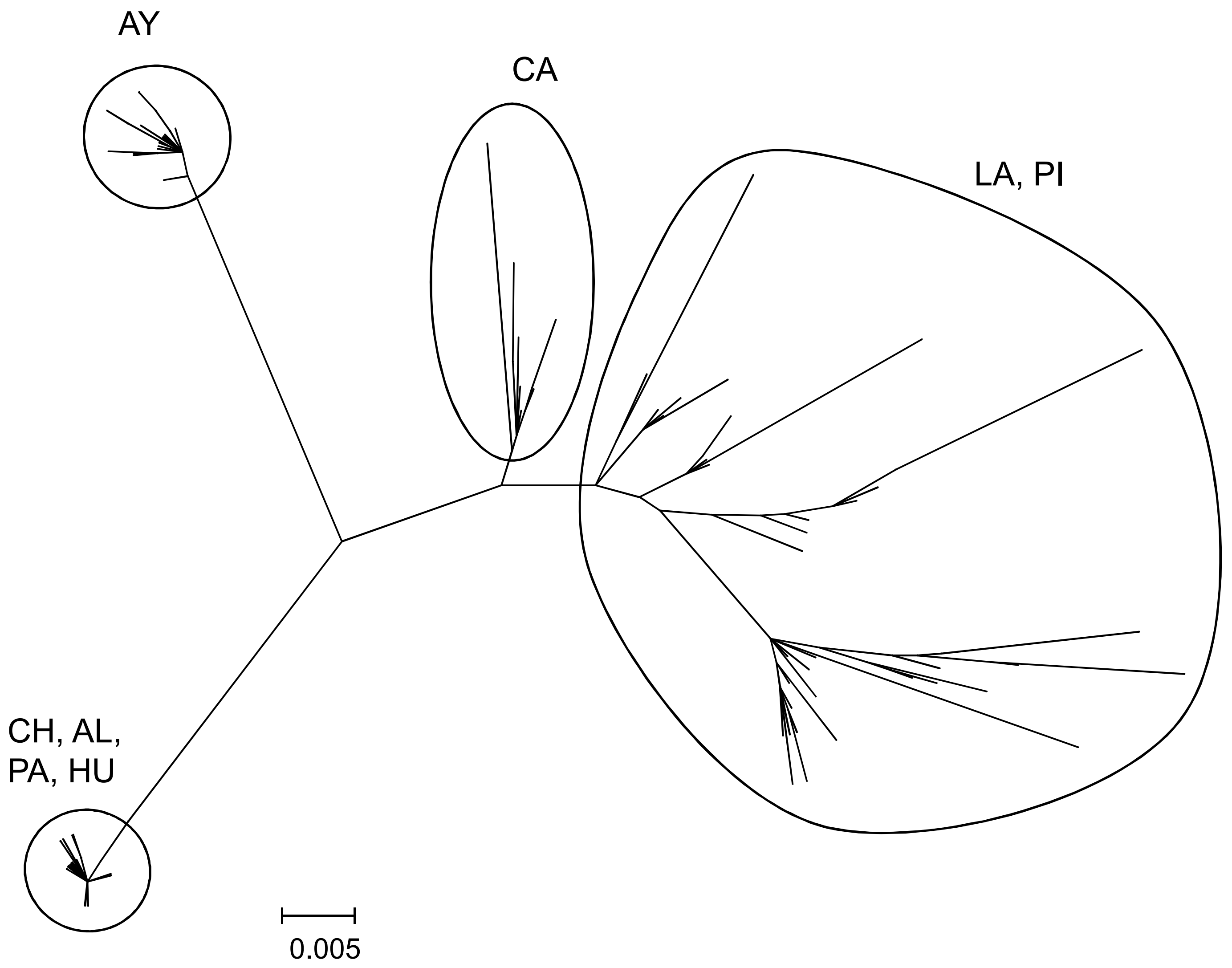

Fig. 7