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▣ ORIGINAL ARTICLE

Genetic Diversity of *Ascaris* in China Assessed Using Simple Sequence Repeat Markers

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Abstract: The giant roundworm *Ascaris* infects pigs and people worldwide and causes serious diseases. The taxonomic relationship between *Ascaris suum* and *Ascaris lumbricoides* is still unclear. The purpose of the present study was to investigate the genetic diversity and population genetic structure of 258 *Ascaris* specimens from humans and pigs from 6 sympatric regions in *Ascaris*-endemic regions of China using existing simple sequence repeat data. The microsatellite markers showed a high level of allelic richness and genetic diversity in the samples. Each of the populations demonstrated excess homozygosity (Ho < He, Fis > 0). According to a genetic differentiation index (Fst = 0.0593), there was a high-level of gene flow in the *Ascaris* populations. A hierarchical analysis on molecular variance revealed remarkably high levels of variation within the populations. Moreover, a population structure analysis indicated that *Ascaris* populations fell into 3 main genetic clusters, interpreted as *A. suum*, *A. lumbricoides*, and a hybrid of the species. We speculated that humans can be infected with *A. lumbricoides*, *A. suum*, and the hybrid, but pigs were mainly infected with *A. suum*. This study provided new information on the genetic diversity and population structure of *Ascaris* from human and pigs in China, which can be used for designing *Ascaris* control strategies. It can also be beneficial to understand the introgression of host affiliation.

Key words: *Ascaris lumbricoides*, *A. suum*, genetic diversity, structure, simple sequence repeat

INTRODUCTION

Ascariasis in humans is caused by infection with the soil-transmitted giant roundworm *Ascaris lumbricoides*. Approximately 760 million people are infected with this roundworm worldwide [1]. Although the majority of infections occur in developing countries, especially in Asia and Africa, cases have been reported in developed countries such as Japan, the United States, and Denmark [2-4]. The closely related parasite *Ascaris suum* mainly infects pigs [5]. *A. lumbricoides* and *A. suum* have similar transmission cycles and morphologies [6]. Cross-transmission of certain haplotypes and hybrids have been observed [7].

There has been considerable controversy about the taxonomic relationship between *A. lumbricoides* and *A. suum*. One view is that *A. lumbricoides* and *A. suum* are both valid species that persist in separate transmission cycles with limited gene flow [8]. The second is that *A. lumbricoides* and *A. suum* are different species existing in host-specialist parasite populations, but that there are some cross-infection and hybrids [7]. The third is that *A. lumbricoides* and *A. suum* are actually the same species [6, 9, 10].

Many molecular techniques, such as isoenzyme restriction fragment length polymorphism RFLP analyses of nuclear genes and mitochondrial DNA sequences have been used to study the genetic diversity and population structure of *Ascaris* [9, 11]. However, there are limitations in the analyses of genetic diversity and population structure using markers. For example, nuclear markers and isoenzymes have a low frequency of polymorphisms and mitochondrial DNA is maternally inherited and reflects the evolution of females rather than of the entire population [12]. In addition, the use of a single molecular marker can provide results that are misleading [13]. Therefore, new molecular techniques should be applied to investigate the genetic diversity and population structure of *Ascaris*. Microsatellite markers are regarded as an ideal tool for the examination

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of populations because they are co-dominantly inherited, easily amplified, and abundant [14]. Anderson and colleagues first applied microsatellite markers to understand mating of *A. lumbricoides* [15]. Then, Criscione and colleagues [16] developed and assessed 35 microsatellite markers for *Ascaris*, providing candidate markers for investigation of molecular epidemiology [17,18], mating patterns [5], cross-infection and hybridization [7,16], and genetic diversity [19,20].

Ascariasis is not considered a high-priority disease globally. However, ascariasis is still a public health problem in China [7] and additional prevention and control strategies are needed. To inform these strategies, the fine-scale genetic structure and microepidemiology of *Ascaris* in China must be surveyed. The genetic diversity and population structure of *Ascaris* in China has been examined using nuclear and mitochondrial markers, but rarely using microsatellite markers [8,11,21].

Previously, we determined the frequency and distribution of cross-infection and hybridization of human and pig *Ascaris* in sympatric populations in China [7]. In this paper, we re-analyzed these data (1) to determine the genetic diversity and structure of 12 *Ascaris* populations in China using multiple polymorphic microsatellite markers, (2) to apply traditional epidemiological models to population genetics, and (3) to better understand the relationship between *A. lumbricoides* and *A. suum*.

MATERIALS AND METHODS

Specimens and data collection

Specimens were collected from 12 *Ascaris* populations in humans (H) and pigs (P) in 6 provinces of China (Yunnan [YN], Hainan [HN], Jiangxi [JX], Xinjiang [XJ], Liaoning [LN], and Qinghai [QH]) described with 3-letter codes to indicate these sources. Twenty microsatellite loci in 258 samples were amplified according to methods described by Zhou et al. [7].

Microsatellite and genetic diversity analyses

The frequencies of null alleles were calculated using Micro-Checker ver. 2.2.3 [22]. The number of alleles (Na), allelic richness (Ar), and inbreeding coefficient (Fis) per population were computed using Fstat 2.9.3.2 [23]. GENETIX ver. 4.05.2 was used to calculate expected heterozygosity (He) and observed heterozygosity (Ho) per population [24]. Genepop ver. 4.0.7 was used to test linkage disequilibrium and Hardy-Weinberg equilibrium (HWE) with Bonferroni correction [25].

Population genetic structure analysis

Pairwise fixation index values (Fst) with ENA (excluding null alleles) correction was inferred using the FreeNA package [26]. Population genetic structure was analyzed in Structure ver. 2.3.4 using a Bayesian algorithm [27]. We used admixture ancestry and correlated allele frequency models with 20 runs and 100,000 Markov Chain Monte Carlo (MCMC) repetitions after a burn-in period of 100,000 interactions for each group number K. The appropriate K-value was determined using the method proposed by Evanno et al. [28] in the Structure Harvester program. Arlequin ver. 3.11 was used for a hierarchical analysis of molecular variance (AMOVA) [29].

RESULTS

Microsatellite variation and genetic diversity

The Micro-Checker analysis indicated that all alleles were null except at loci ALGA44, ALAC32, L017-est, and ALTN01. The frequencies of null alleles per locus ranged from 0.0000 to 0.2087 (Supplementary Table S1).

There were 2,435 alleles at 20 microsatellite loci in the 12 populations. The number of alleles observed (Na) per population varied from 143 to 276 (Table 1) and allelic richness (Ar) ranged from 6.7037 to 10.2161. Among sympatric populations of *Ascaris* from human and pigs, the number of alleles and allelic richness were greater in the populations from humans. Expected heterozygosity (He) and observed heterozygosity (Ho) ranged from 0.6209 to 0.7867 and 0.5299 to 0.6575, re-

Table 1. Genetic diversity in 12 populations of *Ascaris*

Population	No.[a]	Na	Ar	He	Ho	Fis
Jiangxi-H[b]	26	276	10.1245	0.7712	0.6283	0.204
Jiangxi-P[c]	15	143	6.8048	0.6209	0.5318	0.178
Xinjiang-H	21	204	8.5370	0.7306	0.5299	0.298
Xinjiang-P	21	156	6.7037	0.6673	0.5331	0.225
Qinghai-H	22	205	8.2204	0.7158	0.6037	0.179
Qinghai-P	21	158	6.8199	0.7000	0.5381	0.254
Hainan-H	22	230	9.4233	0.7645	0.5916	0.249
Hainan-P	21	196	8.2144	0.7369	0.5560	0.269
Liaoning-H	25	231	8.9309	0.7588	0.6106	0.216
Liaoning-P	20	164	7.0032	0.6710	0.5851	0.153
Yunnan-H	21	252	10.2161	0.7836	0.6575	0.185
Yunnan-P	23	220	9.1046	0.7867	0.5917	0.269

[a]No. of individuals sampled in each population, Na, no. of alleles observed; Ar, allelic richness; He, expected heterozygosity; Ho, observed heterozygosity; Fis, inbreeding coefficient.
[b]H, human *Ascaris*.
[c]P, pig *Ascaris*.

spectively. In all populations, the expected heterozygosity was greater than the observed heterozygosity and the Fis per population varied from 0.153 to 0.298.

The genotypic disequilibrium for each pair of 20 microsatellite loci was examined and significant linkage disequilibrium was identified in 6 loci: loci ALGA20 and ALGA15, ALGA32 and ALAC32, ALAC32 and L010, ALGA32 and ALAC01, ALGA15 and ALAC01, and L010 and ALAC01 Supplementary Table S2).

Microsatellite analysis revealed that there were 148 population-loci that deviated significantly from HWE at 20 microsatellite loci in the 12 *Ascaris* populations (Supplementary Table S3). When all loci were considered, deviation from HWE was detected in all populations (Supplementary Table S4).

Population differentiation

The pairwise Fst in the 12 populations ranged from 0.0065 to 0.1620, with a global value of 0.0567. The greatest genetic variation was observed in the populations HN-H and JX-P, whereas the least differentiation was in XJ-H and JX-H. The Fst in human *Ascaris* populations ranged from 0.0065 to 0.0496, with a mean of 0.0263. The Fst in *Ascaris* populations in pigs ranged from 0.0478 to 0.1271, with an average of 0.0810. The Fst in *Ascaris* populations in humans and pigs ranged from 0.0242 to 0.1620, with a mean of 0.0819 (Table 2).

Genetic relationships and population genetic structures

The 258 *Ascaris* specimens were further examined for genetic relationships using a Bayesian model in the software Structure. Possible population K-values from 1 to 12 were analyzed. The Bayesian clustering analysis showed that as K increased, InP (D) increased, then decreased (Supplementary Fig. S1). Subsequently, we calculated the relationship between Δ K and K, using a method described by Evanno et al. [28]. The clear maximum value of Δ K was at K=3, which indicated that the 12 populations could be differentiated into 3 groups (Supplementary Fig. S2). These groups are shown with red, green, and blue in Figure 1. Populations JX-P, XJ-P, QH-P, and LN-P had the highest membership coefficients (JX-P, 0.915; XJ-P, 0.928; QH-P, 0.872; LN-P, 0.800) in the second cluster (green). The majority of individuals from populations XJ-H, YN-H, and YN-P belonged to cluster 1 (red), whereas most individuals from JX-H, HN-H, and LN-H belonged to cluster 3 (blue) (Supplementary Table S5).

The AMOVA indicated greater genetic differentiation within populations than between populations. The genetic difference between *Ascaris* from pigs and *Ascaris* from humans was 1.9854%, with 3.9398% between populations in a group and 94.0749%

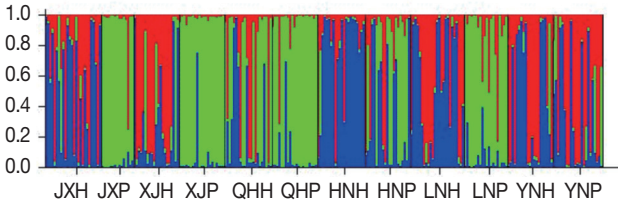

Fig. 1. Population genetic structure at K=3 based on microsatellite data from *Ascaris* sampled in different geographical regions. H, human *Ascaris*; P, pig *Ascaris*; JX, Jiangxi; XJ, Xinjiang; QH, Qinghai; HN, Hainan; LN, Liaoning; YN, Yunnan.

Table 2. Pairwise Fst values (below the diagonal) and probabilities (above the diagonal) over 20 loci in the 12 *Ascaris* populations

Pop	JX-H[a]	JX-P[b]	XJ-H	XJ-P	QH-H	QH-P	HN-H	HN-P	LN-H	LN-P	YN-H	YN-P
JX-H[a]		*	***	**	**	*	***	*	**	*	**	**
JX-P[b]	0.1165		*	*	*	*	NS	*	*	*	*	*
XJ-H	0.0065	0.1307		*	**	*	***	*	**	*	**	**
XJ-P	0.0909	0.0616	0.0956		**	*	*	*	*	*	*	*
QH-H	0.0244	0.0938	0.0242	0.0391		*	*	*	*	*	*	*
QH-P	0.0807	0.0892	0.0942	0.0478	0.0595		*	*	*	*	*	*
HN-H	0.0147	0.1620	0.0169	0.1080	0.0407	0.1062		*	**	*	**	*
HN-P	0.0488	0.1271	0.0536	0.0574	0.0557	0.0965	0.0567		*	*	*	*
LN-H	0.0268	0.1317	0.0215	0.1003	0.0496	0.0981	0.0250	0.0578		*	***	*
LN-P	0.0974	0.0730	0.1208	0.0619	0.0769	0.0662	0.1150	0.1096	0.1077		*	*
YN-H	0.0272	0.1414	0.0258	0.1016	0.0437	0.0943	0.0276	0.0498	0.0206	0.1013		**
YN-P	0.0242	0.1191	0.0298	0.0884	0.0504	0.0802	0.0304	0.0480	0.0307	0.0887	0.0255	

JX, Jiangxi; XJ, Xinjiang; QH, Qinghai; HN, Hainan; LN, Liaoning; YN, Yunnan; NS, not significant.
[a]H, human *Ascaris*.
[b]P, pig *Ascaris*.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 3. Analysis of genetic variance in the *Ascaris* populations

Source of variation	Sum of squares	Variance components	Percentage of variation	Fixation indices
Two groups (different hosts)				
Among groups	61.077	0.1579	1.9854%	FCT = 0.0196 ($P < 0.0001$)
Among populations within groups	207.202	0.3133	3.9397%	FSC = 0.0402 ($P < 0.0001$)
Within populations	3,711.708	7.4806	94.0749%	FST = 0.0593 ($P < 0.0001$)
Three groups (structure analysis)				
Among groups	91.159	0.1550	1.9557%	FCT = 0.0196 ($P < 0.0001$)
Among populations within groups	177.119	0.2909	3.6700%	FSC = 0.0374 ($P < 0.0001$)
Within populations	3,711.708	7.4806	94.3743%	FST = 0.0563 ($P < 0.0001$)

within populations. The structure analysis of the 3 genetic groups revealed limited variation between groups (1.9557%) and considerable variation within populations (94.3743%) (Table 3).

DISCUSSION

Microsatellite DNA molecular markers are widely used in population genetics research because of their high frequency of polymorphisms [30]. However, ubiquitous null alleles can cause allele numbers and frequencies to be underestimated, affecting assessments of genetic diversity [31]. A null allele is an allele of one locus that is not amplified, which can affect the accuracy of certain parameters based on the proportion of heterozygotes and, in particular, the accuracy of the inbreeding coefficient. In addition, higher null allele frequencies can reduce estimates of the genetic diversity within populations, causing fixation indices to be overestimated and the extent of gene flow to be underestimated in the analysis [32]. In the present study, the frequencies of null alleles per locus ranged from 0.0000 to 0.2087 (Supplementary Table S1). Null alleles in *Ascaris* populations in China could be a result of inbreeding (see discussion below). Inbreeding can accelerate the homogenization of populations, increasing the frequency of null alleles. When the null allele frequency is less than 0.2, they do not affect the accuracy of the data analysis [32]. Although there were various frequencies of null alleles in this study, accuracy of the results was not affected.

High genetic diversity was observed in the *Ascaris* populations at 20 simple sequence repeat (SSR) regions (Table 1). This result was similar to that based on mitochondrial data [21]. Among sympatric populations of *Ascaris* from human and pigs, the number of alleles and allelic richness was greater in the populations from human, which may because of cross-transmission (human infections with *Ascaris* from pigs) and hybridization [2-4,7,18]. In all populations, the expected heterozygosity was higher than the observed heterozygosity and Fis values were positive. The same results were obtained for populations of *Ascaris* in southwestern Uganda [20]. Positive Fis values indicate an excess of homozygotes [16]. The heterozygote deficiency (Ho < He, Fis > 0) demonstrated in these *Ascaris* populations may due to inbreeding. Polyandry [5], hybridization [7], Wahlund effects [16], or null alleles [20] can also result in such a deficiency.

When genetic variation is studied at 2 or more loci simultaneously, allele frequency is insufficient to indicate the extent of genetic variation in natural populations, so the non-random association of alleles at different sites must also be considered [33]. Linkage disequilibrium refers to the non-random association of alleles at genetic loci [34]. The decay of linkage disequilibrium is affected by many factors, including genetic drift, natural selection, mutation, and gene flow [35]. Linkage disequilibrium was apparent in this study, but not all associations were identified. Significant associations between pairs of loci were also not tested in populations of *A. lumbricoides* from Nepal [16] and similar results were found in insect species [36].

The microsatellite analysis revealed a departure from HWE for a number of loci in the *Ascaris* populations (Supplementary Table S3). Taking all loci into account, all populations deviated from HWE (Supplementary Table S4). Similar results were reported for *Ascaris* populations in southwestern Uganda [20]. A lack of heterozygotes may be a universal phenomenon in *Ascaris* populations. Criscione et al. [16] also found a departure from HWE caused by heterozygote deficiency by developing and assessing microsatellite markers in *A. lumbricoides*. In almost all cases, a departure from HWE was also observed in southwestern Uganda [20]. Using Micro-Checker software, we detected null alleles. Thus, departures of populations from HWE might have resulted from heterozygote deficiency and/or null alleles.

Pairwise Fst values were divided into 4 categories: Fst < 0.05

indicated that genetic differentiation among populations was very low; 0.05 < Fst < 0.15 signified moderate genetic differentiation among populations; 0.15 < Fst < 0.25 indicated that genetic differentiation was high; and Fst > 0.25 expressed a very high level of genetic differentiation [37]. When population structuring in *Ascaris* from humans was investigated, there was little evidence for genetic differentiation between worm populations (Fst = 0.0264) (Table 2). However, when populations from humans were compared with populations from pigs, genetic differentiation was moderate (Fst = 0.0819), as it was between populations from pigs (Fst = 0.0810). Differentiation between populations of *Ascaris* from China, measured by Fst and based on mitochondrial DNA, was previously shown to vary greatly [8]. Fst based on microsatellite data suggested that there was gene flow in *Ascaris* populations (either within human- or pig-derived populations or between the 2 species), similar to that shown in our previous research [21]. Despite the wide geographical area over which specimens were collected, genetic differentiation was limited and, similar to the results of previous studies [9,16], no clear host or geographical patterns were identified. There was evidence for panmixia or high gene flow between worm populations in 2 villages in southwestern Uganda based on the haplotype data and microsatellite analysis, despite their physical separation [20].

In population genetics studies, the question of how to objectively identify and divide homogeneous populations has plagued researchers [27]. This problem was solved by Structure. Then, the problem was how to objectively determine the cluster values (K) in cluster analyses. Evanno developed a ΔK method to calculate the possible values for the cluster K. The Bayesian clustering analysis showed that, with an increase in K, the values of lnP (D) first increased and then decreased (Supplementary Fig. S1). This result indicated there was weak differentiation among the 12 worm populations. The optimal cluster was difficult to be determined using Pritchard's methods based on lnP (D) [27]. This may have been because, in highly mixed worm populations, a gene frequency gradient was formed. In this case, the value of K was also vague and inaccurate according to lnP (D). To determine the appropriate K value, the method of Evanno was used. In this study, the ΔK was largest at K = 3 (Fig. 1). Therefore, the 12 *Ascaris* populations could be divided into 3 larger groups.

The result of the Structure analysis revealed that there were 3 clusters of *Ascaris* in China and that each cluster consisted of several populations. Populations from pigs (JX-P, XJ-P, QH-P, LN-P) constituted one group. The rest of the populations were divided into 2 genetic groups that did not represent their host or geographic distributions. The genetic structure could be explained by the hypothesis that the 3 groups represent *A. suum*, *A. lumbricoides*, and *Ascaris* hybrids, respectively. Animal experiments have shown that mice can be infected with eggs of *A. suum*; however, pigs were not susceptible to infection with eggs of *A. lumbricoides* [38]. In addition, humans can be infected by *A. suum* [2-4,18]. This information may explain why cross-infection and hybridization can occur in humans. We propose that people in China become infected with *A. lumbricoides*, *A. suum*, and a hybrid of the 2 species, but that pigs are not susceptible to this hybrid. Of course, this hypothesis requires testing with animal experiments.

The AMOVA analyses show variation in *Ascaris* population, similar to what was shown by Cavallero et al. [9]. Our results confirmed that gene flow between populations of *Ascaris* was strong and that population differentiation was weak. The genetic variation among the 3 groups was relatively small, but statistically significant (Table 3).

Based on results of Fst statistics, a Bayesian clustering analysis, and AMOVA using microsatellite data, worm population differentiation was low and gene flow was high between the populations. These findings are consistent with those of a previous Bayesian clustering study [7]. The absence of differences could be a result of complex transmission mechanisms. Although there are no confirmed drug resistance genes in *Ascaris*, more attention should be paid to the high degree of gene flow in *Ascaris* [39].

Conclusively, the results of this study provided additional insights into the genetic diversity and population structure of *Ascaris* from humans and pigs in China. This knowledge can be useful for treatment and control of ascariasis. It might also be beneficial in understanding the co-evolution of hosts and parasites and the introgression of host affiliation and/or drug-resistance genes between different parasite populations.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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Supplementary Table S1. Null allele frequency per locus

Locus	Null allele frequency
1	0.0000
2	0.1722
3	0.1453
4	0.1842
5	0.1603
6	0.1053
7	0.2071
8	0.1326
9	0.0981
10	0.1924
11	0.1095
12	0.0896
13	0.1988
14	0.0000
15	0.0000
16	0.2024
17	0.2087
18	0.1146
19	0.0000
20	0.0464

Supplementary Table S2. Analysis of disequilibrium of pairwise loci across all populations

Locus pair	Chi2	df	*P*-value
Loci-ALGA44 & Loci-ALGA32	8.5041	24	0.9985
Loci-ALGA44 & Loci-ALGA31	17.4453	24	0.8291
Loci-ALGA32 & Loci-ALGA31	32.4170	24	0.1170
Loci-ALGA44 & Loci-ALGA20	15.4340	24	0.9074
Loci-ALGA32 & Loci-ALGA20	21.8617	24	0.5875
Loci-ALGA31 & Loci-ALGA20	20.0654	24	0.6931
Loci-ALGA44 & Loci-ALGA48	17.5742	24	0.8232
Loci-ALGA32 & Loci-ALGA48	10.6075	24	0.9915
Loci-ALGA31 & Loci-ALGA48	17.0356	24	0.8471
Loci-ALGA20 & Loci-ALGA48	38.6087	24	0.0300
Loci-ALGA44 & Loci-ALGA15	6.6071	24	0.9998
Loci-ALGA32 & Loci-ALGA15	23.2441	24	0.5054
Loci-ALGA31 & Loci-ALGA15	13.6759	24	0.9536
Loci-ALGA20 & Loci-ALGA15	Infinity	24	Highly sign.
Loci-ALGA48 & Loci-ALGA15	8.5954	24	0.9983
Loci-ALGA44 & Loci-L001-est	24.1556	24	0.4527
Loci-ALGA32 & Loci-L001-est	16.8932	24	0.8532
Loci-ALGA31 & Loci-L001-est	12.3693	24	0.9754
Loci-ALGA20 & Loci-L001-est	15.1043	24	0.9177
Loci-ALGA48 & Loci-L001-est	14.3721	24	0.9378
Loci-ALGA15 & Loci-L001-est	13.2844	24	0.9612
Loci-ALGA44 & Loci-ALAC08	21.6364	24	0.6010
Loci-ALGA32 & Loci-ALAC08	14.8728	24	0.9244
Loci-ALGA31 & Loci-ALAC08	37.8652	24	0.0358
Loci-ALGA20 & Loci-ALAC08	21.3611	24	0.6173
Loci-ALGA48 & Loci-ALAC08	17.9917	24	0.8034
Loci-ALGA15 & Loci-ALAC08	9.4690	24	0.9964
Loci-L001-est & Loci-ALAC08	19.5046	24	0.7246
Loci-ALGA44 & Loci-ALAC09	27.0478	24	0.3022
Loci-ALGA32 & Loci-ALAC09	11.0259	24	0.9888
Loci-ALGA31 & Loci-ALAC09	26.7457	24	0.3164
Loci-ALGA20 & Loci-ALAC09	7.4000	24	0.9995
Loci-ALGA48 & Loci-ALAC09	15.8017	24	0.8951
Loci-ALGA15 & Loci-ALAC09	13.5450	24	0.9563
Loci-L001-est & Loci-ALAC09	28.3226	24	0.2467
Loci-ALAC08 & Loci-ALAC09	22.3991	24	0.5555
Loci-ALGA44 & Loci-ALAC07	28.4986	24	0.2396
Loci-ALGA32 & Loci-ALAC07	30.2567	24	0.1764
Loci-ALGA31 & Loci-ALAC07	20.5125	24	0.6673
Loci-ALGA20 & Loci-ALAC07	15.9380	24	0.8903
Loci-ALGA48 & Loci-ALAC07	12.2115	24	0.9774
Loci-ALGA15 & Loci-ALAC07	28.3669	24	0.2449
Loci-L001-est & Loci-ALAC07	37.5971	24	0.0381
Loci-ALAC08 & Loci-ALAC07	28.3836	24	0.2442
Loci-ALAC09 & Loci-ALAC07	16.7202	24	0.8603
Loci-ALGA44 & Loci-L007	13.1700	24	0.9632
Loci-ALGA32 & Loci-L007	22.1386	24	0.5710
Loci-ALGA31 & Loci-L007	14.4012	24	0.9371
Loci-ALGA20 & Loci-L007	21.5863	24	0.6039
Loci-ALGA48 & Loci-L007	17.9327	24	0.8063

(*Continued to the next*)

Supplementary Table S2. Continued

Locus pair	Chi2	df	*P*-value
Loci-ALGA15 & Loci-L007	21.6289	24	0.6014
Loci-L001-est & Loci-L007	30.8648	24	0.1577
Loci-ALAC08 & Loci-L007	18.1151	24	0.7974
Loci-ALAC09 & Loci-L007	28.2192	24	0.2509
Loci-ALAC07 & Loci-L007	27.6889	24	0.2734
Loci-ALGA44 & Loci-L008	9.9716	24	0.9947
Loci-ALGA32 & Loci-L008	3.7162	24	1.0000
Loci-ALGA31 & Loci-L008	28.2567	24	0.2494
Loci-ALGA20 & Loci-L008	32.0852	24	0.1249
Loci-ALGA48 & Loci-L008	20.3630	24	0.6760
Loci-ALGA15 & Loci-L008	13.9275	24	0.9483
Loci-L001-est & Loci-L008	19.6521	24	0.7164
Loci-ALAC08 & Loci-L008	22.2623	24	0.5636
Loci-ALAC09 & Loci-L008	8.0675	24	0.9990
Loci-ALAC07 & Loci-L008	23.5493	24	0.4876
Loci-L007 & Loci-L008	29.6724	24	0.1958
Loci-ALGA44 & Loci-ALTN02	23.1204	24	0.5127
Loci-ALGA32 & Loci-ALTN02	15.9042	24	0.8915
Loci-ALGA31 & Loci-ALTN02	26.7059	24	0.3183
Loci-ALGA20 & Loci-ALTN02	18.9105	24	0.7567
Loci-ALGA48 & Loci-ALTN02	23.7495	24	0.4760
Loci-ALGA15 & Loci-ALTN02	18.2233	24	0.7920
Loci-L001-est & Loci-ALTN02	37.8181	24	0.0362
Loci-ALAC08 & Loci-ALTN02	22.9674	24	0.5217
Loci-ALAC09 & Loci-ALTN02	28.9376	24	0.2225
Loci-ALAC07 & Loci-ALTN02	22.6168	24	0.5425
Loci-L007 & Loci-ALTN02	32.6113	24	0.1125
Loci-L008 & Loci-ALTN02	54.2653	24	0.0004
Loci-ALGA44 & Loci-ALAC32	4.4543	24	1.0000
Loci-ALGA32 & Loci-ALAC32	Infinity	24	Highly sign.
Loci-ALGA31 & Loci-ALAC32	18.6912	24	0.7683
Loci-ALGA20 & Loci-ALAC32	22.2961	24	0.5616
Loci-ALGA48 & Loci-ALAC32	2.9702	24	1.0000
Loci-ALGA15 & Loci-ALAC32	15.7122	24	0.8982
Loci-L001-est & Loci-ALAC32	15.3986	24	0.9086
Loci-ALAC08 & Loci-ALAC32	7.9486	24	0.9991
Loci-ALAC09 & Loci-ALAC32	12.2437	24	0.9770
Loci-ALAC07 & Loci-ALAC32	17.8333	24	0.8110
Loci-L007 & Loci-ALAC32	27.1513	24	0.2975
Loci-L008 & Loci-ALAC32	19.2166	24	0.7403
Loci-ALTN02 & Loci-ALAC32	16.9535	24	0.8506
Loci-ALGA44 & Loci-L017-est	19.6068	24	0.7189
Loci-ALGA32 & Loci-L017-est	21.4184	24	0.6139
Loci-ALGA31 & Loci-L017-est	36.1007	24	0.0537
Loci-ALGA20 & Loci-L017-est	9.8421	24	0.9952
Loci-ALGA48 & Loci-L017-est	16.8971	24	0.8530
Loci-ALGA15 & Loci-L017-est	16.9634	24	0.8502
Loci-L001-est & Loci-L017-est	24.6890	24	0.4228
Loci-ALAC08 & Loci-L017-est	13.5092	24	0.9570
Loci-ALAC09 & Loci-L017-est	9.0927	24	0.9974
Loci-ALAC07 & Loci-L017-est	20.5073	24	0.6676

(*Continued to the next page*)

Supplementary Table S2. Continued

Locus pair	Chi2	df	*P*-value
Loci-L007 & Loci-L017-est	28.3265	24	0.2465
Loci-L008 & Loci-L017-est	23.2879	24	0.5029
Loci-ALTN02 &Loci-L017-est	20.0166	24	0.6958
Loci-ALAC32 &Loci-L017-est	17.9394	24	0.8059
Loci-ALGA44 & Loci-L010	16.4681	24	0.8704
Loci-ALGA32 & Loci-L010	37.6349	24	0.0378
Loci-ALGA31 & Loci-L010	26.9364	24	0.3074
Loci-ALGA20 & Loci-L010	28.3507	24	0.2455
Loci-ALGA48 & Loci-L010	10.6590	24	0.9912
Loci-ALGA15 & Loci-L010	21.1388	24	0.6305
Loci-L001-est & Loci-L010	15.3285	24	0.9108
Loci-ALAC08 & Loci-L010	34.0256	24	0.0842
Loci-ALAC09 & Loci-L010	21.3587	24	0.6175
Loci-ALAC07 & Loci-L010	29.0234	24	0.2193
Loci-L007 & Loci-L010	18.4968	24	0.7783
Loci-L008 & Loci-L010	13.0566	24	0.9652
Loci-ALTN02 & Loci-L010	20.6774	24	0.6577
Loci-ALAC32 & Loci-L010	Infinity	24	Highly sign.
Loci-L017-est & Loci-L010	18.9125	24	0.7566
Loci-ALGA44 & Loci-ALAC01	24.9654	24	0.4076
Loci-ALGA32 & Loci-ALAC01	Infinity	24	Highly sign.
Loci-ALGA31 & Loci-ALAC01	20.9465	24	0.6419
Loci-ALGA20 & Loci-ALAC01	25.5918	24	0.3742
Loci-ALGA48 & Loci-ALAC01	12.2147	24	0.9774
Loci-ALGA15 & Loci-ALAC01	Infinity	24	Highly sign.
Loci-L001-est & Loci-ALAC01	11.5777	24	0.9843
Loci-ALAC08 & Loci-ALAC01	20.7899	24	0.6511
Loci-ALAC09 & Loci-ALAC01	13.8861	24	0.9492
Loci-ALAC07 & Loci-ALAC01	24.1816	24	0.4513
Loci-L007 & Loci-ALAC01	10.6138	24	0.9915
Loci-L008 & Loci-ALAC01	11.8527	24	0.9815
Loci-ALTN02 & Loci-ALAC01	24.9774	24	0.4070
Loci-ALAC32 & Loci-ALAC01	25.8442	24	0.3611
Loci-L017-est & Loci-ALAC01	20.0661	24	0.6930
Loci-L010 & Loci-ALAC01	Infinity	24	Highly sign.
Loci-ALGA44 & Loci-ALGA47	9.9470	20	0.9691
Loci-ALGA32 & Loci-ALGA47	6.6504	20	0.9977
Loci-ALGA31 & Loci-ALGA47	18.0108	20	0.5867
Loci-ALGA20 & Loci-ALGA47	9.9094	20	0.9698
Loci-ALGA48 & Loci-ALGA47	9.9166	20	0.9697
Loci-ALGA15 & Loci-ALGA47	3.0464	20	1.0000
Loci-L001-est & Loci-ALGA47	13.6566	20	0.8475
Loci-ALAC08 & Loci-ALGA47	17.8753	20	0.5956
Loci-ALAC09 & Loci-ALGA47	9.2253	20	0.9801
Loci-ALAC07 & Loci-ALGA47	9.7258	20	0.9729
Loci-L007 & Loci-ALGA47	16.8049	20	0.6656
Loci-L008 & Loci-ALGA47	2.7796	20	1.0000
Loci-ALTN02 & Loci-ALGA47	8.1918	20	0.9905
Loci-ALAC32 & Loci-ALGA47	3.9804	20	1.0000
Loci-L017-est & Loci-ALGA47	5.2393	20	0.9996
Loci-L010 & Loci-ALGA47	10.8331	20	0.9504
Loci-ALAC01 & Loci-ALGA47	28.7703	20	0.0924

(*Continued to the next*)

Supplementary Table S2. Continued

Locus pair	Chi2	df	*P*-value
Loci-ALGA44 & Loci-ALTN01	0.2910	24	1.0000
Loci-ALGA32 & Loci-ALTN01	7.2364	24	0.9996
Loci-ALGA31 & Loci-ALTN01	14.3650	24	0.9380
Loci-ALGA20 & Loci-ALTN01	7.1605	24	0.9996
Loci-ALGA48 & Loci-ALTN01	5.5361	24	1.0000
Loci-ALGA15 & Loci-ALTN01	6.8773	24	0.9998
Loci-L001-est & Loci-ALTN01	8.9051	24	0.9978
Loci-ALAC08 & Loci-ALTN01	0.0000	24	1.0000
Loci-ALAC09 & Loci-ALTN01	12.9868	24	0.9663
Loci-ALAC07 & Loci-ALTN01	17.2990	24	0.8356
Loci-L007 & Loci-ALTN01	10.7575	24	0.9906
Loci-L008 & Loci-ALTN01	5.7782	24	1.0000
Loci-ALTN02 & Loci-ALTN01	12.5295	24	0.9733
Loci-ALAC32 & Loci-ALTN01	16.1392	24	0.8830
Loci-L017-est & Loci-ALTN01	13.6322	24	0.9545
Loci-L010 & Loci-ALTN01	6.6142	24	0.9998
Loci-ALAC01 & Loci-ALTN01	4.4814	24	1.0000
Loci-ALGA47 & Loci-ALTN01	18.3393	20	0.5651
Loci-ALGA44 & Loci-ALTN04	22.6123	24	0.5428
Loci-ALGA32 & Loci-ALTN04	3.1532	24	1.0000
Loci-ALGA31 & Loci-ALTN04	16.8710	24	0.8541
Loci-ALGA20 & Loci-ALTN04	22.5058	24	0.5491
Loci-ALGA48 & Loci-ALTN04	21.0223	24	0.6374
Loci-ALGA15 & Loci-ALTN04	4.1450	24	1.0000
Loci-L001-est & Loci-ALTN04	17.5523	24	0.8242
Loci-ALAC08 & Loci-ALTN04	15.5787	24	0.9027
Loci-ALAC09 & Loci-ALTN04	3.2069	24	1.0000
Loci-ALAC07 & Loci-ALTN04	5.6282	24	1.0000
Loci-L007 & Loci-ALTN04	19.7936	24	0.7085
Loci-L008 & Loci-ALTN04	8.0347	24	0.9991
Loci-ALTN02 & Loci-ALTN04	13.4982	24	0.9572
Loci-ALAC32 & Loci-ALTN04	12.1620	24	0.9780
Loci-L017-est & Loci-ALTN04	14.3658	24	0.9380
Loci-L010 & Loci-ALTN04	6.4726	24	0.9999
Loci-ALAC01 & Loci-ALTN04	8.4718	22	0.9957
Loci-ALGA47 & Loci-ALTN04	3.5992	20	1.0000
Loci-ALTN01 & Loci-ALTN04	10.1631	24	0.9938

Supplementary Table S3. Hardy-Weinberg exact test at 20 simple sequence repeat loci in 12 populations of *Ascaris*

Locus	JX-H[a]	JX-P[b]	XJ-H	XJ-P	QH-H	QH-P	HN-H	HN-P	LN-H	LN-P	YN-H	YN-P
ALGA44	0.0073*	0.1396	0.0188*	0.5070	0.1920	0.1361	0.4512	0.0014*	0.0049*	0.0760	0.0812	0.0617
ALGA32	0.0000*	0.0001*	0.0000*	0.0007*	0.0000*	0.0439*	0.0315*	0.0193*	0.0000*	0.0950	0.0074*	0.0014*
ALGA31	0.0487*	0.0129*	0.0000*	0.0000*	0.6615	0.0078*	0.0883	0.0006*	0.1605	0.4345	0.0000*	0.0000*
ALGA20	0.0000*	0.0000*	0.0000*	0.0000*	0.0150*	0.0228*	0.0000*	0.0480*	0.0000*	0.5246	0.0000*	0.0000*
ALGA48	0.0000*	0.1965	0.1404	0.0000*	0.0021*	0.0108*	0.0439*	0.0082*	0.0134*	0.0003*	0.1248	0.0077*
ALGA15	0.0204*	0.2561	0.0000*	0.0132*	0.0000*	0.8902	0.0000*	0.0028*	0.0737	0.9330	0.9274	0.0000*
L001-est	0.0000*	0.0143*	0.0107*	0.0007*	0.0000*	0.0000*	0.0000*	0.0002*	0.0624	0.0000*	0.0003*	0.0000*
ALAC08	0.1173	0.8685	0.0011*	0.0000*	0.0000*	0.0001*	0.0000*	0.0007*	0.0000*	0.5502	0.4550	0.0000*
ALAC09	0.0030*	1.0000	0.7472	0.1036	0.5979	0.4574	0.0000*	0.1780	0.0000*	0.6259	0.1493	0.0238*
ALAC07	0.0357*	0.0014*	0.0000*	0.4102	0.0038*	0.0144*	0.0000*	0.0003*	0.0000*	0.0002*	0.0881	0.0007*
L007	0.0158*	-	1.0000	0.0862	1.0000	0.0544	0.0088*	0.0129*	0.0026*	1.0000	0.5477	0.0003*
L008	0.9556	0.4129	0.0310*	0.0288*	0.8758	0.0000*	0.0000*	0.1765	0.0021*	0.0277*	0.4577	0.0000*
ALTN02	0.0021*	0.0000*	0.0000*	0.0000*	0.0767	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
ALAC32	0.8492	0.2787	0.6952	0.2093	0.5180	0.0851	0.1063	0.0776	0.0000*	0.3334	0.9521	0.0750
L017-est	0.8141	0.5085	0.0258*	0.3784	0.0258*	1.0000	0.7146	0.1488	0.2104	0.3077	1.0000	0.0002*
L010	0.0000*	0.0275*	0.0000*	0.0119*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0200*	0.0000*	0.0000*
ALAC01	0.0000*	0.0303*	0.0000*	0.0059*	0.0000*	0.0054*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
ALGA47	0.0000*	0.0158*	0.0195*	0.0359*	0.0000*	0.0019*	0.0823	0.0020*	0.0005*	0.0912	0.0000*	0.0000*
ALTN01	0.0829	0.7487	0.4877	0.8735	0.7729	0.1027	0.1883	0.0029*	0.6467	0.7381	0.7758	0.1127
ALTN04	0.0393*	0.5054	0.5174	0.1853	0.8413	0.1462	0.0653	0.0397*	0.0068*	0.0136*	0.0000*	0.4581

JX, Jiangxi; XJ, Xinjiang; QH, Qinghai; HN, Hainan; LN, Liaoning; YN, Yunnan.
[a]H, Human *Ascaris*.
[b]P, Pig *Ascaris*.
*$P < 0.05$.

Supplementary Table S4. Table Hardy-Weinberg exact tests for each population

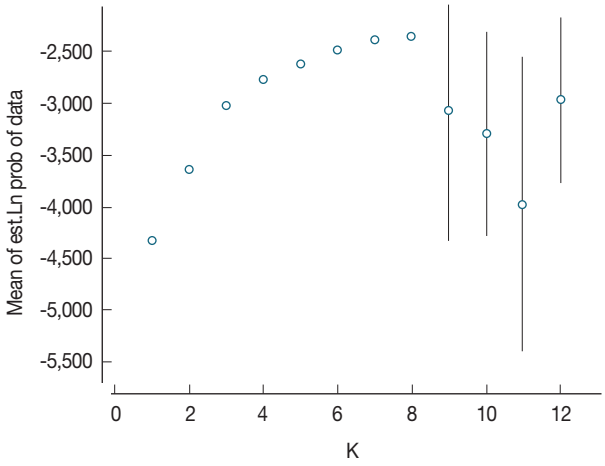
Population	*P*-value	S.E.
JX-H[a]	0.0000	0.0000
JX-P[b]	0.0000	0.0000
XJ-H	0.0000	0.0000
XJ-P	0.0000	0.0000
QH-H	0.0000	0.0000
QH-P	0.0000	0.0000
HN-H	0.0000	0.0000
HN-P	0.0000	0.0000
LN-H	0.0000	0.0000
LN-P	0.0000	0.0000
YN-H	0.0000	0.0000
YN-P	0.0000	0.0000

JX, Jiangxi; XJ, Xinjiang; QH, Qinghai; HN, Hainan; LN, Liaoning; YN, Yunnan.
[a]H, Human *Ascaris*.
[b]P, Pig *Ascaris*.

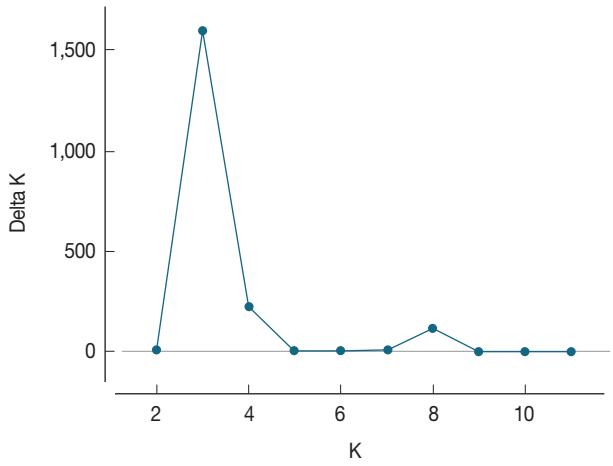
Supplementary Table S5. Proportion of membership coefficients with K = 3 in each population

Population	Red	Green	Blue
JX-H[a]	0.395	0.066	0.539
JX-P[b]	0.062	0.915	0.023
XJ-H	0.600	0.133	0.267
XJ-P	0.018	0.928	0.054
QH-H	0.291	0.485	0.224
QH-P	0.060	0.872	0.068
HN-H	0.189	0.038	0.773
HN-P	0.227	0.429	0.344
LN-H	0.451	0.021	0.528
LN-P	0.132	0.800	0.068
YN-H	0.509	0.054	0.437
YH-P	0.577	0.107	0.316

JX, Jiangxi; XJ, Xinjiang; QH, Qinghai; HN, Hainan; LN, Liaoning; YN, Yunnan.
[a]H, Human *Ascaris*.
[b]P, Pig *Ascaris*.



Supplementary Fig. S1. Relationship between lnP (D) and K-values from the STRUCTURE analysis.



Supplementary Fig. S2. Number of inferred clusters (K) with the highest probability determined by applying Evanno's Δ K method.