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Evidence of Restricted Maternal Gene Flow of Purana (Old) Population in the Suburbs of Sigiriya, Sri Lanka

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Abstract

Introduction

Based on historical records suggesting that the ancestry of the *Purana* (old) population living in the suburbs of Sigiriya in Sri Lanka can be traced back to the times of the Sinhalese Kings of the 5th century A.D. (1,450 YBP) and studies reporting that the *Purana* are biometrically different from the rest of present Sri Lankans and other populations in the world, we investigate the maternally inherited Mitochondrial DNA (mtDNA) of *Purana* population in the suburbs of Sigiriya to better understand genetic affinities of them to present day Sri Lankans.

Materials and Methods

Forty six *Purana* inhabitants belonging to *Purana* pedigrees were recruited in the study. Mitochondrial DNA was extracted, amplified and sequenced. Polymorphisms of mtDNA hypervariable segment I (HVS –I) between nucleotide positions 16,004 – 16,411 were identified using CLUSTALX option of MEGAVA 4.0 sequence alignment software. In order to investigate genetic affinity of *Purana* population, Arlequin software version 3.11 and analysis of molecular variance (AMOVA) were applied using reported similar genetic data of present Sri Lankans such as Sinhalese, Sri Lankan Tamils, Indian Tamils, Sri Lankan Moors and *Vedda*. Genetic relationships of *Purana* population with other Sri Lankans were further explored by phylogenetic analysis.

Results and Analysis

Genetic dissimilarity among groups was higher (2.81%) when populations were grouped into two as modern and *Purana* than grouping them according to their ethnic basis (0.00%). This indicates a restricted mtDNA flow between the two groups *(Purana* and rest of the Sri Lankans) that made *Purana* population was maternally isolated from the rest of Sri Lankans.

Conclusion

Detailed phylogenetic analysis of the study revealed that they are maternally more related to Sri Lankan Tamil than to any other present Sri Lankans.

Keywords: Mitochondrial DNA, Maternal Inheritance, *Purana* population, Sigiriya Sri Lanka

Introduction

Mitochondrial DNA (mtDNA) provides a potent tool for studying human evolution, in view of characteristics such as a maternal mode of inheritance, a mutation rate ~10x higher than nuclear DNA, presence in high copy number, and lack of recombination[1,2,3,4]. As mtDNA is strictly maternally inherited, sequence variation evolves through the sequential accumulation of base substitutions along radiating maternal lineages[1,3,4,5]. Polymorphisms which occurred thousands of years ago results in the geographic structuring of mtDNA haplogroup distributions therefore it is possible to investigate an individual's matrilineal ancestry [6,5].

Mitochondrial DNA is composed of two regions: the D-loop control region (the non-coding segment of mtDNA), and the coding region, and that while the coding region is much larger, we more often study the polymorphisms in the D-loop region[7]. The D-loop region is 1124 bp in length and is made up of hypervariable segments I and II (HVS - I and HVS - II)[8]. In human mitochondrial genome, HVS - I is found between the nucleotide positions 16, 024 to 16,365 and HVS II from positions 73 to 340[7].

The HVSs reveal about 3 % variability between individuals. Within the HVSs, the polymorphic sites are not distributed uniformly. Because of high number of polymorphic sites, the analysis of HVS - I and II enable to study matrilineal ancestry and explore patterns of female movement and interactions in the past[9]. The HVSs are highly valuable markers in terms of population genetics or phylogenetic studies[10,11].

The first mtDNA study on *Vedda* in Sri Lanka a minority indigenous group of people in Sri Lanka was done by Harihara[12]. Since then several national level genomic studies have been conducted representing major ethnic groups and *Vedda* in Sri Lanka[13]. The Genome Variation Database containing information on Single Nucleotide Polymorphisms (SNPs) found in major ethnic groups in Sri Lanka (i.e. Sinhalese, Tamils and Moors) has been established[14]. The genetic database of Sri Lankan population, containing information including genotype frequencies of 34 genomic variations encompassing 14 medically important genes, has been reported[15].

“The ancestry of the present-day *Purana* inhabitants of villages *Talkote, Pidurangala, Diyakepilla, Nagalaweva, Alakolaweva, Ilukweva, Kosgahaela* in Sigiriya area has been a subject of debate. There are two potential hypotheses regarding their ancestry can be tested. They are descendants of the prehistoric hunter-gatherer populations from the region or descendants of contemporary *Vedda* community in Sigiriya region.

The Purana populations in Sigiriya represented by having *Purana* surnames such as *Aluthgedara, Gamagedara, Undiyagedara, Beddegedara, Millagahagedara, Kongahagedara* etc, maintain their caste system by strictly practicing their marriages among themselves. They are

considered as isolated breeding units with extended family endogamy. They trace their ancestry to the times of the Sinhalese Kings of the 5th century A.D. (1,450 YBP)[16]. Therefore, one hypothesis is that the *Purana* population have been occupying this locality for quite a long time and might have preserved the genetic signatures from ancient pre-Sinhalese settlers due to their practice of endogamy. Exploring the genetics of the *Purana* population of Sigiriya is important it's that their unique genetic signature (if there is one) will become admixed with other Sri Lankans and the current population structure (if there is any) will change. Studying mtDNA of the HVS - I from the *Purana* population in the suburbs of Sigiriya is important to explore the genetic structure and to construct the phylogenetic tree of the *Purana* population.

Materials and Methods

The present study analyses the mtDNA HVS-I control region of the *Purana* population in Sigiriya suburbs. The ethical clearance to study certain anthropological measures and collection and analysis of blood samples of the *Purana* population in Sigiriya has been granted by the Ethics Review Committee of Faculty of Medical Sciences, University Sri Jayewardenepura, Sri Lanka (Ref No: 491 / 10).

Study population and selection criteria

Forty-six individuals from *Purana* populations living in *Nagalaweva, Diyakepilla, Pidurangala* and *Talkote* at Sigiriya suburbs, who gave their informed consent, were included in DNA study. The *Purana* pedigrees such as *Gamagedara, Undiyagedara, Liyanagedara, Beddedara, Millagahagedara, Kongahagedara* and *Aluthgedara* of the subjects of the present study identified as *Purana* pedigrees and confirmed according to *Purana* pedigree recorded in *Bandaranayake et al*., (1994) [16]. Each pedigree was traced back to at least three generations prior to the recruitment to the study.

Blood sample collection and DNA extraction

Capillary blood was collected from 46 adult individuals from *Purana* villages in Sigiriya suburbs. Samples were taken from the fingertips of each individual onto labelled filter paper from each selected individual under strict sterile conditions. A diameter of four millimetres (4 mm) of blood stain from filter paper was used to extract DNA from the leukocyte fraction of the blood according to Walsh *et al*., (1991)[17].

PCR amplification of the human mtDNA HVS - I

The hypervariable segment - I (HVS - I) of the D-loop control region between the positions L16,000 and H16,450 of the human mtDNA was amplified by PCR using the following primer pair targeting a 446 bp DNA fragment[18].

PCRs were performed in 50 µl reaction volumes using 5 µl of extracted

mitochondrial DNA for each reaction in GeneAmp 9,600 thermal cycler (Applied Biosystems) using stranded PCR amplification protocol.

Automated DNA sequencing of mtDNA HVS - I

Each PCR product was purified by following the methods described in the MinElute PCR product purification kit (QIAGEN, Germany). Automated cycle sequencing was performed in an ABI 310 genetic Analyzer (Applied Bio systems, USA). Each automated DNA cycle sequencing mixture contained 1µl of 10 µM forward primer/ reverse primer 4 µl of cleaned PCR product, 2.5 µl nuclease free water, 1.5 µl sequencing buffer and 1µl Big Dye terminator solution (http://www.appliedbiosystem.com).

Determination of nucleotide sequence variation

Sequence data retrieved from the data collection software of ABI 310 instrument were aligned with the human mitochondrial DNA revised Cambridge Reference Sequence (GenBank accession number – NC 012920 Andrews et al., 1999)[19] using CLUSTALX option of MEGAVA 4.0 sequence alignment software[20]. Mitochondrial DNA HVS -I sequences were aligned using CLUSTALX option of MEGAVA 4.0 sequence alignment software [20]. Additional sequences from modern Sri Lankan reported by Illeperuma, (2009) [13] were also included in the study for genetic comparison. Arlequin software version 3.11 was used for the calculation of haplotype frequencies, AMOVA, pair wise Fst values and associated probability values were estimated from 10,000 mutations calculated using the software ARLEQUIN Version 3.11 [21].

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Construction of phylogenetic tree of the Purana population using mtDNA markers

The genetic relationship among Purana population and major ethnic groups in Sri Lanka was explored by drawing Neighbour-joining tree based on DA distances by using the reported data of

Illeperuma (2009) [13]. The distance matrices namely Nei's DA [22] were calculated using GENDIST option in PHYLIP v3.5c software package [23] using mtDNA HVS -I sequences. Neighbour – joining tree was constructed using DA distances and its robustness was established by bootstrap re-sampling procedure. The option NEIGHBOUR in PHYLIP v3.5c was used to draw the phylogenetic trees from the distance matrices created by GENDIST.

In this method, statistical bootstrap involving 1000 replicates was carried out using SEQBOOT option PHILIP v3.5c. Finally, a consensus of 1000 Neighbour-joining trees was drawn using CONSENSE option of PHILIP v3.5 [23] and viewed using the program TREEVIEW.

Results and Analysis

Mitochondrial HVS - I sequence polymorphisms of the Purana population at Sigiriya suburbs

Each sequence comprised of 407 nucleotides spanning nucleotide positions (bp) 16,004 – 16,411 of the human mtDNA. The variable positions recorded upon compared with rCRS were presented in Table 1.

Table 1. Mitochondrial HVS - I sequences of 46 samples of the Purana population aligned with rCRS. Only variable positions are given. Positions are numbered accorded to rCRS.

	1111111111111111111111111111111111111111111111111111111111111111
	6666666666666666666666666666666666666666666666666666666666666666
	0000000000000111111111222222222222222223333333333333334444
	0345678888899223458888012333345667777899011122245555668900 11
	4721950134503696843479943014925060248945418905742368121989 01
rCRS	CAGACTAACGCTTTGTCTACCTTCCATCCCGCCCAGCACCTTAGCTCCTCTCGTTACTCC
SITH_143	.....T..C.T.....C.....G
SITH_145	.....C.....T.C.....C.....G
SITH_155	.....T.....C....C..TCG.
SITH_184	.....TT.....T..C.T.....C.....G
SITH_180	.....T..C.T.....C.....G
SITH_181	G.....C.....T.....G
SITH_195	G.....T.....CC.....C.....G
SITH_179	.....C.....T.....
SITH_166	.....T.....CC.....C.....G
SITH_157	.....T.....CC.....C.....G
SITH_159	.....T.....CC.....C.....G
SITH_201	.....T...A.....T.....G
SINA_214	.....T...T.....A.....AT.....C..T..G
SINA_216	.....CT...T.....TC.....G
SINA_219	.....T...T.....A.....AT.....C.....G
SINA_217	.....CT...T.....TC.....
SINA_221	.....CT...T.....TC.....G
SINA_218	.....T.....CT...T.....TC.....G
SINA_215	....T.....C.....A.....T.....G
SINA_223	.....T.....G
SINA_224	..A.T.....A.....T.....TC.G
SINA_220	....T.....A.....T.....TC.G
SINA_222	....T.....A.....T...T .....TC.G
SIPI_120	.....C.C.....C..T.....T.....T.....G
SIPI_125	.....T..C.T.....C.....G
SIPI_56	.....T.....T.....C.....G
SIPI_42	...G.....T.....C.....TG.....C.....
SIPI_39	.....C.....T.....T.A .....A....C.....
SIPI_61	.T.....T.....T.....C.....G
SIPI_36	.....T..T...T..G..G.....C.....G....
SIPI_109	.....T..C.T.....C.....
SIPI_137	.....T.....T.....C.....G
SIPI_140	.....A.....T.....G
SIPI_141	.....A.....T.....G
SIPI_138	.....C.....T.....T.A.....A....C.....G
SIPI_32	.....T.....T.....C.....G
SIPI_52	.....C..T....T.....C.....TC.C..CGG
SIDI_17	...G.....C.....T.A.....CT.....G
SIDI_20	...G.....T.....CG.T.....C.....
SIDI_12	...G.....C.....T.A.....CT.....G
SIDI_13	.....CC.....T.....T.....
SIDI_21	...G.....C.....T.A.....CT.....G
SIDI_4	.....CCTT.....C.....T.A.....CT.....
SIDI_8	.....CCTT.....T.....A.....G
SIDI_5	.....CCTT.....C..C..T.....T.....
SIDI_14	.....CCTT.....C.....T.A.....CT.....G

Nucleotide position of humanmtDNA

SITH - Sigiriya, *Talkote*
SINA - Sigiriya, *Nagalaweva*
SIPI - Sigiriya, *Pidurangala*
SIDI - Sigiriya, *Diyakepilla*

Comparison of mtDNA HVS - I sequence polymorphisms of the *Purana* population with modern populations in Sri Lanka

The sequences of the mtDNA HVS - I in 46 individuals belonging to the *Purana* population living at Sigiriya suburbs was compared with reported data available for major ethnic groups: Sinhalese (n = 55), Sri Lankan Tamils (SL Tamils) (n = 40), Indian Tamils (IN Tamils) (n = 34) and Sri Lankan Moors (SL Moors) (n = 55) [13]. Arlequin software version 3.11 was used for the calculation of haplotype frequencies, mean number of pair wise differences, nucleotide diversity and gene diversity among *Purana* and modern Sri Lankans [21] (Table 2).

Table 2. Genetic indices of mtDNA HVS - I of the Purana population and modern Sri Lankan populations

Genetic Indices	*Purana*	Sinhalese	SL Tamils	IN Tamils	SL Moor
Number of haplotypes	28	46	28	34	31
Mean number of pair wise difference	7.459903 ±3.549980	5.989226 ±2.899290	4.819231 ±2.402766	5.601329 ±2.742017	3.734007 ±1.913702
Nucleotide diversity	0.020780 ±0.010978	0.016683 ±0.008959	0.013424 ±0.007435	0.015603 ±0.008482	0.010401 ±0.005914
Gene diversity	0.9720 ±0.0103	0.9939 ±0.0046	0.9628 ±0.0193	0.9878 ±0.0080	0.9542 ±0.0170

Gene diversity of the *Purana* population was low (0.9720 ± 0.0103) with compared to Sinhalese (0.9939 ± 0.0046) and IN Tamils (0.9878 ± 0.0080). However, diversity was higher than that of SL Tamils (0.9628 ± 0.0193) and SL Moors (0.9542 ± 0.0170).

Haplotype frequencies of the *Purana* population and modern populations in Sri Lanka

The Purana population living at Sigiriya suburbs showed the highest percentage of population specific mtDNA haplotypes:- Single unique, Multiple unique and Non unique (24 haplotypes out of 28 – prevalence of 86%). Fourteen percent of haplotypes that were shared among individuals between populations (non unique haplotypes) of the Purana populations shared among Sinhalese (50%), SL Tamils (25%) and SL Moors (25%) (Table 3).

Table 3. Haplotype sharing statistics of the Purana population among modern Sri Lankan populations

Haplotype	Sinhalese	SL Tamil	IN Tamil	SL Moor	*Purana*
Single unique	27	15	20	15	17
Multiple unique	06	01	02	02	07
Non unique	13	12	12	14	04

Inter population and intergroup mtDNA genetic variation of the Puranapopulation with modern population

Intergroup mtDNA genetic variation was analyzed using Analysis of Molecular Variance (AMOVA) by grouping them on the basis of their ethnicity, *Purana* and modern status using the data of present study with the data published for the modern Sri Lankan population groups [13].

Table 4. Inter population mtDNA genetic variation of the *Purana* and modern Sri Lankan populations

Grouping	Source of variation	Percentage variation	F_{ST}
Purana & modern groups	Among groups	2.81	
Group 1 – *Purana*	Among populations in groups	0.74	0.03556
Group 2 – Sinhalese	Within p opulation	96.44	
SL Tamil / IN Tamil			
SL Moor			
Ethnic groups	Among groups	0.00	
Group 1 – *Purana*	Among populations in groups	1.91	0.01869
Group 2 – Sinhalese	Within population	98.13	
Group 3 – SL Tamil / IN Tamil			
Group 4 – SL Moor			

The percentage of genetic variation was higher (2.81) when comparing *Purana* against the rest of the Sri Lankan population than grouping the four populations (0.00). This difference between *Purana* and the other populations was clearly affected by its elevated F_{ST} (0.03556) compared to 0.01869.

Table 5. Population pair wise F_{ST} values (below the diagonal) and their significance level (above the diagonal)

	Sinhalese	IN Tamils	SL Tamils	SL Moors	*Purana*
Sinhalese		0.71171±0.0516	0.01802±0.0121	0.38739±0.0490	0.00901±0.0091
IN Tamils	-0.00322		0.00901±0.0091	0.45946±0.0515	0.00000±0.0000
SL Tamils	0.01817	0.02115		0.00000±0.0000	0.01802±0.0121
SL Moors	0.00072	0.00021	**0.03504**		0.00000±0.0000
Purana	0.02447	**0.02899**	0.02476	**0.03909**	

Note – Highly significance P values (P< 0.00005) are in bold face

The lowest pair wise FST has been observed among IN Tamils and purana populations (0.02899, P<0.00000). The highest significant pair wise was among Moors and *purana* population. Other values are not significant.

Phylogenetic analysis of mtDNA for the Purana population

Genetic relationships of the Purana population with modern Sri Lankans and *Vedda* (Adivasis) groups reported by Illeperuma (2009) [13] were further explored by drawing neighbour joining trees based pair wise DA distances between populations[22]. An un –rooted neighbour joining (NJ) tree was constructed on the basis of DA matrices and visualized by TREEVIEW 1.6.1.

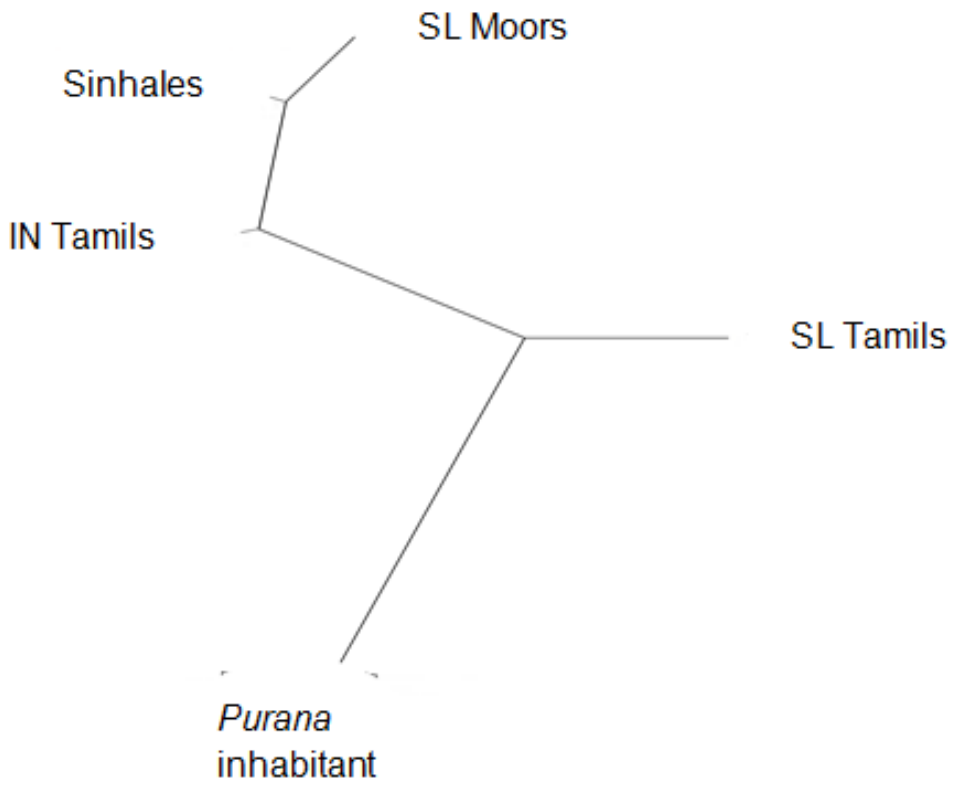

Fig 1. Neighbour – joining tree based on DA distance values of mtHVS - I sequence variation of the *Purana* population and four modern Sri Lankan populations

The significant observation of the DA based neighbour – joining tree was the clustering of Sinhalese, Indian Tamils and the Sri Lankan Moors together in a single clade on the tree compared to others. Although the *Purana* population was isolated in the tree, the genetic distance between the *Purana* population and SL Tamils was low compared with that of others.

Upon inclusion of the aboriginal *Vedda* population to the analysis, the genetic distance of *Purana* to Sri Lankans was high other than the SL Tamil and *Vedda.*

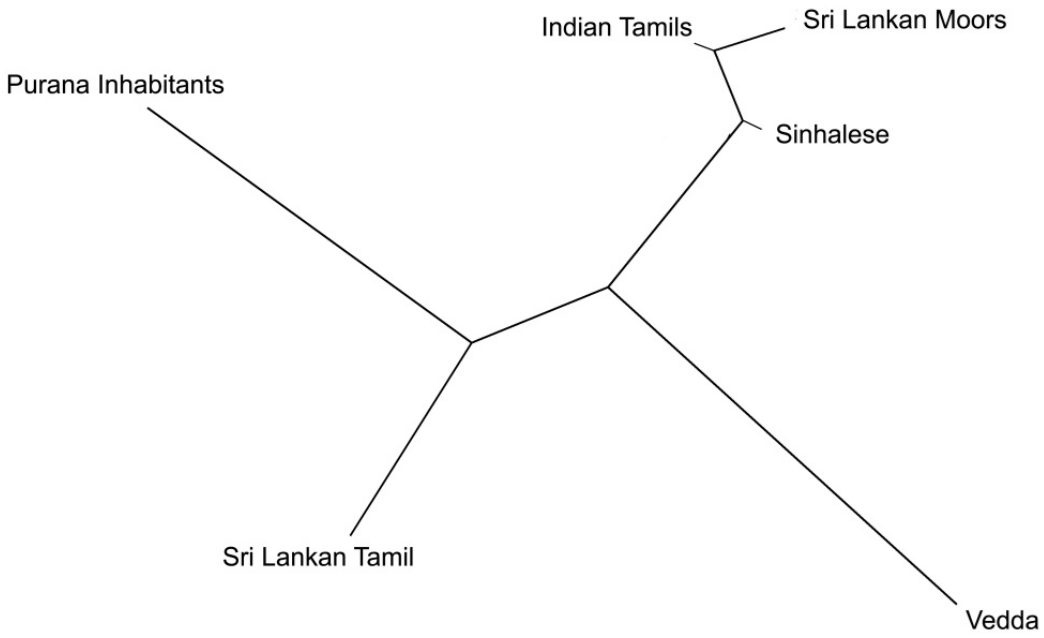

Fig 2. Neighbour – joining tree based on DA distance values of mtHVS - I sequence variation of the Purana populations, *Vedda* and four modern Sri Lankan populations

Note – Close affiliation of the *Purana* population with the SL Tamils was observed. *Purana* population was closer to SL Tamils than *Vedda* and to other Sri Lankans.

Each sequence comprised of 407 nucleotides spanning nucleotide positions (bp) 16,004 – 16,411 of the human mtDNA. The variable positions recorded upon compared with rCRS were presented in Table 6.3.

Discussion

A high percentage of population specific haplotypes observed in the *Purana* population in comparison to modern Sri Lankan populations reported by Illeperuma (2009) [13] suggested that the maternal gene flow of the *Purana* population was restricted among them (Table 3.). This may be a reflection of the endogamous caste system and intra-group marriages that are common among *Purana* populationPurana population at Sigiriya suburbs presently belonging to Govigama and Nekathi castes make marriages restricted to the same caste[24]. Therefore presently maternal gene flow has taken place among the *Purana* population in Sigiriya suburbs and gene flow among other Sri Lankans was limited. The isolation and endogamy observed with mtDNA is also consistent with the reported morphological and morphometrical variations such as dominant mesocephalic cephalic phenotype (35%), leptoprosopic facial phenotype (38%), mesorrhine nasal phenotype (56%), blood group O (46%) etc when

Purana population is compared with present Sri Lankans. The effects of isolation of the *Purana* population were further demonstrated by their other morphometrical and morphological findings [25,26].

Intergroup mtDNA genetic variation was analyzed using AMOVA by grouping them on the basis of their ethnicity, *Purana* and modern status using the data of present study with the data published for the modern Sri Lankan population groups [13]. When populations were grouped according to their ethnicity, AMOVA revealed a 98.13% variation within populations is higher and the variability among four ethnic populations was only 1.91 % (Table 4). This was in agreement with the AMOVA results of HVS - I polymorphisms of the four major ethnic groups in Sri Lanka (Sinhalese, Sri Lankan Tamils, Indian Tamils and Sri Lankan Moors) [13]. Among Sri Lankan ethnic populations, the percentage variation of AMOVA was high (98.97%) within population and the variability among four ethnic populations was 1.03 % [13]. The AMOVA analysis of HVS - I showed less differentiation (FST -0.01869) among the four ethnic populations in this study (Table 5.). This might be due to a higher amount of admixture among them in their maternal linage.

The *Purana* population was reported to be isolated from 5th Century A.D. from the rest of the Sri Lankan population [27]. Therefore, grouping of the population for AMOVA was done with reference to modern and *Purana* basis and increased genetic variation could be identified among groups (2.81%) (Table 4.). Differentiation among populations when grouped by the basis of the origin of the population was higher (F_{ST} - 0.03556) than among population grouped according to ethnicity (F_{ST} - 0.01869) (Table 4.). Bandaranayake *et al*., (1994) [27] recorded that the marriages of the *Purana* inhabitants have been continuously to occur only within each *Purana* community due to strict practicing caste system. Hence present findings demonstrate the genetic consequences of practiced endogamy of the *Purana* population at Sigiriya suburbs in population isolation.

Population pair wise F_{ST} values and their related statistical significances (Table 5.) confirm that the *Purana* population at Sigiriya suburbs was genetically differentiated from the rest of the various modern Sri Lankan tested. The DA distance based neighbour – joining tree (Fig 1. and Fig 2.) further justifies this isolation. High DA distance of the *Purana* population compared to the other Sri Lankans is an indicative of a recent possible occurrence of a population bottleneck. Genetic distance values are known to increase rapidly when bottleneck has occurred [28]. Reduced population size, cultural isolation due to strict practicing caste system are probably the other reasons for the occurrence of the *Purana* population in Sigiriya suburb as an outlier in mitochondrial neighbour – joining phylogeny.

D_A based neighbour joining tree showed that the *Purana* population

were genetically closer to Sri Lankan Tamils than to any other population probably because of an occurrence of increased maternal gene flow among them than other respective populations in Sri Lanka (Fig 2). The antiquity of the *Purana* population has been reported as old as 5th Century AD (1,450 YBP) [27]. The Purana population those who had lived long before, might have made a close association with the Tamils those who migrated from India at the historic time. In Mahavansa, Tamil adventurers such as Elara invaded the Anuradhapura area around 2,100 – 2,200 YBP. In the 9th and 10th centuries, *Pandya* and *Chola* incursions into Sri Lanka culminated in the *Chola* annexation of the island, which lasted until the latter half of the 11th century. Tamil soldiers from Southern parts of India were brought to Anuradhapura between the 7th and 11th centuries by the Tamil dynasty. Such a large influx of Tamils those who came from India in historic time tend to be intermixed with Sri Lankans living in dry zone (Anuradhapura and Polonnaruwa kingdoms).

The present study explores the genetic affinity of the *Purana* population at Sigiriya suburbs in respective to the genetic variation seen only in the HVS - I of the mitochondrial genome. As the mtDNA represents the maternal gene flow, the finding of the present study restricts to the maternal lineage of the studied subjects. Hence the findings of the present study should be further verified with genetic data derived from nuclear and male-specific Y chromosomal DNA markers which may furnish information regarding patrilineal relationships.

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