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# Study of morphology, morphometry and mitochondrial DNA polymorphisms of prehistoric skeletal remains of *Potana* and *Purana* population in Sigiriya, Sri Lanka

BA

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# Study of morphology, morphometry and mitochondrial DNA polymorphisms of prehistoric skeletal remains of *Potana* and *Purana* population in Sigiriya, Sri Lanka

By

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**Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Doctor of Philosophy in Anatomy on November 2013.**

# Declaration

The work described in this thesis was carried out by me under the supervision of,

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A report on this has not been submitted in whole or in part to any university or any other institution for another Degree or Diploma.

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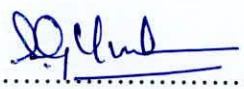
Date

# Declaration by the Supervisors

We certify that the statement made by the candidate is true and that this thesis is suitable for submission to the University of Sri Jayewardenepura for the purpose of evaluation.



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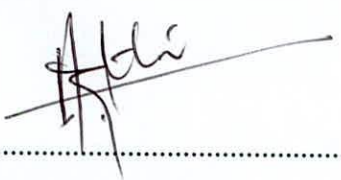

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| µl | microlitre |
| µM | micromole |
| A.D. | Ano domini |
| aDNA | ancient DNA |
| AMOVA | Analysis of molecular variance |
| ANOVA | Analysis of variance |
| B.C. | Before Christ |
| BMI | Body mass index |
| bp | base pairs |
| CVDs | Cardiovascular diseases |
| D-loop | Displacement loop |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxyribonucleic acid triphosphate |
| E log | East longitude |
| EDTA | Ethylene-diamine-tetraacetic acid |
| Hb | Haemoglobin |
| HCl | Hydrochloric acid |
| Hp | Haptoglobulin |
| HVS | Hyper variable segment |
| HVS - I | Hyper variable segment I |
| HVS - II | Hyper variable segment II |
| IAA | Isoamyl alcohol |
| KCl | Potassium chloride |
| $M_1$ | First molar teeth |
| $M_2$ | Second molar teeth |
| $M_3$ | Third molar teeth |
| $MgCl_2$ | Magnesium chloride |
| mtDNA | mitochondrial DNA |
| MW | Molecular weight |
| N lat | North latitude |
| NaCl | Sodium chloride |
| NaOH | Sodium hydroxide |
| NHANES | National Health and Nutrition Examination Survey |
| NIH | National Institutes of Health |
| PCR | Polymerase chain reaction |
| rCRS | revised Cambridge Reference Sequences |
| RFLP | Restriction fragment length polymorphism |
| rpm | round per minute |
| rRNA | ribosome RNA |
| SD | Standard deviation |

| | |
|---|---|
| SNPs | Single nucleotide polymorphism |
| STEPS | STEPwise approach to Surveillance |
| STR | Short tandem repeats |
| TBE | Tris base, Boric acid and EDTA |
| Tf | Transferine |
| tRNA | transport RNA |
| WC | Waist circumference |
| WHO | World Health Organization |
| WHR | Waist hip ratio |
| Y BP | Years before present |

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# Study of morphology, morphometry and mitochondrial DNA polymorphisms of prehistoric skeletal remains of *Potana* and *Purana* population in Sigiriya, Sri Lanka

**K. M. Chandimal**

## ABSTRACT

This thesis explores morphology, morphometry and mitochondrial DNA polymorphisms of prehistoric and the *Purana* population at Sigiriya suburbs. The first part of the thesis contains the findings of morphometry and morphology of three human skeletons excavated from *Potana* Sigiriya that has been radio carbon dated as 4,000 years before present (YBP). The gender, age, stature, cause of death, health status, food habits and the way of life of *Potana* population have been explored. One skeleton unearthed from context no 10 of the excavation presently displayed at National Museum, Colombo is of female sex and age at death is around 25–35 years. The calculated stature of the individual is 170 cm. Other skeleton found at the same context presently displayed at Sigiriya Museum is of male sex and determined age at death is around 25–30 years. The calculated stature of the male is 173.61cm. The skeleton excavated in context no 03 presently stored at Osteology Laboratory, Postgraduate Institute of Archaeology, University of Kelaniya is determined as female and age at death is around 35 – 40 years. In general, the prehistoric population that lived in *Potana* is further identified as a hunter gatherer population that shared many biometrics and socioeconomic characteristics among contemporary prehistoric populations that lived in Sri Lanka such as *Bellan bandi Palassa*, *Batadomba lena, Beli lena, Fa Hien lena* etc.

Moreover archaeological remains contain genetic information that has capability to elucidate recent evolutionary history of humans. Ancient DNA (aDNA) recovered from archaeological remains is in minute amounts because of degradation which limits the successful retrieval of valuable historical genetic information. The preservation of ancient DNA (aDNA) in Sri Lanka has been reported to be low (Reed *et al.,* 2003). In this study, an attempt was made to extract DNA from ancient human bones using reported aDNA extraction protocols (Newman *et al.*, 2002; Mohendasan *et al.,* 2004; Ariffin *et al.,* 2007: DNA investigation Kit – QIAGEN, Germany). Mitochondrial DNA (mtDNA) of ancient bone samples taken from the prehistoric skeleton (4,000 YBP) excavated from *Potana* Sigiriya presently displayed at Sigiriya Museum along with the historic skeleton dated as 1,000 YBP displayed at Polonnaruwa Museum was extracted and analyzed. Genetic information of a 231 bp long mtDNA fragment was successfully recovered from human skeletons dated 1,000 YBP and 4,000 YBP excavated from Sri Lanka. Analysis revealed that the mtDNA haplotypes of ancient bone samples of 1,000 YBP and 4,000 YBP were unique.

The present human population living in Sigiriya suburbs with the *Purana* surnames such as *Aluthgedara, Gamagedara, Undiyagedara, Millagahagedara, Kongahagedara* etc are considered as the *Purana* population whose ancestry could be traced back to the times of Sinhalese Kings of 5$^{th}$ Century A.D (1,450 YBP). The quantitative (morphometrical) and qualitative (morphological) anthropological traits of the *Purana* populations (n=313) representing *Purana* villages: *Pidurangala, Thalkote, Nagalawewa and Diyakepilla* at Sigiriya suburbs were investigated. The quantitative anthropological traits such as height, cranial index, facial index, nasal index and qualitative traits such as skin colour, hair type and colour etc of the *Purana* population

revealed that the *Purana* populations living in these four villages' posses increased variations. Results analyzed by ANOVA showed higher variations of most of studied phenotypic characteristics (morphometrical) among *Purana* female populations living in four villages in Sigirya suburbs. Principle component analysis (PCA) confirmed that *Purana* male populations of *Talkote* and *Diyakepilla* are closer in terms of morphometrical characteristics while the inhabitants at *Pidurangala* and *Nagalaweva* are quite isolated from the rest of the population. In contrast to male population, PCA analysis of female populations living in four villages are morphometrically placed in different clusters. The gender, pedigree or caste system, geographical location and socioeconomic status have been identified as limiting factors for the extent of phenotypic divergence among the *Purana* population.

When comparing the present findings and other documented studies based on morphological and morphometrical traits of population groups in Sri Lanka, the analyzed percentage distribution of each blood groups (A, B, AB, O and Rh) of Sinhalese, Tamils, Muslims, Burgers and the *Purana* population showed that the *Purana* population was phenotypically different from the rest of Sri Lankans. The individual quantitative traits (stature, cranial length, cranial breadth, facial height, facial length etc) and qualitative anthropological traits (skin colour, hair type and colour) of the *Purana* population were observed to be different when compared with other Sri Lankans.

Polymorphisms in the mitochondrial DNA hypervariable segment - 1 (HVS -1) of the *Purana* population was studied to explore the genetic affinity of them to modern Sri Lankans. The results of AMOVA confirmed that the four *purana* populations living in

four villages were genetically isolated from each other and the rest of Sri Lankans and maternal gene flow was limited to them. This isolation was also collaborated with findings of morphological / morphometrical variations of the *Purana* population compared to modern Sri Lankans as observed in the percentage distribution of each blood group in the *Purana* population and modern Sri Lankans. The effects of isolation of the *Purana* population further demonstrated their other morphometrical and morphological findings.

Pedigree or caste system and geographical location are suggested as the limiting factors for restricted female gene flow among the *Purana* populations. Phylogenetic analysis based on mtDNA HVS - I polymorphism data revealed that the *Purana* population in Sigiriya was genetically closer to Sri Lankan Tamils than the *Vedda* and other modern Sri Lankans. This may have been as a result of genetic isolation and genetic drift. The genetic evidence did not support for existence of any genetic affinity between the *Purana* population and prehistoric population that lived in *Potana* Sigiriya. As the mtDNA represents the maternal gene flow, the finding of the present study restrict 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.