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RESEARCH NOTE

Tracing the genetic root of Trinket cattle: an endangered cattle population of a small isolated island of Nicobar group of islands

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Abstract. Trinket cattle are the inhabitant of a small island called Trinket, which is one of the picturesque islands of Andaman. This herd is thought to be of Danish leftover during their dynasty in Nicobar archipelago. When the island was abandoned by foreign invaders, indigenes utilized the animals for the purpose of meat. As a result, the cattle became semi-feral in nature. After the Great Sumatra earthquake and tsunami of Indian Ocean in 2004, Trinket island was left abandoned by indigenes and the cattle became totally feral in nature. To trace the genetic root of the cattle, this study has been undertaken based on the sequence information of the mitochondrial D-loop and cytochrome b gene. The genomic DNA was extracted from the blood samples of the Trinket cattle and was used for amplification of mitochondrial markers, and the sequence information was generated by Sanger sequencing. The analysis of sequence information revealed that the Trinket cattle belongs to *Bos indicus* (I) haplotype, sub-haplotype I2. The presence of I2 sub-haplotype in Trinket cattle may be due to the expansion of this I2 haplotype towards Southeast Asian countries. This is a novel input for the formulation of breeding strategy towards conservation of eco-friendly sustainable livestock in the isolated island ecosystem.

Keywords. Trinket cattle; Andaman and Nicobar islands; Danish colony; tsunami; haplotype; *Bos indicus*.

Introduction

This is a historic story of a herd of cattle which were reared for milk once upon a time, now feral in nature and seen in Trinket island of Nicobar archipelago, Andaman and Nicobar Islands, India. To elaborate the tale further, let us recapitulate the history of Andaman and Nicobar Islands (ANI), an unique union territory of India. ANI are situated in the Bay of Bengal, spanning latitude 6°45'N to 13°4'N and longitude 92°12'E to 93°57'E. ANI comprise of 574 picturesque islands, of which only 36 are inhabited by human.

Unique location of Nicobar Islands is known since time immemorial because these islands are situated in the sea route between India and Southeast Asia and regarded as the finest natural harbour. Historians say that in the past traders and passing sailors used it as a resting harbour for replenishment of food and water (Kloss 1903). When the sailors from western countries developed barter relationships with

the Nicobarese, the indigenous tribe of Nicobar archipelago, contact with the western world started (Knoll and Hiery 2010).

Historical indenture suggests that the Nicobar Islands were colonies of Denmark, Austria, Britain and Japan before they became part of Indian Union in 1950 and was declared Union Territory in 1956. The Portuguese and French Jesuit attempted to propagate Christianity in Nicobar Islands during 14th, 15th and 17th centuries. But the islands were colonized by Danish in 1756 and they named them as 'Frederick islands' and founded 'New Denmark' as their capital on the island of Car Nicobar. Because of adverse climatic conditions (Kloss 1903) the missionaries died and the Danish left the hope of colonization (1756–1768). Thereafter, from 1778–1784, the Nicobar Islands were occupied by Austrians and the same went for British possession from 1789–1796, and 1807–1814, in two different spells. Further, the islands were left abandoned from 1814–1831 and then reclaimed by Danish people

(Knoll and Hiery 2010). The Danish colonization continued until 16th October, 1868 when they sold the rights to Britain. During initial span of Danish colonization (1756–1768), a herd of cattle were introduced in Trinket Island for the purpose of milk (Kloss 1903). They converted a considerable portion of forest into grassland for feeding of the cattle. When Danish colony was abolished, the herd of cattle became semi-feral in nature as the Nicobarese (the indigenes of these islands) rather prefer meat and not milk. Infographic of Kloss (1903) suggested that in Trinket Island a herd of 50 semi-wild cattle roamed, which seemed to be descendants of European cattle that were slaughtered by these indigenes during their festivals for the purpose of meat.

The semi-feral cattle became totally feral when there was change of landscape of Andaman and Nicobar islands after 26 December 2004 due to the Great Sumatra earthquake and Indian Ocean tsunami. Trinket Island was the worst affected island and the land was reduced manifold, leaving only slender sandbars with drowned houses and coconut trees as markers. In the existing structure of the island, the slender sandbars are constantly washed away and reconstructed by the actions of waves (Thakkar and Goyal 2006). Therefore, after 26 December 2004, all the indigenes left the Trinket Island and shifted to Kamorta, leaving the cattle in wildness.

In a recent survey, it has been found that around 150 of the descendants of the cattle are present in the Trinket island (Annual Report 2018–19 <https://ciari.icar.gov.in>). The genetic lineage of the cattle introduced by the Danish was not properly known. It was hypothesized that the cattle were exotic in nature and belonged to *Bos taurus* lineage (Kundu et al. 2010). But, recently, the hypothesis faced severe challenge due to phenotypic resemblance of these cattle with *Bos indicus* (Annual Report 2018–19 <https://ciari.icar.gov.in>). To resolve the issue of genetic root of Trinket cattle, the present study has dealt with the mitochondrial DNA (mtDNA) sequence analysis of the cattle, as mtDNA has 5–10 times higher evolutionary rate than nuclear genes. In addition, mtDNA control region (mtDNA D-loop) has an evolutionary rate which is faster than the other mitochondrial regions (Bronstein et al. 2018).

For the conservation of Trinket cattle and to save these extremely vulnerable species from extinction, formulation of a scientific breeding plan is the need of the hour. The identification of genetic lineage of the cattle has huge practical implication in sketching the right breeding plan. Therefore, this study aimed at tracing the genetic root of Trinket cattle. Besides, to know the physiological status of the animals, physical parameters of the cattle have been worked out during the present investigation.

Materials and methods

Study area

Trinket island (latitude: 8°05′19.20″N and longitude: 93°34′29.99″E), one of the 24 islands that make the

Nicobar chain, has an area of 12.25 km². With the permission of the Tribal Council, Nicobar, the island was approached by boat during high tide for collection of samples of Trinket cattle.

Collection of samples

Three cattle were captured by the indigenes. Initially, body measurements of cattle were taken. Subsequently, blood samples were taken from clinically healthy animals without any pathological abnormalities.

Physical parameters of cattle

Phenotypic characteristics (body length, height at withers, punch girth, head length, tail length, ear length and horn length) were recorded using an ordinary tape. Body weight was deduced following standard methodology (Sawanon et al. 2011).

Extraction of DNA samples from blood and amplification of mitochondrial D-loop and cytochrome b (Cytb) sequence

DNA from the blood was extracted using a commercial blood DNA extraction kit (GCC Biotech, India). D-loop and Cytb were amplified using the primers described earlier by Yang et al. (2014) and Kim et al. (2013), respectively. Amplified products were purified and sequence information was generated by dideoxy fingerprinting.

Analysis of sequence information

Initially, a comparison was made between the sequence information of Trinket cattle with other haplotypes in terms of polymorphism and population size changes using DnaSp v 6.12.01 (Rozas et al. 2017). The sequence information was submitted to the GenBank with accession numbers (MH730662–MH730664 for D-loop, MH714782–MH714784 for Cytb). Sequence alignment of D-loop region and Cytb was done using MEGA package v X (Kumar et al. 2018). To investigate the genetic relationship among mitochondrial sequences, the unrooted neighbour-joining (NJ) phylogenetic trees were constructed. Tajima's relative rate test was performed to know the equality of evolutionary rate between sequences (Tajima 1993). Phylogenetic analysis was done by NJ method with 1000-bootstrap replications. For constructing the phylogenetic tree with D-loop sequence of Trinket cattle, a total of 17 sequence information T1 (LC013968), T2 (AB117049), T3 (V00654), T4 (LC013966), I1 (AB268579, FJ971088), I2 (AB268559, EU199870), P (JQ437479, GU985279, AY337527, DQ124389, AY998840) and other Indian cattle breed (Tharparkar HQ234743, Red Sindhi HQ234730, Sahiwal L27733, Nellore AY126697)

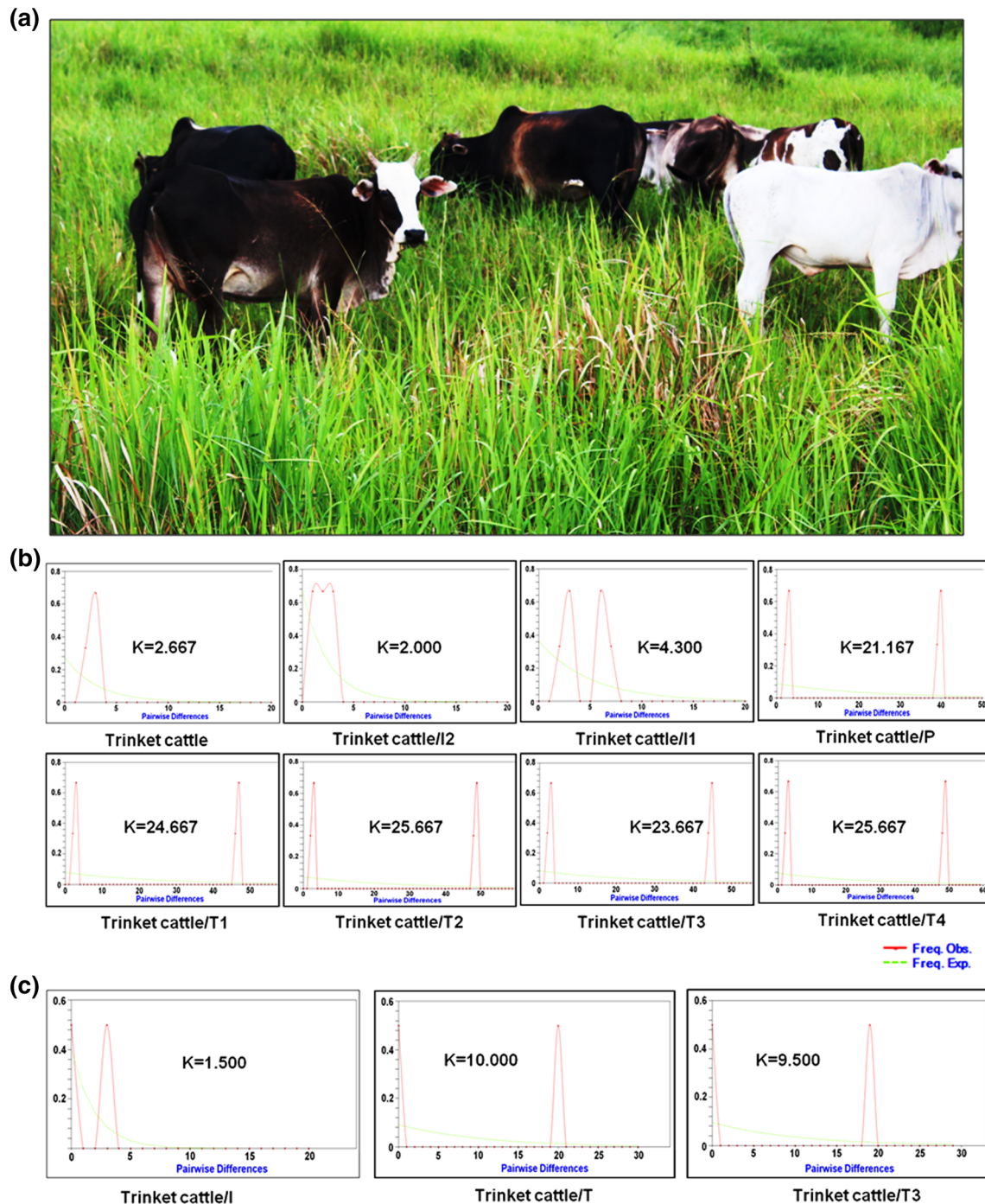


Figure 1. (a) Trinket cattle in Trinket Island of Andaman and Nicobar Islands. (b) Average number of pairwise difference in nucleotides (k value) of D-loop sequence indicates that Trinket cattle is closely related to I2 subhaplotype ($k = 2.0000$); x -axis indicates pairwise difference and y -axis indicates frequency. (c) Average number of pairwise difference in nucleotides (k value) of CytB sequence indicates that Trinket cattle is closely related to I subhaplotype ($k = 1.5000$); x -axis indicates pairwise difference and y -axis indicates frequency.

were taken into consideration. For Cytb sequence phylogenetic analysis, available sequence information of T and T3 haplotypes and sub haplotypes (AY885283, V00654), P haplotype (DQ124389, JQ437479, GU985279), I haplotype (EU177870, FJ971088, EU177868) and three distinct breeds of cattle (Holstein Friesian DQ124413, Ongole AF 531473, Nellore AY 126697) were considered.

Results

The cattle were of medium size, horned, strong and not docile in nature. Bulls looked very strong, vigorous with medium sized humps, penis, naval flap and dewlap. The colour of the animals varied from white, black and brown (figure 1a). The average body weight of the cattle was

Table 1. Polymorphic sites, DNA polymorphism and population size changes of Trinket cattle and different haplotypes of cattle.

Compared between	No. of variable polymorphic sites	Singleton variable sites	Parsimony informative sites	Haplotype diversity (Hd) \pm SD	Nucleotide diversity (Pi)
Based on D-loop sequences					
Trinket	4	4	0	1.000 \pm 0.272	0.00294
Trinket/I2	4	4	0	1.000 \pm 0.177	0.00221
Trinket/I2	9	9	0	1.000 \pm 0.177	0.00496
Trinket/T1	48	46	2	1.000 \pm 0.177	0.02726
Trinket/T2	50	48	2	1.000 \pm 0.177	0.02836
Trinket/T3	46	44	2	1.000 \pm 0.177	0.02615
Trinket/T4	50	48	2	1.000 \pm 0.177	0.02836
Trinket/P	41	39	2	1.000 \pm 0.177	21.167
Based on CytB sequences					
Trinket	0	0	0	0.000 \pm 0.000	–
Trinket/I	3	3	0	0.500 \pm 0.265	0.00132
Trinket/T	20	20	0	0.500 \pm 0.265	0.00877
Trinket/T3	19	19	0	0.500 \pm 0.265	0.00833

287.93 \pm 9.28 kg. Paunch girth, head length, tail length, ear length and horn length were 172.38 \pm 2.87 cm, 43.56 \pm 3.12 cm, 92.67 \pm 3.14 cm, 18.82 \pm 1.32 cm and 15.38 \pm 0.95 cm, respectively.

Mitochondrial D-loop sequence for Trinket cattle varied from 917 to 918 bp. When the sequence information of D-loop between Trinket cattle and I2 sub-haplotype was compared, no parsimony informative sites could be found (table 1). Comparison between sequence information of D-loop sequence of Trinket cattle revealed variation of four nucleotides in terms of polymorphic and singleton variable. Whereas, polymorphic and singleton variables was not found when sequence information of CytB of three Trinket cattle was considered. Nucleotide diversity (Pi) was least (Pi = 0.00221) between Trinket cattle and I2 sub-haplotype (based on D-loop sequences). When CytB sequences were considered, Pi was least (Pi = 0.00132) between Trinket cattle and I haplotype. However, we could not compare our data with I2 sub-haplotype of CytB due to the nonavailability of information in public database. The details of information on polymorphic sites, DNA polymorphism and population size changes in Trinket cattle and different haplotypes, and sub-haplotypes of cattle on the basis of sequence information of D-loop and CytB has been depicted in table 1. Further analysis of data on average number of pairwise differences (k) indicated that k value ranged between 2.0000–25.667 when D-loop sequences of Trinket cattle was compared with other haplotypes and sub-haplotypes of cattle. The minimum k value was observed (k = 2.0000) when sequence information of Trinket cattle was compared with I2 haplotype (figure 1b). Similarly, the least k value (0.00132) of CytB was observed when sequence information Trinket cattle was compared with I haplotype (figure 1c). Thus, the result indicated that the Trinket cattle was related to *B. indicus* (I haplotype) and may be clustered more precisely to I2 sub-haplotype.

The mitochondrial sequence analysis revealed that the Trinket cattle not only clustered with I2 haplotype (AB268559, EU177870) but shared the same cluster with Red Sindhi cattle (HQ234730) when sequence of D-loop was analysed (figure 2). When CytB sequence information was analysed, the group of cattle shared the same cluster with I haplotype (EU177869–70), Nellore (AY126697) and Ongole (AF531473) breed (figure 2). However, we could not compare our dataset of CytB with I2 sub-haplotype due to the nonavailability of data in the public domain.

Discussion

Conservation of native endangered livestock breeds is one of the priorities of Government of India (<http://dahd.nic.in/related-links/chapter-vii>). To set up a long-term goal for sustainable livestock production and food security, conservation and management of locally adapted native livestock breeds is extremely important. Trinket cattle is a highly endangered cattle breed of Andaman and Nicobar Islands, adapted to the microenvironment of Trinket island and demand serious efforts for the conservation of the genetic pool. Tracing the genetic root of Trinket cattle is very vital from the angle of conservation and heritage of humanity, which is necessary since these animals have survived against environmental challenge in this island ecosystem after tsunami. Formulation of a distinct breeding plan for the cattle breed is the most crucial step towards conservation of the breed and in this context, unravelling the genetic lineage or makeup of the breed is most essential.

Trinket cattle exhibited a very low haplotype diversity and nucleotide diversity (table 1) since they are inhabited in isolated island and indigenes never used other breed of cattle for their breeding. The result of the present study indicated that the Trinket cattle belong to *B. indicus* mitochondrial lineage. On the contrary, previous studies with Brahman

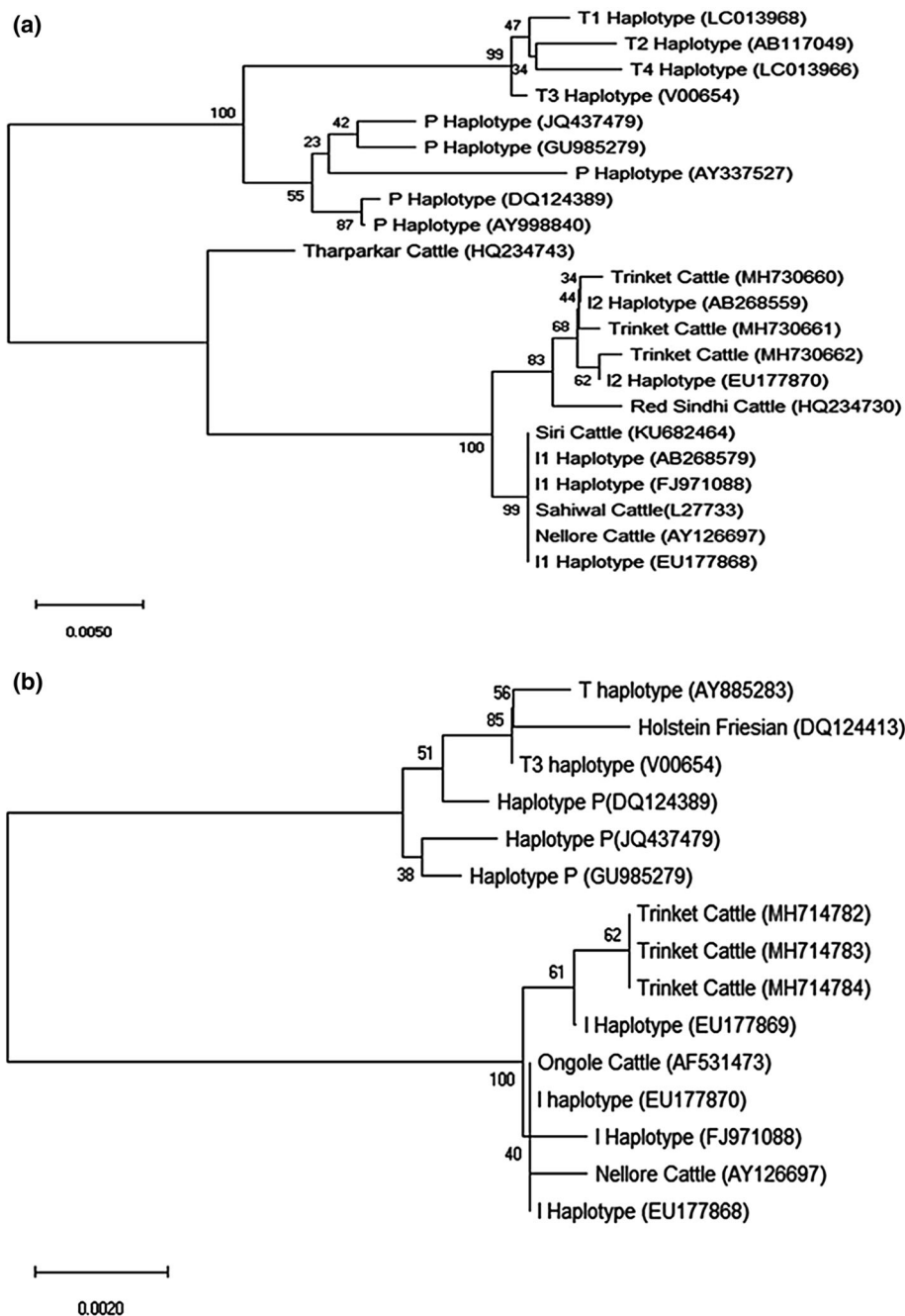


Figure 2. Phylogenetic tree based on (a) D-loop sequence; (b) CytB sequence (total open reading frame considered) in MEGA-based NJ method using 1000-bootstrap replications.

cattle indicated that, high genetic diversity in terms of haplotype and nucleotide diversity was suggestive of presence of both mitochondrial lineage of *B. taurus* and *B. indicus* (Kai-xing *et al.* 2006).

This has been documented in the literature that domestication of cattle started with human civilization and agricultural activities, which included rearing of both *B. taurus* and *B. indicus* (Beja-Pereira *et al.* 2006). T3 haplogroup of *B. taurus* originated from Europe, T1 haplogroup from Africa, and T and T2 haplogroup from North East (Troy *et al.* 2001).

Archaeological documents suggested that ancestor of modern zebu cattle (*B. indicus*) was *B. primigenius namadicus* (Meadow 1996). This has been hypothesized that *B. p. namadicus* was the inhabitant of Indian subcontinent during Pleistocene and Holocene periods and few survived during Neolithic times to give rise to *B. indicus* (Van Vuure 2005). *B. indicus* falls into two haplogroups I1 and I2 (Lai *et al.* 2006). Expansion of I1 haplotype occurred 13,600 years ago and I2 haplotype occurred 8200 years ago. Albeit I2 haplotype was brought into the domestic pool after

incorporation of I1 haplotype in the Indus valley but later on diffused to south India, Southeast Asia and southern China during the course of commercial exchanges.

During the period of study, identification of haplotype of Trinket cattle has been done on the basis of three samples which represent 2% of the total population of the animals present in this island. The sample size is quiet reasonable because not more than 10% of any one herd or village population should be sampled and in any case no more than five animals should be sampled from any herd to carry out molecular diversity study (FAO 2011).

In conclusion, the presence of I2 haplotype in Trinket island may be due to the outcome of commercial exchange either by indigenes or Danish in the past since this island was a sea route between Indian subcontinent and Southeast Asia. This information on the genetic root of the cattle breed will be useful for formulation of long-term breeding policy towards conservation of the endangered gene pool.

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