Journal of Genetics (2022)101:5
https://doi.org/10.1007/s12041-021-01353-8

RESEARCH NOTE

Genetic diversity in mitochondrial DNA D-loop region of indigenous pig breeds of India

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Received 25 August 2020; revised 15 September 2021; accepted 11 October 2021

Abstract. India is home for at least 18 indigenous pig breeds; however, the genetic diversity of Indian pig, *Sus scrofa domesticus*, population is poorly known. Here, the hypervariable region (HVR) of mitochondrial DNA D-loop (~487 bp) of 214 pigs representing five indigenous and three exotic breeds was sequenced and analysed with reference sequences from other countries. A total of 54 segregating sites among the sequences revealed 56 different haplotypes. Two, 11, eight, seven and six haplotypes were identified with some haplotype sharing in indigenous breeds: Doom, Ghungroo, Mali, Niang-Megha and Tenyi-Vo, respectively. Population pairwise differences (PhiST) (0.409) were found significant ($P < 0.001$), and variance within breeds (59.1%) was more than that of among breeds (40.9%). Similar topology was noted in phylogeny and median-joining network. Indian domestic pigs from this study were found to possess unique and highly differentiated haplotypes on network analysis. The diverse haplotypes and phylogenetic lineages identified here is the first report on Indian pig breeds that need to be further explored by complete mitochondrial DNA sequencing and analysis. These findings provide indicative insights for conservation and optimum utilization of the porcine genetic resources.

Keywords. haplotype; hypervariable region; mitochondrial DNA; indigenous pigs.

Introduction

Indigenous domestic pigs (*Sus scrofa domesticus*) are the mainstay of Indian piggery sector that is contributing significantly towards livelihood and nutritional security of resource-scarce small-holders (www.fao.org/dad-is/en). East and northeast India accommodates two-thirds of the domestic pig population, and hosts 13 of 18 indigenous pig (prospective) breeds of the country (Banik *et al.* 2017). These indigenous pigs possess wide phenotypic diversity and many important attributes, such as early sexual maturity (Kumaresan *et al.* 2011), good quality bristles (Narayana *et al.* 2014) and climate-resilience (Vashi *et al.* 2018), although production economics may rate them poor *vis*-a-*vis* commercial exotic pigs. Following the recommendations of the National Commission on Agriculture (https://krishikosh.egranth.ac.in), exotic breeds of pigs (live animals, and frozen semen in recent past) have been imported for genetic improvement of indigenous stock and piggery developmental programmes in India. Of the exotic breeds, Large White Yorkshire (LWY), Hampshire and Duroc pigs have found wide acceptance and usage. Nevertheless, pig population in India witnessed a significant decline during the past two decades, from 13.52 million in 2003 to 10.29 million in 2012 to 9.06 million in 2019 (Livestock Census 2019). The decline has been mainly due to diminishing population of native pigs, widening of the demand-supply gap of pork, and disease epidemics (Banik *et al.* 2017). Direct competition of indigenous pigs with exotic pigs might also drastically reduce their population size, and inbreeding depression might increase the risk of their extinction (Fang and Andersson 2006).

Rongala Laxmivandana and Yoya Vashi contributed equally to this work.

Supplementary Information: The online version contains supplementary material available at https://doi.org/10.1007/s12041-021-01353-8.

Published online: 12 January 2022

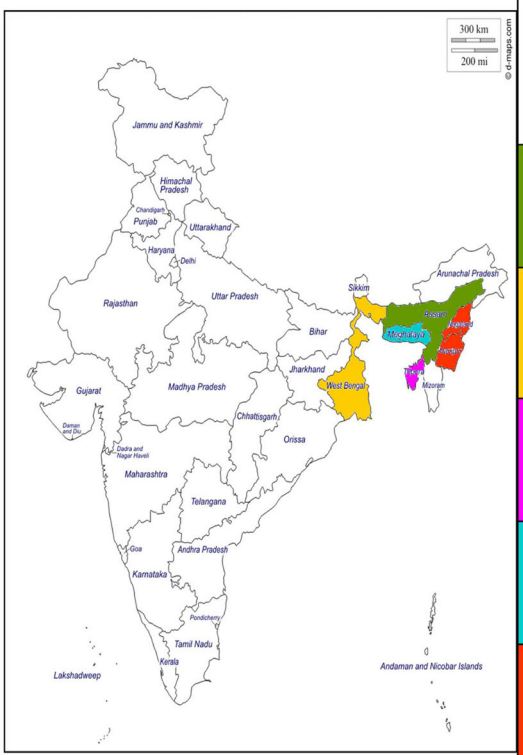
Previous studies on matrilineal/mitochondrial DNA (mtDNA) demonstrated a considerable population genetic structure of various pig breeds from different countries (Larson *et al.* 2005; Fang and Andersson 2006). The phenotypic diversity of Indian pig breeds are fairly known (Banik *et al.* 2017); however, their genetic diversity is less understood. This study has aimed to understand the molecular variation in mtDNA D-loop, the hypervariable region (HVR), for deciphering the genetic structure of indigenous pig breeds of India.

Materials and methods

The D-loop HVR (~487 bp) of mtDNA of 214 pigs comprising of five indigenous breeds, namely Doom, Ghungroo, Mali, Niang-Megha and Tenyi-Vo found across eastern and north-eastern India (figure 1) – the centre of pig production, and three exotic breeds, namely LWY, Hampshire and Duroc, originally imported for genetic improvement programmes and that are most popular, were sequenced and analysed.

The experiment was designed as per the recommended guidelines (http://cpcsea.nic.in) and was approved by the Institutional Animal Ethics Committee. Genomic DNA was isolated from blood samples (collected from jugular vein of the animals) using DNA Isolation kit for Mammalian Blood (Roche, USA). Amplification and sequencing was carried out using primers (MacKay *et al.* 1986; Jiao *et al.* 2009) L99 (forward: 5′-CCAAAGCTGAAATTCTAACTAAA-3′) and H451 (reverse: 5′-GGTGAGATGGCCCTGAAGTAAG-3′) for generation of a product of ~487 bp (procedure described in electronic supplementary material at http://www.ias.ac.in/jgenet/). Original sequences obtained were submitted to GenBank (accession nos. MK102353–MK102565, table 1 in electronic supplementary material).

The nucleotide sequences were aligned by CLUSTAL-W with other reference sequences retrieved from GenBank (listed in table 1 in electronic supplementary material), and the genetic distances among the sequences were calculated using maximum composite likelihood model in MEGA v.X (Kumar *et al.* 2018). Phylogenetic analysis was conducted using MEGA v.X (Kumar *et al.* 2018), maximum-likelihood tree was constructed with 100 bootstrapping replications. FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) was used for the tree visualization. The haplotypes were identified using DnaSP v.6 (Rozas *et al.* 2017). The haplotype network was constructed by median-joining network method (epsilon = 0) using PopART v.1.7 (Leigh and Bryant 2015). The PhiST, molecular variance (AMOVA) and haplotype frequency of the pig breeds were also analysed (Leigh and Bryant 2015).



S.No	Name of the breed	Latitude and longitude of the sampling location/native tract	No. of animals included and haplotypes identified	Haplotype (No. of the animal samples, percent (%) contribution)
1	Doom	between 24°8' and 28° 2' north and 89°42' and 96° east, Assam.	10 and 2	Hap_1(7, 70), Hap_10 (3,30).
2	Ghungroo	between 24° and 27° north and 88° and 89° east, West Bengal.	74 and11	Hap_8 (51, 69), Hap_23 (7, 9.4), Hap_9 (4, 5.4), Hap_2 (2, 2.7), Hap_11 (2, 2.7), Hap_25 (2, 2.7), Hap_43 (2, 2.7), Hap_3 (1, 1.35), Hap_18 (1, 1.35), Hap_24 (1, 1.35), Hap_26 (1, 1.35).
3	Mali	between 22°56' and 24° 32' north and 90°09' and 92°20' east, Tripura.	40 and 8	Hap_2 (23, 57.5), Hap_1 (8, 20), Hap_11 (3, 7.5), Hap_20 (2, 5), Hap_9 (1, 2.5), Hap_15 (1, 2.5), Hap_23 (1, 2.5), Hap_30 (1, 2.5).
4	Niang-Megha	between 25° and 26°42' north and 89°52 and 94°42' east, Meghalaya.	31 and 7	Hap_11 (13, 41.9), Hap_6 (7, 22.6), Hap_2 (6, 19.4), Hap_21 (2, 6.5), Hap_1 (1, 3.2), Hap_4 (1, 3.2), Hap_28 (1, 3.2).
5	Tenyi-Vo	between 26°62' and 27° 42' north and 98° and 96° east, Nagaland and Manipur.	30 and 6	Hap_2 (14, 46.7), Hap_11 (8, 26.7), Hap_3 (5, 16.7), Hap_7 (1, 3.3), Hap_13 (1, 3.3), Hap_22 (1, 3.3).

Figure 1. Geographical location and haplotype frequency of indigenous breeds of pigs.

Results and discussion

The phylogeny of the mtDNA D-loop sequences of the pig breeds of this study and other published sequences (table 1 in electronic supplementary material) is depicted in figure 2. The monophyletic position noted in this study as the macro clade containing D-loop sequences of domestic, wild, Asian and non-Asian pigs was also noted previously using partial and complete mtDNA sequences (Larson *et al.* 2005, 2007; Wu *et al.* 2007; Yu *et al.* 2013). Four distinct clades, with bootstrap support of > 70%, containing sequences of (i) many of the exotic pigs, (ii) Indian wild pigs, (iii) Doom pigs, and (iv) some Ghungroo pigs, apart from the other sequences (figure 2) were noted. The ratio of transition to transversion (T_s/T_v) in the mtDNA D-loop sequences of this study was found to be greater (3.85:1) than the critical value of 2.0 (Knight and Mindell 1993), indicating the transition preference. Similar observation was made earlier by Jiao *et al.* (2009). In this study, PhiST were found to be 0.409 ($P < 0.001$); a significant mtDNA D-loop variation was

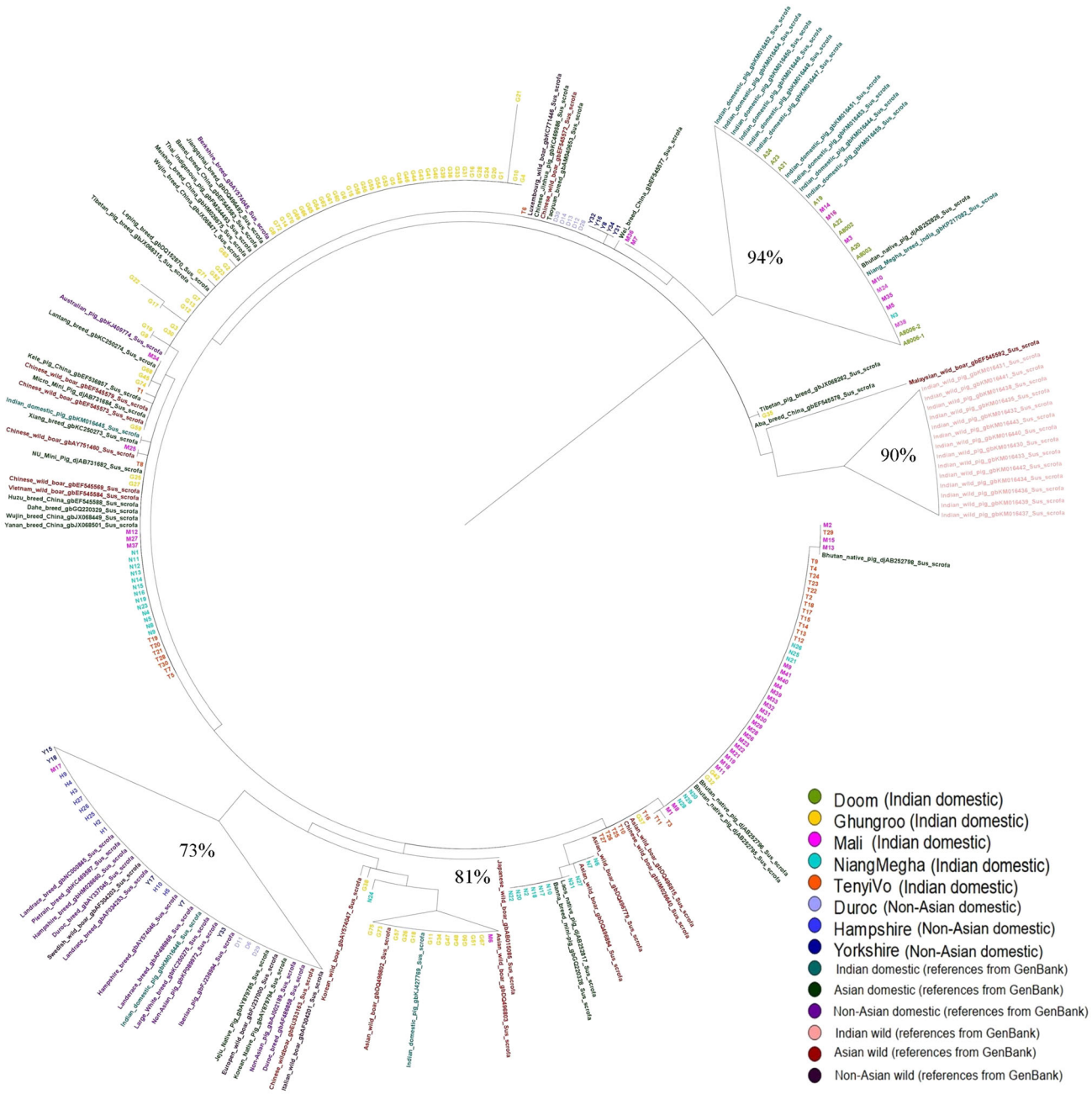


Figure 2. Maximum likelihood phylogenetic tree showing distribution of studied sequences. Sample labelled as A, D, G, H, M, T, N and Y denotes Doom, Duroc, Ghungroo, Hampshire, Mali, Tenyi-Vo, Niang-Megha and Large White Yorkshire pig breeds, respectively, followed by animal numbers. Clades showing > 70% bootstrap support are mentioned as node label.

found within (59.1%) and among (40.9%) the breed sequences of the pigs. However, the variation among the breeds was insufficient to illustrate the breed with respect to segregation.

Within breeds, the genetic distance was found to be highest in Mali (1.1%), followed by Ghungroo (0.9%), Niang-Megha (0.7%), Tenyi-Vo (0.4%) and Doom (0.2%) (table 2 in electronic supplementary material). Niang-Megha and Tenyi-Vo, the semi-domesticated pig breeds included in this study showed low genetic distance (0.6%) between them, while Doom pigs revealed high genetic distance (1.7–2.1%) with other indigenous pig breeds. The genetic distance between Indian wild and domestic pigs (table 2 in electronic supplementary material) was found to be 3.5%, while the distance within the groups was noted as 1.9% and 1.1%, respectively (table 2 in electronic supplementary material).

Further, each mtDNA sequence was allocated to a haplotype through detection of the haplotype-specific mutation motifs (figure 1 in electronic supplementary material). A total of 54 segregating sites among the sequences revealed 56 different haplotypes. A median joining network was constructed for the 56 haplotypes identified based on mtDNA D-loop sequences to depict the relationship among the pig populations (figure 2 in electronic supplementary material). Most of the Indian domestic pig breeds of this study shared the haplotypes among each other and some with other Asian pigs. This is consistent with the phylogenetic pattern discerned in the tree of the present study (figure 2). The topology of the network (figure 2 in electronic supplementary material) showed multiple star-like appearances suggesting expansion of the corresponding haplotypes. This is in agreement with observation that was made by studies using partial and complete mtDNA sequences of pig population from other countries (Larson *et al.* 2005; Wu *et al.* 2007). The mtDNA analysis of this study showed that Indian domestic pigs possess unique and highly differentiated haplotypes, similar to those found in Indian wild pig (figure 2 in electronic supplementary material). However, no haplotype sharing with any other domestic pig breeds was noted in Indian wild pigs while haplotype sharing was noted in Indian domestic pigs (Hap_3, 7, 9, 11, 15, 21, 23, and 24) of this study with East Asian/Chinese wild pigs, but not with Indian wild pigs. Most of these Asian pig populations formed a branch/haplogroup different from most of the exotic pig populations (listed in table 1 in electronic supplementary material). Similar findings were reported by previous studies from other regions (Larson *et al.* 2005; Wu *et al.* 2007).

Although most of the haplotypes present only in non-Asian pig populations were clustered into one haplogroup (figure 2 in electronic supplementary material), Asian type mtDNA was noted in non-Asian haplogroup and vice versa. The introgression of pigs between Asian and non-Asian countries was reported in previous studies (Fang and Andersson 2006).

Larson *et al.* (2005) demonstrated that basal lineages of *S. scrofa* occurred in Island South East Asia (ISEA); an initial dispersal from this area into the Indian subcontinent was followed by subsequent radiations into East Asia and a final progressive spread across Eurasia into western Europe (Larson *et al.* 2005). In agreement with this, phylogenetic analysis in our study also showed that some basal lineages/nodes closer to root are sequences of pigs from Malaysia/ISEA and Indian subcontinent (figure 2). However, Larson *et al.* (2007) has also highlighted the complexities associated with Holocene human migration and the translocation of animal species in ISEA and Pacific (Larson *et al.* 2007).

Hap_1, Hap_8, Hap_2, Hap_11 and Hap_2 were found predominant in breeds (geographic locations): Doom (Assam), Ghungroo (West Bengal), Mali (Tripura), Niang-Megha (Meghalaya) and Tenyi-Vo (Nagaland and Manipur), respectively (figure 1). The mtDNA haplotypes of indigenous pigs identified here revealed sympatric distribution with respect to their sampling locations. Within east and northeast India, hap_2 was found predominant in both Mali and Tenyi-Vo pigs from Tripura and Nagaland/Manipur respectively, while the matrilineal pool of Ghungroo from West Bengal contained many lineages from other regions (figure 1). This may suggest potential derivation/dispersal from the matrilineal pool of one region to the other regions, caused due to the migration. Similar observation has been made in previous studies from other countries (Wu *et al.* 2007).

This is the first report that shows presence of highly differentiated mtDNA haplotypes in Indian pig breeds and their phylogenetic lineages. Despite the similarity of the results with complete mtDNA based studies carried out elsewhere, present findings are to be explored further using complete mtDNA sequencing of indigenous pigs and their analysis. The dispersal of the five indigenous breeds should also be studied further. The genetic diversity indicated in this study shall have implications for conservation decision and optimum utilization of the porcine genetic resources.

Acknowledgements

This work was supported by the Department of Biotechnology, Govt. of India through Institutional Biotech Hub Project (BT/O4/NE/2009), in part by the Indian Council of Agricultural Research through Extramural Research Project (AS/8/20/2015-ASR-IV), and ICAR-National Research Centre on Pig, Guwahati. The authors acknowledge Dr Anil K. Das, Dr Gagan Bhuyan and Mr Kailash Choudhury for their help in collection of biological samples, and Dr David Peris for providing help in using PopART software.

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Corresponding editor: T. N. C. VIDYA