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Copy number variation regions in Nellore cattle: Evidences of environment adaptation

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ABSTRACT

The aim of the present study was to analyze the distribution of CNV regions (CNVRs) as well as to address hypothesis about the natural/artificial selection process in Nellore cattle. A total of 399,361 CNVs were identified, using the PennCNV algorithm, in 3794 Nellore cattle (*Bos taurus indicus*) genotyped with the Bovine HD BeadChip array. The *default* quality control was applied and 2902 samples and 195,873 CNVs remained. The medium CNV length size was 54,744 bp with a maximum of 870,000 bp and a minimum of 3000 bp. The CNVRs were generated by overlapping the 195,873 identified CNVs using the CNVRuler program. There was a higher incidence of CNVRs on BTA19 (24.26%), BTA23 (18.68%), and BTA25 (18.05%). The chromosomes that showed a lower incidence of CNVR were BTA29 (1.63%), BTA13 (9.72%), and BTA8 (9.72%). According to the type, 38.5%, 28.5% and 33.0% of the CNVRs were characterized as insertion, deletion and mixed (insertion and deletion in the same region), respectively. The 9805 CNVR estimated in the present study covered approximately 13.05% of the cattle genome (UMD_3.1, 2,649,685,063 bp) and overlapped with 5495 genes. These genes have functions described as involved in biological processes that might be related to the environmental adaptation of the subspecies to tropical areas, such as regulation of vasodilatation, immune system response, hair follicle morphogenesis, among others. This study confirms the existence of large structural variations in the Nellore cattle genome and contributes to understanding the differences between cattle subspecies. Besides, it can also work as a guideline for future studies in which structural variations are present.

1. Introduction

The identification of new DNA variations affecting quantitative traits is important for beef cattle genetic improvement trough genomic selection. The sequencing of the bovine genome identified more than 27 millions single nucleotide variants and 3,828,041 insertions and deletions (Stafuzza et al., 2017). In the last years, the focus of the scientific community was to identify SNP markers related to phenotypic variation so as to assess genetic variation for genetic improvement. Several forms of DNA variations, i.e. structural genomic variations, may contribute to phenotypic variation (Redon et al., 2006) since they encompass deletions, inversions, mobile-element transpositions, translocations, and copy number variations (CNVs) (Tattini et al., 2015). The CNVs are defined as a DNA segment with 1 kb or more in length and it presents a variable number of copies, insertions, deletions, translocations or inversions, when compared to a reference genome (Feuk et al., 2006; Scherer et al., 2007).

Several studies have shown the importance of the CNVs for many traits in domestic animals, like cattle (Liu et al., 2008; Seroussi et al., 2010; Seroussi et al., 2012a, 2012b; Cicconardi et al., 2013;), horses (Rosengren-Pielberg et al., 2008), pigs (Paudel et al., 2013; Paudel et al., 2015), sheep (Fontanesi et al., 2011a; Liu et al., 2013;), chickens

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(Crooijmans et al., 2013; Wang et al., 2010), and dogs (Alvarez and Akey, 2012; Salmon Hillbertz et al., 2007). A considerable number of CNV maps in cattle were developed using different methods, such as comparative genomic hybridization arrays (aCGH) (Lai et al., 2005; LaFramboise et al., 2009; Pinto et al., 2011), SNP genotyping arrays (Oliveira Junior et al., 2014; da Silva et al., 2016a; da Silva et al., 2016b) and high-throughput next-generation sequencing (Liu et al., 2010; Hou et al., 2011b; Bickhart et al., 2012;) showing that the same genetic variation may be studied from different source of information.

Despite of having a common ancestor, *Bos taurus indicus* and *Bos taurus taurus* cattle have undergone separate evolution for numerous hundred thousand years (Machugh et al., 1997a, 1997b). As a result of their domestication process, *Bos taurus indicus* or zebu animals can be categorized into tropical cattle, having its own phenotypic and genetic differentiation based on the adaptation and evolutionary traits that they possess (Chan et al., 2010). Thus, the zebu cattle have a better adaptation upon high temperatures and a better ability to carry lower burdens of cattle tick. Brazil is one of the largest Nellore's breeder and also a noticeable genetic exporter to others tropical and subtropical regions, contributing whether through purebred selection within the breed or through crosses with local breeds, mainly with European origin. Due to the importance of the Nellore breed and considering the scarce of genomic characterization studies, the aim of the present study was to analyze the distribution of CNV regions (CNVRs) as well as to address hypothesis about the natural/artificial selection process.

2. Material and methods

2.1. Samples

A total of 3794 Nellore animals (1759 sires and 2035 dams) were used in the present study. The animals were raised in 10 farms located in the Southeast, Northeast, and Midwest regions of Brazil. The farms belonged to three beef cattle breeding programs (CRV-Paint, DeltaG, and Nellore Qualitas). These breeding programs used as selection criteria growth, finishing, and sexual precocity traits. This study was approved by the ethic committee of the Faculty of Agrarian Sciences and Veterinary, São Paulo State University (UNESP).

2.2. CNV and CNVR detection

The animals were genotyped with the Bovine HD BeadChip array (High-Density Bovine Bead Chip - Illumina), that contains 777,962 SNP markers. SNP markers with unknown genomic position, located on sex chromosomes, monomorphic and with excess of heterozygosity were removed, as were those with minor allele frequency (MAF) lower than 0.05 and call rate lower than 90%. Samples were also edited for call rate lower than 90%. The CNVs identification was carried out with the SNP data file from the GenomeStudio 1.0 software. For CNVs detection, the PennCNV algorithm was used (Wang et al., 2007). This software incorporates multiple sources of information based on a hidden Markov model for CNV detection from the high-density genotyping data. The PennCNV is the most commonly used algorithm for CNV studies, since it presents a low rate of false positives (Winchester et al., 2009; Tsuang et al., 2010; Eckel-Passow et al., 2011).

Initially, the PennCNV algorithm was used without any quality control parameter so as to obtain the number of CNVs in overall samples (3974 animals). To maintain the quality of the samples, the default PennCNV's quality control was applied, eliminating samples with standard deviation for LRR (log R ratio) higher than 0.30; BAF (frequency of allele B) higher than 0.05; and a value of the waves factor higher than 0.01 (Liu et al., 2013).

After the CNVs identification, the CNVRs were generated by overlapping the CNV samples using the CNVRuler program (Kim et al., 2012). First, individual CNVs are merged into CNVRs, which are genomic regions covering CNVs overlapping by at least 1 bp (Redon et al., 2006). This process is simple and straightforward, but it may overestimate the size of CNVRs when any of the overlapping CNVs are extremely long. In order to minimize this possibility, the CNVRuler provides the option to assess base-by-base the regional density of the participating CNVs and trim the low-density areas. Genomic areas with density lower than 10% were excluded ("recurrence 0.1"). The recurrence trims a CNVR based on its frequency to avoid false positive predictions, and it defines more robust limits of the start and end regions. The option “Gain/Loss separated regions” was applied to evaluate the type of the event (gain, loss) in each region. Overlapping "gain" and "loss" CNVRs were merged into single regions to account for genomic regions in which both events can occur ("mixed" CNVRs).

2.3. Annotation (genes and enrichment)

Gene content in CNVRs was assessed thought the Ensembl tool VEP database (Bos taurus genes UMD3.1). The genes overlapping CNVRs were subjected to an enrichment gene ontology (GO) analysis with PANTHER and Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 tool (Huang et al., 2009). The enrichment GO analysis determined whether a particular GO was statistically over-represented by the fold enrichment value (≥ 2.0). The enrichment analysis was carried out for the subset of CNVs whose frequency was higher than 1%.

2.4. Overlap statistics

Overlap analysis was carried out using the Bioconductor package regioneR (Gel et al., 2016). This package implements a general framework for testing overlaps of genomic regions based on permutation sampling.

3. Results

3.1. CNV and CNVR discovery

The CNV calling on the UMD3.1 assembly identified a total of 399,361 CNVs for the 3794 samples. After the PennCNV default quality control filtering, a total of 2902 animals were kept for subsequent analyses. A total of 195,873 CNVs were identified with an average, maximum and minimum length size of 54,744, 870,000, and 3000 bp, respectively. The CNV length class size with the highest frequency was between 10 and 30 kb (Fig. 1).

The CNVRs mean length size was 3,528,473 bp, ranging from 520 to 1,476,546 bp. The highest rates of CNVRs coverage were found on BTA19 (24.26%), BTA23 (18.68%) and BTA25 (18.05%), and the lowest on BTA29 (1.63%), BTA13 (9.72%), and BTA8 (9.72%) (Fig. 2).

The CNVRs obtained in this study were compared to previous results also reported in Nellore cattle, and a total of 5155 (52.6%) CNVRs were identified as overlapping with those reported by Da Silva et al. (2016a, 2016b) (Fig. 3).

A total of 9805 CNVRs scattered in all 29 chromosomes were obtained from the CNVs calls. These CNVRs covered approximately 13.05% of the total length of the bovine genome (UMD_3.1,

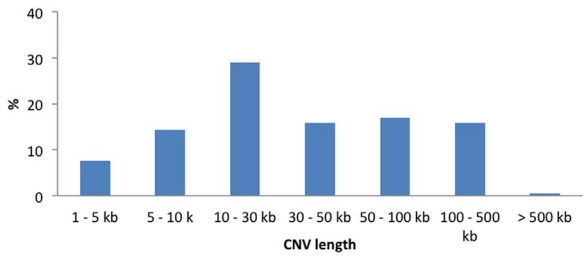

Fig. 1. Range of distribution of CNVs, expressed in kb.

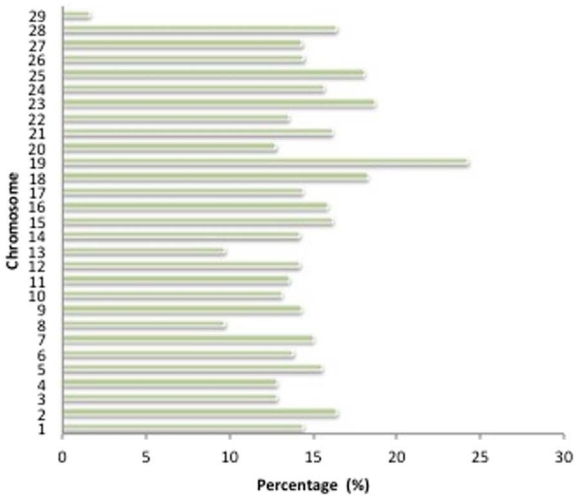

Fig. 2. Percentage of chromosome coverage by CNVRs.

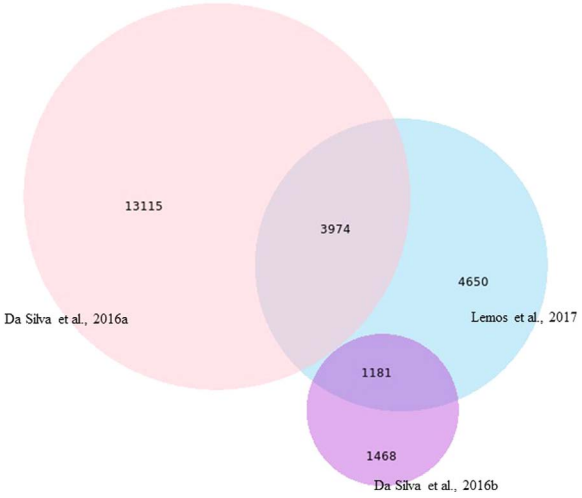

Fig. 3. Venn diagram representing the overlapping CNVRs in Nellore cattle.

2.649.685.063 bp) (Table 1). From the 9805 CNVRs obtained, 38.5% of them were characterized as insertion regions of copy number, 28.5% as deletion regions, and 33.0% showed both events in the same region (insertion and deletion). The BTA28 showed the highest percentage of insertion (47.87%); meanwhile, the highest percentages of deletion and both events were identified on BTA12 and BTA19, respectively (Fig. 4).

3.2. Gene content and gene ontology

The gene content of CNVRs derived from the UMD3.1 autosomes was investigated. The 9805 non-redundant CNVRs identified in the autosomes overlapped with 5495 known genes. Among them, 15% of the CNVRs were within coding regions, suggesting that exon regions were duplicated or deleted. In addition, 2% of the CNVRs were in upstream (1%) or downstream (1%) regions, which were very close to genes regions. Besides, 17% were in intragenic regions, 19% of the regions were found in introns and 18% in non-coding exon regions (5 'and 3' UTR), evidencing the importance the CNVRs (Fig. 5).

The PANTHER accessions were assigned to the overlapped genes for CNVRs identified on UMD3.1. Multiple categories were statistically significant. This set of copy number variable genes encompasses a wide spectrum of molecular functions, biological processes, cellular components, panther protein classes, and pathways. Several GO terms were found to be significantly over-represented, which we highlight

Table 1
CNVR distribution, coverage size and coverage percentage of chromosomes by CNVR detected across Nellore genome.

Chromosome	Chromosome size (bp)	Number of CNVR	CNVR mean size (bp)	CNVR Coverage (bp)	% Coverage
1	158,337,067	732	31,496.55	23,055,476	14.56
2	137,060,424	584	33,764.03	19,718,197	14.39
3	121,430,405	559	29,669.44	15,395,899	12.68
4	120,829,699	515	29,891.89	15,394,327	12.74
5	121,191,424	477	39,081.58	18,641,916	15.38
6	119,458,736	475	34,775.71	16,585,218	13.88
7	112,638,659	469	32,827.07	16,518,465	14.67
8	113,384,836	435	32,851.60	10,701,231	9.4
9	105,708,250	387	33,408.40	14,290,463	13.52
10	104,305,016	385	38,737.20	13,107,356	12.57
11	107,310,763	371	28,844.28	14,913,824	13.90
12	91,163,125	355	31,988.35	11,355,867	12.46
13	84,240,350	352	37,525.45	7782,113	9.24
14	84,648,390	335	33,898.29	11,355,929	13.42
15	85,296,676	316	35,129.38	12,929,053	15.16
16	81,724,687	278	36,158.40	11,100,887	13.58
17	75,158,596	278	32,282.02	10,052,037	13.37
18	66,004,023	272	53,521.74	10,949,639	16.59
19	64,057,457	268	36,134.05	14,557,915	22.73
20	72,042,655	267	41,009.88	8,947,404	12.42
21	71,599,096	226	34,434.12	9,683,926	13.53
22	61,435,874	226	41,343.97	8,128,130	13.23
23	52,530,062	194	32,929.14	9,343,739	17.79
24	62,714,930	194	37,253.85	9,388,255	14.97
25	42,904,170	193	42,114.66	7,222,553	16.83
26	51,681,464	188	34,903.89	7,227,248	13.98
27	45,407,902	177	46,287.58	5,736,358	12.63
28	46,312,546	171	33,545.95	6,561,933	14.17
29	51,505,224	126	57,321.84	819,203	1.59

apoptotic process (GO:0006915), biological regulation (GO:0065007), development process (GO:0032502), growth (GO:0040007), immune system process (GO:0002376), metabolic process (GO:0008152), reproduction (GO:0000003), as well as several enriched biological processes.

The same gene dataset was used in the DAVID tool (Huang et al., 2009) so as to analyze the GO functional categories of the protein coding genes located in the CNVs (Table 2). The most enriched GO categories are related to biological regulation process and are shown in Table 2.

4. Discussion

A total of 195,873 CNVs, with an average length size of 54,744 bp, maximum of 870,000 and a minimum of 3000 bp were identified. The high number and distribution of CNVs in the Nellore genome was also evidenced by Da Silva et al. (2016). These authors detected a total of 68,007 CNVs using the high-density SNP array (BovineHD Genotyping BeadChip) from 1717 Nellore animals. Similarly, Zhou et al. (2016), in a sample of 2230 Nellore cattle identified a total of 992,350 CNVs using a multivariate method supplied by Golden Helix SVS 8.3.0 (Golden Helix, Bozeman, MT, USA http://www.goldenhelix.com).

Several studies have highlighted the distribution and characterization of CNVs in the genome of various animals' species (Fadista et al., 2010; Liu et al., 2010; Hou et al., 2011a). Fadista et al. (2010) reported 304 CNVs regions in 20 animals from Holstein, Simmental, Red Danish, and Hereford breeds. From these preliminary results, new evidences of CNVs in cattle were observed with the use of bovine HapMap (over 500 animals of different breeds), which allowed the identification of 79 candidate deletions (Matukumalli et al., 2009). Bickhart et al., (2012) characterized the genome of three taurine breeds (Angus, Holstein, and Hereford) and one indicine breed (Nellore). The authors observed that the number of CNVRs within the taurine breeds was higher than those

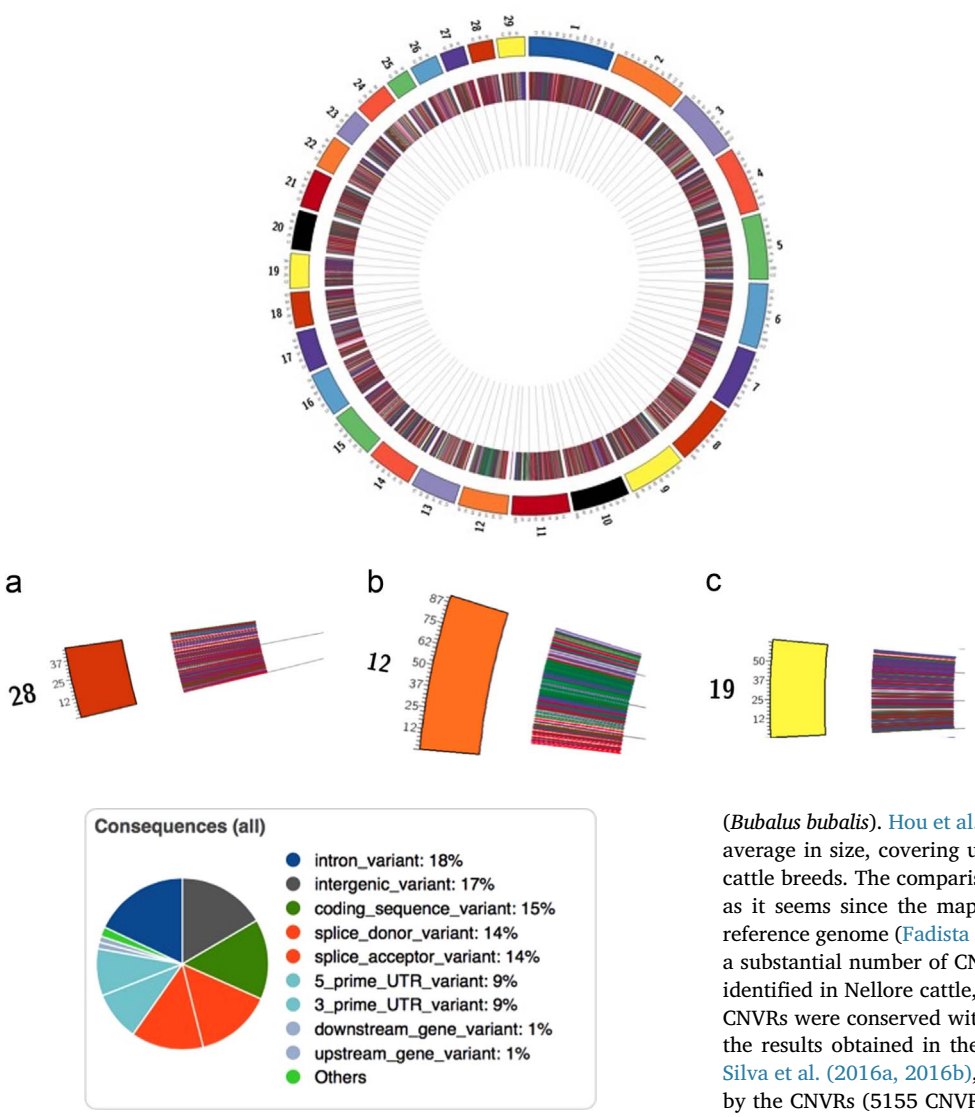

Fig. 4. Comprehensive circular map of autosomal copy-number variants in *Bos indicus*. From the outside to the inside of the external circle: Chromosome name; genomic location (in Megabases); bars depicting the CNV regions (loss in green, gain in red, and both events in purple). Figs. 4a, 4b and 4c are a zoom of the chromosome that showed the highest percentage of insertion regions (BTA28, BTA12 and BTA19, respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. Graphic representation of CNVRs distribution on the bovine genome.

between taurine and indicine breeds. Moreover, the Nellore breed presented the highest CNVs' diversity among the studied breeds, suggesting that the genetic difference between taurine and indicine breeds might be due to the number of CNVs and its diversity.

By comparing the results discussed above with those of the present study, it possible to note a significant difference in the number of CNVs identified. This divergence may occur due to the use of different methodologies for identifying the CNVs, i.e. different platforms or genotyping algorithms (Table 3). Current methodologies used to infer variations in the number of copies from SNP data tend to lead to some errors at the time of detection (Castellani et al., 2014; Redon et al., 2006). Besides, a number of factors may influence the accuracy of the CNVs detection, such as the breakpoint, the inclusion of batch effects, the stratification of the population, experimental differences, and the robustness of the statistical model (Dellinger et al., 2010). The SNP array used can also interfere with the number of CNVRs discovery, increasing or decreasing the detection sensitivity (Hou et al., 2012).

The proportion of the genome covered by CNVRs was higher than those reported in the literature even for Nellore animals, with values ranging from 0.68% to 5.85% (Fadista et al., 2010; Liu et al., 2010; Zhou et al., 2016). Zhang et al. (2014) identified 486 CNVRs covering 2.45% of the cattle genome in 24 taurine samples, together with 161 CNVRs in two yaks (*Bos grunniens*) and 163 CNVRs in three buffaloes (*Bubalus bubalis*). Hou et al. (2011b) detected 682 CNVRs with 204.4 kb average in size, covering up to 4.6% (139.9 MB) of the genome of 21 cattle breeds. The comparison between CNVs and CNVRs is not as easy as it seems since the mapping in each study is based on a different reference genome (Fadista et al., 2010). Notwithstanding the foregoing, a substantial number of CNVRs were overlapped with previous CNVRs identified in Nellore cattle, suggesting that an important portion of the CNVRs were conserved within the Nellore breed over time. Comparing the results obtained in the present study with those presented by Da Silva et al. (2016a, 2016b), 52.6% of the genomic regions encompassed by the CNVRs (5155 CNVRs) were overlapped in both studies.

The higher proportion of the genome covered by CNVRs in this study may be explained by the large number of samples available for analysis, the methodology applied for identifying the CNV and CNVRs, and also due to the sub-specie (*Bos taurus indicus*) used in the present study.

Preliminary analysis showed that the number of CNVRs identified increased as the number of samples raised. However, it is important to consider the possibility of a bias due to the reference genome, mainly from *Bos taurus taurus* cattle. A *Bos taurus indicus* public reference genome would better guide the indicine genetic studies. Several studies characterizing CNVs in cattle have been performed, and most of them were carried out in taurine (Salomon-Torres et al., 2016; Jiang et al., 2012; Liu et al., 2010) and some in indicine cattle (Zhou et al., 2016; Da Silva et al., 2016a, 2016b). Besides, results have shown that more CNV loci have been reported and predicted in indicine breeds compared to European taurine breeds, which is consistent with the known breed divergence and history (Liu et al., 2010). Recent studies reported that CNVs evolved 2.5 folds faster than SNPs and helped to promote a better adaptation upon different environments (Paudel et al., 2015), being in accordance to the cattle sub-speciation as a result of their domestication process over time.

The relationship between the chromosome size and the number of CNVRs is straightforward (Fig. 6). Although some chromosomes revealed a higher proportion of CNVRs than others (relative to their size), the correlation between the number of detected CNVRs and the chromosome size was 0.96. This result was in accordance with that reported

Table 2
Gene ontology enrichment.

Category	Term	Count	P-Value	Fold Enrichment	FDR
GOTERM_BP_DIRECT	negative regulation of cAMP-dependent protein kinase activity	4	2,4E-2	5,2	3,5E1
GOTERM_BP_DIRECT	histone deubiquitination	4	2,4E-2	5,2	3,5E1
GOTERM_BP_DIRECT	regulation of vasodilation	3	9,7E-2	5,2	8,3E1
GOTERM_BP_DIRECT	positive regulation of histone methylation	3	9,7E-2	5,2	8,3E1
GOTERM_BP_DIRECT	positive regulation of cardiac muscle cell proliferation	3	9,7E-2	5,2	8,3E1
GOTERM_BP_DIRECT	protein localization to endosome	3	9,7E-2	5,2	8,3E1
GOTERM_BP_DIRECT	regulation of proteasomal ubiquitin-dependent protein catabolic process	4	5,2E-2	4,2	6,1E1
GOTERM_BP_DIRECT	cellular response to extracellular stimulus	4	5,2E-2	4,2	6,1E1
GOTERM_BP_DIRECT	amino acid transmembrane transport	4	8,9E-2	3,5	8,1E1
GOTERM_BP_DIRECT	signal transduction by protein phosphorylation	8	5,5E-3	3,2	9,3E0
GOTERM_BP_DIRECT	cellular response to cAMP	5	7,5E-2	2,9	7,5E1
GOTERM_BP_DIRECT	somitogenesis	5	7,5E-2	2,9	7,5E1
GOTERM_BP_DIRECT	hair follicle morphogenesis	5	7,5E-2	2,9	7,5E1
GOTERM_BP_DIRECT	negative regulation of JAK-STAT cascade	6	4,3E-2	2,8	5,4E1
GOTERM_BP_DIRECT	retrograde vesicle-mediated transport, Golgi to ER	7	3,6E-2	2,6	4,8E1
GOTERM_BP_DIRECT	protein tetramerization	6	8,6E-2	2,4	7,9E1
GOTERM_BP_DIRECT	regulation of autophagy	6	8,6E-2	2,4	7,9E1
GOTERM_BP_DIRECT	negative regulation of cyclin-dependent protein kinase activity	10	1,5E-2	2,4	2,4E1
GOTERM_BP_DIRECT	protein autophosphorylation	9	3,4E-2	2,2	4,6E1
GOTERM_BP_DIRECT	MAPK cascade	9	3,4E-2	2,2	4,6E1
GOTERM_BP_DIRECT	fatty acid biosynthetic process	7	9,0E-2	2,1	8,1E1
GOTERM_BP_DIRECT	chloride transmembrane transport	11	2,3E-2	2,1	3,4E1
GOTERM_BP_DIRECT	cell cycle arrest	9	5,8E-2	2,0	6,5E1
GOTERM_BP_DIRECT	heart development	12	2,5E-2	2,0	3,5E1
GOTERM_BP_DIRECT	ubiquitin-dependent protein catabolic process	17	1,1E-2	1,9	1,8E1
GOTERM_BP_DIRECT	protein stabilization	12	5,8E-2	1,8	6,5E1
GOTERM_BP_DIRECT	cell proliferation	15	3,2E-2	1,8	4,3E1
GOTERM_BP_DIRECT	transcription from RNA polymerase II promoter	25	7,8E-3	1,7	1,3E1
GOTERM_BP_DIRECT	negative regulation of apoptotic process	25	2,7E-2	1,5	3,8E1
GOTERM_BP_DIRECT	intracellular protein transport	20	6,6E-2	1,5	7,0E1
GOTERM_BP_DIRECT	positive regulation of transcription from RNA polymerase II promoter	50	3,0E-3	1,5	5,1E0

Gene ontology (GO) categories significantly over represented, with false discovery rate (FDR).

by Da Silva et al. (2016a) for Nellore cattle (0.98). The BTA1 exhibited the highest number of CNVRs (692) and CNVRs mean length size (46.68 kb), while BTA25 the lowest number of CNVRs (131) and CNVRs mean length size (79.37 kb).

Cicconardi et al. (2013) found 1522 CNVs in Italian Friesian cattle breed, in which 27.52% of them were insertion and 75.29% deletion regions. Da Silva et al. (2016a) found 4801 CNVRs of mixed events in the Nellore genome, while the number of deletions and insertions were 212 and 2306, respectively. In the present study, from the total of CNVRs obtained (9805), 38.5% of them were characterized as insertion regions of copy number, 28.5% as deletion regions and 33.0% showed both events in the same region (insertion and deletion). Compared to previous studies (Fadista et al., 2010; Bae et al., 2010; Seroussi et al., 2010; Liu et al., 2010; Hou et al., 2010; Cicconardi et al., 2013; Da Silva et al., 2016b), the results obtained in the present study pointed out the presence of a higher number of CNVRs and insertion regions. The insertion or deletion events of one or more copies of a gene could result in a proportional reduction or absence of the gene product, and it is determined according to the distance that the CNVRs are from the gene (Iafrate et al., 2014). The present study revealed a large proportion of insertion regions, thus, a higher proportion of genes affected by the CNVRs were observed when compared to other studies (Hou et al., 2011a; Bae et al., 2010; Cicconardi et al., 2013).

The GO analyses showed several enriched terms for the CNVRs gene list. One of them was the immune system response (GO:0002376), which is defined as the process involved in the development or functioning of the immune system, i.e. an organismal system for calibrated responses to potential internal or invasive threats. Some authors reported over-represented genes related to the immune system and sensory response in cattle and humans (Nguyen et al., 2006). This fact suggests that the survivability benefit can be achieved with the increased dosage of genes related to the immune and environmental sensory response (Stothard et al., 2011; Liu et al., 2010).

Indicine breeds are favored in tropical regions due to their tolerance to heat and parasites resistance, being the focus of several studies that aims to compare their immunological response upon various environmental factors. In this prerogative, O'Kelly (1980) found higher resistance of indicine crossbreeds raised in tropical conditions, mainly to *Haemonchus* and *Cooperia*, when compared to taurine purebred animals. The animal's resistance to internal and external parasites, which stands

Table 3
List of CNVs detection studies in cattle using different approaches.

Study	Method	Algorithm	Samples	Bovine sub-specie	CNV
Hou et al. 2012a	SNP chip (777 kb)	PennCNV	674	*Bos taurus*	443
Coccinard et al., 2013	SNP chip (648 kb)	PennCNV; QuantiSNP	2654	*Bos taurus & Bos indicus*	4839
Salomón-Torres et al. (2015)	SNP chip (648 kb)	PennCNV; QuantiSNP	12	*Bos taurus*	77
Yang et al., 2015	SNP chip (770 kb)	PennCNV; CNVPartition	792	*Bos taurus*	263
Wang et al., 2015	SNP chip (54 kb)	PennCNV	231	*Bos taurus x Bos indicus*	433
Zhang et al., 2015	aCGH	seg-MNT	24	*Bos taurus & Bos indicus*	356
Zhou et al. (2016)	700,000 SNP probes	Golden Helix SVS 8.3.0	2230	*Bos indicus*	992,350
Da Silva et al., 2016	SNP chip (777 kb)	PennCNV;	1717	*Bos indicus*	68,000
This study	SNP chip (777 kb)	PennCNV; CNVRuler	3794	*Bos indicus*	195,873

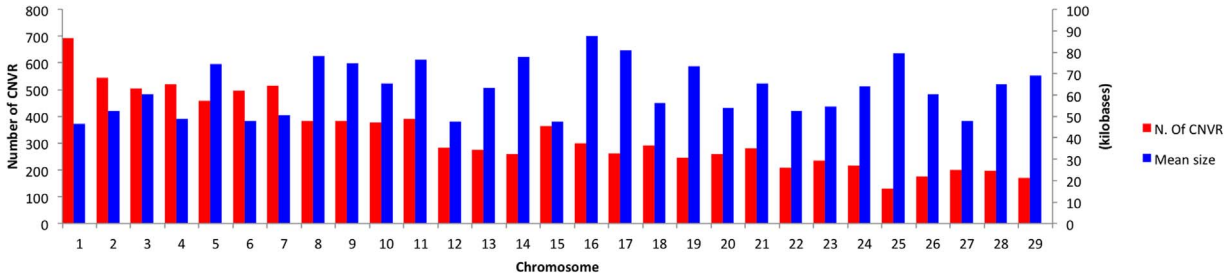

Fig. 6. Number and mean length size of copy number variation regions by chromosome.

for the immune response system efficiency, was “acquired” by natural selection in the Indian subcontinent (O’Kelly, 1980). Up to date, few studies have enlighten the mechanisms underlying the resistance and adaptation traits, thus, the natural selection and domestication process (breed divergence) has been speculated as the start point to explain those acquired traits over time.

The regulation of vasodilatation (GO:0045909) had a 5.2-fold enrichment through DAVID analyzes. This term is defined as any process that modulates the frequency, rate or extent of increases in the diameter of blood vessels. Indicine cattle show a higher thermo tolerance when compared to taurine cattle. Although the physiological basis for this fact has not yet been identified, one possibility is that the density of arteriovenous anastomoses is higher in indicine cattle (Finch, 1985). These structures have lower resistance to flow than vascular passages, it involves capillary networks and facilitates the blood flow to the skin during heat stress (Hales et al., 1978), being a more efficient way to dissipate heat. Another enriched term associated with heat tolerance was hair follicle morphogenesis (GO:0031069) (Finch et al., 1984). The indicine cattle have thin, short, smooth and shiny hair coats. The light color hair reflects a large proportion of solar incident radiation and also acts to reduce heat exchange via radiation (Hutchinson and Brown, 1969; Cardoso et al., 2015). To better dissipate the heat, indicine cattle also have a higher hair angle and a low hair density per skin area (Cruz et al., 2016). All these physiological mechanisms are highly associated with the term pointed out, evidencing its better environmental adaptation.

Terms related to performance were also identified (Panther analyses), such as growth (GO:0040007), defined as the increase in size or mass of an entire organism or a part of an organism. It has been previously reported in a CNV study done by Stothard et al. (2011) with taurine animals. This term could appear due to the selection for weight in Nellore cattle over the last decades; or even due to the lower weight gain that indicine breeds still have when compared to taurine breeds. The term reproduction (GO:0000003) was also significant. It is defined as the production of new individuals that contain some portion of genetic material inherited from one or more parent organisms (Stothard et al., 2011; Ashburner et al., 2000). It is fully described that indicine cattle are outstanding in longevity when compared to taurine breeds, especially regarding their aggregate culling rates for death, unsoundnesses, and injuries. (Rohrer et al., 1988a, 1988b). The CNVRs herein identified may be associated with these phenotypes and differences between subspecies.

Some others relevant biological processes were enriched by the gene list, such as fatty acid biosynthesis process (GO:0006633). For definition, fatty acid biosynthesis are the chemical reactions and pathways resulting in the formation of a fatty acid, any of the aliphatic monocarboxylic acids that can be liberated by hydrolysis from naturally occurring fats and oils (Ashburner et al., 2000). An important group of genes belonging to the ELOVL family are responsible for performing the fatty acids biosynthesis and it has been previously linked to beef fatty acid profile in Nellore cattle (Lemos et al., 2016). The authors, working with the same group of animals of this study, associated the *ELVOL5* gene with the arachidonic fatty acid, which is a polyunsaturated fatty acid present in the phospholipids membranes of the body's cell. This fatty acid is abundant in the brain, muscles and liver, and it is the product of their oxygenation and it can mediate or modulate inflammatory reactions (Smith et al., 1979; Samuelson, 1991). The enrichment of these terms could reflect a selection for muscle mass production with improved fatty acid profile, associated with a better resistance of these animals to inflammatory processes.

Biological regulation process (GO:0065007), development process (GO:0032502), apoptotic process (GO:0006915), among others, were enriched in DAVID analyses, further confirming the Panther results. The results of the functional analyses were in agreement with those reported in previous CNV studies in cattle (Fadista et al., 2010; Seroussi et al., 201; Hou et al., 2012; Cicconardi et al., 2013; Da Silva et al., 2016b). Recently, Da Silva et al. (2016a) reported 4097 CNVRs located within genomic regions containing 10,399 annotated genes, revealing important GO categories, i.e. metabolic and cellular processes, biological regulation, immune system process, response to stimulus, cell signaling, reproduction, and growth. Therefore, it is important to emphasize the importance of a deep investigation within these regions, as well as their validation, which can disclose about important mechanisms that underlie and affect economically important traits.

Understanding the genetic basis for productive and functional traits in zebu cattle have a great economic and biological importance. The broad identification of CNVs segregating within the breed provided crucial information to infer that CNVs may be subject to natural and/or artificial selection. The variations in the number of copies of the Nellore cattle genome brings important information to identify genetic variations associated with phenotypes of interest, such as production and meat quality traits, which studies are often restricted to the use of SNPs arrays. The information obtained in this study should support to conduct further association and fine mapping studies that aim to understand the genetic mechanisms and biological pathways underlying the productive and functional traits in Nellore cattle.

5. Conclusion

The present study revealed a high variability of CNVs in the Nellore (*Bos taurus indicus*) cattle genome. The gene enrichment analysis of the CNVRs revealed biological processes that might be involved in the environmental adaptation of the subspecies to tropical areas. In this regard, contributions to understand differences between cattle subspecies, as well as, can be used as s guideline for future studies with structural variations were presented.

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Competing interests

The authors declare that they have no competing interests.

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