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ORIGINAL RESEARCH

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Size does matter: Parallel evolution of adaptive thermal tolerance and body size facilitates adaptation to climate change in domestic cattle

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Summary

- Reduction in body size is a responce to warming climate.

- Parallel evolution in adaptative tolerance to heat stress in dwarf cattle breeds (DCB) and standard size cattle breed (SCB).

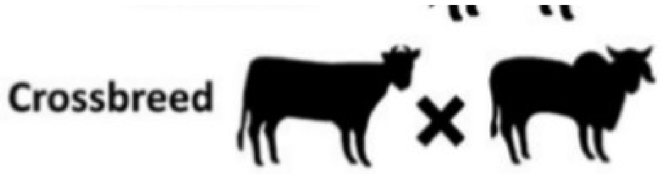
- Measured physiological, hematological, biochemical and gene expression and compared molecular phylogeny using mitochondrial genome analysis.

- Demonstrate that combine functional, physiological and evolutionary approaches delineate adaptative potential and plasticity in domestic species.

- SCB acclimatize in short term, but reach their tolerance limit under prevailnig tropical conditions

- DCB is adapted to warmer climate by:
  - increasing of emoglobin concentartion
  - reducing cellular and body size

- Larger breeds are preferred in intensive agriculture, but native smaller breeds show climatic resilience.



Bos taurus indicus
Kasargode
Weight: 50-130 kg
Height: 61-90 cm

Bos taurus indicus
Vechur
Weight: 50-130 kg
Height: 61-90 cm

Bos taurus taurus
crossbreed
Weight: 300-375 kg
Height: 120-150 cm

FROM LOCAL POPULATION, NONPREGNANT, 10 ANIMALS EACH.
GRAZED DURING DAY AND HAUSED AT NIGHT. FREE OF INFECTIONS. USED FOR 10 DAYS IN SUMMER, GRAZING FROM 8-14 (THI= 75-83). AFTER MORNING MILKING, WATER AND GRASS WERE GIVEN.

Adaptation: complex and energetic process caused by mutations.

Acclimatization: changes in physiology including gene expression.

Variations in size are considered as adaptive response to climate warming.

Heat tolerance: combining rectal temperature and respiratory rate, or by heat shock protein/factor (HSP/HSF) and reactive oxygen species at molecular level.

Biomarker for heat stress: HSP 70, but also ATP1A1, GAPDH and ACTB

Mitocondria by environmental stimuli modify gene expression througt mitonuclear communication and controlling metabolism.

Experimental spot: Kerala (Kerala veterinary and animal sciences university farm), tropical humid climate. Max temperature 35°C → farms 93% SCB and 6% DCB

Phenotypic changes are confined locally, not all species have decreased in size because of heat dissipation other reason can be changes in food aviability or hunting.

Diversity of traits and their interaction

PD= phylogenetic diversity
(measure evolutionary history among taxa)

FD= functional diversity
(measure functional traits)

Hypothesis: increas in temperature and humidity = adaptative change in physiology and genetic architecture that leads to dwarf breeds.

Measuraments: change in phenotypic and genotipic traits, combining physiological and phylogenic approach.

Aims:

- understand physiological basis
- demostrate evolutionary origins of different heat adaptation/ acclimatization

Metereological data:

Ta °C= ambient temperature

RH %= relative humidity

SR W/m”= intensitive of solar radiation

WS m/s= wind speed

Heat stress assessment:

THI= temperature humidity index (combined effect of humidity and temperature) used to monitor heat stress. Generally = 72. *THI = (1.8 × Ta + 32) – (0.55 – 0.0055 × RH) × (1.8 × Ta – 26)*

HLI= heat load index (measure body heat), in crossbreads from 70 to 96. *HLI = 8.62 + (0.38 × RH) + (1.55 Ta – 0.5 WS) + [e2.4–WS]*

AHL= accumulated heat load (duration of exposure), determed by HLILT(HLI threshold which cattles dissipate heat). HLIUT(HLI threshold where cattles gain heat) and M(measuraments/h). *AHL = IF [HLI < HLILT, (HLI– HLILT)/M], IF [HLI > HLIUT, (HLI– HLIUT)/M, 0)]*

AHLI= accumulated heat load index (all AHI during the day)

Physiological measuraments:

Every 30 minutes from 8 to 14, recorded:

- RT (rectal temperature),
- RR(respiration rate).
- HTC= (heat tolerance coefficient) RR/23+RT/38.3.
- PR (pulse rate) recorded for 1 minute

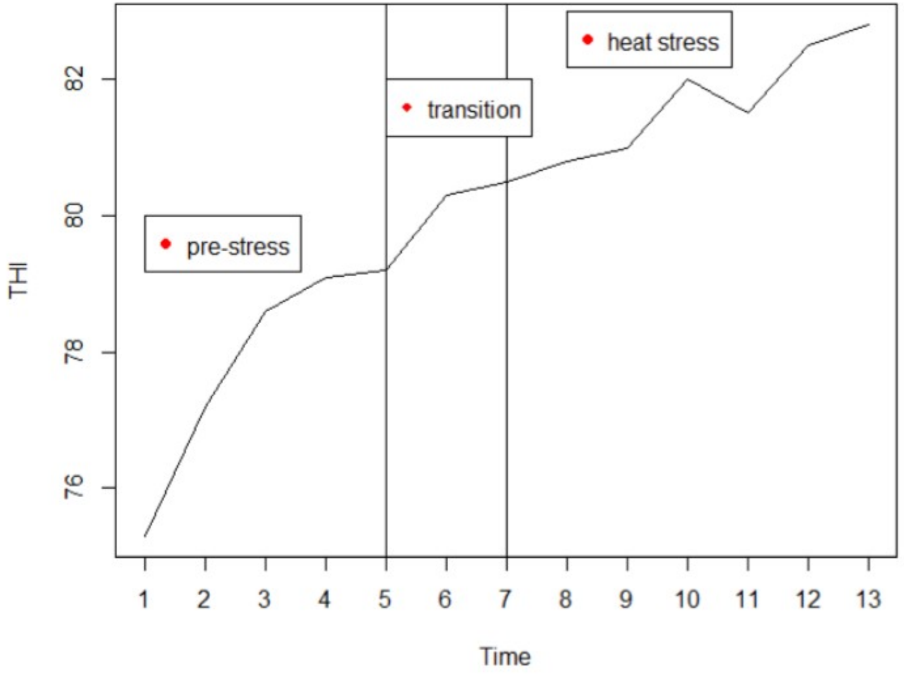
From 8 collected 5 ml of blood evry 2 hour using 5ml of EDTA as anticoagulant, also collected blood without EDTA centrifugated and storaged at -20°C t determine cortisol concentration.

Analysis:

- Q-RT-PCR
- Mitochondrial genome sequencing
- Statistical analysis: R 3.5.1
- Phylogeny

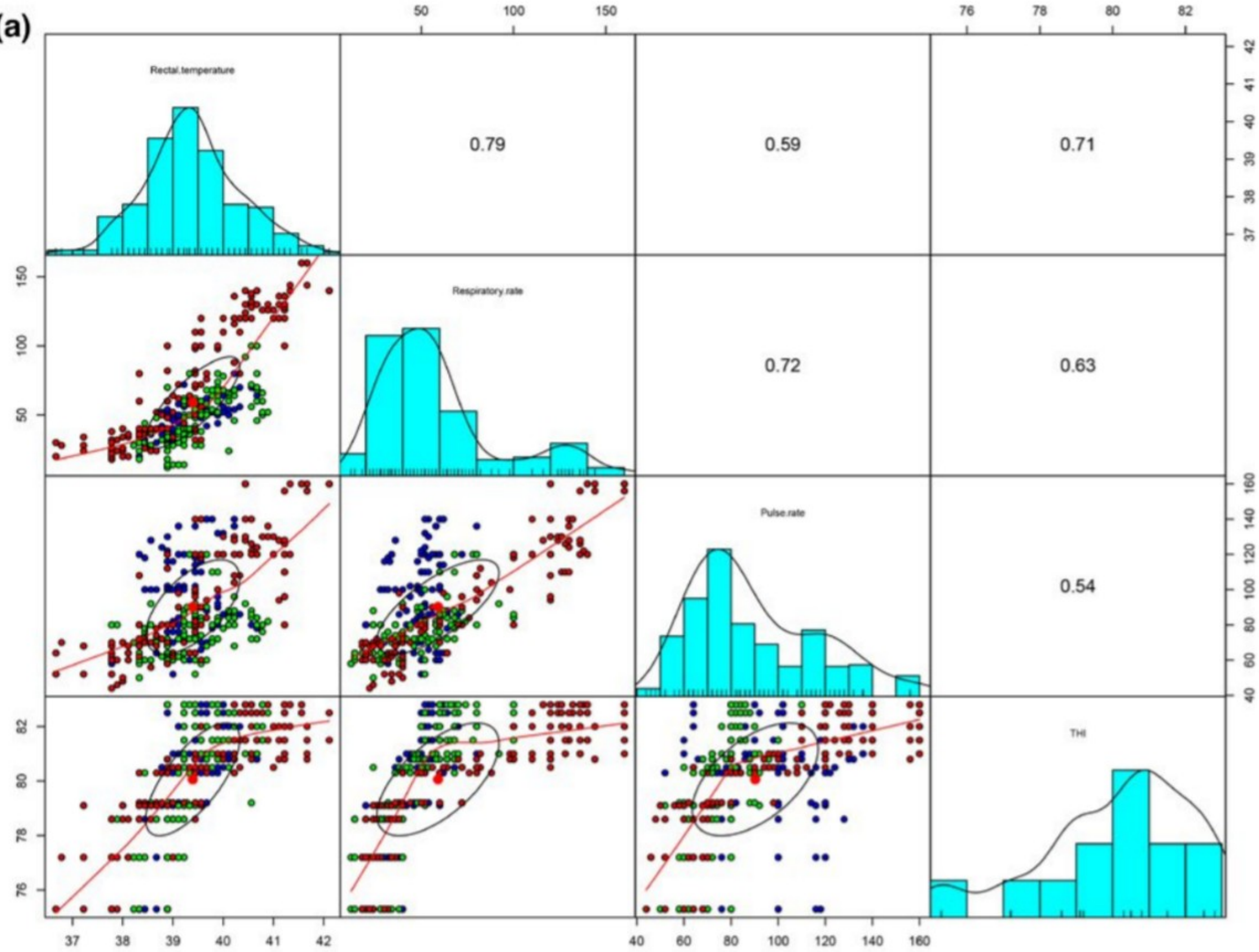
RESULTS:

Mean THI



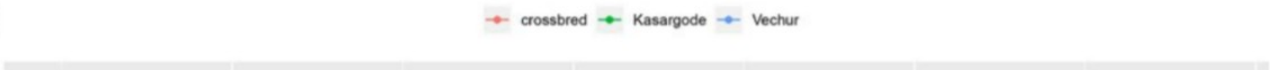
Supplementary figure 2.Temperature Humidity Index (THI) during the study period at half-hour intervals beginning at 8.00 am (1=8.00 am, 2=8.30 am….13=2.00 pm). Pre-stress (8.00 am to 10 am- THI= 75.3 to 79.2, transition (10 am to 11 am-THI=79.2 to 80.5, heat stress-11 am to 2 pm-THI= 80.5 to 82.8).

(a)



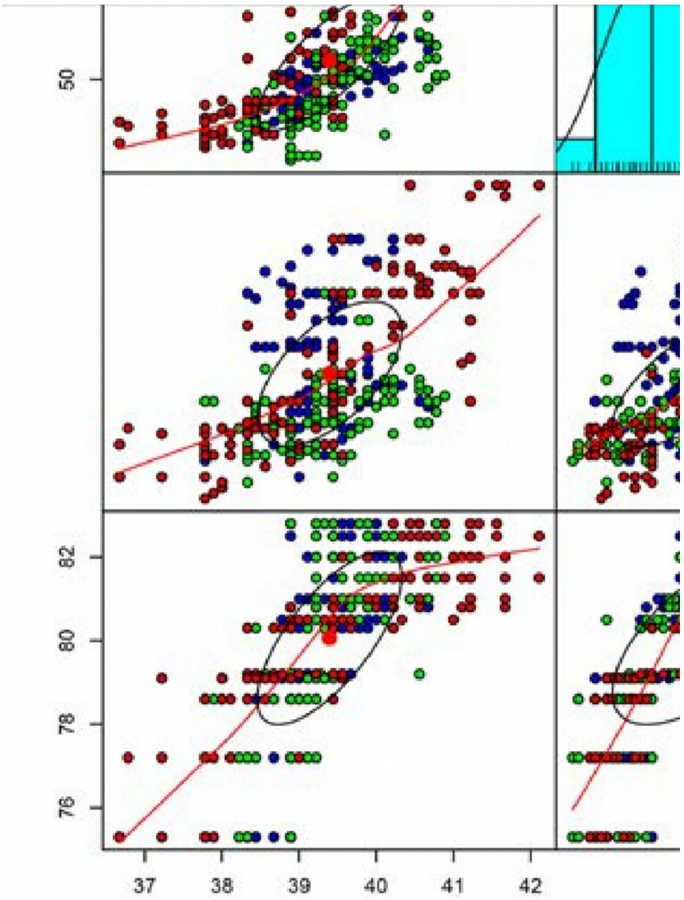
Rectal.temperature
Respiratory.rate
Pulse.rate
THI
0.79
0.59
0.71
0.72
0.63
0.54

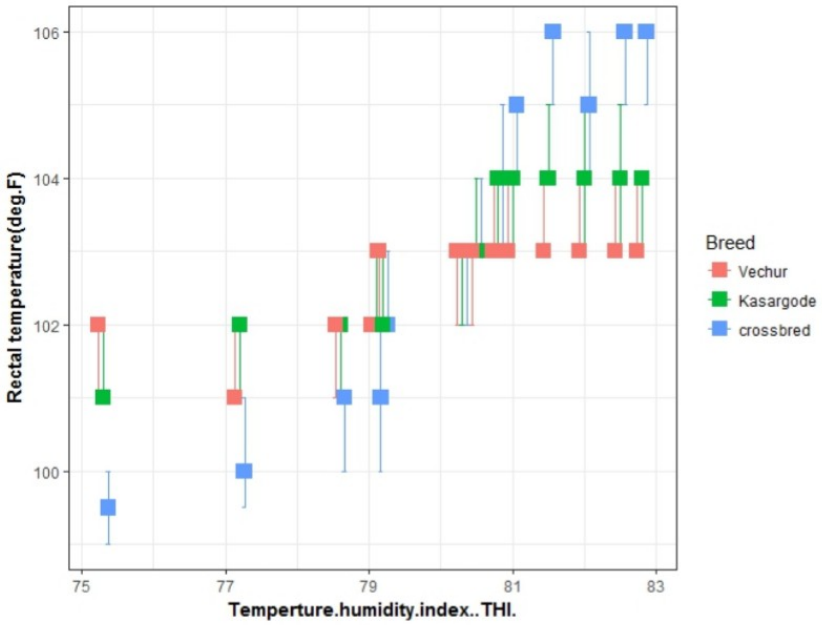
(b)



crossbred
Kasargode
Vechur

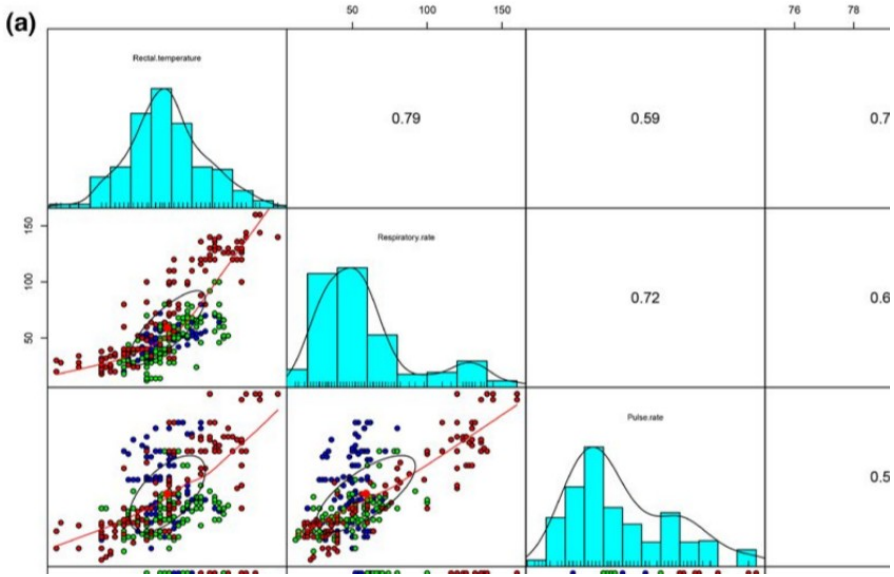
RT

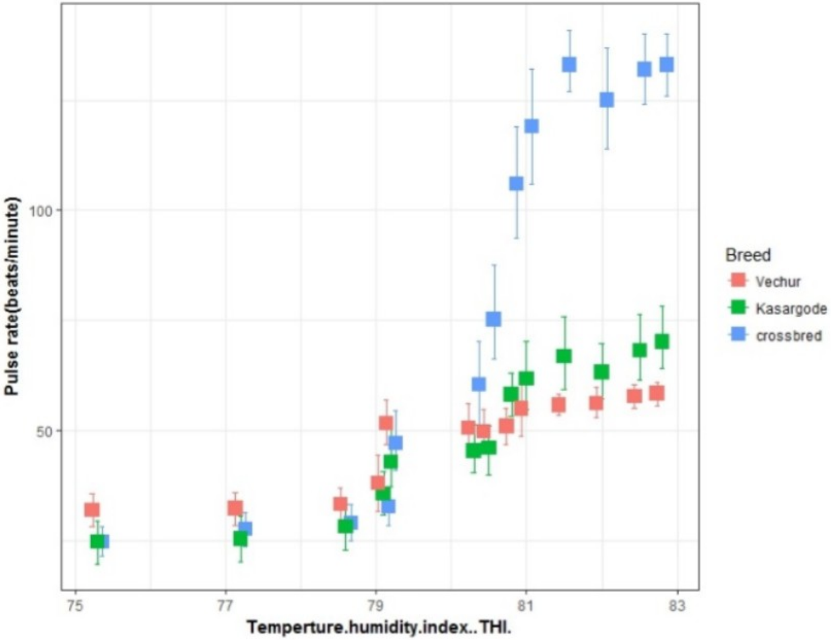




Supplementary figure 3. Mean rectal temperature of Vechur, Kasargode and crossbred cattle plotted as a function of temperature humidity index with successive progression of stresswith residuals plotted.

PR

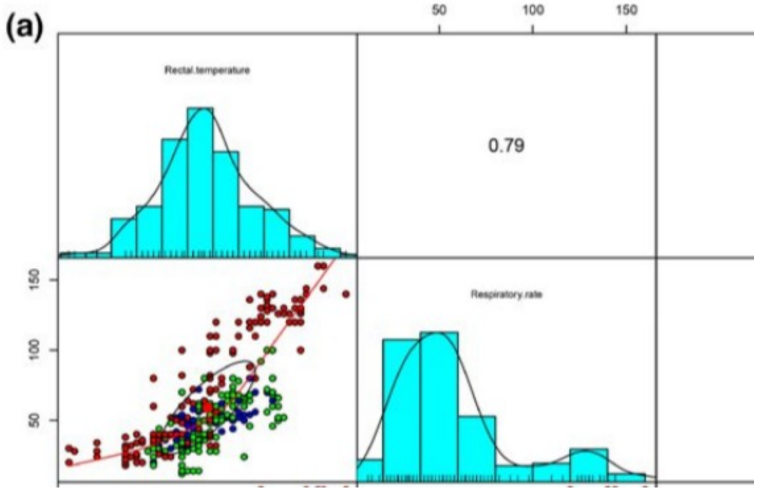


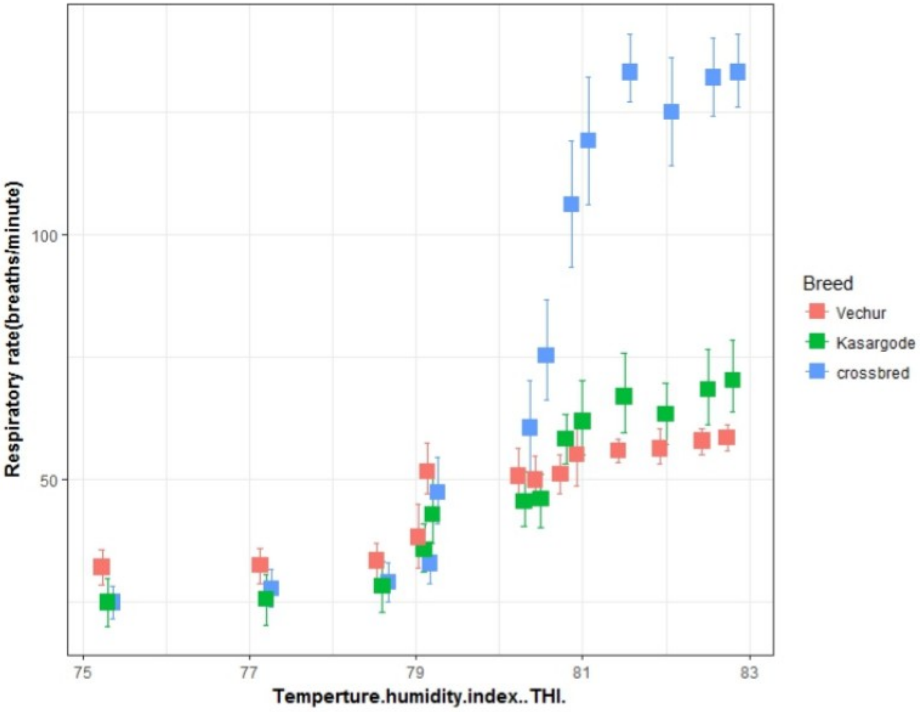


Supplementary figure 4. Mean pulse rate of Vechur, Kasargode and crossbred cattle plotted as a function of temperature humidity index with successive progression of stress

Vechur: relied more in PR to dissipate heat.

RR



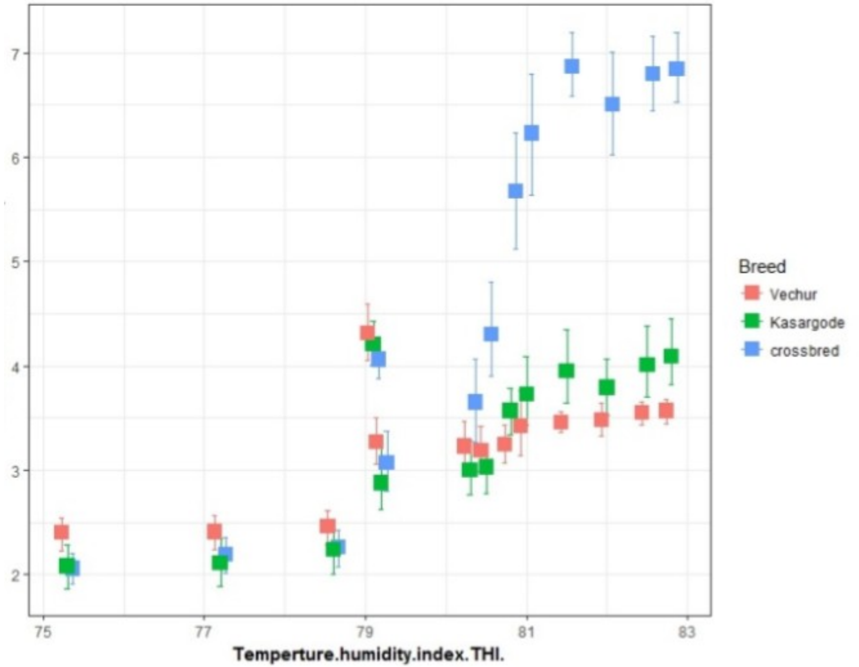


Supplementary figure 5. Mean respiratory rate of Vechur, Kasargode and crossbred cattle plotted as a function of temperature humidity index with successive progression of stress. Note that crossbred animals were unable to return to normalcy while Vechur and Kasargode animals did.

Kasargode: relied on RR to dissipate heat

HTC:

combining RT and RR. Index of tolerance



Supplementary figure 6. Mean heat tolerance coefficient (HTC) of Vechur, Kasargode and crossbred cattle plotted as a function of temperature humidity index with successive progression of stress. Note the significantly low HTC in Vechur, Kasargode and high HTC in crossbred during heat stress.

HLI:

In SCB = 90, in DCB not reach the threshold.

HLI = 8.62 + (0.38 × RH) + (1.55 Ta – 0.5 WS) + [e2.4–WS]

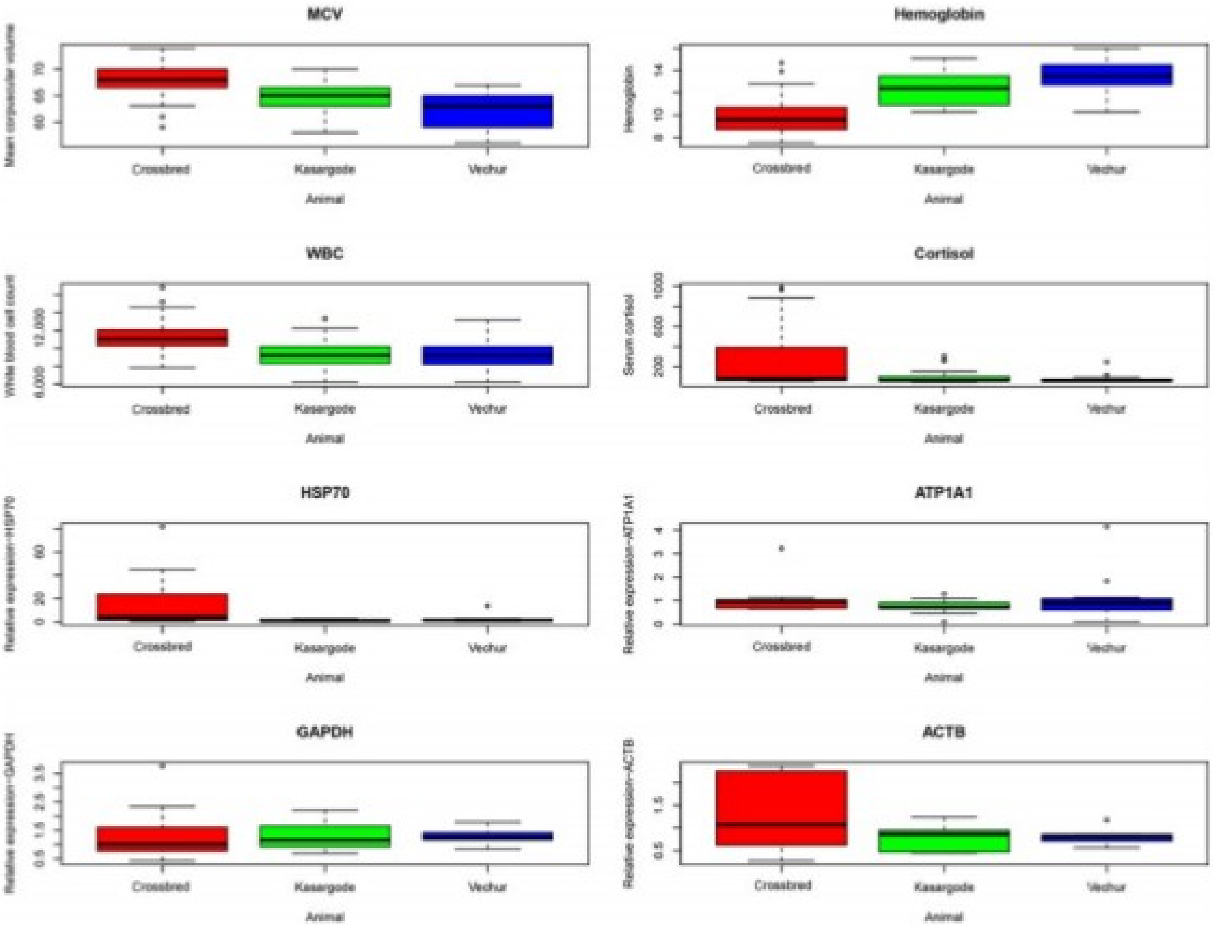
AHL:

In SCB = evident, signal of thermal strain (open mouthed breating,salivating), in DCB= not evident

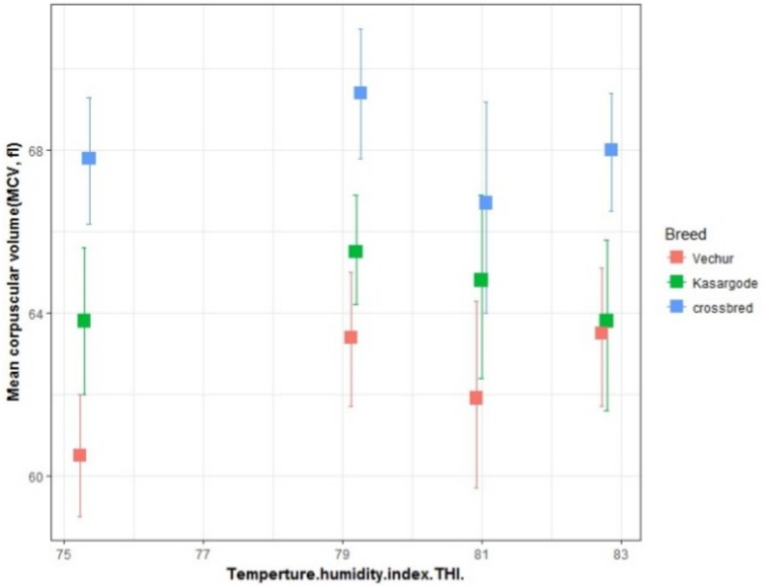
AHL = IF [HLI < HLILT, (HLI–HLILT)/M], IF [HLI > HLIUT, (HLI– HLIUT)/M, 0)]

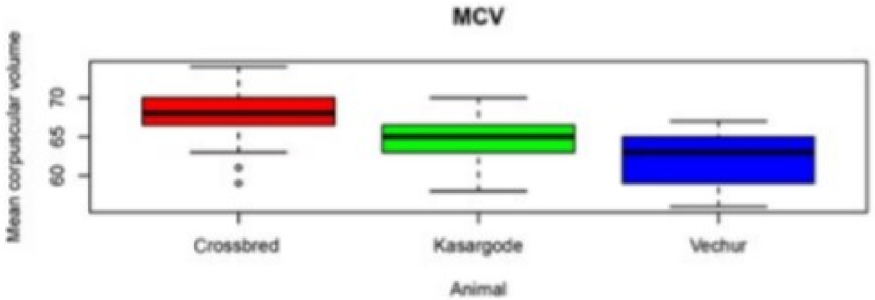
(HLILT is the HLI threshold below cattle will dissipate heat, HLIUT is the HLI threshold above cattle will gain heat and M the number of measurements per hour)

Hematological parameters



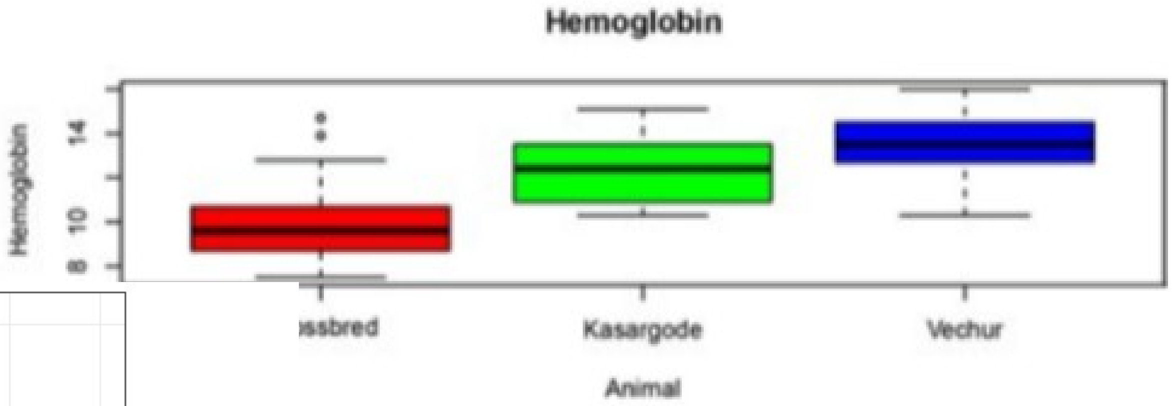
MCV

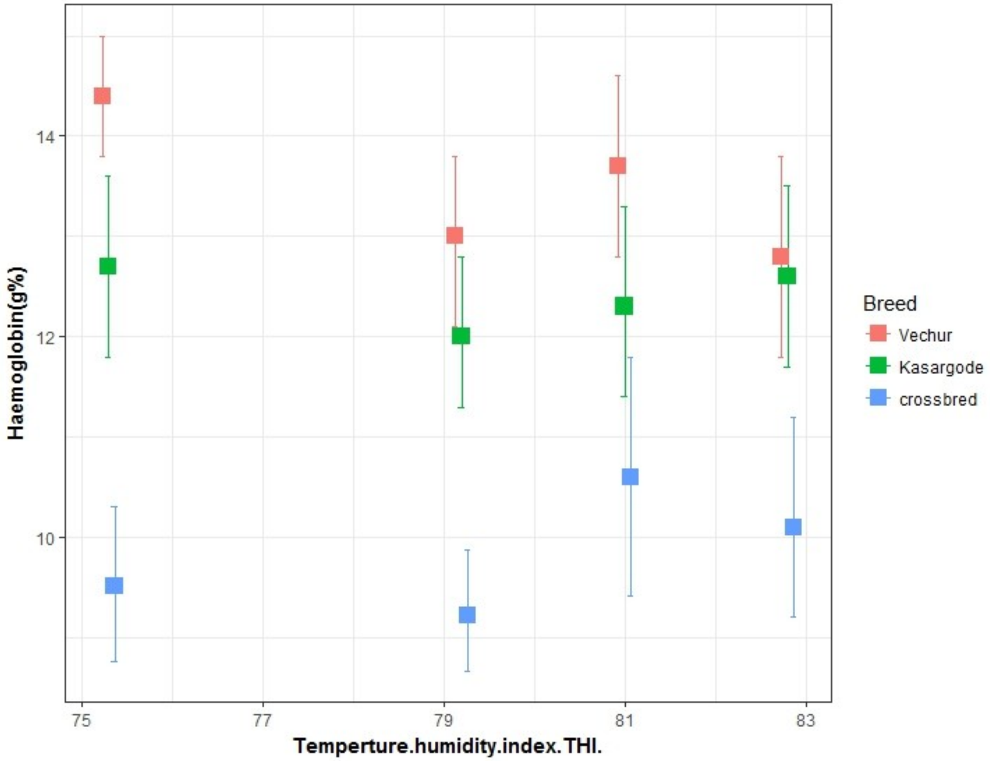




Supplementary figure 7. Mean Corpuscular Volume (MCV) of Vechur, Kasargode and crossbred cattle plotted as a function of temperature humidity index with successive progression of stress. Note low MCV of Vechur and Kasargode and higher MCV in crossbred indicating small red blood cell size in dwarf cattle, which we propose as a mechanism of dwarfing.

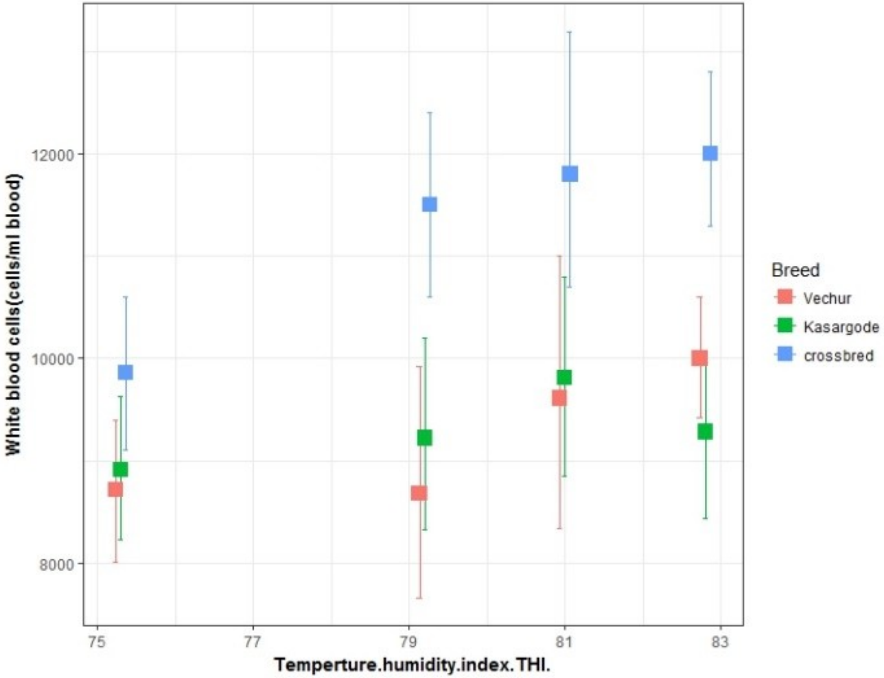
Hemoglobin



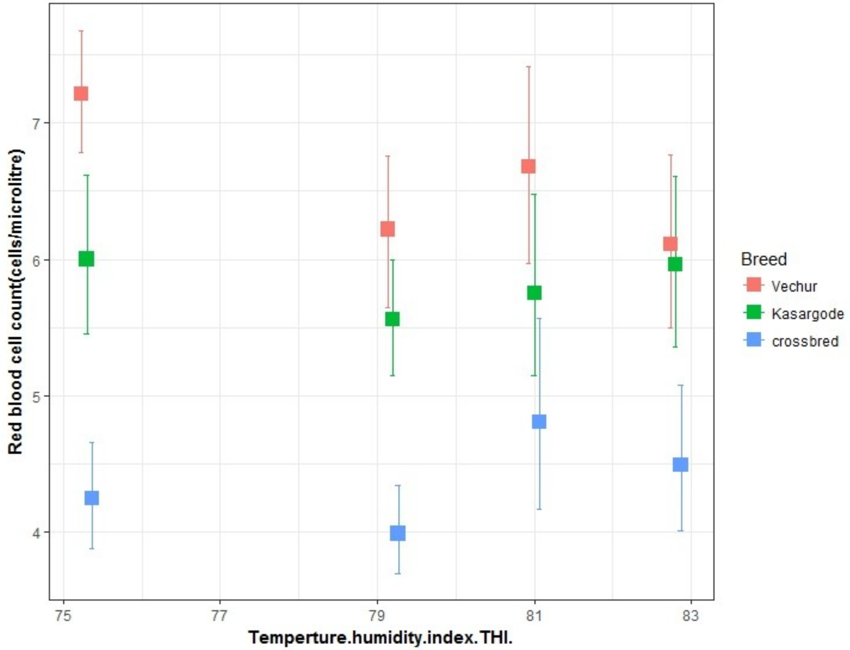


Low hemoglobin level= low protection against oxidative demage, more vulnerable to stress.

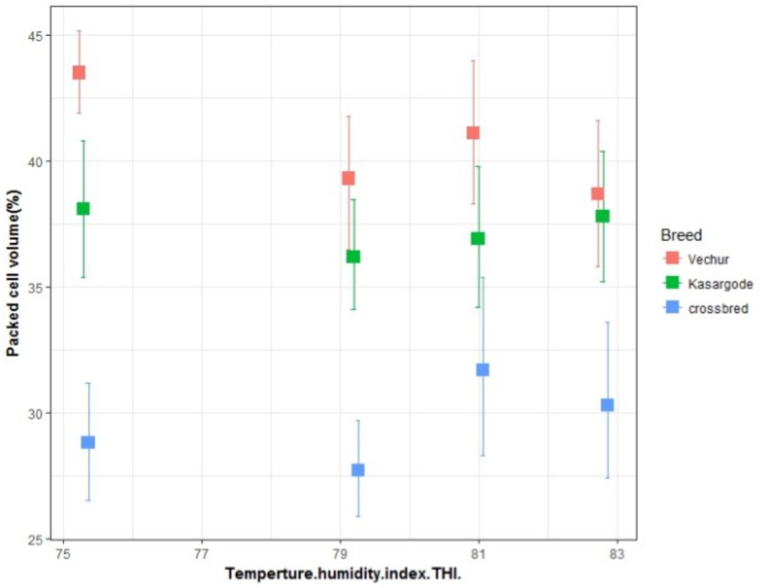
WBC


White blood cells(cells/ml blood)
12000
10000
8000
75
77
79
81
83
Temperture.humidity.index.THI.
Breed
Vechur
Kasargode
crossbred

RBC

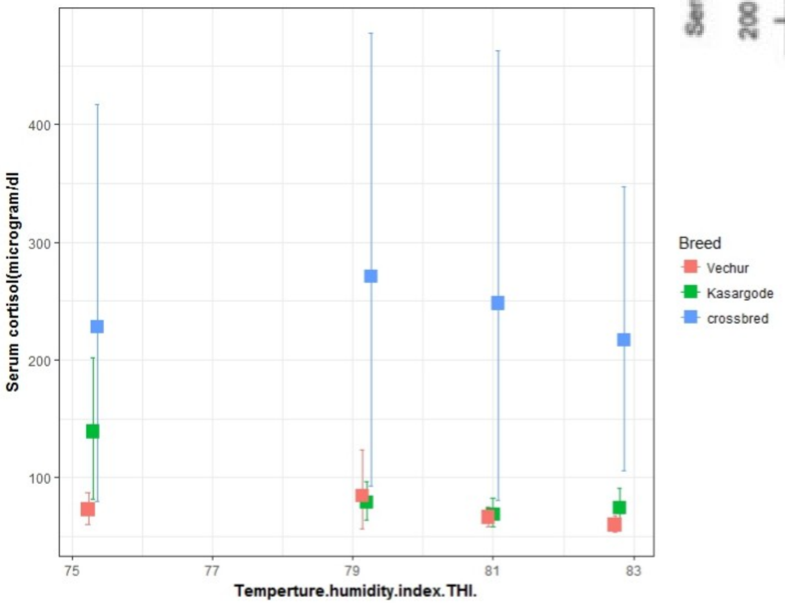

Red blood cell count(cells/microlitre)
7
6
5
4
75
77
79
81
83
Temperture.humidity.index.THI.
Breed
Vechur
Kasargode
crossbred

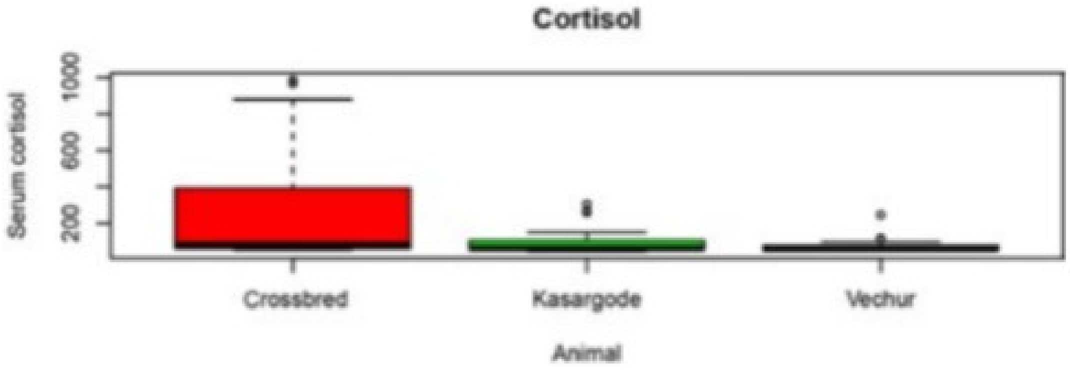
PCV



Supplementary figure 13. Mean packed cell volume (PCV) Vechur, Kasargode and crossbred cattle plotted as a function of temperature humidity index with successive progression of stress.

CORTISOL





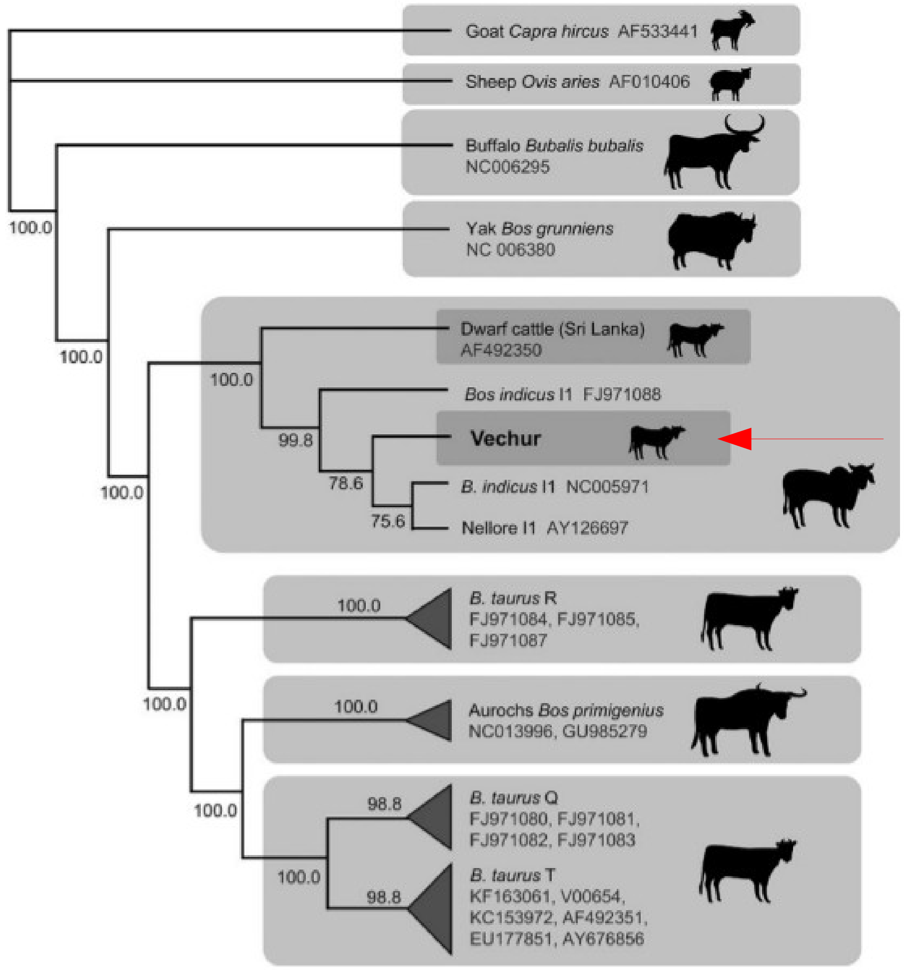
Supplementary figure 14. Mean serum cortisol of Vechur, Kasargode and crossbred cattle plotted as a function of temperature humidity index with successive progression of stress. Note the significantly high serum cortisol in crossbred during heat stress.

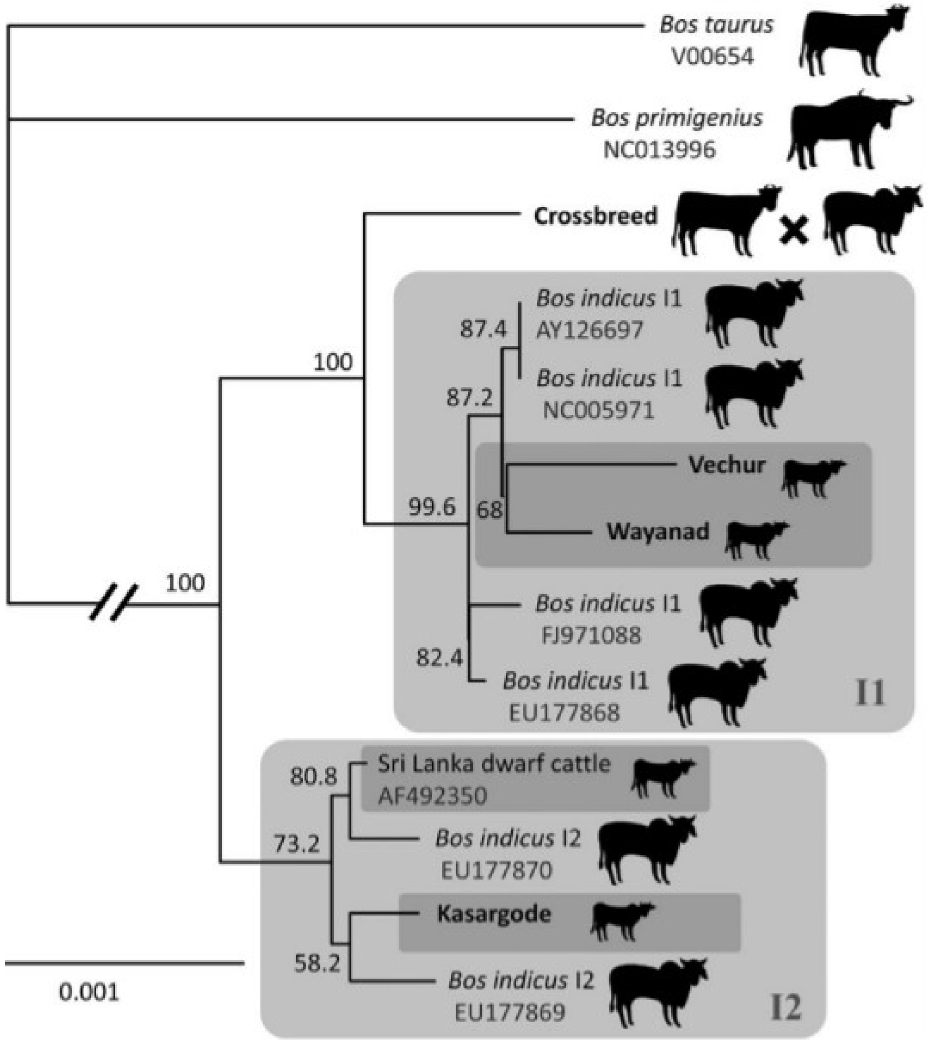
Maximum-likelihood phylogeny used:

- sheep and goat as outgroup,
- mitogenome of Vechur cattle and other <u>B. Indicus and B.Taurus</u> cattles.

Is noted:

Vachur fell into <u>B. Indicus</u> clade





The maximum likelihood phylogram of B.indicus and B. Taurus (outgroup) shows:

- Vechur and Wayanad (from Kerala different area) clustered in Indicus 1 haplotype I1
- Kasargode in indicus 2 I2, closely related to SCB

DISCUSSION

- Warm climate caused decline in body size of domestic cattle, DCB more tolerant and adapted. Smaller individuals are better to dissipate heat.

- SCB acclimatized through alterations in physiological, hormonal and gene expression profiles.

- DCB evolutionary changes evidenced by molecular phylogenetic analysis using mitochondrial genome.

- Genetic changes in mitochondrial genome are associated with cellular and body size and hemoglobin concentration.

- DCB enhanced tolerance increasing hemoglobin concentartion, high hemoglobin concentartion associated with high metabolic rate at high temperatures driven by oxygen demand. Low hemoglobin level= low protection against oxidative demage, more vulnerable to stress.

- Two breed similar environments condition, phenotypic similarity use different physiological end ematologycal startegies.
- Evolution in different niches determined different thermoregulatory patterns.
- Different physiological factors contribuiting to thermal limits in dose-dependent manner and the capacity to cope with varying microclimates.
- Changes in body size have repercussions in thermal biology and energetics, body size affect energy to mantenance, growth and production.


Thanks for your attention