



Body Size Effect in *Eothenomys miletus* of Different Regions from Hengduan Mountain Regions: Roles of Growth Factor Contents

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ABSTRACT

Background: Growth factors are active substances secreted by a variety of cells, acting as messengers to regulate cell migration, proliferation or differentiation, which had important regulatory roles in the growth of individual cells, tissues or organs. Growth factors can regulate the proliferation and differentiation ability of cells, which keep the internal environment stable and affect the body size.

Methods: In order to investigate the influence of the changes of growth factors contents on body size in *Eothenomys miletus* from Hengduan mountain regions, *E. miletus* in Dali (DL), Jianchuan (JC), Lijiang (LJ), Xianggelila (XGLL) and Deqin (DQ) were collected and their 7 morphological indicators and 9 growth factors, including insulin-like growth factor1 (IGF1), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), growth hormone release inhibitory factor (GRIF), platelet-derived growth factor (PDGF), transcription elongation factor1 (TCEA1), a disintegrin and metalloprotease17 (ADAM7), branched-chain amino acid transaminase1 (BCAT1), high mobility group protein1 (HMG1) were measured.

Result: The results showed that body size of *E. miletus* among five regions had significant differentiated, body mass, body length, forelimb length, hind limb length, tail length, IGF, bFGF, EGF, PDGF, TCEA1 contents in DL, JC and LJ were greater than those of in XGLL and DQ and the contents of GRIF, ADAM7, BCAT1 and HMG1 were also different, decreasing with the increasing of regional latitude. Moreover, the increases in the contents of growth factors lead to the increase of body size in *E. miletus*, which does not support Bergman's law. Regional differences in body size of *E. miletus* in different regions may be related to the changes in body mass, morphological indicators and the content of growth factors. Moreover, growth factors may affect the body size of *E. miletus* by affecting the proliferation of bone, epidermis, muscle and differentiation of cells.

Key words: Cell proliferation, Growth factors, Hengduan mountain regions, Morphological differentiation.

INTRODUCTION

Body size of an animal is affected by environmental factors and its own genetic factors, a series of signal pathways and genes in the body regulate the size of animal cells, tissues or organs (Rather *et al.*, 2022). Growth factors are active substances secreted by a variety of cells, acting as messengers to regulate cell migration, proliferation or differentiation, which have an important regulatory role in the growth of individual cells, tissues or organs (Wang *et al.*, 2022). Some studies have shown that insulin-like growth factor1 (IGF-1) is considered to be the key growth factor for the bone and body development of mammals. IGF-1 deficient mice showed growth retardation, mainly manifested in bone structure reduction and it has been proved that IGF-1 has effects from birth to adolescence, resulting in an increase in the size of various tissues (Wang *et al.*, 2021). Basic fibroblast growth factor (bFGF) can activate Wnt signal and promote the proliferation of basic fibroblasts (Wang *et al.*, 2017). bFGF is an important regulator of bone development and bone remodeling, which can regulate appropriate bone development and body segment size (Ito *et al.*, 2022). Epidermal growth factor (EGF) can promote the migration, proliferation and differentiation of various cell types (Alan *et al.*, 2022). High fat diet increased the expression of EGF and its receptor in adipose tissue macrophages, leading to

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insulin resistance and obesity (Cao *et al.*, 2022). Branched-chain amino acid transaminase1 (BCAT1) can promote cell proliferation, the decrease of BCAT1 damage the growth rate of muscle cells (Ouyang *et al.*, 2022). Platelet-derived growth factor (PDGF) is an effective mitogen for fibroblasts and mesenchymal cells, which can stimulate fibroblast proliferation, cytokine production and extracellular matrix

generation, PDGF-BB can activate the proliferation and migration of mouse muscle stem cells, enhance muscle regeneration and delay the differentiation of myotubes, PDGF-BB and PDGF-AB can enhance the migration of myoblasts (Patricia *et al.*, 2017). Transcription elongation factor A1 (TCEA1) can promote the transcription extension of genes and also regulate fatty acid synthesis or β -The ex-pression of oxidation-related genes significantly, promoted the lipid accumulation of AML12 hepatocytes (Shang *et al.*, 2022). Moreover, there are many growth factors, such as disintegrin metalloproteinase 17 (ADAM7), high mobility group protein 1 (HMG1), which act together with other growth factors to promote the proliferation and growth of individual cells (Kaneko *et al.*, 2022). Growth hormone release inhibitory factor (GRIF) is a peptide hormone that regulates the endocrine system (Heo *et al.*, 2022), participates in the regulation of growth hormone and insulin, promotes the growth of animals and participates in the metabolic regulation of the body (Svendsen and Holst, 2021).

Body size of animals plays an extremely important effect in their habitat adaptation (Bai *et al.*, 2019). When the fitness of environment is low, the survival of species may be threatened, the study of body size is helpful to understand the evolution and classification of animals (Shakya *et al.*, 2022). Hengduan Mountain is a typical north-south trend mountain in China, with special geological landforms and rich biodiversity, as the altitude increases, the temperature decreases and the precipitation increases. *E. miletus* is an endemic species in Hengduan Mountain, which lives in shallow tunnels on the surface (Hou *et al.*, 2020). In previous studies, it was found that there were significant differences in body mass among the five regions, which decreased with the increase of latitude and altitude, which did not supporting Bergman's law (Ren *et al.*, 2020). In the present study, we speculated that the changes of growth factors may also affect the body size of *E. miletus* of different regions. Therefore, this study selected 9 growth factors: IGF-1 and bFGF that affect bone development; EGF that affects the growth of epidermal tissue, BCAT1 and PDGF that affect the growth of muscle; TCEA1, ADAM7, HMG1, which can affect cell proliferation and GRIF, which can affect the secretion of other hormones and affect animal body size. Moreover, 7 morphological indicators were measured to explore the effect of growth factors on the body size, further verification of the anti Bergmann's law of the body size of the *E. miletus*.

MATERIALS AND METHODS

Subjects and experimental design

The experimental animals were captured in Dali (DL), Jianchuan (JC), Lijiang (LJ), Xianggelila (XGLL) and Deqin (DQ), in Yunnan Province, China, in the summer of 2022 and a total of 38 adult healthy adult *E. miletus* in non-breeding period. The sample number and climate characteristics of each sampling point see Table 1. All procedures were licensed under and approved by Animal Care and Use Committee of School of Life Science, Yunnan Normal University. This study was approved by the Committee (13-0901-011).

Measurement of morphology

Body mass was measured by an electronic balance (LT502, accurate to 0.01 g); using vernier caliper to measure the body length (BL), tail length (TL), ear length (EL), ear width (EW), forelimb length (FL1) and hind limb length (HL1).

Measurement of growth factor contents

After capturing the animals, they are taken back to the local epidemic prevention station for flea extermination and sterilization and then weighed for the body mass (BM). After the determination of BM, pentobarbital sodium (50 mg/kg) was used for anesthesia and execution and serum was taken, put it in a refrigerator at 4°C for 1 hour, centrifuge it at 4°C for 4 000 r/min for 30 minutes, draw the serum into a 2 ml centrifuge tube and store it in a refrigerator at -80°C. The serum levels of IGF1, bFGF, EGF, GRIF, PDGF, TCEA1, ADAM17, BCAT1 and HMG1 were determined by ELISA kit. IGF1 ELISA kit (kit number: JM-01604R2), bFGF ELISA kit (kit number: JM-01464R2), EGF ELISA kit (kit No.: JM-01457R2), GRIF ELISA kit (kit No.: JM-11013R2), PDGF ELISA kit (kit number: JM-01483R2), TCEA1 ELISA kit (kit number: JM-11014R2), ADAM17 ELISA kit (kit number: JM-10582R2), BCAT1 ELISA kit (kit number: JM-11009R2) and HMG1 ELISA kit (kit number: JM-01893R2), which were all produced by Jiangsu Jingmei Biotechnology Co., Ltd, measure the indicators according to the user manual.

Statistical analysis

SPSS26.0 software was used to test the normality of the data. All the data conformed to the normal distribution. Difference of BM was analyzed by One-way ANOVA, other morphological indicators and growth factor contents were analyzed by one-way ANCOVA with body mass as the covariate. Redundancy analysis (RDA) was used to test the

Table 1: Main conditions for five populations of *Eothenomys miletus*.

Region	Sample number	Longitude and latitude	Altitude/m	Annual average temperature/°C	Precipitation/mm
DL	6	100°42'49" E, 24°90'30" N	2217	19.70	597.00
JC	7	99°75'03" E, 26°43'95" N	2590	13.90	987.30
LJ	8	100°22'90" E, 26°87'53" N	2478	12.60	975.00
XGLL	8	99°83'16" E, 27°90'73" N	3321	5.50	984.20
DQ	9	99°03'75" E, 28°35'14" N	3459	4.70	633.70

correlation between growth factors and morphological indicators. Multiple linear regressions were used to study the main effects of growth factors on various morphological indicators. The results were expressed as mean $\pm$ SE, $P < 0.05$ indicating significant differences.

RESULTS AND DISSICUSION

Morphological indicators

BM, BL, TL, EL, EW, FL1 and HL1 of *E. miletus* in low latitude and high temperature regions were greater than those in high latitude and low temperature regions (Fig 1 and Fig 2).

BM, BL, TL, EL, EW, FL1 and HL1 in *E. miletus* from the five regions were significantly different (BM: $F=11.504$, $P<0.001$; BL: $F=7.458$, $P<0.001$; TL: $F=15.550$, $P<0.001$; EL: $F=43.174$, $P<0.001$; EW: $F=10.112$, $P<0.001$; FL1: $F=10.097$, $P<0.001$; HL1: $F=9.940$, $P<0.001$).

Growth factor contents

The contents of IGF1, bFGF, GRIF, PDGF, TCEA1, ADAM7, BCAT1 and EGF, HMG1 in the five regions were significantly different (IGF: $F=8.237$, $P=0.007$; bFGF: $F=22.034$, $P<0.001$; EGF: $F=6.951$, $P=0.012$; GRIF: $F=10.495$, $P=0.003$; PDGF: $F=9.840$, $P=0.003$; TCEA1: $F=13.495$, $P=0.001$; ADAM7:

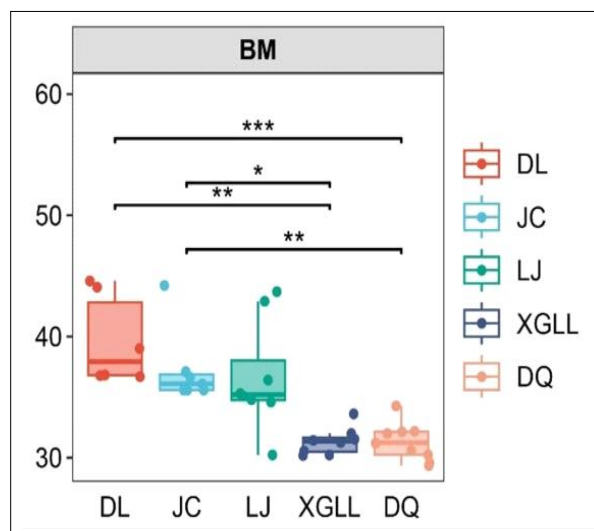


Fig 1: Analysis of the body mass of *E. miletus* in five regions.

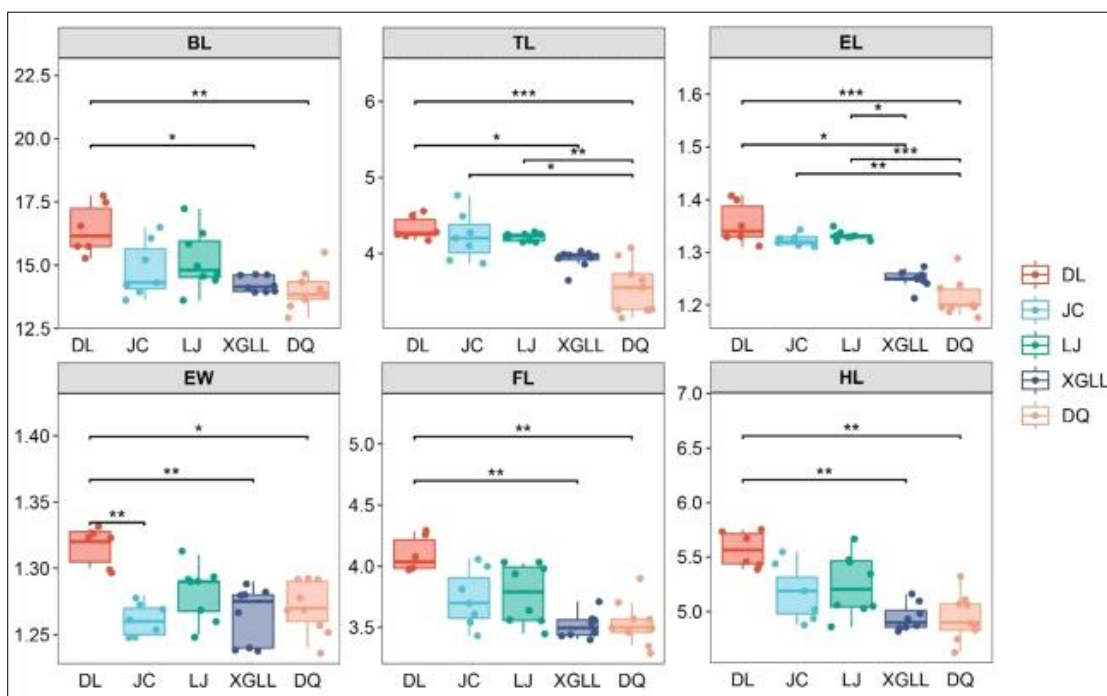


Fig 2: Analysis of morphological indicators of *E. miletus* in five regions.

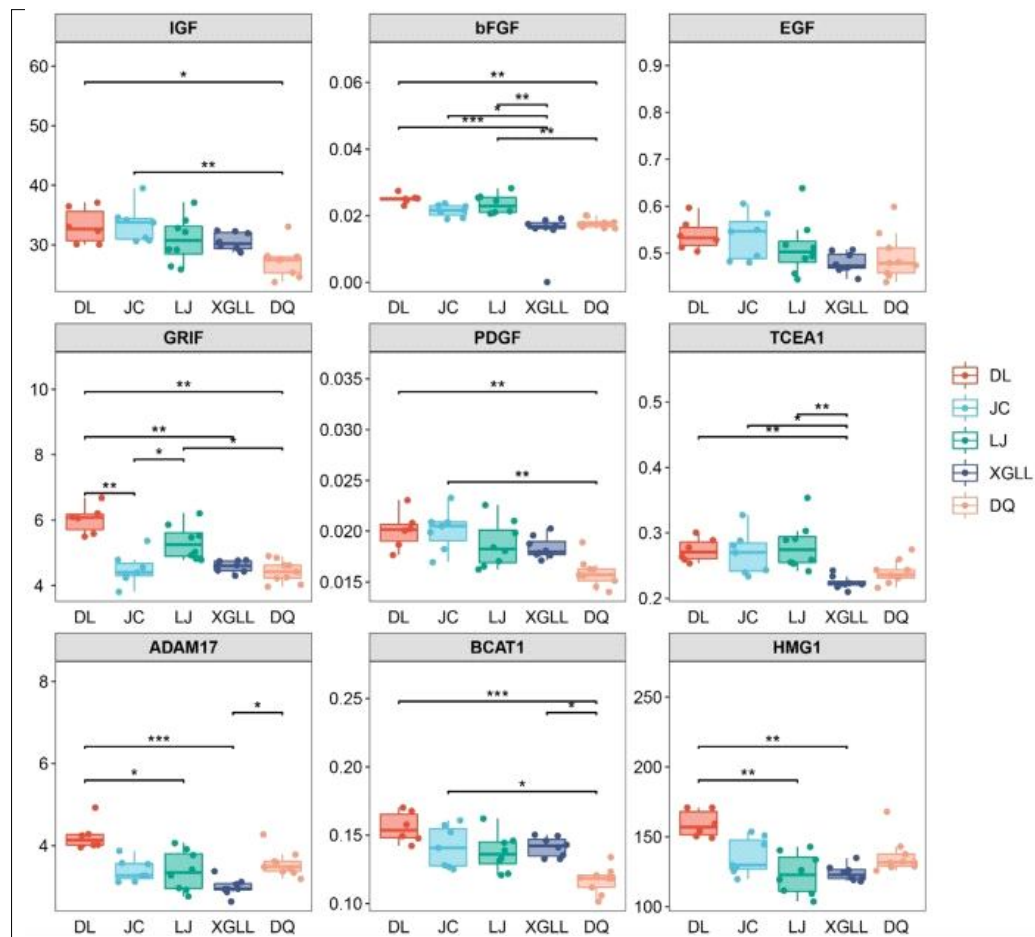


Fig 3: Content analysis of 9 growth factors in *E. miletus* from five regions.

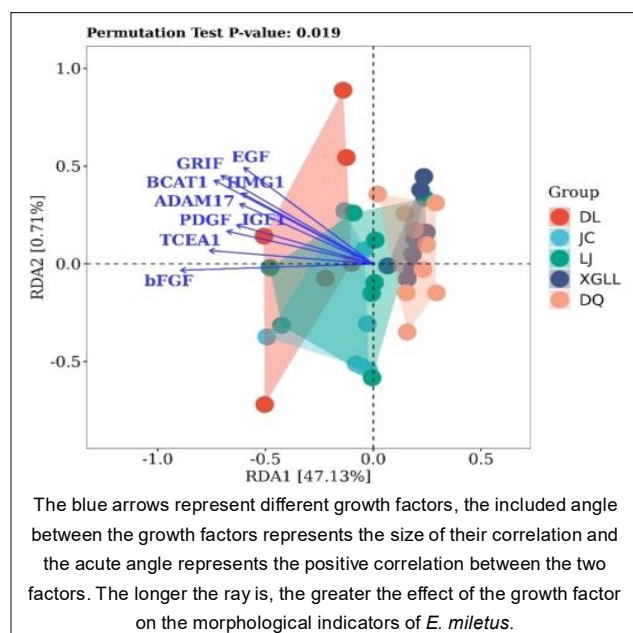


Fig 4: Redundancy analysis of growth factors and morphological indicators.

$F=7.722$, $P=0.009$; $BCAT1$: $F=11.821$, $P=0.001$; $HMG1$: $F=7.372$, $P=0.010$). The contents of IGF , $bFGF$, EGF , $PDGF$ and $TCEA1$ in DL , JC and LJ were higher than those in $XGLL$ and DQ . The contents of $GRIF$, $ADAM7$, $BCAT1$ and $HMG1$ also had differences and decrease with the increase of regional latitude (Fig 3).

Redundancy analysis

The samples of $XGLL$ and DQ were gathered together, which showed that the species in the two regions have high similarity and the samples of DL , JC and LJ also overlap to some extent. The results of the Permutation test showed that $IGF1$, $bFGF$, EGF , $GRIF$, $PDGF$, $TCEA1$, $ADAM7$, $BCAT1$, $HMG1$ were significantly correlated with BM , BL , TL , EL , EW , $FL1$ and $HL1$ of the *E. miletus* ($P=0.019$). It can be seen from the figure that there is a correlation between the growth factors and the morphological indicators of *E. miletus* and there was also a positive correlation between the growth factors and $BCAT1$ and $HMG1$ were very significantly positive correlation (Fig 4).

Multiple linear regression analysis

BM , BL , $FL1$ and $HL1$ of *E. miletus* were positively correlated with $IGF1$, $bFGF$, EGF , $GRIF$, $PDGF$, $TCEA1$, $ADAM7$,

BCAT1, HMG1 and increase with the content of growth factors. Tail length has no significant correlation with EGF, TCEA1, ADAM7 and HMG1, but has positive correlation with IGF1, bFGF, GRIF, PDGF and BCAT1; Ear length was not significantly correlated with ADAM17 and HMG1, but positively correlated with IGF1, bFGF, EGF, GRIF, PDGF, TCEA1 and BCAT1. Ear width was not significantly correlated with IGF1, EGF, PDGF and TCEA1, but positively correlated with bEGF, GRIF, ADAM7, BCAT1 and HMG1 (Fig 5-8).

Body size of animals is affected by many factors, thus affecting the adaptability of animals. In the current study, it was found that the morphological indicators of *E. miletus* in five regions had differentiated, which showed significant differences. BM, BL, TL, EL, EW, FL1 and HL1 of *E. miletus* in DL, JC and LJ were greater than those in XGLL and DQ, which does not conform to Bergmann's law, which is consistent with our previous study (Ren *et al.*, 2020).

Growth factors and their receptors play a role in regulating cell proliferation or differentiation, thus affecting the growth of its tissues and organs (Li *et al.*, 2023). IGF1 can promote the activation, proliferation and differentiation of osteoblasts, enhance the function of osteoblasts (Zhao *et al.*, 2012), promote bone formation, increase bone mass and bone density (Cabrera *et al.*, 2017). Some research results showed that the up-regulation of IGF1 expression can promote bone growth, thereby improving the activity of growth plates and promote longitudinal and radial bone growth (Yan *et al.*, 2016). In our study, significant differences of IGF1 content were found in *E. miletus* from five regions. The content of IGF1 in DL, JC and LJ was higher than that of in XGLL and DQ. Previous study found that BM and BL of *E. miletus* in the five regions of Hengduan Mountain were different and there was differentiation in the morphological indicators of the whole body skeleton (Liao *et al.*, 2023). It showed that IGF1 can promote the growth of the bone and

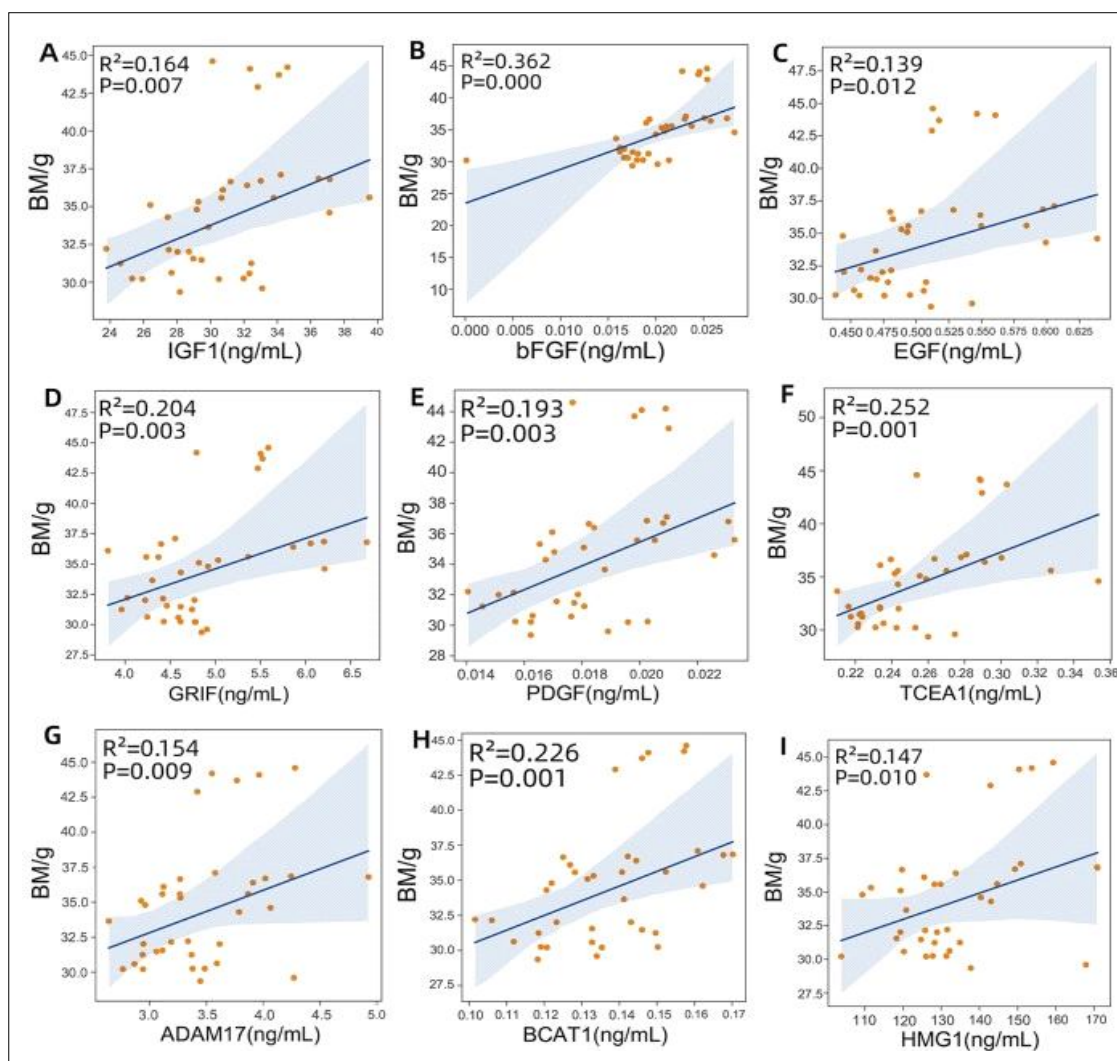


Fig 5: Regression analysis of growth factors and body mass.

cause the difference of body size (Tabnak *et al.*, 2023), which can regulate the growth and differentiation of cells and the formation of tissues and organs (Shah *et al.*, 2022; Ramakrishnan *et al.*, 2023). In addition to participating in the regulation of bone development, bFGF is also a heavy growth factor that activates Wnt signaling pathway. Research has found that bFGF activates Wnt signaling pathway by increasing the expression of CATENIN-BETA, TCF1, TCF3 and LEF, Cell proliferation activity in Wnt/ β -Catenin signal pathway is enhanced after being activated (Fu *et al.*, 2022). In this study, bFGF content in DL, JC and LJ was higher than that in XGLL and DQ and may also be higher than that in XGLL and DQ in terms of the expression of Wnt pathway and cell proliferation activity. EGF can promote cell proliferation and accelerate the growth of epidermal cells by binding with its homologous receptors (Zambarda *et al.*, 2022). In our study, EGF has a positive correlation with the BM, BL, FL1 and HL1 of *E. miletus* from five regions, indicating that EGF

may cause differences in the content of epidermal tissue in *E. miletus*. BCAT1 can catalyze the catabolism of branched chain amino acids (BCAAs) (Francois *et al.*, 2022), which is the key enzyme of amino acid metabolism and can promote the growth of muscle cells. PDGF is essential for the maturation of smooth muscle cells, fibroblasts, glial cells and other cells. It can promote the proliferation of myoblasts, induce angiogenesis and help skeletal muscle regeneration (Liu *et al.*, 2022). There were significant differences in PDGF and BCAT1 in the five regions, BM, BL, FL1 and HL1 increased with the increase of PDGF and BCAT1 content, which may have an impact on the muscle mass in *E. miletus*. Body size is controlled by the number or the size of cells, which together control the size of organs (Ren *et al.*, 2014). TCEA1 can combine with RNA polymerase II to restore the activity of the sites blocked by transcription and continue transcription, improve the efficiency of transcription and promote cell differentiation. ADAM17 is an important member

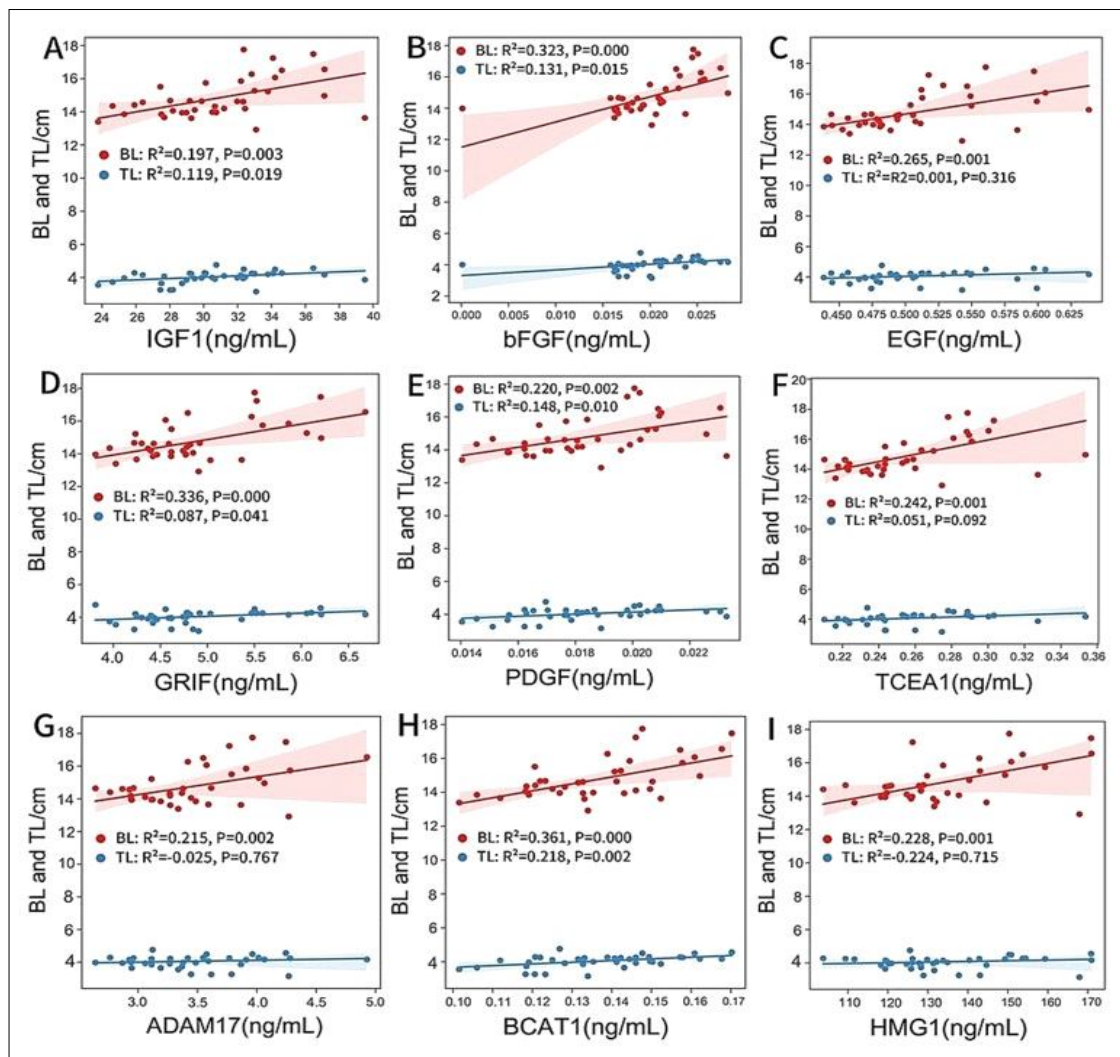


Fig 6: Regression analysis of growth factors and body length and tail length.

of the metalloproteinase family. It can activate or release the growth factors, growth factor receptors or extracellular functional areas of cytokines that bind to the cell membrane by cutting them, thus regulating a variety of biological behaviors of cells, such as proliferation, transfer, etc. (Li and Yang, 2021). HMG1 is a highly conserved progenitor protein, which plays a role in the interaction between protein and DNA (Liu *et al.*, 2009). In the current study, the contents of TCEA1 and ADAM17 in DL, JC and LJ were higher than those in XGLL and DQ. It is possible that the activities of cell differentiation and proliferation in these three regions are stronger than those in the latter two regions, while the contents of HMG in DL, JC and DQ were higher and the contents of LJ and XGLL were relatively small, corresponding to the large body mass of DL and JC and HMG1 promotes cell proliferation and affects body size. Although DQ has more HMG1, it may be caused by individual differences of wild animals. GRIF is an inhibitory hormone,

which can inhibit the secretion of insulin and reduce the response of pancreatin and cholecystokinin (Heo *et al.*, 2022). Cholecystokinin (CCK) acts on the feeding central nerve to inhibit the production of appetite and promote satiety (Pérez-Arana *et al.*, 2023), the GRIF contents in DL, JC and LJ were higher than that in XGLL and DQ. It may be that the feeling of fullness of *E. miletus* in these three regions were weaker than that in the latter two regions, which increases the food intake and affects the body size change.

According to the results of RDA analysis, bFGF had the greatest impact on the morphological indicators of *E. miletus*. bFGF can not only promote the growth and development of bones, but also activate the Wnt signal pathway that regulates the body size of animals, which may be two important aspects of the impact on the size of *E. miletus*. In the current study, 9 growth factors were positively correlated with BM, BL, FL1 and HL1 and there was also a correlation between the 9 growth factors, indicating that

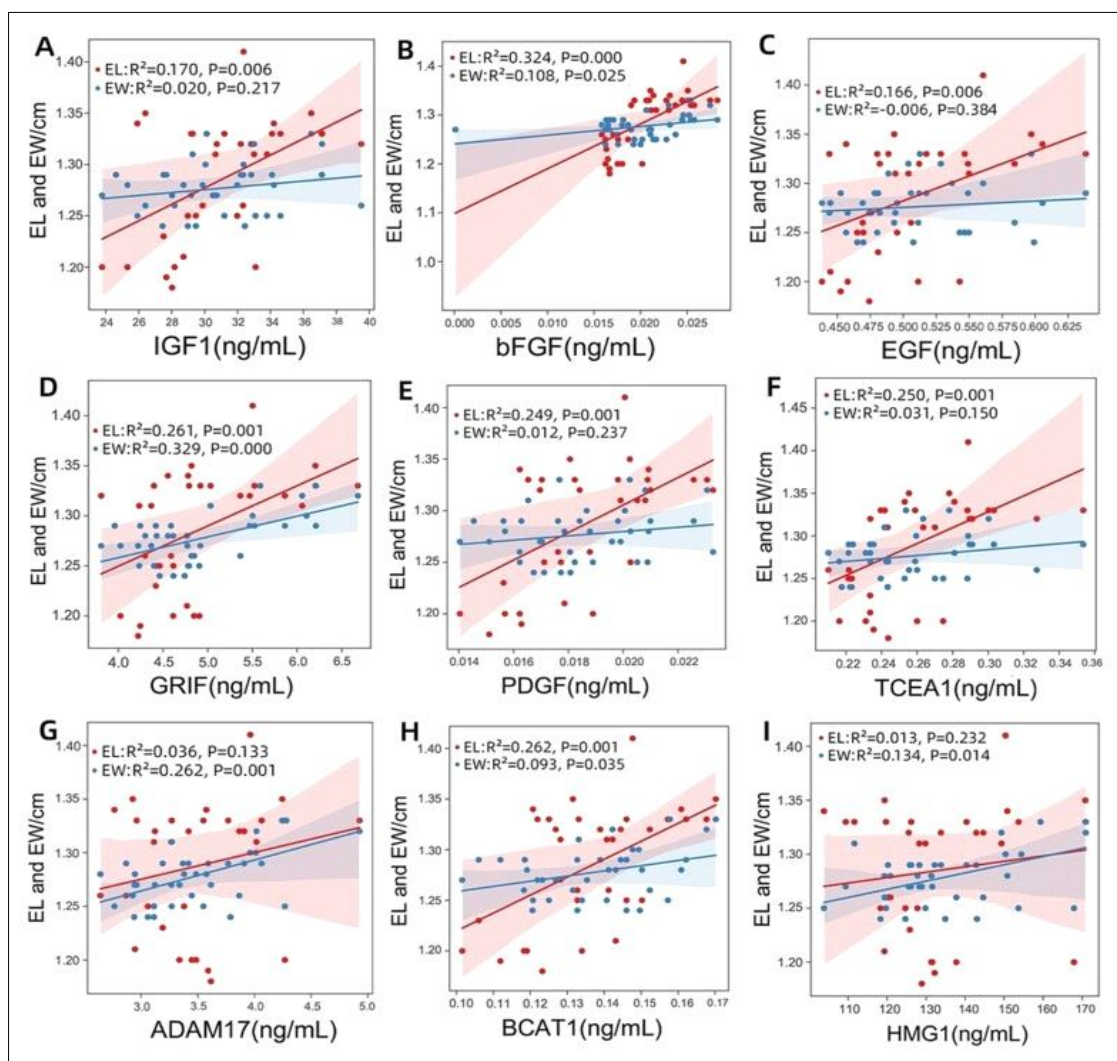


Fig 7: Regression analysis of growth factors and ear length and ear width.

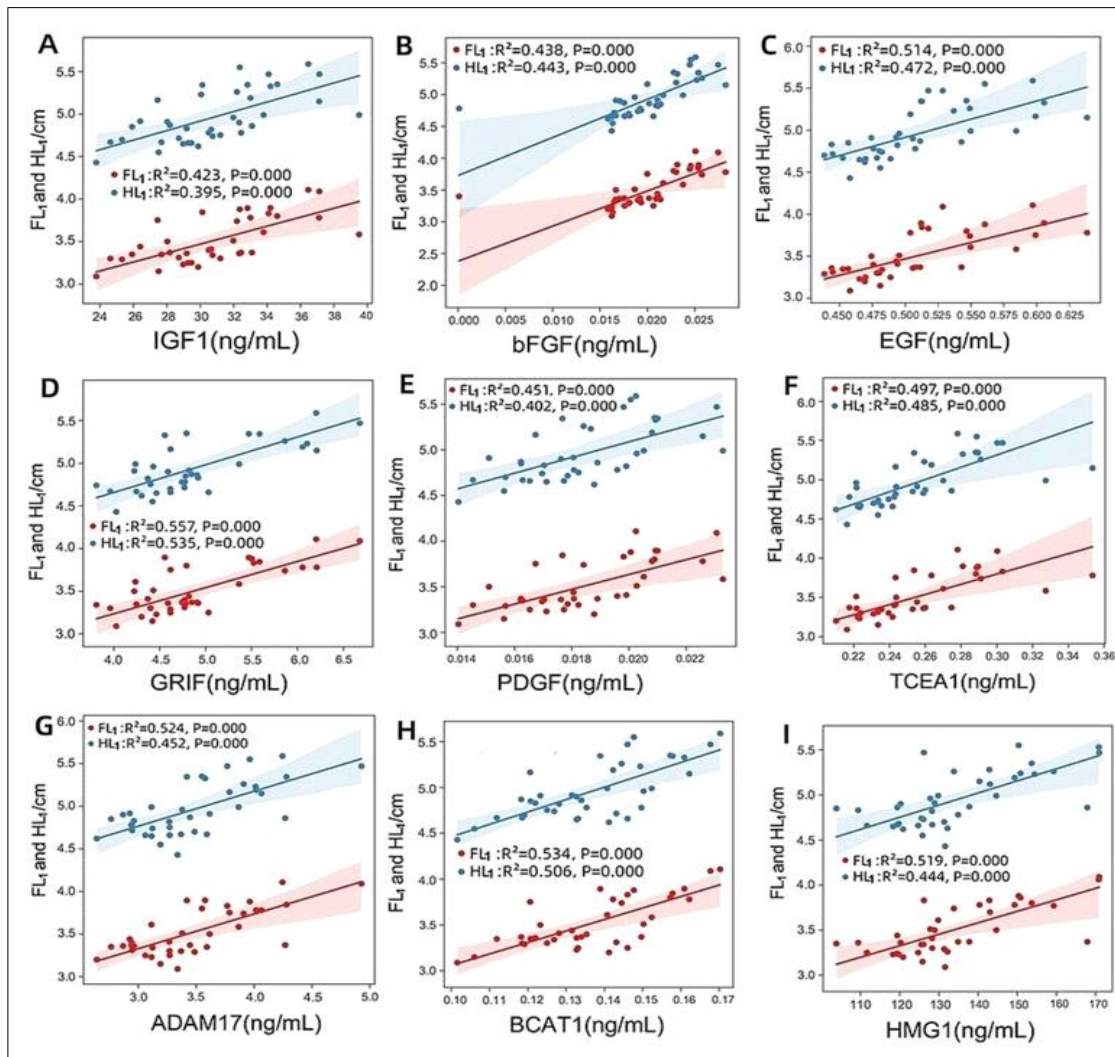


Fig 8: Regression analysis of growth factors and forelimb length and hind limb length.

the 9 growth factors may not act alone, but together to affect the body size of the *E. miletus*, further support for anti Bergman's law.

CONCLUSION

In conclusion, body size of the *E. miletus* in the five regions of Hengduan Mountain had differentiated. BM, morphological indicators, IGF, bFGF, EGF, PDGF, TCEA1 contents of the *E. miletus* in DL, JC and LJ were greater than those in XGLL and DQ. The content of GRIF, ADAM7, BCAT1 and HMG1 also showed regional differences. With the increase of regional latitude, the content of 9 growth factors decreased and the content of 9 growth factors increased, resulting in the increase of body size in *E. miletus*, which does not support Bergman's law. Moreover, these 9 growth factors affect the proliferation of bone, epidermis, muscle and differentiation ability cells through interaction and ultimately affect the body size of the *E. miletus*.

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Conflict of interest

The authors declare no conflict of interest.

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