นิพนธ์ต้นฉบับ

ปริมาณและองค์ประกอบของโปรตีนในแย้อีสาน *Leiolepis rubritaeniata* จากภาคตะวันออกเฉียงเหนือของประเทศไทย

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บทคัดย่อ

ความเป็นมาและวัตถุประสงค์: การศึกษานี้มุ่งเน้นเพื่อเติมเต็มช่องว่างขององค์ความรู้เกี่ยวกับคุณค่าทางโภชนาการของแย้อีสาน (*Leiolepis rubritaeniata*) และศักยภาพที่อาจเป็นแหล่งโปรตีนท้องถิ่น โดยมีวัตถุประสงค์เพื่อวิเคราะห์ปริมาณโปรตีนรวมและองค์ประกอบของกรดอะมิโนในตัวอย่างแย้อีสานจากภาคตะวันออกเฉียงเหนือของประเทศไทย

วิธีการ: เก็บตัวอย่างแย้ จากพื้นที่ตำบลคำโคกสูงและตำบลบะยาว อำเภอวังสามหมอ จังหวัดอุดรธานี โดยใช้วิธีดักด้วยบ่วงและขุดรูเพื่อจับ จากนั้นทำการุณยฆาต และเก็บเนื้อตับในเอทานอลเข้มข้น 95% แล้วเก็บรักษาที่อุณหภูมิ –20 °C เพื่อใช้ในการวิเคราะห์ดีเอ็นเอ สำหรับการวิเคราะห์องค์ประกอบโปรตีนและกรดอะมิโน ได้แยกเก็บตัวอย่างกล้ามเนื้อออกเป็นสองส่วน ได้แก่ (1) ส่วนลำตัว ซึ่งเก็บจากกล้ามเนื้อหัว คอ ลำตัว ขาหน้า และขาหลัง และ (2) ส่วนหาง ซึ่งเก็บเฉพาะกล้ามเนื้อหาง นำตัวอย่างทั้งหมดเข้าสู่กระบวนการวิเคราะห์ทันทีหลังการเก็บ ตัวอย่างอ้างอิงทั้งหมดถูกเก็บรักษาไว้ในคลังสัตว์มีกระดูกสันหลัง มหาวิทยาลัยขอนแก่น การศึกษานี้ได้รับการอนุมัติด้านจริยธรรมจากคณะกรรมการกำกับดูแลการใช้สัตว์เพื่อการทดลองทางวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น (เลขที่อ้างอิง 660201.2.11/318 [50]) การจำแนกชนิดของตัวอย่างแย้ใช้วิธีการทางสัณฐานวิทยาและการวิเคราะห์ยีนไมโตคอนเดรียล ND2 โดยใช้ไพรเมอร์ METF6 และ CO1R1 ทำการขยายและจัดลำดับดีเอ็นเอโดยบริษัท Pacific Science การจัดเรียงลำดับนิวคลีโอไทด์ทำในโปรแกรม SeaView และสร้างแผนภูมิญาติเชิงวิวัฒนาการด้วยวิธี Maximum Parsimony (MP) ในโปรแกรม PAUP* และ Bayesian Inference (BI) ในโปรแกรม MrBayes โดยเลือกแบบจำลองการแทนที่นิวคลีโอไทด์ที่เหมาะสมที่สุดด้วยโปรแกรม MrModelTest และคำนวณค่าความแตกต่างระหว่างคู่ลำดับ (Pairwise sequence divergence) ด้วยโปรแกรม MEGA X การจำแนกชนิดโดยใช้สัณฐานวิทยาใช้ความยาวจากปลายจมูกถึงรูเปิดทวาร (Snout–vent length: SVL) ความยาวหาง จำนวนเกล็ด จำนวน Femoral และ Precloacal pores และแบบแผนของลวดลาย การวิเคราะห์โปรตีนรวมดำเนินการสามซ้ำ ด้วยวิธีของ Kjeldahl โดยใช้เครื่อง Gerhardt KT 8s และคำนวณปริมาณโปรตีนจากเปอร์เซ็นต์ไนโตรเจนตามสูตรของ Horwitz และ Latimer สำหรับการวิเคราะห์องค์ประกอบกรดอะมิโน ใช้เทคนิคโครมาโทกราฟีแบบแลกเปลี่ยนไอออน โดยใช้เครื่องวิเคราะห์กรดอะมิโนอัตโนมัติ SCION Artemis 6000 ซึ่งตรวจวัดด้วยปฏิกิริยา

ninhydrin หลังการอนุพันธ์แบบ post-column

ผลการศึกษา: การวิเคราะห์ระดับโมเลกุลโดยใช้ยีนไมโตคอนเดรียล ND2 ยืนยันว่าตัวอย่าง *Leiolepis* จากจังหวัดอุดรธานีเป็น *L. rubritaeniata* ผลการวิเคราะห์ทางสายวิวัฒนาการ พบว่าลำดับดีเอ็นเอตัวอย่าง (รหัส PV746783 และ PV746784) จัดอยู่ในกลุ่มเดียวกับลำดับของ *L. rubritaeniata* จาก GenBank โดยมีความแตกต่างทางพันธุกรรมต่ำและแยกออกอย่างชัดเจนจาก *L. reevesii* และ *L. glaurung* ตัวอย่างจากจังหวัดอุดรธานีมีลักษณะสัณฐานวิททยาคล้ายคลึงกับ *L. rubritaeniata* ในลักษณะสำคัญ เช่น ขนาด ลักษณะเกล็ดและรูปแบบสี รวมถึงแถบสีด้านข้างลำตัวซึ่งเป็นลักษณะจำแนกเฉพาะ แม้ว่าจะมีขนาดลำตัวใหญ่กว่าเล็กน้อย แต่ขนาดลำตัว จำนวนเกล็ดบริเวณริมปาก และจำนวน Femoral pores อยู่ในช่วงจำนวนที่พบภายในชนิด ผลการวิเคราะห์โปรตีนรวมพบว่าปริมาณโปรตีนในกล้ามเนื้อหางสูงกว่ากล้ามเนื้อส่วนลำตัวอย่างมีนัยสำคัญในทั้งสองเพศ ซึ่งยืนยันโดยการทดสอบ *t*-test แบบจับคู่ ($p < 0.05$) อย่างไรก็ตาม ไม่พบความแตกต่างของปริมาณโปรตีนระหว่างเพศ ผลการวิเคราะห์องค์ประกอบกรดอะมิโนพบว่ากล้ามเนื้อหางของเพศเมียมีปริมาณกรดอะมิโนรวมสูงที่สุด โดยกรดกลูตามิก กรดแอสปาร์ติก และไลซีนเป็นกรดอะมิโนที่พบในปริมาณสูงสุด ขณะที่ซีสเทอีนเป็นกรดอะมิโนที่มีปริมาณต่ำสุดในทุกตัวอย่าง ผลการศึกษานี้ยืนยันสถานะทางอนุกรมวิธานของ *L. rubritaeniata* ในจังหวัดอุดรธานี และยังแสดงให้เห็นถึงความแตกต่างระหว่างส่วนของร่างกายในด้านปริมาณโปรตีนและองค์ประกอบกรดอะมิโน ซึ่งอาจมีความสำคัญทั้งในเชิงชีววิทยาและนิเวศวิทยา

สรุป: การศึกษานี้เป็นครั้งแรกที่รายงานข้อมูลทางโภชนาการอย่างครบถ้วนของแย้ *L. rubritaeniata* โดยพบว่ามีปริมาณโปรตีนและกรดอะมิโนจำเป็นในระดับสูง โดยเฉพาะในเนื้อเยื่อหาง ซึ่งแสดงให้เห็นถึงศักยภาพทางโภชนาการของแย้อีสานชนิดนี้ และอาจสนับสนุนการนำมาใช้เป็นแหล่งโปรตีนท้องถิ่น รวมถึงเป็นข้อมูลพื้นฐานสำคัญเพื่อสร้างความเข้าใจด้านชีววิทยาและคุณค่าทางโภชนาการในสัตว์เลื้อยคลานและสัตว์สะเทินน้ำสะเทินบกในอนาคต

คำสำคัญ: กรดอะมิโน; แย้; โปรตีนรวม; *Leiolepis rubritaeniata*; ยีน ND2

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ORIGINAL ARTICLE

Protein Content and Composition in *Leiolepis rubritaeniata* from Northeastern Thailand

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ABSTRACT

Background and Objectives: The genus *Leiolepis* (family Agamidae), commonly known as butterfly lizards, is widely distributed across Southeast Asia and typically inhabits dry, open areas with sandy soils and sparse vegetation. Among the ten recognized species, *L. rubritaeniata* is notable for its distinctive red-striped flanks and occurrence in northeastern Thailand. Although previous studies have explored its taxonomy, distribution, and behavior, limited information is available on its physiological and nutritional biology, particularly regarding protein content and amino acid composition. Proteins are essential macromolecules involved in tissue development, immune response, metabolism, and energy balance, and reptiles living in seasonal environments may develop adaptations to cope with fluctuating food availability. In some rural communities, agamid lizards are also consumed as supplementary sources of protein, making their nutritional value relevant from both ecological and ethnobiological perspectives. Despite this importance, most reptilian nutrition studies have focused on snakes and turtles, leaving small lizard species such as *L. rubritaeniata* understudied. This research aims to confirm the species identity of *L. rubritaeniata* specimens collected from Udon Thani Province using morphological and ND2 gene sequence analyses and to determine and compare the total protein content and amino acid composition in the body and tail tissues of male and female individuals. The findings will provide baseline data that support further studies in reptilian nutritional ecology, physiological adaptation, and conservation planning.

Methodology: Specimens of *L. rubritaeniata* were collected from Kham Khok Sung and Bayao Subdistricts in Wang Sam Mo District, Udon Thani Province, using snares and excavation methods. Following euthanasia, liver tissues were preserved in 95% ethanol at –20 °C for DNA analysis. For protein and amino acid composition analysis, muscle samples were collected separately from two regions: (1) the body, comprising the head, neck, trunk and limbs, and (2) the tail, consisting exclusively of tail muscle. All samples were promptly processed for analysis. All specimens were deposited in the Khon Kaen Vertebrate Collection (KKUC), and the study received ethical approval from the Institutional Animal Care and Use Committee of Khon Kaen University (Ref. no. 660201.2.11/318 [50]). Species identification was based on morphology and mitochondrial ND2 gene sequences, amplified using METF6 and CO1R1 primers and sequenced by Pacific Science. Sequences were aligned in SeaView and phylogenetic trees constructed using Maximum Parsimony in PAUP* and Bayesian Inference in MrBayes, with model selection via MrModelTest. Pairwise sequence divergences were calculated in MEGA X. Morphological identification included snout-vent length (SVL), tail length, scale and pore counts, and coloration

traits. Crude protein was analyzed in triplicate using the Kjeldahl method with a Gerhardt KT 8s system, and protein content was calculated from nitrogen percentage following Horwitz and Latimer's formula. For amino acid composition, hydrolyzed samples were analyzed using ion-exchange chromatography and an automatic amino acid analyzer (SCION Artemis 6000), with detection via post-column derivatization and ninhydrin reaction.

Main Results: Molecular analyses based on the mitochondrial ND2 gene confirmed that the *Leiolepis* specimens from Udon Thani Province belong to *L. rubritaeniata*. Phylogenetic analyses using Maximum Parsimony and Bayesian Inference revealed that the new sequences (PV746783 and PV746784) clustered strongly with published *L. rubritaeniata* sequences in GenBank, supported by minimal genetic distances and clear separation from *L. reevesii* and *L. glaurung*. Morphologically, the Udon Thani Province specimens closely resembled *L. rubritaeniata* in key traits such as size, scalation, and coloration patterns, including diagnostic flank markings. Although slightly larger, their body measurements, labial counts, and femoral pore numbers fell within the species' intraspecific variation. Crude protein analysis showed significantly higher protein content in the tail than in the body in both sexes, confirmed by paired *t*-tests ($p < 0.05$), while no significant sex-based differences were observed. Amino acid profiles showed that female tail tissue contained the highest total amino acid content, with glutamic acid, aspartic acid, and lysine being the most abundant. Cysteine was consistently the least abundant across all samples. These molecular, morphological, and nutritional findings provide robust evidence for the identification of the specimens as *L. rubritaeniata* and highlight notable tissue-specific differences in protein quantity and amino acid composition, with possible biological and ecological significance.

Conclusion: This study confirms the identification of *L. rubritaeniata* specimens from Udon Thani Province, northeastern Thailand, through both molecular and morphological analyses. Mitochondrial ND2 gene sequencing placed the specimens firmly within the *L. rubritaeniata* clade with strong nodal support, and their external morphological traits were consistent with those of known *L. rubritaeniata* populations. Crude protein analysis revealed that tail tissues contained significantly higher protein levels than body tissues in both sexes, while no significant differences were found between males and females for either body or tail protein content. Amino acid profiling showed that glutamic acid, aspartic acid, and lysine were the most abundant amino acids across all samples, with the highest total amino acid content observed in female tail tissues. These findings provide evidence for the taxonomic status of *L. rubritaeniata* in Udon Thani and also offer new insights into its nutritional composition, particularly the higher protein and amino acid content in tail tissues, which may have implications for both biological understanding and potential utilization in reptiles and amphibians.

Keywords: Amino acids; Butterfly lizard; Crude protein; *Leiolepis rubritaeniata*; ND2 gene

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Introduction

The genus *Leiolepis* (family Agamidae), commonly known as butterfly lizards, comprises a group of agamid lizards widely distributed across Southeast Asia. These lizards inhabit a range of open and semi-arid environments, often characterized by sandy soils and sparse vegetation. Currently, the genus *Leiolepis* comprises ten recognized species (Uetz *et al*., 2025): *L. belliana* (Hardwicke and Gray, 1827), *L. boehmei* Darevsky and Kupriyanova, 1993, *L. guentherpetersi* Darevsky and Kupriyanova, 1993, *L. guttata* Cuvier, 1829, *L. ngovantrii* Grismer and Grismer, 2010, *L. ocellata* Peters, 1971, *L. peguensis* Peters, 1971, *L. reevesii* (Gray, 1831), *L. rubritaeniata* Mertens, 1961, and *L. triploida* Peters, 1971. Among the ten recognized species, *L. rubritaeniata* is particularly notable for its striking red-striped lateral markings and its preference for dry, open habitats in northeastern Thailand and neighbouring regions (Mertens, 1961; Köhler & Thammachoti, 2023). Over recent decades, this species has been the focus of several ecological and taxonomic studies (Wanchai *et al*., 2024), with research addressing its geographic distribution (Nabhitabhata *et al*., 2004; Chuaynkern & Chuaynkern, 2012; Poyarkov *et al*., 2023), and behavioral ecology (Köhler & Thammachoti, 2023). Despite these efforts, relatively little is known about the physiological and nutritional biology of this species, particularly regarding its protein content and composition. This knowledge gap is significant, as nutritional attributes, especially protein profiles, are vital to understanding reptilian ecology, health, and adaptability to changing environments (Dolly & Tardieu, 2023; John & Jones, 2024).

Proteins are vital macromolecules that underpin key biological functions such as tissue growth, immune competence, enzymatic reactions, and energy metabolism (Hoffman & Cawthorn, 2012). In ectothermic vertebrates like reptiles, protein requirements vary depending on life stage, habitat conditions, diet composition, and seasonal activity cycles. Species such as *L. rubritaeniata*, which inhabit environments with pronounced seasonal fluctuations, may experience nutritional stress when prey availability decreases during dry periods. These conditions can lead to physiological adaptations related to nutrient storage and metabolic regulation (Santos *et al*., 2018; van Wijngaarden *et al*., 2020). Therefore, assessing protein content and composition in this species provides valuable insight into its nutritional ecology and functional biology. From an ethnobiological perspective, understanding the protein profile of *L. rubritaeniata* is also important, as agamid lizards in Southeast Asia are occasionally consumed for subsistence or traditional purposes. In some rural communities, wild monitor lizards and *Agama* species are regarded as supplementary sources of

protein (Hartman *et al*., 2012). Reliable data on their nutritional value, particularly protein content, can help inform sustainable wildlife use and support food security initiatives. However, to ensure that such usage does not negatively impact wild populations, it is important to consider the conservation status of the species. *L. rubritaeniata* is currently listed as Least Concern (LC) on the IUCN Red List due to its wide distribution, adaptability to disturbed habitats, and continued presence in many protected areas (Cota *et al*., 2018). Although the species faces significant exploitation across much of its range and has experienced population declines, it still persists at low densities even in areas with ongoing harvesting and is therefore considered to face no immediate risk of extinction. In Thailand, the species has been evaluated under the name *L. reevesii rubritaeniata* and classified as Near Threatened (NT) by the Office of Natural Resources and Environmental Policy and Planning (2017). Despite this status, it is not currently included in the list of protected animals under the Wild Animal Conservation and Protection Act (Wild Animal Conservation and Protection Act, 2019). On a broader scale, comparative studies of protein composition among reptile species contribute to identifying species-specific adaptations and physiological markers relevant to environmental stress or overall health (Santos *et al*., 2018; van Wijngaarden *et al*., 2020). However, most research on reptilian nutrition has focused on captive diets or commercially significant taxa such as snakes and turtles (Dolly & Tardieu, 2023; John & Jones, 2024), leaving smaller agamids like *L. rubritaeniata* underrepresented in the scientific literature. Generating baseline data on the protein composition of *L. rubritaeniata* will not only enhance understanding of its physiology but also strengthen the comparative framework for reptilian nutritional science. Such information can support future research in ecological physiology, conservation biology, and sustainable resource management.

This study aims to quantify the total protein content and analyse the protein composition of *L. rubritaeniata* collected from Udon Thani Province, northeastern Thailand. Species identification was confirmed through morphological assessment and genetic analysis of the NADH dehydrogenase subunit 2 (ND2) gene, which verified that the specimens from Udon Thani Province correspond to *L. rubritaeniata*. By establishing foundational knowledge on the nutritional biochemistry of this species, the research contributes to both ecological and conservation perspectives. The findings are expected to support further investigation into reptilian physiological adaptations, guide sustainable wildlife use, and potentially inform conservation management of agamid species in habitats undergoing environmental change.

Materials and Methods

Specimens and Ethics statements

Specimens were collected from Kham Khok Sung and Bayao Subdistrict, Wang Sam Mo District, Udon Thani Province of northeastern Thailand. The specimen was captured using a snare and by digging into its burrow. After euthanasia, the liver tissue was preserved in 95% ethanol and stored at –20°C for DNA extraction. Meat samples for protein content and composition analysis were taken from all parts of the body except the tail, and from the entire tail. The meat samples were promptly taken for analysis. The specimens were stored in the Khon Kaen Vertebrate Collection (KKUC) at Khon Kaen University, located in Khon Kaen Province, northeastern Thailand. This study was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, in accordance with the Ethical Guidelines for Animal Experimentation of the National Research Council of Thailand (reference no. 660201.2.11/318 [50]).

Specimen identification

The *Leiolepis* specimens were identified at the species level using both molecular techniques and morphological characteristics. Genomic DNA was extracted from liver tissue sample using NucleoSpin® Tissue (Ciontech Laboratorues, Inc., CA, USA). Approximately 600 base pairs of the NADH dehydrogenase subunit 2 region (ND2) from mitochondrial DNA (mtDNA) using primer METF6 (5'–AAGCAGTTGGGCCCATACC–3') and CO1R1 (5'– AGRGTGCCAATGTCTTTGTGRTT–3') (Macey *et al*., 1997; Grismer & Grismer, 2010; Wanchai *et al*., 2024). The PCR conditions were adapted from Wanchai *et al*. (2024), amplification of 50 µl PCR consisting of an initial denaturation at 95°C for 2 minutes, followed by 32 cycles of denaturation at 95°C for 35 seconds, annealing at 50°C for 35 seconds, and extension at 72°C for 150 seconds, with a final extension at 68°C for 5 minutes. The PCR products were purified using the GF-1 PCR Clean-Up Kit (Vivantis, Inc., Malaysia) and sent to Pacific Science (Bangkok, Thailand) for DNA sequencing. DNA sequences were aligned using SeaView version 4. Reference sequences used in this analysis were downloaded from GenBank (Supplementary material 1). Phylogenies were built using Maximum Parsimony (MP) and Bayesian inference (BI). A heuristic search for the most parsimonious tree was conducted using PAUP* 4.0a 169 (Swofford, 2022) with 1,000 replicates, utilizing the TBR (tree bisection-reconnection) branch swapping option and treating gaps as missing data. The Bayesian analysis parameter model was estimated from the dataset using MrModelTest 2.2 (Nylander, 2004). The best-fit nucleotide substitution models for ND2 genes based on the Akaike information criterion was GTR+I+G (-lnLs=4765.992; p-

inv=0.221137; gamma shape=0.649944), respectively. BI was performed using MrBayes 3.2.7 (Ronquist *et al*., 2012). Markov chains were executed for 10 million generations, with trees sampled every 1,000 generations. The initial 25% of samples were discarded as burn-in, leading to a potential scale reduction factor of < 0.005. To compute the posterior probabilities of tree nodes, a consensus of the sampled trees was established using majority rule at 50%. In MEGA X, uncorrected pairwise sequence divergences (*p*-distance) were computed (Kumar *et al*., 2018).

For morphological identification, two morphometric characters were measured: snout-vent length (SVL) and tail length (TaiL). Three meristic characters were counted: number of supralabial scales, ventral scales at midbody, and femoral pores. In addition, five coloration traits were observed: dorsal pattern, mid-dorsal stripe, dorsolateral stripe, anterior flank pattern, and posterior flank pattern.

Protein analysis

Crude protein in meat samples was determined using the Kjeldahl method (Sáez-Plaza *et al*., 2013) with a Gerhardt KT 8s system. The analysis was performed in triplicate, and the average was calculated. Meat samples were dried at 70°C for 10 hours and grind finely with pestle and mortar, following Manapim (2006), and then baked at 103±2°C for 1–2 hours to remove moisture. After cooling in a desiccator, approximately 1 g of the dried sample was digested with 20 ml of concentrated H_2SO_4 and a catalyst at 400°C for 70 minutes until a clear green solution formed. Ammonia was distilled into an Erlenmeyer flask containing 80 ml of 4% H_3BO_3, and nitrogen content was determined by titration with 0.1 HCl using an automatic titrator. Total protein was calculated using the equation from Horwitz and Latimer (2000). Total protein content was calculated using the method described by Horwitz and Latimer (2000). The percentage of nitrogen (%N) was determined using the formula: $\%N = [(A - B) \times M \times 1.4007] / W$, where A is the volume of acid (ml) used for titration of the sample, B is the volume of acid (ml) used for titration of the blank, M is the molarity of the acid used for titration, and W is the weight of the sample in grams. The protein content was then obtained by multiplying the nitrogen percentage by a conversion factor: % Protein = %N × factor.

Protein composition analysis

Approximately 100 mg of dry, crushed meat sample was placed in a DigiTube with 10 mL of 6 mol/L hydrochloric acid solution and heated at 110°C in a DigiPREP Block Digestion system for 22 hours (adapted from Černíková *et al*., 2015). After cooling to room temperature, a 200 µL aliquot of the hydrolyzed sample was transferred to a 1.5 mL tube and dried at 60°C. The residue was dissolved in 1,000 µL of 0.12 N

citrate buffer (pH 2.20, Sykam GmbH, Germany) and diluted 5x. The hydrolyzed sample was then centrifuged at 10,000 rpm for 10 minutes and filtered through a 0.2 μm membrane. Amino acid content was determined by ion-exchange chromatography (150 mm x 4.6 mm Cation Separation Column, K06/Na) with post-column derivatization and spectrophotometric detection of ninhydrin reaction products, using an automatic amino acid analyzer (SCION Artemis 6000, Goes, Netherlands), following the manufacturer's standard procedure.

Results

Identity of specimens

The combined Maximum Parsimony (MP) and Bayesian Inference (BI) analyses based on the ND2 mitochondrial gene clearly resolved the phylogenetic relationships among *Leiolepis* species, with each species forming distinct and well-supported clades (Figure 1). The two new sequences generated in this study, PV746783 and PV746784, were placed firmly within the *L. rubritaeniata* clade with strong nodal support (MP = 100%, BI = 1.00), clustering closely with previously published sequences (PP987942–PP987939, AB357553), which confirms their identification as *L. rubritaeniata*. The pairwise uncorrected *p*-distances (Table 1) support this placement, with a value of 0.00 between PV746783 and PV746784, indicating genetic identity, and values ranging from 0.03 to 0.05 when compared to other *L. rubritaeniata* sequences, reflecting low intraspecific variation. In contrast, the genetic distances between the new sequences and *L. reevesii* ranged from 0.05 to 0.06, while distances to *L. glaurung* were notably higher, from 0.11 to 0.13, highlighting clear genetic divergence and supporting species-level separation. These findings confirm that the specimens from Udon Thani Province are *L. rubritaeniata* and provide molecular evidence reinforcing the distinctiveness and monophyly of this species relative to other closely related taxa.

Based on molecular analysis of the mitochondrial ND2 gene (Figure 1), the *Leiolepis* specimens from Udon Thani Province are closely related to *L. rubritaeniata*, *L. reevesii*, and *L. glaurung*, with phylogenetic placement most strongly supporting assignment to *L. rubritaeniata*. Morphologically (Table 2 and Figure 2), the *Leiolepis* specimens from Udon Thani Province are assigned to *L. rubritaeniata* based on their close resemblance in external characteristics, and they are clearly distinguishable from *L. reevesii* and *L. glaurung* by differences in size, scalation, and coloration patterns. The snout–vent length (SVL) of males from Udon Thani Province (128.9 ± 7.84 mm) overlaps with that of *L. rubritaeniata* (103.4 ± 10.14 mm), though the Udon Thani specimens are slightly larger. Similarly, SVL in females is also greater in the Udon Thani population (106.3 ± 7.72 mm) than in

L. rubritaeniata (88.6 ± 4.78 mm), yet the values fall within an expected range of intraspecific variation. Tail length in both sexes is longer in the Udon Thani specimens, but again falls within overlapping ranges.

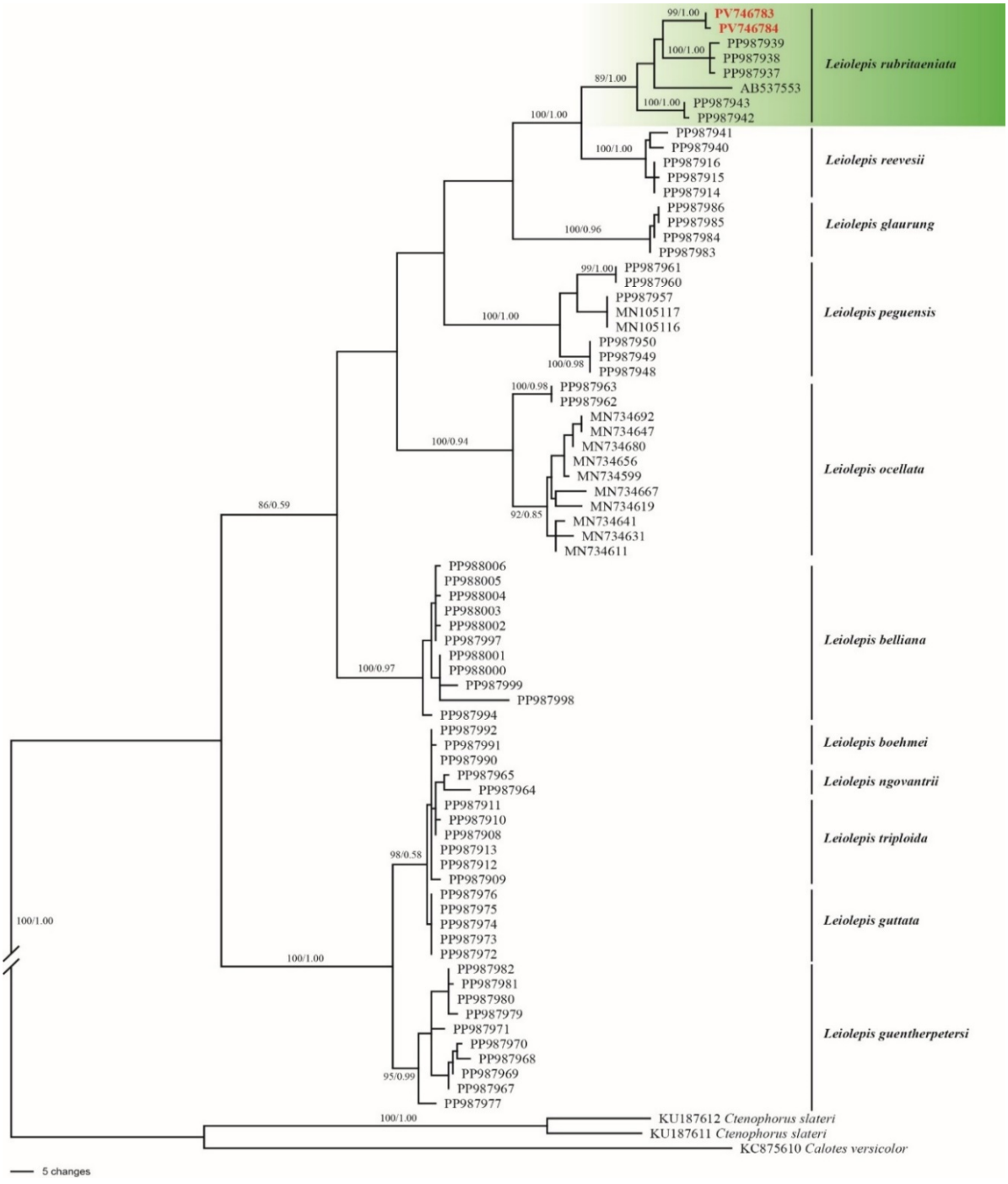

Figure 1 Maximum Parsimony (MP) and Bayesian Inference (BI) tree based on ND2 mitochondrial gene sequences of *Leiolepis* species. Support values are shown at each node as MP bootstrap values (left) and BI posterior probabilities (right). Only values ≥70% for MP and ≥0.70 for BI are shown. *Ctenophorus slateri* and *Calotes versicolor* were used as outgroups.

Table 1 The pairwise uncorrected *p*-distances of the *Leiolepis* species ND2 gene in the clade of *L. glaurung*, *L. reevesii*, and *L. rubritaeniata* in phylogenetic analysis.

No.	GenBank no.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	PV746783	*L. rubritaeniata*	–																
2	PV746784	*L. rubritaeniata*	0.00	–															
3	PP987938	*L. rubritaeniata*	0.03	0.04	–														
4	PP987937	*L. rubritaeniata*	0.03	0.04	0.00	–													
5	PP987943	*L. rubritaeniata*	0.04	0.04	0.04	0.04	–												
6	PP987942	*L. rubritaeniata*	0.04	0.04	0.04	0.04	0.00	–											
7	PP987939	*L. rubritaeniata*	0.04	0.04	0.00	0.00	0.04	0.04	–										
8	AB537553	*L. rubritaeniata*	0.05	0.05	0.05	0.05	0.05	0.05	0.05	–									
9	PP987941	*L. reevesii*	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.07	–								
10	PP987915	*L. reevesii*	0.05	0.05	0.06	0.05	0.06	0.06	0.06	0.07	0.01	–							
11	PP987914	*L. reevesii*	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.07	0.01	0.00	–						
12	PP987940	*L. reevesii*	0.05	0.05	0.06	0.05	0.06	0.06	0.06	0.07	0.01	0.01	0.01	–					
13	PP987916	*L. reevesii*	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.07	0.01	0.00	0.00	0.01	–				
14	PP987986	*L. glaurung*	0.13	0.13	0.13	0.13	0.12	0.11	0.13	0.12	0.12	0.13	0.12	0.13	0.12	–			
15	PP987985	*L. glaurung*	0.13	0.13	0.13	0.13	0.12	0.11	0.13	0.12	0.12	0.13	0.12	0.13	0.12	0.00	–		
16	PP987984	*L. glaurung*	0.13	0.12	0.13	0.13	0.12	0.11	0.13	0.12	0.12	0.12	0.12	0.12	0.12	0.00	0.00	–	
17	PP987983	*L. glaurung*	0.12	0.12	0.12	0.13	0.11	0.11	0.13	0.12	0.12	0.12	0.12	0.12	0.12	0.00	0.00	0.00	–

Table 2 Comparison of morphological and meristic characters between *Leiolepis* specimens from Udon Thani Province and *L. rubritaeniata*, *L. reevesii*, and *L. glaurung*. Values are present as mean, standard deviation, minimum, and maximum. Data sources: a – Wanchai *et al*. (2024) and b – Phan *et al*. (2022).

Characters	Udon Thani	*L. rubitaeniata*	*L. reevesii*	*L. glaurung*
SVL in males	128.9±7.84 (116.6–144.2), *n*=13	103.4±10.14 (87.0–120.0), *n*=22[a]	127.3±20.80 (94.1–167.1), *n*=10[b]	142.7±17.56 (123–170), *n*=6[a]
SVL in females	106.3±7.72 (94.6–123.8), *n*=24	88.6±4.78 (82.0–97.0), *n*=8[a]	87±21.21 (72–102), *n*=1[a]	136.3±13.91 (120–151), *n*=4[a]
TaiL in males	274.6±19.84 (229.6–302.1), *n*=10	197.5±40.96 (88.0–238.0), *n*=10[b]	212.5±71.45 (53.0–309.0), *n*=10[b]	–
TaiL females	235.5±17.63 (205.9–272.4), *n*=12	–	–	–
Supralabials	10.0±0.81 (8–12), *n*=33	9.5±0.82 (7–11), *n*=30[a]	9.3±0.50 (9–10), *n*=4[a]	9.9±0.74 (9–11), *n*=10[a]
Infralabials	10.4±1.03 (8–12), *n*=33	9.6±0.85 (8–12), *n*=30[a]	9.8±0.50 (9–10), *n*=4[a]	11.2±0.79 (10–12), *n*=10[a]
Ventral scales	36.0±2.45 (34–39), *n*=4	32.6±1.63 (27–35), *n*=30[a]	32.3±1.50 (30–33), *n*=4[a]	28.3±0.50 (28–29), *n*=9[a]
Femoral pores in males	16.6±1.17 (15–18), *n*=10	16.6±0.96 (14–19), *n*=22[a]	17, *n*=1[a]	18.2±1.33 (17–20), *n*=6[a]
Femoral pores in females	16.4±0.66 (15–17), *n*=23	16.4±1.06 (15–18), *n*=8[a]	16.5±0.71 (16–17), *n*=2[a]	18.8±0.96 (18–20), *n*=4[a]
Dorsal pattern	Black reticulate pattern forming a spot with a central dot	Black reticulate pattern forming a spot with a central dot	Black reticulate pattern forming a spot with a central dot	Dot
Mid-dorsal stripe	Absent	Absent	Absent	Present
Dorsolateral stripe	Absent	Absent	Absent	Present
Anterior flank pattern	4 black bands	4 black bands	20 black bands	2 black bands
Posterior flank pattern	Black reticulate pattern forming a spot with a central dot	Black reticulate pattern forming a spot with a central dot	–	Dot

The numbers of supralabials (10.0 ± 0.81 in Udon Thani vs. 9.5 ± 0.82 in *L. rubritaeniata*) and infralabials (10.4 ± 1.03 vs. 9.6 ± 0.85) are similar. Ventral scale counts (36.0 ± 2.45 in Udon Thani vs. 32.6 ± 1.63 in *L. rubritaeniata*) are slightly higher in the Udon Thani specimens, but not diagnostically distinct. The number of femoral pores in males (16.6 ± 1.17 in Udon

Thani vs. 16.6 ± 0.96) and females (16.4 ± 0.66 vs. 16.4 ± 1.06) are identical. In terms of coloration and pattern, the dorsal and posterior flank patterns in Udon Thani specimens show a black reticulate pattern forming a spot with a central dot, identical to that of *L. rubritaeniata*. Both populations also exhibit 4 black bands in the anterior flank, and lack mid-dorsal and dorsolateral stripes. These consistent similarities in meristic characters and coloration patterns strongly support the assignment of the Udon Thani specimens to *L. rubritaeniata*.

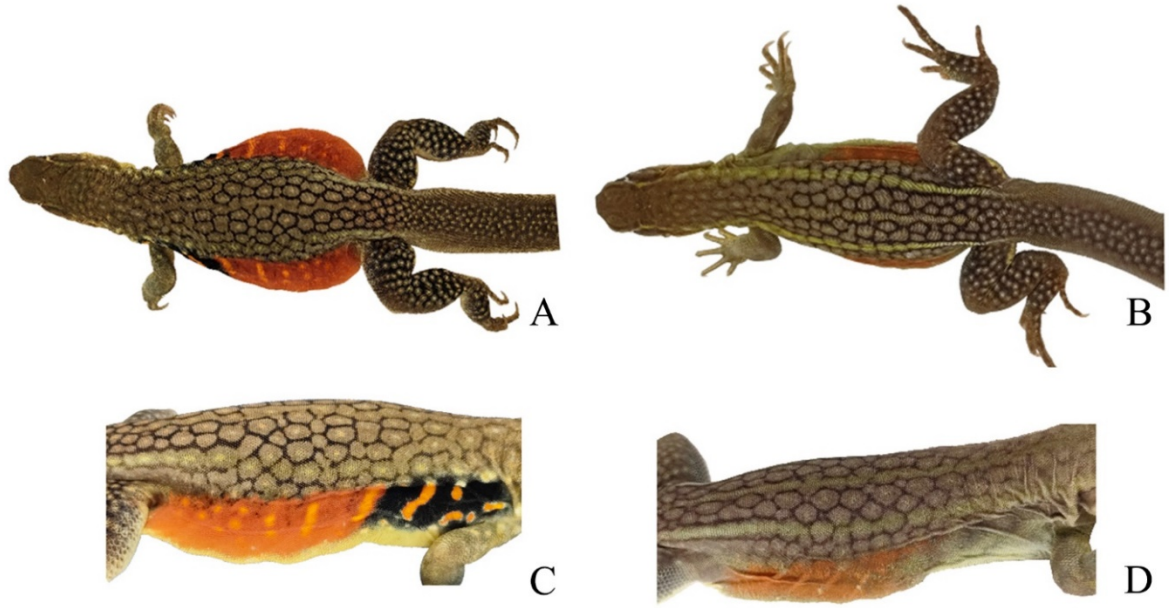

Figure 2 Life photographs showing differences color patterns in dorsal (A and B) and flank (C and D) patterns between adult male (A and C) and female (B and D) *Leiolepis rubritaeniata*. Photographs not to scale.

Crude protein

The comparison of crude protein levels in the body and tail of male *L. rubritaeniata* (Table 3), measured using the Kjeldahl method (Sáez-Plaza *et al*., 2013), reveals a significant difference in protein content. The body protein content is 78.75 ± 1.36 g/100 g dry weight, ranging from 77.34 to 80.06 g/100 g (*n*=3), while the tail protein content is higher at 88.41 ± 1.69 g/100 g, with values ranging from 86.67 to 90.04 g/100 g (*n*=3). This indicates that the tail of male *L. rubritaeniata* has a notably higher protein content compared to the body. The greater variability in tail protein content, indicated by the larger standard deviation (1.69 g/100 g) compared to the body (1.36 g/100 g), may reflect more diversity in the tail samples. This difference could suggest an anatomical or functional distinction between the body and tail that influences protein distribution. A paired *t*-test analysis would be required to confirm whether this difference is statistically significant.

Table 3 Mean, standard deviation, minimum, and maximum values of crude protein content (g/100g dry weight basis) of body and tail of male and female specimen from Udon Thani Province in Thailand, derived from three replicates.

Parts of body tested (Total dry weight mass/sex)	**Crude protein content (g/100g)**		***t*-test statistics**
	male	**female**	
Body (5 g)	78.75±1.36 (77.34–80.06)	76.68±1.03 (76.04–77.87)	$p = 0.104$
Tail (5 g)	88.41±1.69 (86.67–90.04)	86.33±3.57 (82.27–88.94)	$p = 0.412$
Total	83.58±5.47 (77.34–90.04)	81.51±5.79 (76.04–88.94)	$p = 0.537$

Similarly, in female *L. rubritaeniata*, the crude protein content in the body is 76.68 ± 1.03 g/100 g, with a range from 76.04 to 77.87 g/100 g (n=3), while the tail protein content is higher at 88.33 ± 3.57 g/100 g, with values ranging from 82.27 to 88.94 g/100 g (n=3). This suggests that, similar to males, the tail of female *L. rubritaeniata* contains significantly more protein than the body. The tail also exhibits higher variability in protein content, as indicated by the larger standard deviation (3.57 g/100 g) compared to the body (1.03 g/100 g). This difference may be attributed to biological or anatomical factors influencing protein distribution. A paired *t*-test analysis would be necessary to confirm if this difference is statistically significant.

The paired *t*-test analysis of crude protein levels in the body and tail of male *L. rubritaeniata* showed a statistically significant difference in protein content ($p < 0.05$). This suggests that the protein content in the body and tail differs, with the observed variation unlikely to be due to random chance. Therefore, the data supports the conclusion that the protein content in the body and tail of male *L. rubritaeniata* is significantly different. Similarly, the paired *t*-test analysis of crude protein levels in the body and tail of female *L. rubritaeniata* also indicated a statistically significant difference ($p < 0.05$), supporting the conclusion that the protein content in the body and tail of female *L. rubritaeniata* is significantly different.

In contrast, the independent *t*-test analysis of body crude protein levels between males and females of *L. rubritaeniata* showed no statistically significant difference in protein content ($p > 0.05$). This suggests that the crude protein levels in the bodies of both male and female *L. rubritaeniata* are similar, and any observed variation is likely due to random chance rather than a true biological difference. Similarly,

the independent *t*-test analysis of tail crude protein levels between males and females revealed no statistically significant difference in protein content ($p > 0.05$), indicating that the crude protein levels in the tails of both male and female *L. rubritaeniata* are comparable, with any observed variation likely due to random chance rather than a genuine biological difference.

Protein composition

The amino acid content of body and tail tissues from male and female specimens was analyzed (Figure 3; Table 4), revealing notable differences in protein composition across sexes and body parts. The total amino acid content was highest in the female tail tissue (11.811 mg/100 mg dry weight), followed by the male tail (9.666 mg/100 mg), female body (9.620 mg/100 mg), and male body (9.208 mg/100 mg). Glutamic acid was the most abundant amino acid across all samples, with the highest concentration observed in the female tail (2.049 mg/100 mg). Aspartic acid and Lysine were also present in significant amounts, particularly in the female tail tissue, which showed elevated levels of Aspartic acid (1.224 mg/100 mg) and Lysine (1.115 mg/100 mg). Conversely, Cysteine was consistently the least abundant amino acid across all tissues and sexes, with concentrations ranging from 0.024 to 0.034 mg/100 mg. These results indicate sex-based and tissue-specific variations in amino acid composition, with the female tail showing the highest overall amino acid richness. The higher amino acid content in the female tail tissue suggests its potential biological significance, possibly reflecting differing metabolic or functional demands between sexes and tissues.

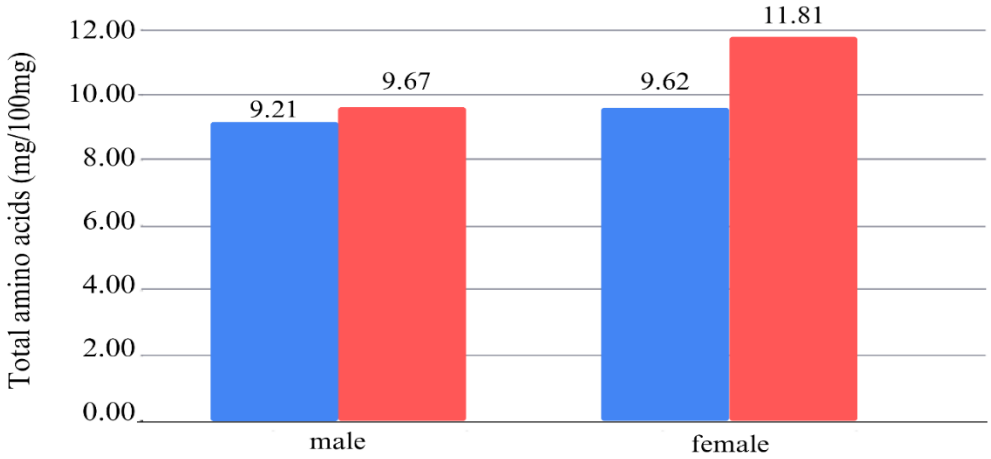

Figure 3 Total amino acid content (mg/100 mg dry weight) in the body (blue) and tail (red) of male and female *Leiolepis rubritaeniata* from Udon Thani Province, Thailand.

The amino acid composition of body and tail tissues from male and female specimens revealed distinct patterns across sexes and tissue types. In males, the total amino acid content was higher in the tail (9.666 mg/100 mg dry weight) compared to the body (9.208 mg/100 mg). Glutamic acid

was the most abundant amino acid in both tissues, with slightly higher levels in the tail (1.637 mg/100 mg) than the body (1.567 mg/100 mg). Aspartic acid (0.986 mg in the tail and 0.952 mg in the body) and Lysine (0.911 mg in the tail and 0.875 mg in the body) were also present in significant amounts. In contrast, Cysteine was the least abundant amino acid, with similar concentrations in both tissues (0.028 mg in the body and 0.024 mg in the tail).

Table 4 Amino acid content (mg/100mg dry weight) of body and tail of male and female *Leiolepis rubritaeniata* from Udon Thani Province in Thailand

No.	Amino acid types	Amino acid content (mg/100mg dry weight basis)			
		Male		Female	
		Body	Tail	Body	Tail
1	Aspartic acid (Asp)	0.952	0.986	1.008	1.224
2	Threonine (Thr)	0.471	0.487	0.487	0.596
3	Serine (Ser)	0.395	0.406	0.450	0.524
4	Glutamic acid (Glu)	1.567	1.637	1.661	2.049
5	Glycine (Gly)	0.524	0.557	0.574	0.682
6	Alanine (Ala)	0.589	0.619	0.637	0.768
7	Cysteine (Cys)	0.028	0.024	0.033	0.034
8	Valine (Val)	0.436	0.453	0.401	0.518
9	Methionine (Met)	0.220	0.251	0.228	0.309
10	Isoleucine (Ile)	0.396	0.414	0.362	0.475
11	Leucine (Leu)	0.779	0.807	0.808	0.990
12	Tyrosine (Tyr)	0.291	0.312	0.301	0.383
13	Phenylalanine (Phe)	0.433	0.454	0.448	0.543
14	Histidine (His)	0.243	0.284	0.233	0.290
15	Lysine (Lys)	0.875	0.911	0.895	1.115
16	Arginine (Arg)	0.614	0.645	0.647	0.796
17	Proline (Pro)	0.396	0.420	0.448	0.517
	Total	9.208	9.666	9.620	11.811

In females, the total amino acid content was significantly higher in the tail (11.811 mg/100 mg) compared to the body (9.620 mg/100 mg). Glutamic acid was again the most abundant amino acid, with elevated levels in the tail (2.049 mg) compared to the body (1.661 mg). Aspartic acid (1.224 mg in the tail and 1.008 mg in the body) and Lysine (1.115 mg in the tail and 0.915 mg in the body) were also prominent. Similar to males, Cysteine was the least abundant amino acid, with concentrations of 0.034 mg in the tail and 0.033 mg in the body.

Discussion

This study found that *L. rubritaeniata* has a total protein content ranging from 76.04 to 90.04 g/100 g dry weight, with an average of 82.55 g. These values align with protein levels reported in other reptiles. For example, Boyd & Goodyear (1971) reported an average protein content of 75.7% in eight reptile species, including *Sceloporus undulatus*, *Cnemidophorus sexlineatus*, and *Anolis* species. In comparison, 15 amphibian species showed a slightly lower average of 74.4%. Protein levels in domestic birds and mammals typically fall within the range of 70 to 80% (Maynard & Loosli, 1962), while edible insects such as crickets contain 58.3 to 71.7% protein (Udomsil *et al*., 2019). Therefore, *L. rubritaeniata* is notable for its relatively high protein content among these groups.

The amino acid profiling revealed significant sex-based and tissue-specific differences. Notably, female tail tissue exhibited the highest total amino acid content, characterized by elevated levels of glutamic acid, aspartic acid, and lysine. These differences may reflect physiological or reproductive demands, particularly in females, and may correspond to specific metabolic or functional roles of the tail in both sexes.

In male specimens, the crude protein content from all body parts excluding the tail averaged 78.75 g/100 g dry weight, while the tail alone averaged 88.41 g/100 g. A similar trend was observed in females, with the body and tail averaging 76.68 g/100 g and 86.33 g/100 g, respectively. These data indicate a consistent pattern of higher protein concentration in the tail across both sexes (Tables 9–10), supported by statistical significance ($p < 0.05$). Such anatomical specialization mirrors findings in other reptiles. In *Crocodylus niloticus*, for example, tail dorsal, shoulder, and leg regions contained significantly more protein than the neck and tail ventral areas (Černíková *et al*., 2015). Similarly, *Tupinambis merianae* showed uniform protein levels across different cuts, with a slight elevation in the hind leg (Caldironi & Manes, 2006). The elevated tail protein levels in *L. rubritaeniata* may therefore relate to muscle mass, energy storage, or defensive behavior, all of which are important in both ecological and functional contexts.

Amino acid composition supported these trends. Glutamic acid was the most abundant non-essential amino acid across all tissues, aligning with patterns seen in other vertebrates, including Nile crocodiles (Černíková *et al*., 2015), pigs (Dai *et al*., 2014; Zhang *et al*., 2021), and turkeys (Essary & Ritchey, 1968). Lysine, the most prominent essential amino acid, also consistently appeared in high concentrations, as observed in proteins from beef, poultry, fish, and amphibians (Beach *et al*., 1943; Zhang *et al*., 2021). These amino acids are crucial for muscle growth and

metabolic regulation, further underscoring the nutritional potential of *L. rubritaeniata*.

From a genetic perspective, this study provides the first integrated analysis of both protein composition and amino acid profiles in *L. rubritaeniata*, validated by morphological and molecular data. The newly sequenced individuals (PV746783 and PV746784) were firmly placed within *L. rubritaeniata* clade with strong nodal support (MP = 100%, BI = 1.00), and showed minimal genetic divergence (0.00–0.05 uncorrected *p*-distance) from previously published sequences. Their distinction from related congeners such as *L. reevesii* (0.05–0.06) and *L. glaurung* (0.11–0.13) (Wanchai *et al*., 2024) further supports species-level integrity and genetic cohesion of northeastern Thai population.

Importantly, the findings of this study highlight *L. rubritaeniata* as a promising candidate for small-scale or community-based farming. The high protein and essential amino acid content, particularly in the tail, suggest that this species may offer significant nutritional benefits. Moreover, the observed consistency in protein content between males and females suggests stable nutritional profiles, which is advantageous for farming practices. In some rural communities, agamid lizards are already consumed as a traditional protein source (Hartmann *et al*., 2012), supporting the cultural and practical feasibility of farming this species.

In recent years, the global shift toward alternative protein sources has highlighted the potential of non-traditional foods such as insects, amphibians, and reptiles to support sustainable nutrition and reduce reliance on conventional livestock (Dolly & Tardieu, 2023; John & Jones, 2024; Nirmal *et al*., 2024). In reptiles, for example, several species have been identified as having high potential for farming to meet growing consumer demand while minimizing pressure on wild populations (Klemens & Thorbjarnarson, 1995; Aust *et al*., 2017; John & Jones, 2024). Among these, *L. rubritaeniata*, a butterfly lizard native to Southeast Asia, emerges as a promising candidate for reptile farming due to its local consumption, high protein content, and biological traits that are favorable for captive breeding. Our study, which provides the first integrated analysis of the species' protein and amino acid profiles, underscores its nutritional value and potential for sustainable farming initiatives aimed at improving food security and reducing harvesting pressure on wild populations. However, the development of *L. rubritaeniata* farming systems should be approached with caution, incorporating ecological assessments, genetic monitoring, and disease management to avoid overexploitation and negative impacts on wild stocks (Kusrini & Alford, 2006; Gratwicke *et al*., 2010). When guided by scientific evidence, farming practices for this species can contribute

meaningfully to both conservation and local economic development.

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