NORTH-WESTERN JOURNAL OF ZOOLOGY 21 (2): 151-157
Article No.: e251502

http://biozoojournals.ro/nwjz/index.html

Morphometric analysis, food composition, and parasitic investigation of the Kashmir rock agama (*Laudakia tuberculata*) from Swat, Pakistan

Zakir ULLAH [1], Wali KHAN [1,*], Arshad ALI [1,*],
Abdur RAHIM [1], Alaa B. ISMAEL [2],
and Ayman A. SWELUM [3]

1. Department of Zoology, University of Malakand, Chakdara, Lower Dir, 18800, Khyber Pakhtunkhwa, Pakistan.
2. Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.
3. Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia.
* Corresponding authors: W. Khan, E-mail: walikha.pk@gmail.com; and A. Ali, E-mail: arshadkotlai1985@gmail.com.

Received: 13 December 2024 / Accepted: 22 June 2025 / Available online: December 2025 / Printed: December 2025

Abstract. This study offers a thorough analysis of the morphometric traits, feeding habits, and parasitic infestations of *Laudakia tuberculata* in the hilly regions of Swat, Pakistan. Morphometric analysis revealed significant sexual dimorphism, where females were larger than males in tibial length (2.23 ± 0.45 cm vs. 1.84 ± 0.68 cm) and snout-vent length (9.57 ± 1.10 cm vs. 8.69 ± 2.88 cm). Furthermore, morphological features were affected by altitude; lower-altitude lizards had longer toes (mean length of 7.44 ± 7.44 cm at lower altitudes vs. 5.99 ± 2.04 cm at higher altitudes). Dietary analysis revealed omnivorous behavior, with all examined lizards consuming a significant proportion of plant material, including leaves (86.1%), seeds (66.1%), as well as invertebrates such as ants (67%), flies (49.4%), beetles (13%), fireflies (7.1%), and other non-nutritive sources (27.5%). Parasitic investigation showed that *Amblyomma nuttalli* tick infestations were 43.5% common, with adults having a higher parasitism rate (38.8%) than juveniles (16%). Tick attachment locations and habitat types did not significantly correlate ($\chi^2 = 0$, df = 1, p-value = 1.00), indicating a generalist attachment behavior. These results highlight the need for further research and offer vital baseline data for comprehending the ecological dynamics of *Laudakia tuberculata.* Our study establishes a platform for further ecological research while providing insights into Pakistan's herpetofauna richness.

Keywords: herpetology, morphometry, diet, ectoparasites, *Laudakia tuberculata*, Pakistan.

Introduction

Squamata, a diverse group of ectothermic tetrapods, account for around one-fourth of the world's vertebrate biodiversity. These species greatly impact the world's ecological, economic, and cultural landscapes (Zug et al. 2001). Despite its importance, the study of Pakistan's reptilian diversity remains relatively understudied, leaving a significant gap in regional and global biodiversity assessments (Rais et al. 1997). Reptiles have a 230-million-year evolutionary history and approximately 11500 identified species worldwide (Uetz et al. 2025). In Pakistan alone, the reported herpetofauna comprises 24 amphibian and over 200 reptile species (Masroor 2012), with 13 endemic reptile species and 9 endemic amphibian species underscoring the region's particular ecological value (Ali et al. 2018).

The Agamidae, notably prominent in Pakistan, comprises seven genera —*Laudakia, Paralaudakia, Japalura, Phrynocephalus, Bufoniceps, Trapelus,* and *Xenagama* —and twenty-three species. *L. tuberculata* is one of the most extensively studied species in this region (Khan 2004, Baig et al. 2012). *Paralaudakia* was historically considered a subgenus of *Laudakia* but was elevated to genus rank based on molecular and morphological data (Baig et al. 2012, Melville et al. 2009). The agamids are widely distributed, with species ranging from eastern Afghanistan and northwestern Pakistan to southwestern Nepal and Tibet, where they thrive at elevations varying from 150 to 3,600 meters (Baig et al. 2012). *Laudakia tuberculata*, or Kashmir rock agama, is highly adaptable to a variety of microhabitats, including rocky crevices and human-occupied structures, indicating a varied survival strategy across seasons (Waltner 1991, Singh & Banyal 2014).

Sexual dimorphism in lizards correlates with differences in ecological roles, influencing body size, head morphology, and locomotor performance (Herrel et al. 1996, 1999). Studies on high-altitude populations of agamid lizards indicate that elevation-related environmental pressures can drive variations in morphometry and physiological traits (Bahuguna 2008). Additionally, parasites, especially ticks, significantly impact physiological stress, affecting the health, immune function, and reproductive success of their hosts (Amo et al. 2004, Keskin et al. 2013a). *L. tuberculata* has a depressed body and head with distinct scale patterns, no gular pouch, and occasionally increased vertebral scales depending on the individual (Boulenger 1885). These lizards exhibit sexual dimorphism and seasonal variations in behavior, especially during the breeding season, which lasts from late April to mid-August. Males exhibit increased aggressive behavior during this phase, as well as a specific courtship ritual that includes a pursuit before copulation (Pandav et al. 2007). The reproductive tactics of these lizards are remarkable, as females dig shallow burrows in sandy riverbanks for their eggs, which have an incubation period of roughly 39.10 days (Bashir et al. 2017). Recent studies revealed that *L. tuberculata* is an omnivore, consuming a wide range of food items, including insects and plant material (Waltner 1991, Kapoor et al. 2024). This nutritional flexibility enables it to adapt to seasonal and environmental changes, thereby improving its overall health and reproductive

performance (Panov & Zykova 2016, Minton 1966).

The main objectives of this research are to perform a morphometric analysis of *Laudakia tuberculata* based on sexual dimorphism and physical characteristics, including snout-vent length, head size, limb proportions, and tail length. Additionally, the study aims to examine the dietary composition of the species, highlighting its omnivorous behavior and seasonal feeding preferences. Further, the study seeks to investigate the prevalence and effects of parasitic infestation, especially by *Amblyomma nuttalli* ticks, on the population's health. The study will finally analyze the ecological implications of these findings, including the roles of food composition and parasitic load in shaping the species' survival and reproductive success in the Swat region. The current study reports results from an examination of the morphometry, dietary preferences, and parasitic infestation of *Laudakia tuberculata* in the mountainous areas of Swat, Pakistan. This study will contribute to a better understanding of the species' ecological role and inform future conservation strategies.

Materials and Methods

Study area

The study was conducted in the Swat District, approximately 236 kilometers north of Islamabad, Pakistan's capital city. This district covers 5,337 km² and has a human population of around 2.31 million (Census 2017). The region has a moderate climate with hot summers and freezing winters, with average summer temperatures of 22°C and winter temperatures of -2°C. The annual rainfall averages 864 mm, reflecting the diverse climatic variables influencing local biodiversity (Jabbar et al. 2008) (Figure 1). The sampling sites in this study were located in four villages within the district, each with the following GPS coordinates: Kotkar (Latitude 35.035°N, Longitude 72.5772°E), Jabe Ghat (Latitude 35.032°N, Longitude 72.582°E), Khair Abad (Latitude 35.031°N, Longitude 72.58°E), and Baaban (Latitude 35.033°N, Longitude 72.585°E).

Sample collection

Between April and August 2021, samples were collected from four villages: Kotkar (n=12), Jabe Ghat (n=19), Khair Abad (n=34), and Baaban (n=20). These sites were chosen due to their accessibility and the known presence of *Laudakia tuberculata*. Common rock agamas were caught using the pitfall trapping technique. Pitfall traps (n=XX) were placed at predetermined locations and checked every 6 hours to ensure the humane treatment of specimens. Each lizard was weighed immediately after being captured using a digital balance (model: Ohaus SPX 822). Each lizard was weighed using an electronic balance immediately after capture, and morphometric data such as snout-vent length, tibial length, head length, head width (breadth), internarial space, interorbital space, and limb measures, including forelimb and hindlimb lengths (from shoulder/hip to digits), were recorded using Mitutoyo digital calipers (model: 530-113). An Olympus CX23 compound microscope (with a 10x objective lens) was used for morphological analysis of the parasite and for detailed measurements. Morphometric measurements of lizard specimens were taken with a Mitutoyo digital caliper (model 530-113) to ensure precision. Specimens were then preserved in 70% ethanol before being examined in the parasitology laboratory of the Department of Zoology at the University of Malakand for further analysis.

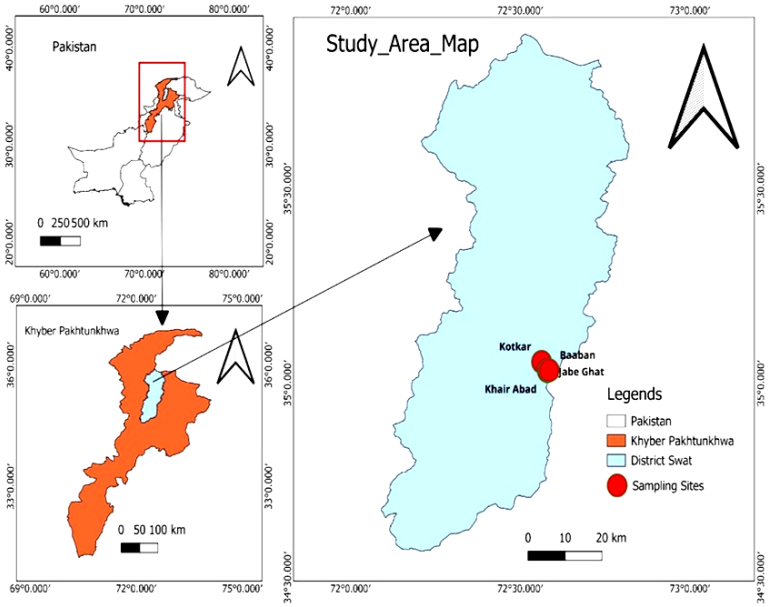

Figure 1. Map of the study area, showing the geographic distribution of the sampling site in district Swat, Khyber Pakhtunkhwa, Pakistan.

Ethical considerations

This research was conducted as part of a graduate thesis project under the supervision of faculty in the Department of Zoology at the University of Malakand. Following institutional academic protocols, ethical approval for fieldwork and specimen collection is embedded within the university's research framework for postgraduate studies. All procedures involving *Laudakia tuberculata* in this study adhered to ethical guidelines for the care and use of reptiles in scientific research. The collection, handling, and examination of specimens were conducted according to protocols designed to minimize harm and stress. Lizards were captured using humane methods (pitfall trapping) and transported carefully to the laboratory for analysis. To ensure ethical compliance, specimens were first anesthetized using an approved euthanasia protocol before preservation. The euthanasia method followed international ethical standards for reptile research, minimizing pain and distress (AVMA Guidelines 2020). After data

collection, specimens were fixed in 10% buffered formalin for morphological analysis and later transferred to 70% ethanol for long-term preservation, following established guidelines (Hoogstraal 1956a, Khan 2004).

Laboratory analysis

In the laboratory, specimens were identified as *Laudakia tuberculata* using taxonomic keys provided by Baig et al. (2012), which offer a comprehensive morphological framework for distinguishing agamid lizards in the region. Ecto-parasites were collected from external body surfaces and stored in 70% ethanol. The lizards were euthanized humanely in accordance with approved ethical guidelines before being dissected to determine their dietary contents. The euthanasia procedure adhered to the AVMA (2020) standards to minimize pain and distress. All Ecto-parasites were dehydrated in ethanol solutions at increasing concentrations (30%, 50%, 70%, 90%, and 100%) before being examined morphologically under a microscope.

Statistical Analysis

The data was analyzed using R software (version 4.3.2). Means and standard deviations were calculated for all morphometric data as part of the statistical analysis. To improve the interpretability of the morphometric data, body length-to-weight ratios were calculated, providing information about the lizards' physical condition and health status. These measures are critical for comprehending the ecological adaptations and variances observed in the examined population.

Results

Morphometric analysis

The morphometric analysis of *L. tuberculata* revealed differences between male and female lizards across several traits, including head size and upper eyelid length. However, many of these differences were not statistically significant ($p > 0.05$). Notably, males had longer heads and upper eyelids than females. These findings are summarized in Table 1 and Figure 4, which present the mean and standard deviation for each trait.

Dietary analysis

The dietary analysis of *L. tuberculata* revealed a varied diet, with insects being the most common food source (100%), followed by leaves (86.1%), seeds (66.1%), ants (67%), and flies (49.4%). Other items like stones (27.5%), beetles (13%), and fireflies (7.1%) were less frequently consumed by the studied species (Table 2).

Table 1. Morphometric analysis of *Laudakia tuberculata* (n=85) found in hilly areas of Swat, Pakistan.

Character	Mean male ± SD	Mean female ± SD	Mean difference	t-value	p-value
Snout-vent length	8.688 ± 2.8787	9.571 ± 1.0965	0.883	-0.973	0.347
Tibial length	1.838 ± 0.6760	2.229 ± 0.4532	0.391	-1.805	0.093
Head length	5.825 ± 8.596	3.229 ± 0.6473	-2.596	1.147	0.270
Head width	1.650 ± 0.5855	1.814 ± 0.4706	0.164	-0.719	0.482
Internarial space	0.373 ± 0.1750	0.400 ± 0.1155	0.027	-0.439	0.668
Interorbital space	1.088 ± 0.3441	1.143 ± 0.3155	0.055	-0.433	0.672
Upper eyelid length	0.338 ± 0.3441	0.314 ± 0.0900	-0.024	0.215	0.832
Upper eyelid width	0.100 ± 0.0000	0.100 ± 0.0000	0.000	0.000	0.000
Snout to naris length	0.238 ± 0.1061	0.271 ± 0.0488	0.033	-0.940	0.355
Naris to eye length	0.550 ± 0.2000	0.643 ± 0.2070	0.093	-1.228	0.230
Arm length	3.438 ± 1.0336	5.014 ± 5.014	1.576	-1.221	0.233
Hand length	1.338 ± 0.4596	2.029 ± 2.029	0.691	-1.082	0.283
Leg length	5.988 ± 2.0371	7.443 ± 7.443	1.455	-0.805	0.432
Foot length	2.350 ± 0.9335	2.871 ± 2.871	0.521	-0.753	0.457

Table 2. Dietary composition of *Laudakia tuberculata* observed in this study

Food items	Number (n=85)	Percentage (%)
Plant material		
Seeds	56	66.1%
Leaves	73	86.1%
Invertebrates		
Total insects	85	100%
Ants	57	67.0%
Flies	42	49.4%
Beetles	11	13.0%
Fireflies	6	7.1%
Other items		
Stones	23	27.5%

Ticks' identification and demographics.

A total of 108 ticks were collected from 85 *L. tuberculata* specimens, identified as *Amblyomma nuttalli* based on morphological features. Ticks were predominantly found in the groin and dorsal regions, with no significant preference for specific body parts (Figure 2, 3A and B).

A habitat-based Pearson's Chi-square test ($\chi^2 = 0$, df = 1, p-value = 1.00) found no significant relationship between tick attachment locations and habitat types, indicating random attachment patterns across all habitats. Table 3 presents the distribution of habitat, indicating that 56 lizards were captured in woodland (36 from uncultivated forests, 19 from cultivated forests), and 29 were taken from non-forested areas, including 14 from uncultivated ground, 1 from human settlement, and four from water-related regions.

Figure 2. Photographs of lizards collected from the sampling sites

Demographics of infestation

Of the 85 lizards examined, 60 were adults, and 25 were subadults. Of these, 51 were male, and 34 were female. It was revealed that 43.529% (n=37) of *L. tuberculata* were infected with *Amblyomma nuttalli,* whereas 56.471% (n=48) were not (Table 4). A total of 108 ticks were collected from lizards, including 46 male ticks (42.6%) and 62 female ticks (57.4%). Out of the total ticks, 48 (44.4%) were subadult ticks, consisting of 21 male subadults (43.8%) and 27 female subadults (56.2%), whereas 60 ticks (55.6%) were adults, comprising 25 male adults (41.7%) and 35 female adults (58.3%). The statistics indicate a greater percentage of female ticks (57.4%) than males (42.6%), and a higher percentage of adult ticks (55.6%) than subadults (44.4%), indicating that adult female ticks are more prevalent, possibly because of their role in reproduction and longer attachment time to the host (Table 5). Regarding gender, the analysis showed that of the 51 male lizards analyzed, 13 (25.5%) had an *Amblyomma nuttalli* infection, and 38 (74.5%) did not. Out of the 34 female lizards, 24 (70.6%) had the infection, and 10 (29.4%) had no infection. About age, out of the 60 adult lizards, 33 (55%) were infected with *Amblyomma nuttalli*, and 27 (45%) had no infection. Conversely, 4 (16%) out of the 25 subadult lizards were infected, while 21 (84%) were not infected. This shows a remarkable age difference in infestation rates (Table 5).

Tick infestation across municipalities

The prevalence of infestation differed slightly across sites: Kotkar (50%, n = 6/12), Jabe Ghat (47.37%, n = 9/19), Khair Abad (38.23%, n = 13/34), and Baaban (45%, n = 9/20) (Table 3). Nevertheless, a chi-square test ($\chi^2 = 5.1006$, df = 2, p-value = 0.07806) revealed that habitat type did not have a statistically significant influence on tick prevalence.

Figure 3. A indicates the location of parasites on the body, while B indicates the parasite specimens of *Amblyomma nuttalli* obtained during the microscopic observation.

Table 3. Habitat preferred by *Laudakia tuberculata* captured in the hilly areas of Swat, Pakistan.

Factors		Cultivated	Uncultivated	Human settlements	Water bodies	Total specimens
Areas	Forest	19	36	1	0	56
	Non forest	10	4	1	4	29
Sexes	Male	12	36	0	3	51
	Female	17	14	2	1	34
Age	Adult	26	33	1	0	60
	Sub Adult	3	17	1	4	25

Table 4. Prevalence of *Amblyomma nuttalli* in *Laudakia tuberculata* (locality, sex, and age-based analysis)

Parameters	Kotkar	Jabe-Ghat	Khair Abad	Baaban	Total (locality)	Male lizards	Female lizards	Total (sex)	Sub-adults	Adults	Total (age)
Number examined	12	19	34	20	85	51	34	85	25	60	85
Number infected	6	9	13	9	37	13	24	37	4	33	37
Number non-infected	6	10	21	11	48	38	10	48	21	27	48

Table 5. Tick demographics in *Laudakia tuberculata.*

Parameters	Total (ticks)	Percentage (%)
Total ticks collected	108	-
Male ticks	46	42.6%
Female ticks	62	57.4%
Subadult ticks	48	44.4%
Male subadult ticks	21	43.8%
Female subadult ticks	27	56.2%
Adult ticks	60	55.6%
Male adult ticks	25	41.7%
Female adult ticks	35	58.3%

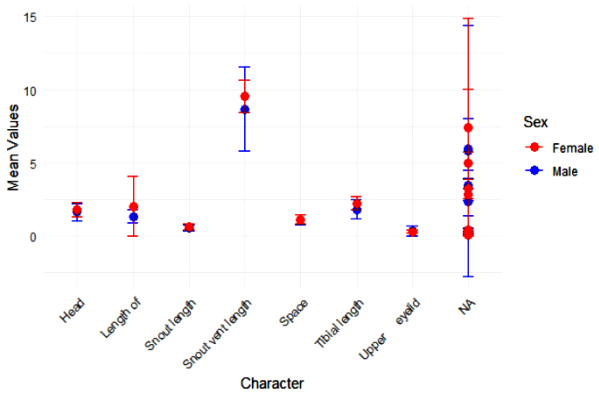

Figure 4. Morphometric analysis of *Laudakia tuberculata* by sex (the mean values and standard deviations of various morphometric characteristics of males and females)

Discussion

The current study was conducted in Swat, a hilly region of Pakistan. A total of 85 *Laudakia tuberculata* lizard specimens were collected and identified as members of the Agamidae family based on current taxonomic classification. The focus of this study was on the morphometric, dietary, and parasitic investigation of L. *tuberculata.*

Morphometric analysis

Significant differences in the morphometric characteristics of male and female lizards were revealed by biometric research. With notable differences in tibial length, head size, and snout-vent length, females were usually larger than males. These results are consistent with Bahuguna's (2008) research on sexual dimorphism in lizards. Furthermore, we found that altitude affects morphological characteristics, supporting Bahuguna (2008) by showing that lizards at lower altitudes had longer toes than those at higher altitudes. The observed sexual dimorphism, in which females are larger than males, may be explained by the reproductive roles females play and by the energy requirements of egg production. Larger females may have access to various food sources than smaller males, according to Rabiu (2019), who suggests that these morphological variations may also influence habitat utilization and foraging behavior. The study's observations of morphological changes with altitude indicate that environmental factors are important in shaping *L. tuberculata*'s morphological traits, including tibial length, head size, snout-vent length, and dorsal scales. Higher-altitude lizards may have evolved smoother dorsal scales to minimize radiation and prevent water loss, while lower-altitude lizards with longer toes may be better suited to negotiating rocky terrains. *L. tuberculata* must be able to adapt to diverse altitudinal conditions to survive and spread throughout many habitats.

Dietary analysis

L. tuberculata's food analysis reveals a diversified diet, with insects being the predominant food source, followed by leaves and invertebrates. This affirms their omnivorous nature, aligns with their ecological resilience, and enables them to tap into an array of food sources. Their ability to eat leaves and seeds also proves their dietary flexibility, which is indispensable for survival across fluctuating environmental conditions, as stated by Minton (1966) and Panov & Zykova (2016). Seasonal variation in the feeding behavior of *L. tuberculata*: more plant material is consumed during periods when insect numbers are low. In other research (e.g., Düsen & Öz 2001), similar seasonal diet variations have been reported, suggesting that lizard foraging habits are influenced by food supply and climate. The data in this research do not provide exact details on daily routine activities, such as feeding periods (e.g., dusk or dawn), and, as such, we do not make any assumptions about when feeding occurs.

The dietary flexibility of *L. tuberculata* is a major factor in its capacity to survive in various environments. Although previous research (e.g., Waltner 1991, Yeboah 1982) indicated that lizards are more active in foraging at specific times of day, our study does not directly confirm this assertion because it lacks data on daily foraging activity. Therefore, we exercise restraint from generalizing about activity times based on our data. However, the dietary observations in our study corroborate those of Kapoor et al. (2024), who reported a predominance of plant matter in the diet of *L. tuberculata* in Himachal Pradesh. This consistency across regions highlights the species' opportunistic feeding behavior and its ability to exploit available food resources effectively.

Parasitic investigation

Our results show that 43.5% of *Laudakia tuberculata* were infected with *Amblyomma nuttalli* ticks. This prevalence is consistent with the results of Amo et al. (2004), who found that adult lizards are more likely than juveniles to become parasitized. Adults may have a higher prevalence since they have been exposed to parasites for a longer period. Our research supports the findings of Pandit et al. (2011), who reported that lizards harbored *Amblyomma* ticks. There is limited information available on tick infestation in reptiles, as noted by Keskin et al. (2013a), who observed that most research on tick infestations in wild animals has focused on rodents and birds, with reptiles receiving relatively less attention. By documenting tick infestations in *L. tuberculata,* our study contributes additional data to this underexplored area. According to Bursali et al. (2012), ticks are essential

carriers of a variety of illnesses that can infect people and other animals. Our results support their claim by confirming the presence of *Amblyomma nuttalli* on *L. tuberculata*, as previously reported by Castro et al. (2019).

Most ticks were found affixed to the lizards' dorsal sides and groins, confirming the observations of Keskin et al. (2013b), who identified comparable attachment sites in Agamidae. Ticks have a well-established involvement in the spread of infectious diseases (Hoogstraal 1956a). The lack of a significant correlation between tick attachment sites and the habitat types our study identified suggests that specific environmental conditions influence tick attachment behavior.

The statistical analysis of the present study did not find a significant correlation between habitat type and the locations of tick attachment, indicating that the behavior of ticks adhering to lizards is not dependent on specific environmental conditions. Tick attachment behavior is consistent across different habitats, suggesting a generalist strategy that could help the ticks survive and expand. This study is important because it shows how diseases carried by ticks can impact lizard populations equally across a variety of habitats, requiring broad-based management approaches to limit tick populations (Hoogstraal 1972).

Conclusions

Research findings provide valuable insights into *L. tuberculata's* natural habits, including its ability to thrive under diverse environmental conditions. The measured sexual dimorphism shows that females are larger than males, possibly because different foraging strategies and habitat use are associated with reproductive activities. The morphological data gathered from the study demonstrate that altitude plays a significant role in shaping adaptations, as reflected in differences in toe length and dorsal scales. The research found that *Amblyomma nuttalli* infests L. tuberculata at high rates because adult lizards become infected more often due to their extended time in the environment. The researchers outline tick-mediated disease transmission as a factor affecting lizard populations and note that this requires further study of tick population dynamics. The feeding behavior of *L. tuberculata* is flexible because the lizards mainly feed on insects but will eat plants if they become scarce. Results show that *L. tuberculata* adjusts its food intake from insects to plants in response to environmental conditions, demonstrating the species' ecological adaptability. The research provides insights into *L. tuberculata* morphology and ecology while showing its parasite behavior across different habitats and environmental factors. Future research should investigate genetic variation in herpetofauna, seasonal parasitic infections, and ecological interactions to strengthen conservation plans in Pakistan.

Acknowledgement

The authors acknowledge the Deanship of Graduate Studies and Scientific Research at Taif University for funding this work.

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