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Degrees of Pectoral Reduction On The Gymnophtalmidae Lizards

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DEGREES OF PECTORAL REDUCTION ON
THE GYMNOPHTALMIDAE
LIZARDS

by

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ABSTRACT

DEGREES OF PECTORAL REDUCTION ON

THE GYMNOPHTALMIDAE

LIZARDS

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The Gymnophtalminae family comprises over 250 lizard species. Many of these microteiids present with reduced limbs that contribute to their snake-like appearance. Several predictions regarding limb reduction arise from genetic and environmental influence; however, there is limited literature supporting the impact of limb reduction on the pectoral girdle. Five Gymnophtalminae species were selected, and Dristhi software was used to perform CT segmentation to view their pectoral bones. *Echinosaura horrida* presents with the most developed pectoral structure, as this is a fully developed quadruped species. Whilst *Calyptommatus leiolepis* presents with the most reduction with a thinner structure and diverges from a traditional girdle. These findings support the hypothesis of reduced limbs reducing pectoral structure as *C. leiolepis* have absent forearms. Likewise, the well-developed lizards present with a well-developed pectoral girdle. Further research

should be conducted to compare pectoral reduction amongst other families to help support this hypothesis.

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CHAPTER 1

INTRODUCTION

The lizards of the Gymnophtalminae are squamates, which are found in a diverse range of habitats that are related to the high morphological variation. The evolution toward a snake-like body structure is a well-known trend in this taxon. This morphological phenomenon is associated with limb reduction, girdle reduction, and body elongation throughout the generations; however, there is limiting evidence regarding the pectoral girdle reduction, which led to the proposal of this experiment.

The pectoral girdle serves as structural stability to the bilateral shoulders of the species. This reinforcement allows for a high degree of motion, promoting both shoulder and limb movement within this area. Mutations or disparities of this girdle impair these functions and distort the appearance of the upper torso (Westphal et al. 2019).

In this experiment, five species were focused on within the Gymnophtalimae family and the pectoral structure was examined. *Echinosaura horrida* and *Leposoma hexalepis* are found in the Cercosauninae subfamily. *Bachia peruana* is part of the cercosauninae subfamily. Both *Tretioscincus aff bifasciatus* and *Calyptommatus leiolepis* are found in the Gymnophtalminae subfamily.

Echinosaura horrida is a lizard species that resides in the Andes tropical lowland rainforest of Northwestern Ecuador. The pectoral girdle of this microteiid comprises the main components: clavicle, interclavicle, epicoracoid, and sternum. The clavicle bone appears as large, triangular, and flat. The interclavicle lies superior to the clavicle and appears with lateral branches that are ¼ of the total length of the interclavicle. The median process of the interclavicle extends between the two clavicles. The sternum is rhomboid-shaped with a large fontanelle in the center (Yanez-Munoz et al. 2021).

Leposoma hexalepis is a lizard species located in the Orinoco Basin in Columbia. The Leposoma genus is found in the tropical forests of South and Central America. This species has elongated dorsal scales and the ventral lanceolate lies diagonally. Limited evidence is found in the reduction of the pectoral girdle of the *L. hexalepis* species (Rodrigues and Borges 1997).

Bachia peruana is coined as the spectacled lizard. Located in Peru and Brazil, this species is found under loose dirt and leaves of the ground. B. peruana appears with forelimbs that is accompanied by a serpent-like body form (Westphal et al. 2019).

Tretioscincus aff bifasciatus lizards reside in South America and are found in the Gymnophtalmidae clade. Their elongated bodies and reduced limbs create a divergence into new lineages of genuses. Tretioscinus genera is a monophyletic group within this clade that experiences changes in the typical lizard morphology (Roscito and Rodrigues 2013).

Calyptommatus leiolepis is the sand-swimmer burrower lizard located in Brazil. In the Calyptommatus genus, the reduction of the pectoral girdle and forelimbs takes precedence. This genus experiences high numbers of structural modifications and yet still retains the ancestral characteristics of the Gymnophthalmidae Squamata. This species of lizard shifts to a snake-like body form that could contribute to a reduction in girdle development (Holovacs et al. 2020).

The osteological differences between these quadruped lizards revealed that limb reduction may be caused by heterochronic changes during the lizard's development (Reilly et al. 1997). These changes occur when there is a variation in the rate of cell line development compared to other cells in the lizard's body. These slight variations result in microevolutions that lead to limb reduction, as shown by Jerez & Tarazona (2009). The differences between the pectoral girdles with the Gymnophtalmidae clade relate to the aftermath of both genetic and environmental differences between each species. The discrepancies arise via changes in the pectoral structure due to limb reduction from limited usage (Westphal et al. 2019). The pectoral structure among the five species varied from highly developed to highly reduced. Each species serves a different role in their respective habitats, therefore leading to varying structural differences among the selected lizard species.

The purpose of this research is to examine the structural differences of the pectoral bones amongst the Gymnophtalmidae species with different degrees of limb reduction. The overall genetic and environmental influence on this clade is then analyzed. With limiting literature for Gymnophthalmids, the need to contribute new data on the pectoral girdle differences of these microteiids is necessary.

Figure 1.1: *Bachia Peruana*, Photo by: Pedro Peloso

Figure 1.2: *Echinosaura horrida*, Photo by Reptilesofecuador.com

CHAPTER 2

METHODOLOGY

CT scans were obtained through an online database, with each scan having an identifiable collection code. *Calyptommatus leiolepis* and *Echinosaura horrida* were obtained through Morphosource.org, where *Calyptommatus leiolepis* was provided by the Miguel Lillo Foundation, while *Echinosaura horrida* was provided by the University of Florida.

Table 2.1: Collection Code for Each Species Obtained

Morphosource.org	Collection Code
Echinosaura horrida	Media 00048792/uf: 166926
Calptommatus leiolepis	Media 00081995/fml:h:30530
UTA	
Leposoma hexalepis	UTA-R 59099
Bachia peruana	UTA-R 18328
Tretioscincus sp.	MBLUZ 1252

The research was conducted using CT scans within the Gymnophthalmidae. CT segmentation was done on five species among these selected clades: *Bachia peruana, Calyptommatus leiolepis, Echinosaura horrida, Lepsoma hexalepis,* and *Tretrioscincus* *sp.* Images of these reptiles underwent three-dimensional segmentation, using the Dristhi software, by isolating each pectoral element (Limaye 2016).

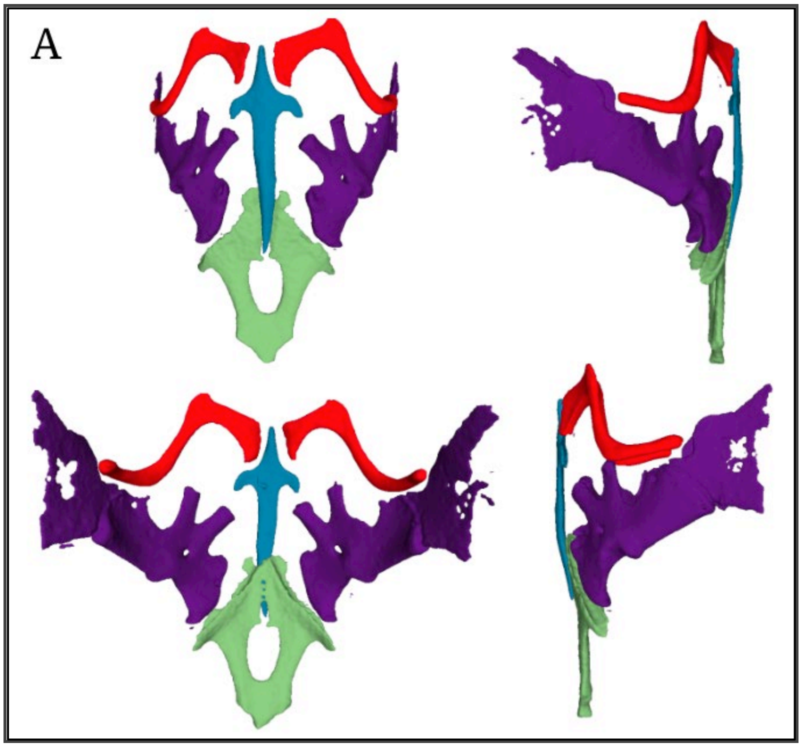
Each bone within the pectoral structure was then analyzed to distinguish any noticeable changes and differences in relation to shape and presence/absence of a feature. Once observations were conducted, findings were then cross referenced with existing literature to reach a broader understanding of the pectoral changes in this clade.

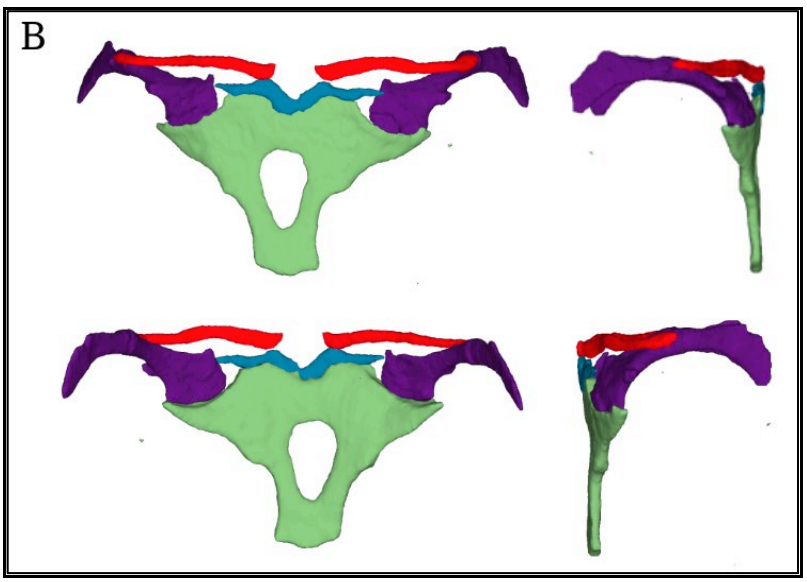
CHAPTER 3

RESULTS

Through processing these scans, a vast range of reduction among the pectoral girdles arose.

The main components of the pectoral girdle consist of the clavicle, coracoid, interclavicle, and sternum. The epicoracoid contacts with the coracoid bone's median and connects laterally to the suprascapular. The coracoid holds the anterior and posterior coracoid fenestrae. The clavicle stretches to the interclavicle. The interclavicle is thin and stretches to the anterior edge of the epicoracoids and the posterior edge of the sternal fontanelle. The sternum contains a presternum with a median sternal fontanelle and two pairs of articulated ribs, that are located medially of the pectoral structure.


A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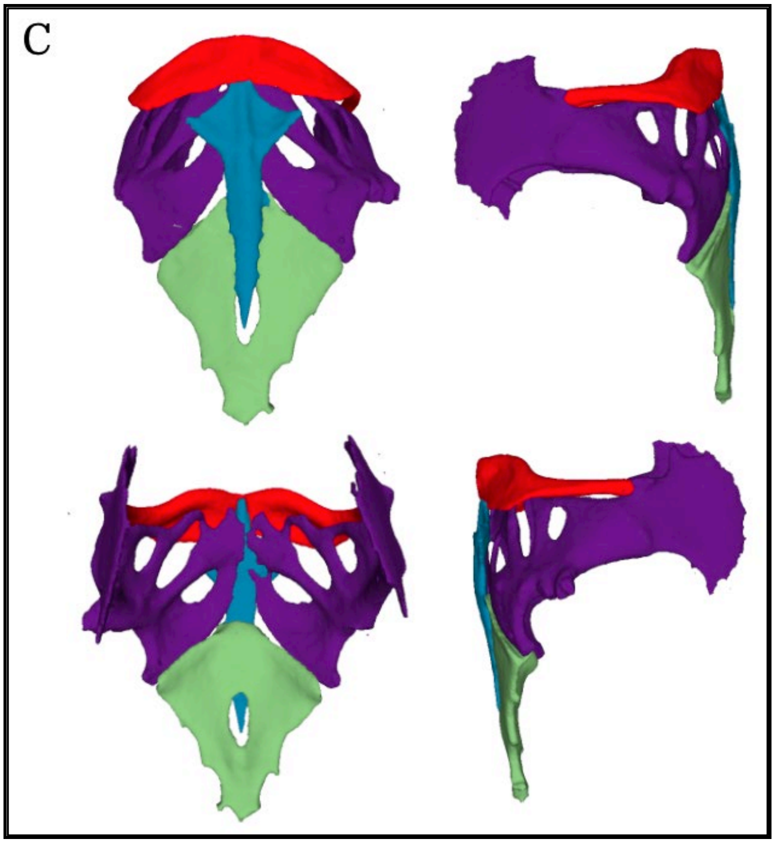
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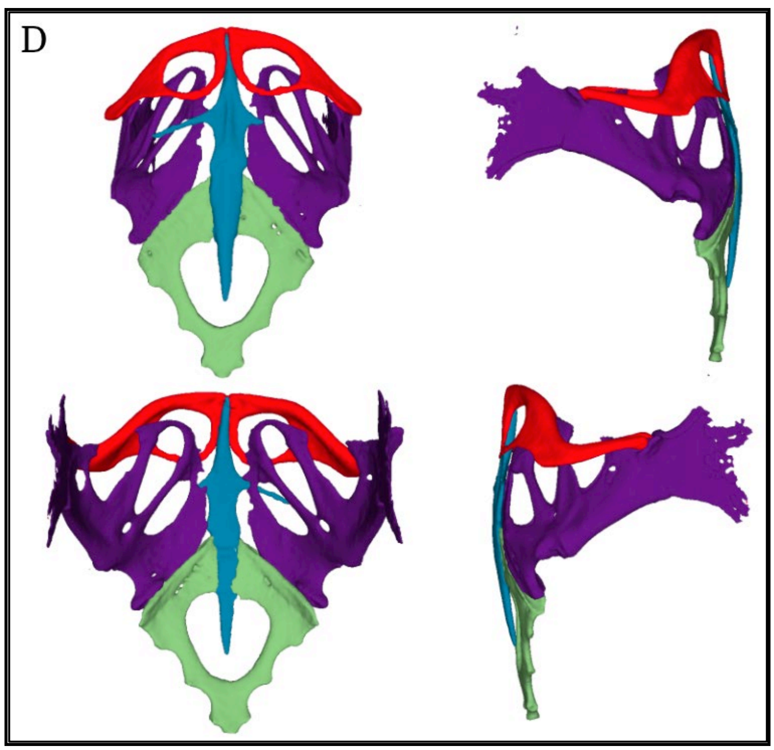
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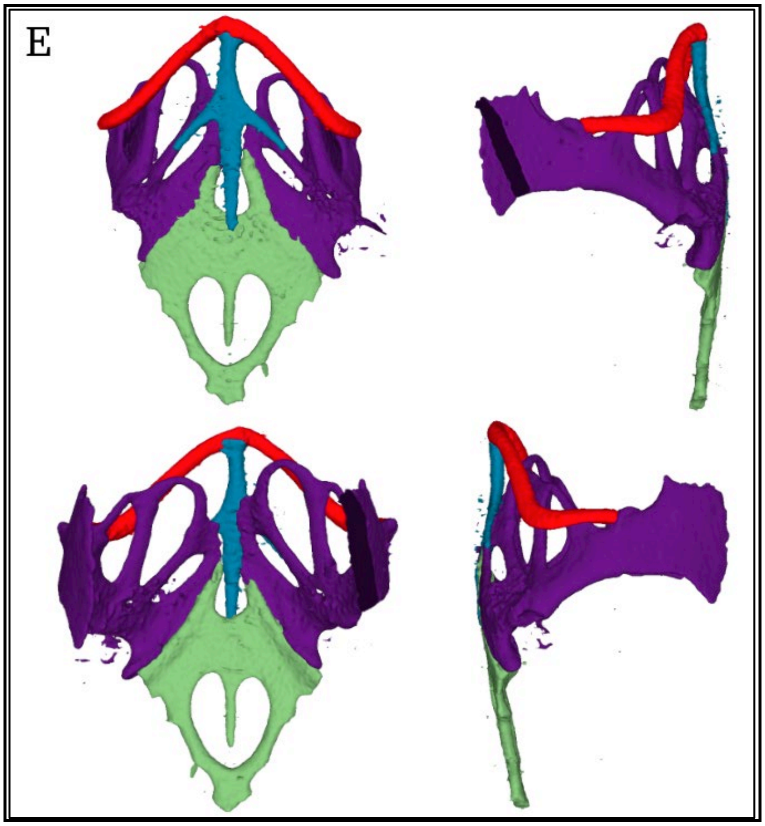

Figure 3.1: CT Segmentations of the Pectoral Girdle Structure within the Gymnophtalminae Lizards (A: *Bachia peruana*, B: *Calyptommatus leiolepis*, C: *Echinosaura horrida*, D: *Leposoma hexalepis*, E: *Tretioscincus sp*.) Each lizard's CT scan was taken in four different views (ventral, dorsal, right lateral, left lateral). Each bone has their own color to distinguish them from each other: Purple: coracoid, Red: clavicle, Blue: interclavicle, Green: sternum.

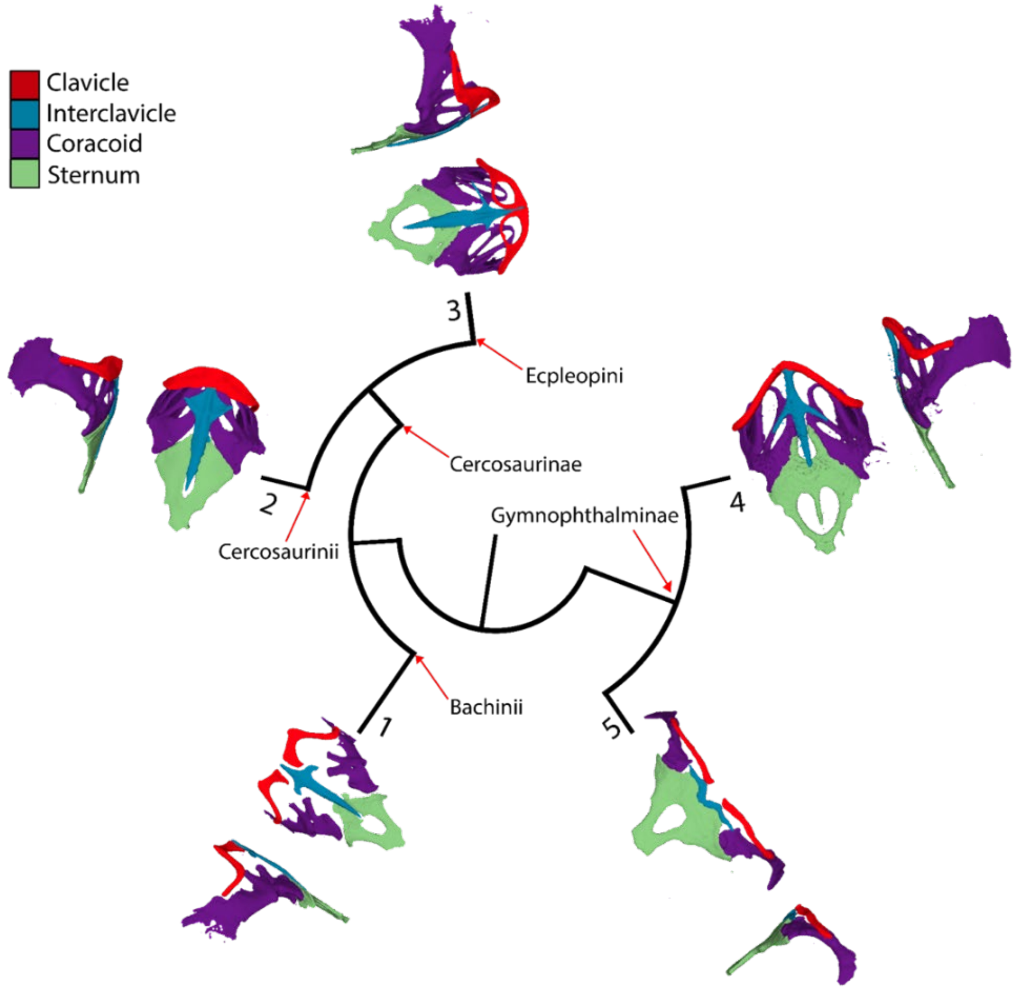

Figure 3.2: Phylogenetic Tree of the Selected Lizards of the Families and Subfamilies within the Gymnophthalmidea Clade
Each species presents with their own CT scan that were processed through Drishti software. The bones of each species' girdle are labeled with the corresponding colors on the legend.

<u>3.1 Coracoid Bone</u>

The coracoid bone exhibits varying levels of reduction throughout the species of interest. *Echinosaura horrida's* coracoid bone appeared as the most developed among the five species where the primary coracoid ray and secondary coracoid ray are present with a thick appearance within the structure. The primary fenestra and the secondary fenestra were both present and fully developed, but the supracoracoid foramen was absent in this bone.

Leptosoma hexalepis's coracoid structure is slightly less developed compared to *E. horrida*, but still retains the primary and secondary coracoid rays; however, these rays appear narrower compared to *E. horrida*. The primary fenestra and secondary fenestra appear larger compared in *E. horrida* as opposed to the previous species. The last feature, the supracoracoid foramen is present among the *L. hexalepis* as opposed to *E. horrida*. *Tretioscincus's* coracoid bone appearance is comparable to *L. hexalepis* due to the presence of coracoid rays and fenestrae. The most prominent discovery is that *L. hexalepis's* primary and secondary coracoid rays are longer, and the primary and secondary fenestrae are slightly larger compared to the previous species. *Bachia peruana* shows a significant reduction compared to the previously discussed species, as the primary and secondary coracoid rays are present, but are not connected with each other to create the fenestrae. *Calyptommatus leiolepis* presents with the most reduced coracoid as the structure appears long and thin with absences of both coracoid rays.

Table 3.1: Coracoid Bone Variations

CORACOID	Primary Coracoid Ray		Secondary Coracoid Ray		Primary Fenestra		Secondary Fenestra		Supracoracoid Foramen		Overall Reduced Level
	Present/ Absent	Size	Present/ Absent	Size	Present/ Absent	Size	Present/ Absent	Size	Present/ Absent	Size	
Bachia peruana	Present	Small, Reduced	Present	Small, Reduced	Absent	N/A	Absent	N/A	Present	Normal	4
Calytommatus leiolepis	Absent	N/A	Absent	N/A	Absent	N/A	Absent	N/A	Absent	N/A	5
Echinosaura horrida	Present	Thick, well developed	Present	Thick, well developed	Present	Normal	Present	Normal	Absent	N/A	1
Leptosoma hexalepis	Present	Thin	Present	Thin	Present	Large	Present	Large	Present	Normal	2
Tretioscincus sp.	Present	Thin	Present	Thin	Present	Large	Present	Large	Present	Normal	3

3.2 Interclavicle Bone

The interclavicle bone of these species presents with significant disparities. *E. horrida* appears with bilateral processes that intersect perpendicularly with the medial process. The top of the interclavicle appears with a strong rhomboid shape. The bottom process is twice as long compared to the top of the structure. *L. hexalepis'* interclavicle presents with a skinnier rhomboid shaped top compared to *E. horrida*. The bilateral structures are significantly thinner compared to *E. horrida*. The top and bottom processes appear to be the same length as the previous species, but significantly more reduced because the processes are thinner and come to a sharper point. *Tretioscincus's* interclavicle is drastically reduced as all four processes are thin throughout the structure. The bilateral processes sit halfway down to the medial process and are angled downwards rather than intersecting perpendicularly. *B. peruana*'s interclavicle is much more reduced compared to *L. hexalepis*, but still retains the rhomboid shape. The bilateral process's ends are significantly shorter and curved. *Calyoptommatus leiolepis's* interclavicle is drastically reduced compared to the rest of the Squamata, as there is no indication of a rhomboid structure nor medial processes. *Calyoptommatus leiolepis* has two bilateral processes resting superiorly to the sternum structure. These processes are both thin and arched.

Table 3.2: Interclavicle Bone Variations

INTERCLAVICLE							
	Lateral Processes		Top Process		Bottom Process		Overall Reduction Ranking
	Length	Reduced?	Length	Reduced?	Length	Reduced?	
Bachia peruana	Short & curved	Yes	Short	Yes	Long	No	4
Calyptommatus leiolepis	Shortest & curved	Yes	N/A	N/A	N/A	N/A	5
Echinosaura horrida	Short & thick	No	Short	No	Long	No	1
Leptosoma hexalepis	Long & thin	Yes	Medium	Yes	Long	No	2
Tretioscincus sp	Long & thin	Yes	Long	Yes	Long	Yes	3

3.3 Clavicle Bone

The clavicle traditionally sits at the top of the pectoral girdle. *E. horrida* appears with the least reduced clavicle. This structure resembles a thick straight band that stretches across the top of the girdle. *Tretioscincus's* clavicle appears thin with a strong upward arch at the middle of the structure. *L. hexalepis's* clavicle also stretches across the pectorals similar to the previous structures; however, prominent reductions occur medially with two large foramina. The lateral processes of *L. hexalepis* appear thin, similar to *Tretioscincus. B. peruana's* clavicle displays a strong upward arch at the medial plane. This clavicle has a significant reduction at the medial plane, with a disconnection between the right and left clavicle Calyoptommatus *leiolepis* displays the most reduction in the clavicle as the bone is thinner to the previous species and has no medial connection between the right and left clavicle.

Table 3.3: Clavicle Bone Variations

CLAVICLE					
	Shape	Width	Foramen	Connected	Overall reduction ranking
Bachia peruana	Arched	Thin	Absent	No	4
Calyptommatus leiolepis	Straight	Thinnest	Absent	No	5
Echinosaura horrida	Straight	Thick	Absent	Yes	1
Leptosoma hexalepis	Straight	Thin	Present, 2	Yes	3
Tretioscincus sp.	Arched	Thin	Absent	Yes	2

3.4 Sternum Bone

The sternum of the Squamata displays the least changes between all the species. *E. horrida* presents with a well-developed rhomboid-shaped sternum to the inferior of the girdle. A small foramen is located centrally of the bone and posteriorly to the interclavicle. *L. hexalepis's* sternum's size is comparable to *E. horrida*. This species' central foramen is much larger compared to *E. horrida*. *Tretioscincus sp.* also presents with a similar rhomboid-shaped sternum. This microteiid displays two foramina stacked on the medial line. The bottom foramen is similar in size compared to *L. hexalepis*., a process extends from the top edge of this foramen to ¾ of the way to the bottom. The top foramen resembles a similar size to *E. horrida's* foramen. The sternum of *B. peruana* is significantly smaller compared to the previously mentioned sternums, but still resembles the rhomboid shape. This species' lateral processes concave slightly inwards. *Calyptommatus leiolepis's* sternum resembles a T-shaped structure, in contradiction to the other microteiid's rhomboid-shaped sternum. The lateral processes are straight and terminate to a sharp point.

Table 3.4: Sternum Bone Variations

STERNUM				
		Foramen		Overall reduction ranking
	Shape	1 or 2	Size	
Bachia peruana	Rhomboid	1	Medium	4
Calyptommatus leiolepis	T-shaped	1	Medium	5
Echinosaura horrida	Rhomboid	2	Small	1
Leptosoma hexalepis	Rhomboid	1	Large	2
Tretioscincus sp.	Rhomboid	1	Top Foramen: Small Bottom Foramen: Large	3

CHAPTER 4

DISCUSSION

Amongst the five lizard species, there is clear evidence of morphological changes in the pectoral girdles. A wide range of well-developed structure to an extremely reduced pectoral structure is evident amongst these five species. Variations most likely occur due to the differences in niches and environmental role each lizard plays in their respected habitat. The reduction of both the clavicle and the interclavicle assists in the snake-like body plan, as it provides higher mobility and flexibility (Russell and Bauer 2008). Likewise, a well-developed tetrapod species depends on a rigid pectoral structure to allow full range of limb movement.

E. horrida appeared as the most developed species amongst the other test subjects, as its pectoral girdle appeared full to scale. *E. horrida's* defined structure coincides with the Ecuadoran aquatic habitat this species resides (Yanez-Munoz et al. 2021). As a tetrapod, *E. horrida* can quickly move through this environment with ease. The highly developed pectoral structure, specifically the clavicle and coracoid, supports the presence of limbs because it allows for stability within the shoulder joint. Because this connection allows for full range of motion of the upper limbs, this lizard can travel swiftly across a terrain.

Furthermore, *C. leiolepis* appears as one of the most reduced gymnophtalidae. This odd structure became distinctly different compared to the rest of the selected microteiids. The clavicle, coracoid, and interclavicle presented with severe reduction through either thinning or splitting of the structure. This divergence concluded in a smaller and lighter framed pectoral area. The compact structure assists in this lizard's high degree of flexion and range of motion, which in turn promotes a snake-like body plan. C. *leiolepis's* natural adaptation coincides with Brazil's burrowing environment, where it can effortlessly slither throughout the terrain (Holovacs et al. 2020). Limbs are still present within the species, but it is severely reduced. With the little usage for a fully developed limb structure, this species naturally selected for limb reduction throughout this genus's generations. Consequentially, this limb adaptation impacted on the pectoral girdle through the layers of reduction.

Additionally, *Bachia peruana* also presents with a peculiar pectoral structure. As being a member in the brachii subfamily, this gymnophtalidae resides amongst the leaves of the Peruvian forest bed, where it depends on a highly flexible body form to travel. Because this microteiid depends on the loose surface debris to travel, the need for limbs is not mandatory. Similar to *C. leiolepis*, this lizard presents with severe reduction of limbs as shown with significantly small legs. With the negative selection from the environment, this pushes the species towards a reduced pectoral structure and snake-like body form.

Within the selected Gymnophtalminae pectoral girdle, the bones that underwent the most reduction are the coracoid and clavicle. The coracoid structure experienced reduction through absence of the rays and fenestrae between some of the species. Likewise, the clavicle either experienced narrowing of the bone, separation of the right and left processes, and or presence of fenestrae. These two bones displayed significant divergence in comparison to the other bones in the girdle. Both the coracoid and clavicle allow for stabilization of the shoulder joint. Since some microteiids present with a reduction or absence of limbs, the need for a stabilized shoulder joint is not required. Species such as the *Bachia peruana* and *Calyptommatus leiolepis* experience a lesser need for limbs due to their burrowing habitat. This environment led to the divergence towards thinner and/or smaller bones, due to limited usage of the upper limbs.

Contrary, the sternum underwent the least reduction among the five selected species. The bone maintained a similar rhomboid structure that is located inferiorly to the coracoids of the lizards. This consistent sternum development is analogous to the sternum's prominent purpose of protecting the internal organs, therefore morphological changes are not needed in this bone. However, slight variations of the sternum were presented in the *C. leiolepis*, as it presented as a "T-shaped" sternum instead. Regardless of the shape, the sternum maintains the similar intent of protecting organs and tissues.

With these polar findings, both genetics and environment play a pivotal role in the adaptation towards either a reduced or a well-developed structure. The case of Gymnophtalmidae exemplifies the trend of limb reduction and snake-like body within the Squamata. Both the niche and the phylogenetic genetic makeup contribute to the changes in pectoral bones. Pectoral reduction occurs distally from the girdles through the loss of limb elements. These reductions allow for further discussion on the overall function and movement within the Squamata. More research should be conducted to examine more structural changes amongst different lizard lineages to gain a better understanding of the adaptation of the clade.

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BIOGRAPHICAL INFORMATION

Thu Tran grew up in Keller, Texas. She attended the University of Texas at Arlington for an Honors Bachelor of Science in Biology with a minor in Psychology. During her time at the university, she participated in leadership programs and organizations including the Honors College, Undergraduate Teaching, and Pre-Health groups. She earned several accolades notably the Josephine Porter Endowment Scholarship and Laurence E. Baker Memorial Scholarships that were both awarded for exquisite demonstration of a proactive role in premedical studies and leadership work at the University of Texas at Arlington. She is set to graduate in Spring 2022.

Post-graduation she hopes to pursue a career in healthcare. She is currently making strides towards this endeavor whether it is volunteering, gaining clinical knowledge, or seeking educational challenges. She frequently volunteers her time at Bob Mann's Medical Clinic at Mission Arlington where she assists in patient care through scheduling and triaging. Additionally, she began a community organization, called The Canister, in 2020, where she organizes donation drives and volunteering events year-round for the local community. In her free time, she enjoys experiencing new cuisine and exploring different media of artistry such as painting and drawing.