# Investigating the Patterns of Convergence in Pectoral Girdle Reduction During the Evolution of Limblessness in *Lerista* (Squamata: Scincidae)

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Geosciences

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December 25, 2018
Blacksburg, VA

Keywords: limb loss, evolution, squamate, skink, lizard

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## ABSTRACT

Over 30 tetrapod groups have evolved a snake-like, elongate, limbless body plan. Studies of the patterns of limb reduction have revealed a close relationship between the reduction of the limbs and body elongation; however, the loss of the skeletal elements that support the limb, the pectoral girdle, has been less thoroughly examined. Here, I use computed tomography to generate three dimensional models of the pectoral girdles of an Australian skink genus, *Lerista*. This group contains pentadactyl species, fully limbless species and many species with intermediate morphologies. I employed a 3D geometric morphometric analysis to compare the shapes of these structures and revealed that the reduction of the pectoral girdle is correlated with the degree of loss in the limbs. The girdle in species with well developed limbs is longer and more narrow than the short, broad girdle of species with highly reduced or absent limbs, but the degree of reduction is only loosely correlated with the degree of reduction in the limbs. Certain events appear to occur concurrently such as the losses of the coracoid foramen and the humerus, but other events such as the loss of the epicoracoid are not consistently associated with any other event. The extent to which limb reduction is associated with the degree of pectoral girdle reduction and the morphology of the girdles appears to be closely associated with subclade, with individuals from closely related clades showing dramatically different degrees of girdle reduction despite similarities in the degree of limb loss. Despite these differences, the patterns in *Lerista* are generally more similar to each other than to those of other lizard groups, and more similar to those of other skinks than to those of other groups of lizards. Though some aspects of

limb loss are common to all lizard groups that have evolved a serpentine body plan, this study shows that, even in closely related groups, this transition does not proceed in exactly the same way.

# Investigating the Patterns of Convergence in Pectoral Girdle Reduction During the Evolution of Limblessness in *Lerista* (Squamata: Scincidae)

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## GENERAL AUDIENCE ABSTRACT

Over 30 tetrapod groups have evolved a snake-like, elongate, limbless body plan. Many studies of the patterns of limb reduction (evolutionarily losing fingers and toes) have revealed a close relationship between the reduction of the limbs and body elongation; however, the loss of parts of the internal skeleton that support the limb (e.g. shoulder blade, collar bone) have been less thoroughly examined. Here computed tomography was used to generate three dimensional models of the pectoral girdle, the array of limb support bones, of the Australian lizard genus *Lerista*. This group contains species that have five fingers, species which are fully limbless (and thus have no fingers), and some that have intermediate numbers of fingers. I quantitatively compared the pectoral girdle shapes, which revealed that the reduction of the pectoral girdle occurs gradually and in a series of steps that are loosely associated with how many fingers are present. The pectoral girdle as a whole shows a more obvious reduction in length than in width, with this length reduction being loosely associated with the number of fingers present. Certain events appear to occur concurrently such as the loss of a small hole in one of the bones through which nerves that control the arm pass and loss of the upper arm bone. However other events, such as the loss of a cartilaginous structure called the epicoracoid is not consistently associated with any other event. The extent to which limb reduction is associated with the extent of pectoral girdle reduction is group dependent, with individuals from closely related groups showing dramatically different degrees of pectoral girdle reduction despite a similarity in the degree of digit loss. Despite these differences, the patterns in *Lerista* are more similar to each other than to

those of other lizard groups, and more similar to those of other skinks than to those of other groups of lizards. Though some aspects of limb loss are common to all lizard groups that have evolved a snake-like body plan, this study reveals that even in closely related groups, this transition does not proceed in exactly the same way.

I dedicate this thesis to my family to thank them for encouraging my curiosity and for allowing me to play with worms in the mud when I was a kid.

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# ATTRIBUTION

This project was conceived of and designed by KK and MRS. Data collection and analyses were conducted by KK. All figures and were done by KK with input from MRS, and writing was done by KK, with advisement from MRS.

# INVESTIGATING THE PATTERNS OF CONVERGENCE IN PECTORAL GIRDLE REDUCTION DURING THE EVOLUTION OF LIMBLESSNESS IN *LERISTA* (SQUAMATA: SCINCIDAE)

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## 1. Abstract

Over 30 tetrapod groups have evolved a snake-like, elongate, limbless body plan. Studies of the patterns of limb reduction have revealed a close relationship between the reduction of the limbs and body elongation; however, the loss of the skeletal elements that support the limb, the pectoral girdle, have been less thoroughly examined. Here, I use computed tomography to generate three dimensional models of the pectoral girdles of the Australian skink genus, *Lerista*. This group contains pentadactyl species, fully limbless species and a number with intermediate morphologies. I employed a 3D geometric morphometric analysis to compare the shapes of these structures and revealed that the reduction of the pectoral girdle is correlated with the degree of loss in the limbs. The girdle in species with well developed limbs is longer and more narrow than the short, broad girdle of species with highly reduced or absent limbs but the degree of reduction is only loosely correlated with the degree of reduction in the limbs. Certain events appear to occur concurrently such as the loss of the coracoid foramen and the humerus, but other events such as the loss of the epicoracoid are not consistently associated with any other event. The extent to which limb reduction is associated with the degree of pectoral girdle reduction and the morphology of the girdles appears to be closely associated with subclade, with individuals from closely related clades showing dramatically different degrees of girdle reduction despite a similarity in the degree of limb loss. Despite these differences, the patterns in *Lerista* are more similar to each other than to those of other lizard groups, and more similar to those of other skinks than to those of other groups of lizards. Though some aspects of limb loss are common to all lizard groups that have evolved a serpentine body plan, this study shows that even in closely related groups, this transition does not proceed in exactly the same way.

## 2. Introduction

Convergent evolution is often seen as evidence of adaptation, and investigating this phenomenon is a way to gain insights into the mechanisms that generate biodiversity and the constraints that influence the evolution of observable phenotypes (e.g. Simpson, 1953; Conway Morris, 2009; Losos, 2018). Similar morphologies often appear in closely related lineages and it is often assumed that these lineages achieve these morphologies via similar pathways (this assumption could be broadly referred to as parallel evolution). Though there are many observed instances of apparent parallel evolution, they are difficult to study as the evolutionary pathways to these phenotypes can only be revealed by extensive and expensive genetic or developmental work or by the presence of extant or fossil intermediate forms. Whether or not parallel evolution should be distinct from convergent evolution is a subject of ongoing investigation and debate(Arendt and Reznick, 2008).

One convergent system that is particularly well-suited for studying this question is the evolution of the elongate, limbless vertebrates. The evolution of this form involves a number of morphological changes, most notably body elongation and the reduction or complete loss of appendages (Gans, 1975; Greer, 1991). Limblessness has evolved independently at least 26 times in squamates (Wiens et al., 2006), at least three times in amphibians (e.g. Caldwell, 2003; Marjanovic and Laurin, 2009), and at least 19 times in teleosts (Jackson et al., 2018). Because limbless tetrapods appear in a wide range of latitudes, and on all continents except Antarctica, there is a large sample size of distantly related limbless taxa that have encountered a wide range of potential selective pressures (Rickelfs et al., 2007, Brandley et al., 2008; Pyron, 2014).

Additionally, the phylogenetic spread of this form is not uniform. By far the group with the highest number of limbless species is Squamata, and of the approximately 26 independent evolutionary limb loss events in this group, the largest proportion have been in the family Scincidae (Brandley et al., 2008; Miralles, et al., 2012). Within this group, there have been at least 31 instances of limb reduction (Greer, 1991), at least eight independent losses of the hindlimb, and 11 complete losses of the forelimb (Brandley et al., 2008). The existence of both closely and distantly related limbless clades may provide a clear idea of the role of phylogenetic signal in the various changes involved in the evolution of limblessness.

Another advantage to studying convergent limb loss in limbless tetrapods is that most relevant squamate groups contain at least some remnants of limb-related structures such as vestigial pelvic spurs or internal structures such as muscles that are homologous with those involved in limb movement in other squamates (Furbringer, 1870; Tsuihiji et al., 2006; Lewis, 2015). Some of these limb loss events occurred many millions of years ago, and in these groups "transitional" or intermediate morphologies are uncommon (Zaher and Rieppel, 1999; Caldwell, 2002; Kearney, 2002; Brandley et al., 2008). However, several groups contain species in various stages of limb reduction (Greer, 1987; 1990; 1991). If intermediate morphologies represent the changes undergone by more highly limb-reduced relatives, it may be possible to reconstruct in detail the nature and order of the changes involved and to compare them with other lineages.

There has been a great deal of work done to that end focusing on digit and limb reduction and body elongation (Gans, 1975; Caldwell, 2002; Brandley et al., 2008). Those studies have found that the degree of reduction in the limb (whether measured by phalangeal count, digit number, or relative limb length) are negatively correlated with body length (measured either as snout-vent length or by number of presacral vertebrae) (Gans, 1975; Greer, 1991). However, the relationship is not perfect. It appears as if there are thresholds of correlation among these traits in which lizards of a certain relative length express certain other traits related to limblessness

(Lande, 1978; Brandley et al., 2008; Morinaga and Bergman, 2017). For instance, species with four or fewer digits always have a total-length to head-length ratio greater than 20 (Brandley et al., 2008).

However, the limbs are not the only structures lost during limb reduction. The internal limb support structures, the pectoral and pelvic girdles, are also reduced and lost in limb-reduced squamates (Cope, 1892; Müller, 1900; Camp, 1923; Stokely, 1947; Kearney, 2002, Miralles et al., 2016; Liniewski et al., 2016). In most lizards, these are modules of multiple bones that serve as an anchor for limb-controlling muscles and as the connection between the appendages and the axial skeleton (Evans, 2008) (Fig. 1). The limb girdles are comprised of bones that serve complex functions and show a great deal of variation among and between different squamate groups (Evans, 2008) (Fig. 2). For the most part, phalanges have a relatively simple morphology, and, in the context of evolutionary limb loss, have been primarily studied in terms of simple presence/absence (Greer, 1991; Shapiro, 2002). The bones of the zeugopod and stylopod have been studied in terms of length and presence/absence (Greer, 1990; Brandley et al., 2008). Using only these simple metrics, especially in developmentally and functionally similar structures, may obfuscate potential variation or similarities that would be apparent using more complex metrics. In other words, investigating limb loss using systems that vary in more than one dimension may have more possible modes of change and may make it possible to more accurately observe similarities and differences between groups. Observing these similarities and differences may provide insight into the similarities and differences in the mode of evolutionary change within and between clades.

Numerous studies have considered limb-girdle morphology when discussing limb loss, but the study of limb girdles presents challenges that do not appear in the study of the limbs and the vertebral column (Cope, 1892; Müller, 1900; Camp, 1923; Stokely, 1947; Kearney, 2002, Liniewski et al., 2016). Digit counts and snout-vent length (SVL) may be obtained from whole specimens, and phalangeal and vertebral counts may be obtained from skeletal specimens or x-ray radiographs (Greer, 1997; Brandley et al., 2008; Andrade et al., 2016). The limb girdles, however, are more difficult to observe. They are internal structures and therefore cannot be observed in whole specimens. In limb-reduced specimens, the girdles often do not articulate with the axial skeleton and therefore specimen skeletonization causes a loss of positional information (Evans, 2008; K. Koeller, personal observation). Girdles are often reduced in terms of structural complexity and are difficult to identify without positional information (Cope, 1892; Müller, 1900; Camp, 1923; Stokely, 1947; Kearney, 2002, Liniewski et al., 2016).

Another challenge to the inclusion of limb-girdle information and to the study of convergent limb loss in general has been an unresolved squamate phylogeny (Gans, 1975; Greer, 1991; Wiens and Slingluff, 2001). Until the advent of molecular phylogenetics, relationships were determined primarily by morphology, but, as limbless squamate groups possess very similar phenotypes, their phylogenetic positions were difficult to accurately determine (Wiens and Slingluff, 2001). Before there was a resolved squamate tree, many studies on limb loss arranged specimens in a morphocline from what was assumed to be the most plesiomorphic state to most derived, or most snake-like. This approach provided information about the general steps and correlates of limb loss but prevented meaningful comparison between different groups (Gans, 1975; Reippel, 1988; Greer, 1991; Caputo et al.,1995; Lee, 1998). Additionally, the girdle elements of many limb-reduced squamates are mere slivers of bone without distinct, identifying characters (Cope, 1892; Müller, 1900; Camp, 1923; Stokely, 1947; Kearney, 2002). Without information on the limb girdles of close relatives, the identification of these elements proved to be difficult (Cope, 1892; Camp, 1923; Stokely, 1947; Kearney, 2002).

To better understand how the limb girdles change in relation to other aspects of the skeleton during limb loss, this study focuses on the Australian skink genus *Lerista*. This group of 'sand swimmers' has between 96-101 species and includes fully pentadactyl, fully limbless species, and many intermediate morphologies representing different stages in the limb loss transition (Fig. 3)(Wilson and Swan, 2013; Cogger, 2014; Couper, Amey and Wilmer, 2016). Approximately 94% of *Lerista* species have fewer than five digits and only approximately 2% are completely limbless, meaning the vast majority of species in this clade represent intermediate conditions between the most plesiomorphic and most derived conditions (Fig.4). Of those, there is a wide range of morphological diversity with at least 12 different combinations of digits (Greer, 1990). Yet these lizards do not represent a single transition. There have been an estimated 27 instances of digit reduction and four independent losses of all digits from a pentadactyl or tetradactyl condition in this genus over at most 13.4 million years (Skinner, et al., 2008). This rapid divergence time and number of independent losses makes this system ideal for comparing limb loss events of closely related species.

The remarkable potential of these lizards to help answer evolutionary questions has not gone unnoticed. The patterns of body elongation, digit loss, and limb proportions as well as their rates of evolution have been studied in some detail (Greer 1987, 1990; Skinner, 2008; Morinaga and Bergman, 2017). The elongation of the body and the reduction of the digits appears to occur in a largely linear fashion, but the length of the limbs compared to SVL is not greatly reduced until the body reaches a certain length relative to skull length (Morinaga and Bergman, 2017). Digits are lost in a predictable pattern (1>5>2>3>4), yet there is at least some variation in the number of phalanges lost, with at least two pes formulae that are developmentally mutually exclusive and imply at least two different pathways of loss (Greer, 1987; 1990). The previous studies cited here, particularly Morinaga and Bergman, 2017, concluded that the transition to limblessness in *Lerista* happens in much the same way in both major clades, with some differences. However, as discussed above, phalangeal counts and forelimb measurements may produce limited useful information applicable to the similarities and differences between lineages and a more confident conclusion may be reached with additional three-dimensional information.

One nearly unexplored aspect of *Lerista* osteology is the pectoral girdle. To date, the pectoral girdle morphology of only two species of *Lerista* has been described (Moch and Senter, 2011). Those two species, *L. stylis* (USNM 128640) and *L. carpentariae* (USNM 128409) were described from X-ray radiographs of single specimens as having only small, unidentifiable slivers of bone (Moch and Senter, 2011). Those two species are closely related, and both have only small, external hind limbs (Moch and Senter, 2011; Table 1; Cogger, 2014; Zheng and Wiens, 2016). In *Lerista*, as with most limbless species, the reduction of the forelimb is positively correlated with that of the hindlimb (Morinaga and Bergman, 2017; Greer, 1990; Brandley et al., 2008), which means *L. stylis* and *L. carpentariae* likely represent more derived, “late” stages of limb loss, but, as there are species with no limbs at all, potentially not the last stage of pectoral girdle loss. About 22% of species of *Lerista* lack forelimbs entirely, and there are several with highly reduced forelimbs. If, as Stokely hypothesized in 1947, the loss of the pectoral girdle is associated with the loss of the forelimb, it is likely that *Lerista* contains a complete, or nearly complete, series of stages of pectoral girdle loss. This series may allow for the identification of elements in this and other groups which have so far been elusive and allow for a detailed examination of the patterns of morphological change.

The identification of the longest persisting girdle elements in limbless taxa has been a subject of interest in previous research, but a largely fruitless pursuit (Cope, 1892, Stokeley, 1947). In most groups, the first element lost is the interclavicle and the last remaining elements have been identified as either the clavicles or scapulocoracoids based largely on positional information. Comparisons of the order of elements lost between independently limbless groups would provide information about whether these events occur in the same way across different groups. In the girdles of highly limb-reduced skinks from other groups, it appears as though the scapulocoracoids are more robust than the clavicles and, based on the medioposterior angle of the remaining girdle elements of *Lerista stylis*, it seems more likely that the scapulocoracoid is the last remaining element in the pectoral girdles of *Lerista* (Stokely, 1947, Moch and Senter, 2011).

In terms of the pectoral girdle morphology, I predict that the pectoral girdles of species with relatively nonreduced limbs will have a conserved morphology and those with highly reduced limbs will have much more variation in pectoral girdle morphology. Pectoral girdle elements serve as attachment points for limb muscles, but if there are reduced limbs that use the muscles less or in different ways, or no limbs at all, there would be no selective pressure to maintain morphological integrity. A reduction in stabilizing selective pressures would mean relaxed selection and the possibility of greater morphological variation. We therefore predict that species with well developed limbs will have similar pectoral girdles and that species with highly reduced limbs will have more variable pectoral girdles. We also predict that these reduced pectoral girdles will be more similar among closely related groups.

## Institutional Abbreviations

AMNH- American Museum of Natural History, New York, New York, USA; MCZ- Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; CAS- California Academy of Sciences, San Francisco, California, USA; CU- Cornell University, Ithaca, New York, USA; USNM- Smithsonian National Museum of Natural History, Washington, D.C., USA; ROM- Royal Ontario Museum, Toronto, Ontario, Canada; UMMZ- University of Michigan, Ann Arbor, Michigan, USA; YPM- Yale Peabody Museum, New Haven, Connecticut, USA., SAM- South Australia Museum, Adelaide, South Australia, Australia, VTPCC- Virginia Tech Paleobiology Comparative Collection

## 3. Materials and Methods

Here, I took advantage of advances in imaging technology and phylogenetics which make the study of internal structures possible (Miralles, 2015). I used geometric morphometrics to incorporate these new findings with known information about relative changes in limb and body lengths. I then used these data to explore the similarities and differences between the independently limbless clades within *Lerista*. In future studies these findings may be used to compare changes in *Lerista* with other squamate groups to answer broader questions about the process of morphological evolution.

**a. Study Species and Specimen Selection:** My total sample was comprised of 34 specimens of *Lerista*, representing 31 species distributed across the genus (Fig. 4; Zheng and Wiens, 2016). I sampled all species available in North American museum collections as well as several from the South Australia Museum, using one specimen per species, with the exception of *Lerista microtis*, for which two were used. One of the specimens, CAS 77518, was identified upon original collection in 1930 as *Lerista timida* but this appears to be a misidentification; based on scale count and body metrics, CAS 77518 appears to be *L. microtis* (Storr, 1991; Cogger, 2014). This sampling of *Lerista* represents 12 of the 15 possible combinations of limb states (meaning forelimb and hindlimb combinations) found in this genus and all forelimb states found in the genus (Table 2). When possible, adults were sampled; ontogenetic stage was estimated through comparison of each specimen's snout-vent length (SVL) with the average adult (Cogger, 2014).

Two outgroups were included in the study: *Ctenotus robustus,* and *Hemiergis peronii*, giving a total of 34 sampled species. Outgroups were chosen based on phylogenetic position and ecology. *Ctenotus robustus* is in the sister genus to *Lerista* (Zheng and Wiens, 2016) and is another Australian skink which shares its wide range with many species of *Lerista* (Cogger, 2014). This primarily insectivorous skink spends some of its time burrowing and some of its time on the surface, thus sharing some ecological traits with *Lerista*, yet all members of *Ctenotus* are pentadactyl (Cogger, 2014). *Hemiergis peronii* was selected because it is an ecological generalist found to be distant from *Lerista* both phylogenetically and geographically; however, *Hemiergis* is tetradactyl, and this genus contains other members with some amount of digit loss (Zheng and Wiens, 2016; Cogger, 2014).

All specimens of *Lerista* were stored in 75% ethanol with the exception of *Lerista kendricki* (CU-11970), which was stored in isopropyl alcohol.

**b. Phylogenetic Sampling:** For this project, one or two specimens were chosen per species to gain a broad understanding of how the limb-loss transition occurs in *Lerista*, whether there are differences between limb-loss events within the genus, and whether the changes have a phylogenetic signal. Though there is a lot to be gained from this broad sampling, it does mean that there are potentially unknown differences in morphology throughout ontogeny and within species. Testing those aspects is beyond the scope of this work.

Phylogenetic analyses were performed using the tree from Zheng and Weins, 2016. The tree was trimmed to include only the specimens relevant to my study in R using the phytools (Revell, 2012), rotl (Michonneau, 2016), caper (Orme, 2018), geiger (Harmon et al., 2015), and phangorn (Schliep, 2011) packages (Appendix A).

**c. Photography:** Specimens were photographed using three different camera lens types. Photos encompassing the whole specimen and its associated labels were taken using a Nikon D-3300 with an AF-S DX VR Nikkor 18-55mm lens with an ML Tools 6'' digital caliper set for a scale bar. A Canon EOS 7D with a Tamron 90mm lens was used to capture close-up features such as the face and manus, providing a record of digit measurements and facial scale counts. A dissection microscope and Nikon D-3300 was used to take photographs of small elements, enabling distinction between claws and scales at the ends of limbs to determine limb state.

**d. X-Ray Computed Tomography:** X-ray radiographs may provide information about presence/absence of bony limb girdle structures, but several components of the girdles are cartilaginous and difficult to see, especially when they are thin or overlap denser elements (Evans, 2008). Specimen placement in x-rays can also drastically affect the appearance of elements and therefore affect perceptions of symmetry, placement, and identity (Moch and Senter, 2011; Andrade et al., 2016). Clearing and staining of specimens is another way to study these internal structures to provide information on structure, composition, and placement, but this approach is destructive and thus not feasible for large scale studies. An alternative to these methods is the use of X-ray Computed Tomography (CT), which results in a three-dimensional digital representation of internal structures that can reveal positional information and eliminate the problem of dense elements obscuring less dense ones, while leaving specimens intact. This method can provide fine details in even small specimens, allowing for the visualization of even very small elements. (Kearney, 2002; Miralles, 2016).

I obtained osteological data using high resolution X-ray computed tomography (CT) to examine pectoral girdles *in situ*. The majority of CT scans were obtained by Michelle Stocker with the Nikon XT H 225 ST high resolution X-ray CT scanner at the SMiF Laboratory at Duke University. Prior to scanning, individuals were wrapped in cheesecloth dampened with their respective storage liquid, and secured in a sealed plastic vial. Specimens were scanned individually or in pairs. Reconstructions on that instrument were performed in Image J (Rashband, 2008). *Lerista praepedita* (MCZ R-187806) was scanned in the Socha Lab at Virginia Tech using a Bruker Skyscan 1172 micro CT scanner. Specimens scanned in the Skyscan scanner were not wrapped in cheesecloth and were scanned individually. *Lerista kalumburu* (WAM R-100188) and *L. apoda* (WAM-R-168495) were scanned at the Museum of Western Australia by Mark Hutchinson using a Bruker Skyscan 1076 micro CT scanner with modified camera, and *L. ameles* (SAMA-R-55806) was scanned at the University of Michigan Museum of Zoology by Erin Westeen using an X-Tek XT H 225ST micro CT scanner.

CT data was postprocessed using Mimics v. 19.0 (Materialise, Leuven, Belgium; http://www.materialise.com/en/medical/software/mimics) at Virginia Tech. Because some structures were more ossified than others, most exported surfaces were comprised of several layers of different densities, capturing not only bone, but calcified cartilage as well, and represent interpretations of appropriate density by the author. Pectoral girdle elements were isolated as individual masks using the segmentation tool as well as the thresholding tool and were converted to 3D objects as .ply files for 3D geometric morphometric analysis. 3D objects were further processed in Meshlab (Cignoni et al., 2011). Using this program, high density objects such as ribs and stomach contents were removed using the select vertexes and delete selected vertices tools. Additionally, multiple meshes of different densities for individual specimens were combined into single objects. All meshes were then exported as nonbinary .ply files.

**e. Measurements:** Whole body measurements were primarily made using an ML tools 6’’ digital caliper set and were rounded up to the nearest tenth of a millimeter. Each measurement was taken three times, and an average was used as the final reported value. Some specimens were preserved in a curled position, preventing accurate measurements with calipers, and the whole-body photos of those specimens were used for measurements in Maya (Autodesk, 2015) using the polyline tool. Some specimens were too distorted from their preservation to be measured in this way so for these specimens and the specimens received from the South Australia Museum, SVL and limb length estimates from Cogger, 2014 were used. In the case of limb measurements, both right and left limbs were measured, with the exception of several specimens with broken, deformed, or missing limbs. These measurements were still made but were not included in limb length averages. Measurements can be found in Table 2.

Pectoral-girdle and skull measurements were made digitally using CT data. Meshlab was used to convert 3D objects from Mimics into .stl files. These objects were measured in Maya (Autodesk, 2015). For pectoral girdle measurements, the distance tool was used to measure the distance between the dorsalmost points of the suprascapulae to obtain width, and from the anteriormost point of the interclavicle to the posteriormost point of the presternum to obtain length. Because the pectoral girdle curves to follow the body wall, absolute width does not capture the true range in size of the pectoral girdles and causes differences between flatter pectoral girdles to be more apparent than more curved girdles (Fig. 5). To account for this, measurements that accounted for the curvature of the girdle were obtained using the EP curve tool in Maya (Autodesk, 2015). This created a curved line that followed the bend of the clavicles from the distal end of one suprascapula to the distal end of the other (Fig. 6). The Arc measurement tool was used to measure the length of the curve. Because of differences in scan quality, the distalmost extents of the suprascapulae were not represented in the 3D objects for some specimens. Therefore, a similar measurement was acquired from the distalmost end of one scapula to the other. A length measurement was acquired in a similar way, although because the apex of the clavicle was offset from the midline in most specimens, a curved line was created between the clavicles with the measurement made between that line and the posteriormost point of the sternum. Each of these digital measurements was made three times, and the average was used in the analysis. Skull measurements were obtained using the measurement tool in Meshlab. Mesh files of nearly complete or complete specimens were loaded into Meshlab and osteoderms were digitally removed from the ventral side of the cervical region. The measurement tool was then used to obtain the distance between the anteriormost point of the atlas to the anteriormost tip of the premaxilla. All measurements were taken three times and averaged. It was noted that some of the specimens were segmented in a space that used micrometers and some were measured in a space that measured in millimeters. Meshlab did not correct for this and some of the measurements were in micrometers and some in millimeters. This was corrected for in the analysis.

**f. Morphocline, Limb State and Limb Rank:** To study the relationships between various aspects of limb reduction such as body elongation and a reduction in phalangeal number, researchers have often arranged specimens in a "morphocline" from most plesiomorphic to most derived. However the various aspects of limb loss are usually not perfectly correlated. I found that determining the metric by which I defined plesiomorphic and derived was difficult and resulted in different arrangements when I used different parameters. I investigated SVL relative to head size, forelimb length relative to SVL and digit number but decided to come up with a ranking system to at least attempt a morphocline. This system was the limb rank. First, I binned specimens based on their limb state. The categories I used were five digits, four digits, three digits, two digits, one digit, stylar, nub, non-protruding, and no limb (Fig.3). A stylar limb has no digits and is longer than it is wide. A "nub" is defined as a limb that is roughly as long as it is wide. These are categories that have been used by Cogger, 2014 and other researchers based on external morphology. These authors have often made a distinction between small limbs and no external limbs, which is useful when identifying species in the field. However, this study concerns internal anatomy and therefore I added the non-protruding category. Specimens with non-protruding limbs have a small humerus that may not be evident externally, or they may have a tiny claw-like structure that does not protrude past the body wall (Fig. 7). I distinguished between a non-protruding humerus and no humerus at all.

After binning specimens based on forelimb state, I binned them further by hindlimb state, then arranged them by the length of the forelimb relative to SVL for all specimens with a protruding limb. For specimens with non-protruding limbs, I ordered them based on the relative length of their hindlimbs. Though there does not seem to be one true metric by which to measure the degree of reduction, the use of limb rank can be used to illustrate a morphocline and is a compromise between using digit counts and relative limb length.

**g. 3-Dimensional Geometric Morphometric Analysis:** The shape analysis was conducted using a geometric morphometric analysis using landmarks that represent homologous anatomical features (Fig. 8) and semilandmarks that follow the curvature of certain regions (Fig. 9). Detailed descriptions of each landmark and guidelines for standardized placement are given in Table 3. Thirty-seven fixed landmarks and 20 semilandmark curves were used and allowed for the inclusion of almost all specimens in the analysis with the exception of specimens with CT scans of poor quality, in which landmarks could not be accurately placed, and highly reduced specimens such as *Lerista apoda* (WAM-R-168495) and *L. carpentariae* (USNM 128409). Landmarks were digitized using Landmark Editor 3.6 (Wiley et al., 2007). To minimize human-introduced bias and errors, specimens were digitized in a random order, and the first ten to be digitized were redigitized. The landmark placement process was done over the course of two consecutive days to reduce human-introduced variation.

A PCA analysis was used to analyze and summarize similarities and differences in the shapes of the pectoral girdles. This analysis was chosen as it would provide an illustration of how similar the specimens were to each other and would allow me to test whether or not well developed pectoral girdles are more similar to each other than are highly reduced pectoral girdles.

All specimens were aligned in a generalized Procrustes analysis in the R package Geomorph (Adams et al., 2014). PCA and phylogenetic PCA analyses were performed in order to test how conserved pectoral girdle anatomy is among these specimens. We also conducted regression analyses using the R package ggplot to investigate the factors associated with the patterns of pectoral girdle morphology observed. In the transition to limblessness, many body dimensions change such as digit counts, phalangeal counts, relative limb length, body size, relative body length, vertebral counts and skull dimensions. For some specimens, measurement information was not able to be obtained and to include these specimens, we summarized limb loss as a rank by categorizing specimens into bins based on forelimb and hindlimb digit counts. Then, within those groups, we ranked specimens by the length of the limbs relative to SVL.

## 4. Results

**a. PCA analysis:** The PCA does not show any distinct clusters of data, but instead, shows a sinusoidal curve through morphospace (Fig. 10). The curve extends from the quadrant defined by positive scores for both PC1 and PC2 (Quadrant II), through the quadrant defined by PC1 positive PC1 and PC2 negative scores (Quadrant IV), across the origin and into the quadrant defined by PC2 positive scores and PC 1 negative scores (Quadrant I). however, if outgroups are ignored, the *Lerista* specimens show a linear trend from Quadrant IV to Quadrant I.

### i. Interpretation of PC axes

PC1 accounts for 48.0% of the observed variance and PC2 accounts for around 12% of the observed variance. The morphospace described by the PC axes appears to be primarily defined by pectoral girdle dimensions. Specimens that plot in PC1 positive space are characterized by narrower and anteroposteriorly longer pectoral girdles that curve ventromedially in a deep 'U' shape. They also have long, thin 't' shaped interclavicles (typified by Hemiergis peronii Fig. 2) and clavicles that curve posteriorly near the medial nexus of the clavicles. PC 1 negative specimens  are characterized by broad and anteroposteriorly short pectoral girdles that have a shallower curve around the body (Fig 10). As there are few specimens that plot in PC1 negative and PC2 negative space or plot in PC2 positive and PC1 positive space (quadrants II and III), it is difficult to differentiate between and interpret the two axes. However, the ratio of pectoral girdle width to pectoral girdle length, when plotted against PC1 results in a plot that closely resembles the PCA plot(Fig. 11). This suggests that the ratio of width to length may be at least part of the variation accounted for in PC2. However, there is not much variation in ratio of length to circumferential width in Lerista and it is clear these dimensions change largely in tandem as the ratio of length to width is always around 1.5, though it does range from 1.2-1.8 and is associated.(Fig.11). Though the shapes of individual elements likely account for a proportion of the variation, it is also likely that some of the variation is merely an artifact of the degree of curvature (Fig.5).

ii. PC correlates

**Forelimb length**

There is a relationship between forelimb length and PC1 scores, both when forelimb lengths are adjusted by skull lengths (Fig. 13) and when taken as a percentage of SVL (Fig. 14). with p values of 0.0002041 and 0.00093 respectively. PC1 is also strongly correlated with both forelimb state and combined forelimb and hindlimb limb state.

**Body size**

Though miniaturization is thought to be associated with the evolution of limblessness in other groups, there does not appear to be a relationship between PC1 and body size by any metric (Fig. 15, Fig. 16) (Morris and Ryan, 1990). PC1 plotted with skull length reveals no linear relationship but shows a pattern of clusters that appear to be associated with digit count. Specimens with skulls above 7mm have either 5 digits on their forelimbs or fewer than 3 and specimens with skulls that are smaller than 7mm have three or four digits on the forelimb, or no limb remnants. This pattern holds true when taxa not included in the PCA analysis are considered, with the exception of *Lerista* labialis, which has the longest skull yet has no forelimbs. There is no clear pattern in the distribution of PC scores when plotted against absolute SVL or SVL relative to skull length. So body size, by any measure, does not appear to correlate with PC1 scores.

### iii. Phylogenetic PCA

Results of the phylogenetic PCA analysis reveal that species do not necessarily group with close relatives (Fig. 17) Additionally for this analysis, species are more likely to occupy similar positions in morphospace to those with similar limb states. However, species with similar limb states that are close relatives are often found close to each other. The phylogenetic signal was significant (K= 0.402, p=.004) meaning that similarities between specimens are largely predicted by shared ancestry.

## 5. Qualitative Observations of Changes in Individual Elements

The geometric morphometric analysis captures variation as a whole but, as this genus shows a loss of elements, homologous landmarks that can be applied to all taxa are limited. Additionally, some specimens were unable to be included in the geometric morphometric analysis due to deformations during preservation or damage incurred during previous investigations. Qualitative observations can still be made from the collected scan data of these specimens even if quantitative data cannot. All pectoral girdles are pictured in ventral view in Figure 18 and they can bee seen plotted on a phylogeny in Figure 19.

**a. Interclavicle**: The interclavicle in most skinks is a thin, cruciform element with anterior and posterior medial processes and two lateral processes that perpendicularly from the medial processes (Fig. 2) (Evans, 2008). In species of *Lerista* with two or more digits, the interclavicle closely resembles that of other scincids; the anterior medial process rests just dorsally to the medial nexus of the two clavicles and runs along the midline until meeting the lateral process at the level of the primary coracoid fenestra. The medial posterior process extends along the midline to a point roughly parallel to the posterior-most point of the coracoids and lies just ventral to the sternum. The lateral projections vary slightly in the angle of projection and position of origin but this position is always parallel to the coracoid fenestrae.

The species with two or fewer digits (e.g. *L. planniventralis*(UMMZ-241013), *L. stylis* (USNM 128640), *L. edwardsae*(YPM R-13679)) exhibit much more variation in the shape of the interclavicle. In this group, it is universally anteroposteriorly shorter and broader, but there is much more variation in the shape, symmetry, length, width, and angle of the lateral projections. In some species (*L. lineopunctulata*(MCZ-R-33261)*, L. gerrardii*(MCZ-R-33258) and *L. baynesi*(FMNH 75489)), the posterior projection overlaps with the sternum, but in the others, the interclavicle is too short to reach the sternum. In some cases, the interclavicle is wider than it is long, especially in highly limb-reduced species such as *L. stylis* and *L. praepedit*a(R-187806). In some species such as *L. lineopunctulata* and *L. kalumburu*(WAM R-100188), the lateral projections of the interclavicle are angled and curved anteriorly and in some (*L. edwardsae, L. labialis*(AMNH 86086)*,* and *L. kendricki*(CU R-0011970)), the lateral projections are angled posteriorly. In others, like *L. praepedita*, and *L. uniduo* (CU R-11945), they are positioned in a straight line, perpendicular to the midline of the body axis. In some species within this limb-reduced group, the interclavicle is remarkably symmetrical, but in some species such as

*L. allanae* and *L. edwardsae*, the interclavicle is asymmetrical. In *L. allanae* (CAS 77099), the points of origin of the lateral projections are even offset and one is farther anterior than the other. In *L. edwardsae*, the interclavicle is broad and roughly diamond-shaped but is irregular. Another interesting feature is the interclavicle fenestra in *L. stylis*. There is no sign of such an opening any other interclavicle in this sample or, as far as we could find, in any other squamate species described in the literature. Three species, *L. apoda* (WAM-R-168495)*, L. karlschmidti* (AMNH 120381), and *L. carpentariae* (USNM 128409) appear to lack interclavicles altogether. However, USNM 128409, the specimen of *L. carpentariae* used for this study appears to have some sort of genetic abnormality and lacks any sign of a pectoral girdle on its right side, and lacks a hind limb on its right side as well and is likely not representative of its species.

The PCA plot of the interclavicle shows that the shape of the interclavicle is more conserved in specimens with higher limb ranks(Fig. 20). However, because the lateral processes of the interclavicle are so thin, it is likely that in lower resolution scans did not register them at a high enough density to make them distinct from the background. This would make them difficult or even impossible to segment so it is likely that some of the variation, especially on the PC1 axis is due to scanning artifacts rather than anatomy. The angle of the lateral projections as well as variation the medial processes were still likely faithfully captured so the true variation among the interclavicles of these specimens is still likely reflected in this plot.

The evolution of the interclavicle in *Lerista* appears to proceed in association with the reduction of the limb. The element becomes anteroposteriorly shorter and the center of the element broadens. Interestingly, the interclavicle appears to become relatively more robust in limb-reduced species and were always easily distinguishable from the background, unlike species with more well developed limbs.

**b. Clavicles:** The clavicles are paired elements that meet medially on the ventral side of the animal. In species with three or more digits, the shape of the clavicles are regular and similar (Fig. 18) in that they arc anteriorly from the ventromedial nexus then curve posteriorly. The clavicle is usually dorsoventrally thin and has few posterior projections. This may be an artifact of scanning as lizard clavicles often have posterior processes of thin bone (e.g. Evans, 2008). Most of the variation in the clavicle shape in morphospace appears to be explained by the curvature around the body and the degree of the anterior curve. Species with three or more digits tend to have well developed pectoral girdles that generally follow the curve of the body ventrally, wrapping around the circumference and almost reaching as far dorsally as the vertebrae. The clavicles follow this curvature and extend nearly halfway around the circumference of the body in species with well developed limbs. In limb-reduced species (e.g. *L. stylis,* (Fig. 18) *L. baynesi*, Fig. 17), the pectoral girdle is smaller and, though it follows the circumference of the body, it does not extend nearly as far, so the degree of the curve is shallower(Fig. 18) In species with well-developed pectoral girdles (e.g. *L. frosti* (MCZ-R-51893)*, L. bougainvilli* (MCZ-R-129545)), the anteriormost point of the clavicle is anterolateral relative to the most medial point (Fig 18. The clavicle curves anteriorly, away from the most ventral point, reaches its anterior apex at a point roughly anterior to the primary coracoid fenestra, then curves posteriorly until curving gradually anterior until meeting laterally with the suprascapulae. In some species with moderately well developed pectoral girdles (e.g. *L. terdigitata* (MCZ-R-131081)*, L. kingi*(CAS 254618)), the anterior apex of the clavicle is more pointed and more medial than in the less limb-reduced species. In species with more reduced pectoral girdles, the most medial point is also the most anterior point. In these species, the clavicles curve posteriorly before leveling out and following the circumference of the body. Even among highly limb-reduced species, the clavicles have a high degree of symmetry (e.g. *L. praepedita, L. edwardsae, L. stylis*).

The PCA plot of the clavicles shows a weak gradient along the PC1 axis from well developed girdles in PC1 positive space and more reduced girdles in PC1 negative space. The species with well developed pectoral girdles are somewhat more tightly clustered than species with less well developed pectoral girdles and considerably more than those with the lowest limb states. This suggests that clavicle shape is more variable in species with more highly reduced limbs.

The evolution of the clavicles appears to proceed gradually from highly arched pectoral girdles to an inverted curve. It appears as though over the course of the transition to limblessness, the anterior apex of the clavicle curve moves more medial until it and the nexus between the clavicles is one in the same. However there are some specimens for which the anterior apex of the clavicles differs dramatically from the mean level, but in others the apex is a mere suggestion (e.g. *L. uniduo*). Unfortunately there are too few representatives from the limb loss event *L. uniduo* represents so it is difficult to tell whether there are clade-level differences in the evolution of this element.

**c. Scapulocoracoid and Suprascapular Cartilage:** The scapulocoracoid is an element comprised of two elements, the scapula and coracoid, that fuse during ontogeny to form the scapulocoracoid(Evans,2008). The coracoid is shaped like a half circle at the posteromedial articulation with the sternum. The coracoid has two rod-like processes that project anteromedially and articulate with the calcified cartilage epicoracoid to form two coracoid fenestrae. The scapula is a flattened, thin, blade-like element that curves around the body and, in some species with well-developed pectoral girdles, is forked and forms a scapular fenestra. At the articulation between these two elements is the saddle-shaped glenoid fossa, a divot into which the head of the humerus fits.

The scapulocoracoid differs dramatically between species with well-developed pectoral girdles and those with more reduced girdles. There appear to be significant differences in overall shape, but these differences are difficult to capture using a geometric morphometric analysis as reduced pectoral girdles retain so few features to which landmarks may be applied. The geometric morphometric analysis is also unable to capture some of the significant loss events that accompany this reduction.

In species with three or more digits, the scapulocoracoid has a well-defined, saddle-shaped glenoid, a coracoid foramen, and thin rays on the coracoid and the scapula. These bony rays articulate with arcs of calcified cartilage which curve anteromedially, dorsal to the clavicle to form a loop called the coracoid fenestra.

One of the most obvious changes to accompany the loss of the limb is the loss of the glenoid fossa. Curiously, the loss of the fossa appears to precede the complete loss of the limb. Several specimens (e.g. *L. allanae, edwardsae, L. lineopunctulata*) possess small humeri that are about as long as they are wide and are about half a millimeter in length. These humeri, at least at our scan resolution, appear to be fused to the scapulocoracoid and there is no evidence of any depression corresponding to the medial end of the humerus. The loss of the humerus does appear to be concurrent with the loss of the coracoid foramen because all specimens in our analysis have either both a humerus and a coracoid foramen and a humerus, or neither(Fig.22).

Other changes relate to the reduction and disappearance of the scapular and coracoid rays. The scapular ray is absent than are the coracoid rays. In some species (e.g. *L. distinguenda*, and *L. gerrardii)*, the scapula appears wide with a small hole, and in species such as *L. baynesi*, and *L. allanae*, the scapula is wide, suggesting that the scapular ray was not actually reduced or lost, rather the space between the scapular blade and the scapular ray was filled in. The coracoid rays are discernable in more highly limb-reduced species, even in *L. ameles*, which possesses no external limbs. In some lower quality scans, it is difficult to determine whether the calcified cartilage portion of the coracoid fenestra is absent or was merely too low density to be segmented. Evidence of a coracoid fenestra exists in species with highly reduced pectoral girdles such as *L. edwardsae, L. allanae*, and *L. ameles*. *L. cinerea*, a species in the same clade as *L. ameles*, has a more well developed pectoral girdle with distinct, though short coracoid rays and wider coracoid fenestrae. This species has clear epicoracoid cartilage which appears to be dorsoventrally flat and anteroposteriorly wide near the anterior apex of the fenestra. Some amount of coracoid cartilage that extends anterior to the lateral processes of the interclavicle even remains in *L. praepedita*, however, in this specimen, the secondary ray is absent and the epicoracoid does not form a loop. In *L. stylis*, there is no such extension so it is unclear how the coracoid fenestrae disappear or whether the differences in the epicoracoid cartilage in these limb-reduced species represent different possible pathways to loss.

Another difference between the limb-reduced species and the more plesiomorphic species is the articulation of the coracoid with the sternum. In species with three or more digits on each forelimb, the curved, posteromedial edge of the coracoid sits in a corresponding groove called the coracoid groove. The scapulocoracoid is kept in this groove largely by the pectoral muscles. In specimens with well developed forelimbs, the coracoid and sternum are tightly articulated, but in species with more reduced forelimbs such as *L. praepedita*, and *L. baynesi*, the epicoracoid is not sitting in the sternal groove, though the sternal groove is still present in both specimens, suggesting the muscles may no longer generate sufficient horizontal forces to maintain the articulation or they may not exist.

The suprascapular cartilage was not uniformly ossified, which made the definition of the lateral edge difficult. There was also considerable variation in scan quality among the specimens and thin or poorly ossified elements are often difficult to detect in low quality scans. Therefore it was difficult to reliably define the shape and length of this element. However, it was still possible, in most specimens to detect the shape and curvature of the anterior and ventral edges as well as the articulation with the scapula. Specimens with more well developed pectoral girdles have roughly fan-shaped suprascapulae, the edges of which curve ventrally and anteriorly away from the articulation with the scapula. In species with more reduced girdles, the suprascapulae are anteroposteriorly narrower.

Some aspects of the evolution of the scapulocoracoid appear to occur in a manner that is can be generalized across *Lerista*. The scapular fenestra closes up, then the coracoid fenestrae become less distinct, and the coracoid becomes disarticulated from the sternal groove. Then the glenoid is lost, followed by the humerus and coracoid foramen. The suprascapula appears to gradually become shorter, anteroposteriorly thinner and less fan-shaped over the course of this transition. While these changes are seen across the group, the mode of the reduction and loss of the coracoid fenestra may be clade specific.

**d. Sternum and Mesosternum:** The sternum in less limb-reduced species is a diamond shaped element of well calcified cartilage that articulates anterolaterally with the scapulocoracoids, posterolaterally with six ribs (three on each side), and posteriorly with the mesosternum. The sternum often has a an oval, or heart-shaped fontanelle located medially and often slightly more posterior than anterior. The mesosternum extends posteriorly in a thin rod from the sternum and forms a diamond shape with a fenestra in the middle. A pair of ribs extend laterally from the lateral corners of the diamond, and another pair extend posteriorly. Like the scapulocoracoid, there is a significant change in the shape of the sternum during the transition to limblessness, and the absence of so many landmarks in highly-reduced girdles makes a meaningful geometric morphometric analysis difficult. However there are other more qualitative observations that can provide insights into how this element has evolved in *Lerista*.

In limb-reduced species, the sternum shape varies considerably and in some species (e.g. *L. allanae*), there is considerable asymmetry. In many limb-reduced taxa such as *L. baynesi*, the sternum is weakly ossified in the middle, and it is difficult to distinguish between low density patches and possible fontanelles, especially around the overlap with the posterior medial process of the interclavicle as in *L. allanae* (Fig. 22). Species with highly reduced pectoral girdles also have a reduced number of rib attachments. It is not clear from this dataset how the transition occurs but it appears as if the posterior-most ribs  of the mesosternum detach and join, forming an arc, then the next pair, and the next pair until there are four remaining rib attachments and no mesosternum as in *L. praepedita*. The change in shape of the sternum precedes the loss of the mesosternum and species like *L. allanae* have clear mesosterna but only very reduced, non-protruding limbs.

## 6. Discussion

**a. Morphospace Occupation:** Pectoral girdle changes: the pectoral girdles of the Lerista species in this sample appear to be confined to a roughly linear area of morphospace. The position of a specimen within this space is correlated with the degree of limb reduction which suggests that this area of morphospace represents the space a lineage transitions through during limb loss. The tendency of relatively distantly related taxa with similar limb states to cluster together suggests there are constraints on the evolution of the pectoral girdle, even when walking-related functional constraints are not a factor. This might mean that the evolution of the overall pectoral girdle occurs in a somewhat predictable way. However, some species such as *L. kalumburu* (WAM R-100188), *L. planniventralis* (UMMZ-241013), *L. distinguenda* (UMMZ-132393), and *L. elegans* (CU R-0012147) plotted within this trend but relatively far away from other species of similar limb states. *L. kalumburu*, which has two digits on each relatively long forelimb, plotted closest to L. praepedita, and other species with no traces of a forelimb. *L. elegans*, *L. distinguenda,* and *L. planniventralis* are all part of the same subclade and, even though their pectoral girdles differ from each other in appearance, their deviation from the others may suggest that the thresholds for certain pectoral girdle changes may differ from clade to clade (Fig. 19).

The clustering of data at the ends of the trend line appears to be different, but not in the way I initially expected. The pectoral girdles with high PC1 positive scores, those with more well developed limbs are less tightly clustered than species with low PC1 negative scores, or those species with more highly reduced pectoral girdles. This suggests that lineages with disparate morphologies converge on a common form. This is curious as one would expect that, without the functional constraint of walking, the limb girdles would be free from locomotion-related selective pressures. One would expect fewer constraints would give rise to a greater variation in morphology.

Even though the PC1 negative specimens cluster more tightly together than PC1 positive specimens, there is a wider range of limb states that occupy it. In other words, each given range of PC1 values in PC1 negative space is likely to have a greater range of limb states than the same range in PC1 positive space. This also means that specimens with more greatly reduced limb states will have greater variation in the degree of pectoral girdle reduction than species with higher limb states. This is perhaps illustrated most effectively with the contrast in morphologies between *Lerista* apoda and *Lerista* ameles (Fig. 23). Both species are within the most basal clade within *Lerista* (Fig. 19), and both specimens lack external and internal remnants of forelimbsand lack external hindlimbs. *L. ameles* has small femoral remnants and the internal pelvic anatomy of *L. apoda* is unknown. Based on the degree of limb reduction in this species, one would expect they would have similarly reduced pectoral girdles, yet *L. ameles* has a much more well developed girdle with all elements present and easily identifiable, including the cartilaginous epicoracoid. In contrast, *L. apoda* has only remnants that are difficult to identify. If the combined limb state predicted pectoral girdle reduction, *L. ameles* and *L. apoda* would be expected to have a similar degree of pectoral girdle reduction, but this is not what is observed.

This pattern of increased variation with decreasing relative limb lengths is what one might expect in a system with few functional constraints that is subject to genetic drift (Lahti et al., 2009). However, it is also possible that certain disparate morphologies were selected for to meet the demands of disparate ecological niches. Species of Lerista occupy a wide range of environments with different substrate types (Kendrick, 1989; Greenville and Dickman, 2005; Amey and Couper, 2009; Cogger, 2014), and the environments they occupy tend to be associated with certain morphologies (e.g., elongate forms are often found in loosely consolidated sand) (Greer, 1990; Skinner, 2008). Even sympatric species may occupy different microhabitats and substrate types and display differing locomotory strategies when encountered with the same environments (Gans and Fusari, 1994). To examine whether or not the variation in morphospace is the result of relaxed selection or a selective response to diverse functional demands may be explored in the future by incorporating ecological data on substrate preferences with our morphological data.

**b. Potential Issues:** One potential problem with using a PCA analysis to investigate determinism in this group is that it is unclear what aspects of morphology are most influential. It is possible that differences in the shapes of individual elements have been obscured by differences in the curvature of the pectoral girdle around the body, which is merely a consequence of the relative size of the girdle. Due to the placement and structure of the pectoral girdle, it is restricted in terms of the dimensions this complex can be reduced in size. In the body cavity, pectoral girdles are restricted to the space in between the body wall and the ribs, meaning they are restricted to a cylindrical space (Fig. 5). They can therefore only be reduced in length and circumferential width. They are also restricted to a ventral position and, as the lateral elements articulate with medially placed ventral elements, any reduction in circumferential width must cause the girdle to extend less far dorsally. This change in circumferential width means a decrease in the curve of the girdle around the body, causing more reduced girdles to appear wider. The Procrustes analysis accounts for differences in rotation and size, but not differences in curvature. This may cause species with similarly curved pectoral girdles to cluster together because of landmarks placed on lateral elements. For example, the landmark placed at the end of the clavicle and the landmark placed at the lateral-most point of the suprascapula may be exactly the same relative distance apart in two specimens but, in reference to the medial points, the lateral end of the suprascapula would appear to be quite different than that of the other, even if they are morphologically similar. The qualitative observations suggest there are major differences in morphology that are associated with limb reduction, but there are also some aspects that appear to be the result of shared ancestry rather than the degree of limb reduction. These clade-specific features may be obscured by the strong signal from the pectoral girdle curvature.

Another potential issue arises from the taxon sampling. We only used one specimen for most species in the PCA analysis, with the possible exception of CAS 77518, which we believe was misidentified as *Lerista timida* and is really *L. microtis*. Having only one specimen per species means we must assume that each specimen is representative of its species which, in the case of reduced, potentially vestigial elements, may not be the case. Previous studies on pectoral girdles in limb-reduced taxa have found intraspecific variation and even intra-individual variation in terms of asymmetry. There are some sister taxa with remarkably similar pectoral girdles such as the clade containing *L. stylis* and the clade containing *L. ameles* (Fig. 24). However, there are also sister taxa that are quite closely related such as the clade containing *L. baynesi*, where each member has very different morphologies, despite somewhat similar limb states(Fig 19). Until intraspecific variation in the pectoral girdles of *Lerista* is examined, the assumption that specimens are representative of their species is cautiously applied.

Qualitative changes: The shape of pectoral girdle morphospace occupied by *Lerista* suggests a largely deterministic evolutionary pathway. However, the consideration of the qualitative characters suggests that pectoral girdle morphology may be predictable based on phylogeny and degree of limb reduction. There are many changes in pectoral girdle elements that appear to be associated with limb reduction such as the change in the curve of the clavicle from anterior to posterior, the reduction in the number of sternal rib attachments and the broadening of the interclavicle. However, certain traits such as the angle of curvature of the clavicles in *L. stylis*' clade, or the wide posterior medial process of the interclavicle in *L. ameles* and *L. cinerea*, as well as the high level of phylogenetic signal, suggest that there are some aspects of pectoral girdle morphology that are products of phylogenetic history(Fig. 24). This is unsurprising when one examines the limb states mapped onto the Lerista tree (Fig. 19. In such evolutionary transitions, one would expect a clade such as this to display a morphocline from pentadactyl to limbless that follows the phylogeny. What we see instead are a number of small clades that closely resemble each other in terms of limb states. Sometimes sister clades have dramatically different limb states and very similar limb states to their closest relatives. The absence of a phylogenetic morphocline may mean that the factors associated with limb loss may not be gradual and may in fact be stepwise. This may also mean that phylogenetic constraints are unique to each subclade and are less influential at the genus level. This may suggest that the independent evolution of limblessness in *Lerista* may have been driven more by ecological factors or subclade-specific mutations rather than an underlying genetic propensity for limb loss in the genus.

**c. Comparisons with Other Limb-reduced Squamates:** The pectoral girdles of a number of other lineages with limbless or limb-reduced species have been described previously (e.g., Cope, 1892; Kearney, 2002; Jerez and Tarzona, 2009; Miralles et al, 2015; Stephenson, 1961; Stokely, 1947; Camp. 1923; Mueller, 1875), and it is clear from those studies that Lerista differs from other lineages in the order of reduction and loss events and other aspects of the limb loss transition. The majority of the species whose pectoral girdles have been described were studied over fifty years ago, and therefore, were not investigated within the framework of molecular-based phylogenies. Because of the high level of morphological convergence in limb-reduced groups, morphology-based phylogenies can lead to the grouping together of unrelated, convergent forms (e.g., Camp, 1923; Schmitz et al., 2005; McGhee, 2011). Here we emphasize qualitative comparisons of the morphologies observed in the various major groups to lay the groundwork for future examination of evolutionary trends.

One of the aspects of pectoral girdle reduction that has received a considerable amount of attention is the order in which elements are lost (Cope, 1892; Camp, 1923; Stokely, 1947; Kearney, 2002; Andrade et al., 2016; Liniewski et al., 2016). Some of the earliest workers proposed a universal order in which elements were lost (Cope, 1892; Stokely, 1947), in which the interclavicle was lost first, and either the clavicles or scapulocoracoid were retained the longest. However, as the phylogenetic relationships among lineages have been elucidated and the identification of highly reduced elements has become possible, it is apparent that there is considerable variation in the order of the loss of elements.

*Lerista* is unusual in that even species with reduced pectoral girdles retain all bony elements, with the exception of *Lerista apoda* and *L. carpentariae. Lerista carpentariae* is missing the interclavicle, but this particular specimen we sampled (USNM 128409) is also missing all appendicular elements on its left side (Fig. 25. It is possible that this absence is related to a morphological anomaly and is not representative of the rest of its species. *Lerista apoda* retains two roughly oval-shaped pectoral girdle elements (Fig. 23), but they are not clearly identifiable with the information we have at present. I hypothesize that the elements are the clavicles, scapulocoracoids and suprascapulae based on the position of the elements relevant to each other and the apparent pattern of clavicle reduction seen in other limb reduced skinks (Miralles et al., 2015). Species from several other squamate groups also retain highly reduced pectoral girdles, but these are usually described as rods or slivers which have often been identified as clavicles or scapulocoracoids (Cope, 1923; Stokely, 1947; Andrade et al, 2016). Among the species of Lerista in our study, L. karlschmidti has the most highly-reduced pectoral girdle with identifiable elements. Its interclavicle and sternum are weakly ossified and faint, whereas the clavicles and scapulocoracoids are more robust (Fig. karlschmidti). In other limb reduced skinks, such as Feylinia currori (Mueller, 1900), Grandidierina rubrocaudata, and Paracontias minimus (Miralles et al., 2015), the interclavicle is absent and the medial ends of the clavicles are located just anterior to the sternum and the clavicles follow the angle of the scapulocoracoids. In Feylinia currori, the clavicles lie just anterior to the scapulocoracoids and do not leave a gap at any point along their length. In Grandidierina rubrocaudata and P. minimus, however, the clavicles articulate medially with the scapulocoracoids and laterally with the suprascapulae, yet have a distinct gap in between those two points.

**d. Location of the Pectoral Girdle and a Reexamination of the Neck-Trunk Boundary:** For most pentadactyl squamates, the boundary between the neck and the trunk is defined by the position of the pectoral girdle with respect to the cervical vertebrae (e.g., Evans, 2008). The cervical vertebrae are traditionally defined as any vertebrae anterior to the first vertebra bearing a rib that attaches to the sternum (Hoffstetter and Gasc, 1969; Evans, 2008). However, in limbless taxa with no sternum, this criterion cannot be used. This means that, by definition, limbless squamates have been excluded from all neck-related discussions, even though they may have an anterior region of vertebrae bearing distinct distinct cervical characters.

Defining the cervical region by the presence of unique osteological characters, such as the presence of hypapophyses, rather than the placement of the pectoral girdle has been suggested as a possible solution (Caldwell, 2000). In pentadactyl squamates, hypapophyses are associated with the cervical vertebrae and end at the anterior extent of the pectoral girdle (e.g., Caldwell, 2000). This is true of squamates with well-developed forelimbs as well as those with reduced or absent forelimbs that have bony pectoral girdles (Caldwell, 2000; K. Koeller, this work).

Hypapophyses are also present in species that lack a pectoral girdle and often extend to a vertebral position that corresponds to the plesiomorphic position of the pectoral girdle, as far as this can be ascertained by observations of close relatives (e.g. amphisbaenians: Caldwell, 2000). In *Lerista*, however, the story appears more complex. Most specimens examined in this study had hypapophyses that ended just posterior to the medial nexus point of the clavicles. However, one specimen of *Lerista apoda* (SAM 23806) examined in this study was observed to have pectoral girdle remnants approximately near vertebra 9, yet it has hypapophyses on at least the first 12 vertebrae (Fig. 26). Our current CT data unfortunately do not comprise a full body scan for this taxon, so the full extent of the hypapophyses is as of yet unknown. It is clear even from the present information that the extent of the hypapophyses is located more posteriorly than the anterior extent of the pectoral girdle in some taxa. Therefore, the mechanisms involved in pectoral girdle positioning and vertebral identity appear decoupled in *Lerista apoda*. This appears to be the case in snakes as well because hypapophyses can extend to the 30th vertebra and yet muscles associated with the pectoral girdle are found associated with the eighth vertebra (Tsuihiji et al., 2006), not much farther posterior than in limbed squamates.

**e. Comment on Methods for Future Comparative Studies:** There have been several recent studies describing pectoral girdle elements in limb reduced taxa using X-ray radiographs (Moch and Senter, 2011; Stanley et al., 2016; Liniewski et al., 2016; Andrade et al., 2016). Those studies provided valuable information about the presence of pectoral girdle elements and their location in situ. However, the methods used in those studies may not be optimal for detecting all elements present or for accurately discerning and describing morphology.

Among the species described by Moch and Senter (2011) were *Lerista stylis* (USNM 128640; Fig. 26) and *Lerista carpentariae* (USNM 128409; Fig. 25). The pectoral girdles of both those specimens were described as “short slivers” that were unidentifiable (Moch and Senter, 2011:pg.3, Fig. 30 c2). We examined the same specimens using X-ray computed tomography and achieved markedly different results. We found *Lerista stylis* (USNM 128658) to have a full complement of bony pectoral girdle elements: interclavicle, clavicles, scapulocoracoids, and suprascapulae, as well as a sternum (Fig. 27). Though all elements were reduced, we were still able to identify each in our CT datasets. *Lerista carpentariae* (USNM 128409) was found in the 2011 study to have pectoral girdle elements on only one side. We found this same result as well, but instead of a mere sliver, we identified the clavicle, scapulocoracoid, suprascapula, and sternum (Fig. 24).

The use of X-ray computed tomography allowed us not only to detect the presence and location of pectoral girdle elements in our specimens but to confidently identify the elements. We were also able to include them in our quantitative analyses and compare them to other specimens digitally. By using x-ray computed tomography to collect our osteological data, we were able to better detect all present elements, discern morphological details, and conduct shape analyses. All three of these factors are essential for meaningful comparative work in the future, and we therefore recommend the use of X-ray computed tomography over X-ray radiographs for such studies.

## 6. Conclusions

The reduction of the pectoral girdle in *Lerista* is strongly associated with the degree of limb loss and the overall changes in morphology may be constrained to a narrow region of morphospace. This suggests that pectoral girdle reduction happens in a predictable way. However, certain aspects of the transition may be clade-specific, suggesting that each limbless lineage is evolving independently and are evolving in response to constraints that are unique to the lineage, rather than a fundamental, *Lerista*-specific tendency towards limblessness.

This study demonstrates that even in closely related taxa, the evolution of convergent morphologies may proceed in different ways. There is a tighter clustering of limb-reduced specimens than those with well developed limbs in pectoral girdle morphospace, which suggests similar pectoral girdle proportions may be achieved from disparate plesiomorphic states, though this is impossible to know for sure without a fossil record.

Additionally, the information gleaned from this project make it clear that Computed Tomography is the best method for obtaining data on the morphology of internal structures as it allows for more detailed anatomical and positional information than alternative methods and allows for quantitative comparisons to be made.

## 7. Future Directions

One of the limitations of this study was the taxonomic sample. Though the species sampled were spread across the tree, the study would have benefitted from a more complete sample, especially from the clade containing *Lerista* uniduo(Fig. big ol tree with girdles), which likely represents an independent instance of limb loss. A more complete taxonomic sample would provide information about how much of the similarity could be explained by phylogenetic signal. Another next step would be to examine intraspecific variation to determine whether the specimens used were representative of their species.

To explore the potential constraints involved in the evolution of limblessness in *Lerista*, it would be interesting to explore the relationships between morphology and ecological factors. There have been detailed surveys of soil composition across Australia and ecological data are known for many species of Lerista (Cogger, 2014). It would be interesting to explore how much variation can be explained by phylogenetic signal and how much can be explained by ecological factors.

## 8. Acknowledgments

This work was completed as part of an MS thesis by KK. I thank Justin Gladman at Duke University for assisting with the CT scanning and scan reconstructions. We thank Joe Martinez and Jose Rosado at the Museum of Comparative Zoology for loaning specimens and for providing additional scans. We also thank Greg Schneider at The University of Michigan Museum of Zoology, David Kirizian at the American Museum of Natural History, Alan Resetar at the Field Museum of Natural History, Lauren Scheinberg at the United States National Museum, Bob Murphy at the Royal Ontario Museum, and Gregory Watkins-Colwell at the Yale

Peabody Museum for loaning specimens included in this study. We are grateful to Mark Hutchinson at the South Australia Museum for providing additional specimen scans. We thank The Society of Vertebrate Paleontology, The Paleontological Society, and Virginia Tech for providing funding for CT scanning to KK. MRS was supported by the Department of Geosciences at Virginia Tech and NSF 1655609. Martha Muñoz and Josef Uyeda provided valuable statistical and analytical advice. I would also like to thank Patrick Gaudet who generously offered to edit this manuscript and who helped me get through the rough patches.

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# 10. Figures

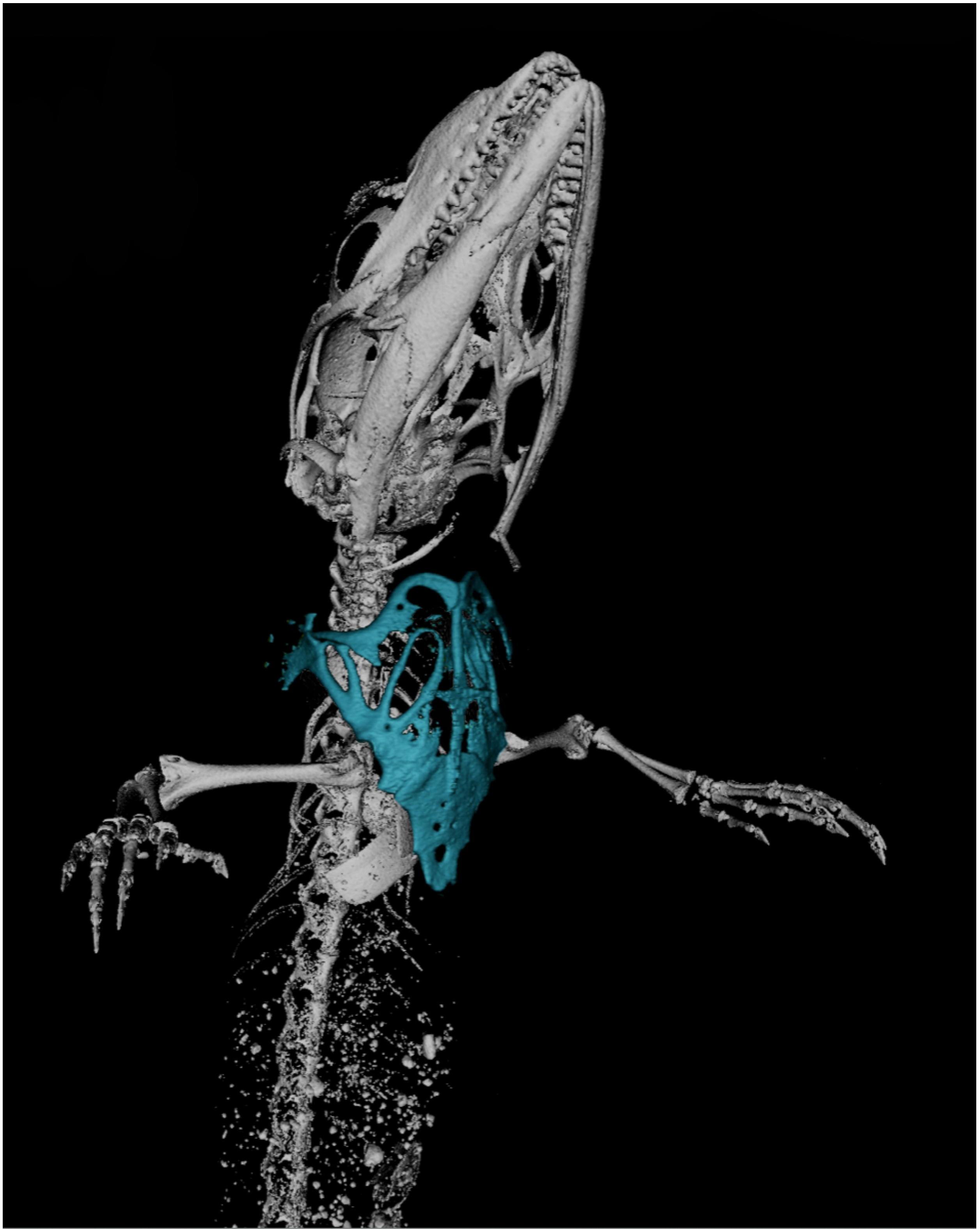


Figure 1. The pectoral girdle, highlighted in blue, is a complex of bones that support the forelimbs, and transfer forces from the appendicular skeleton to the axial skeleton during locomotion.

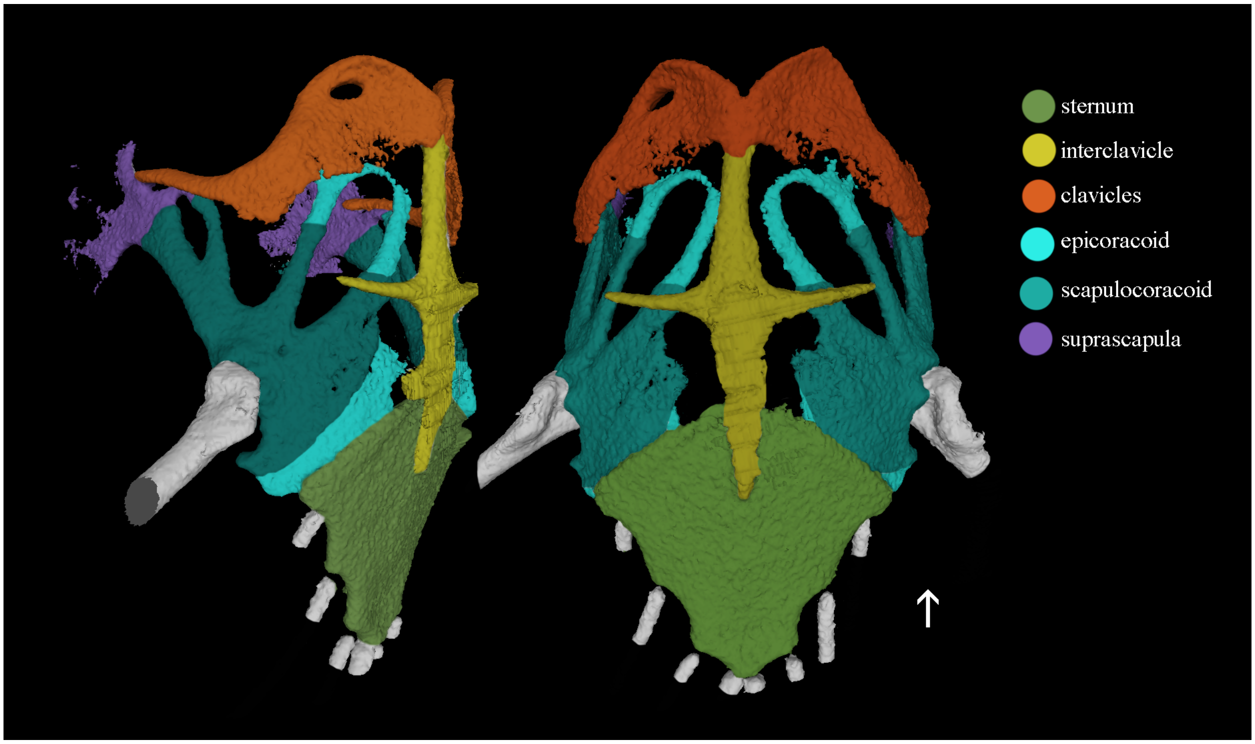

sternum
interclavicle
clavicles
epicoracoid
scapulocoracoid
suprascapula

Figure 2. The pectoral girdle of *Hemiergis peronii* (YPM R-9030), which is likely similar to the plesiomorphic skink condition. This complex of elements is comprised of an interclavicle, sternum, paired clavicles, scapulocoracoids, and paired, cartilaginous epicoracoids and suprascapulae. The right-hand image is in ventral view and the image to the left is a ventrolateral view.

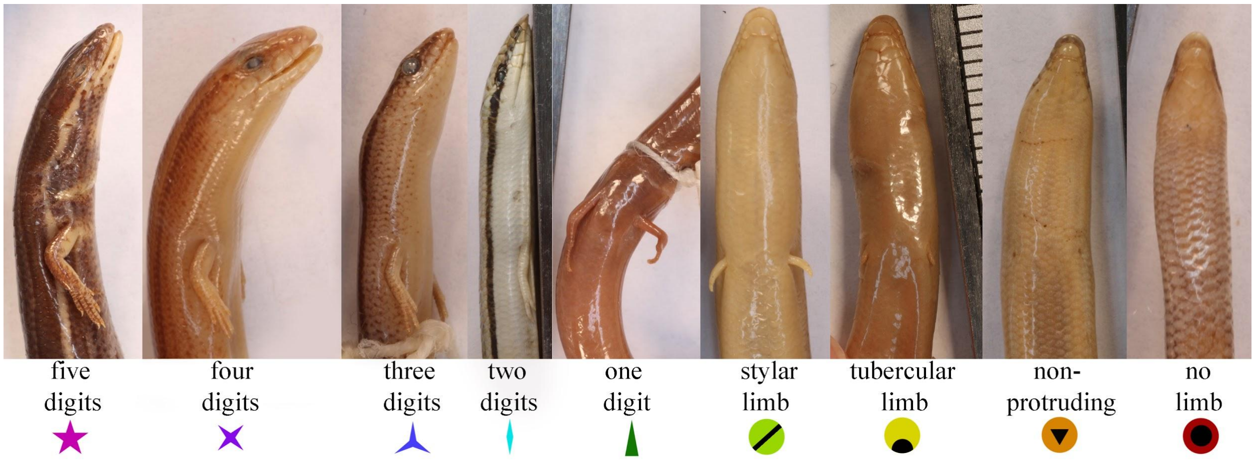

five digits
four digits
three digits
two digits
one digit
stylar limb
tubercular limb
non-protruding
no limb

Figure 3. Species in the genus *Lerista* display a range of morphologies from pentadactyl to fully limbless. Though most previous studies have grouped species based on external limb morphology, I chose to consider internal morphology as well. Some species have no external limb, but still have a small humerus. The difference between a small nub and a non-protruding limb is therefore due to a difference in length of the humerus. Some species do not have any sign of a forelimb and in this study, I make a distinction between those that have no forelimb and those that do, regardless of the length of the limb.

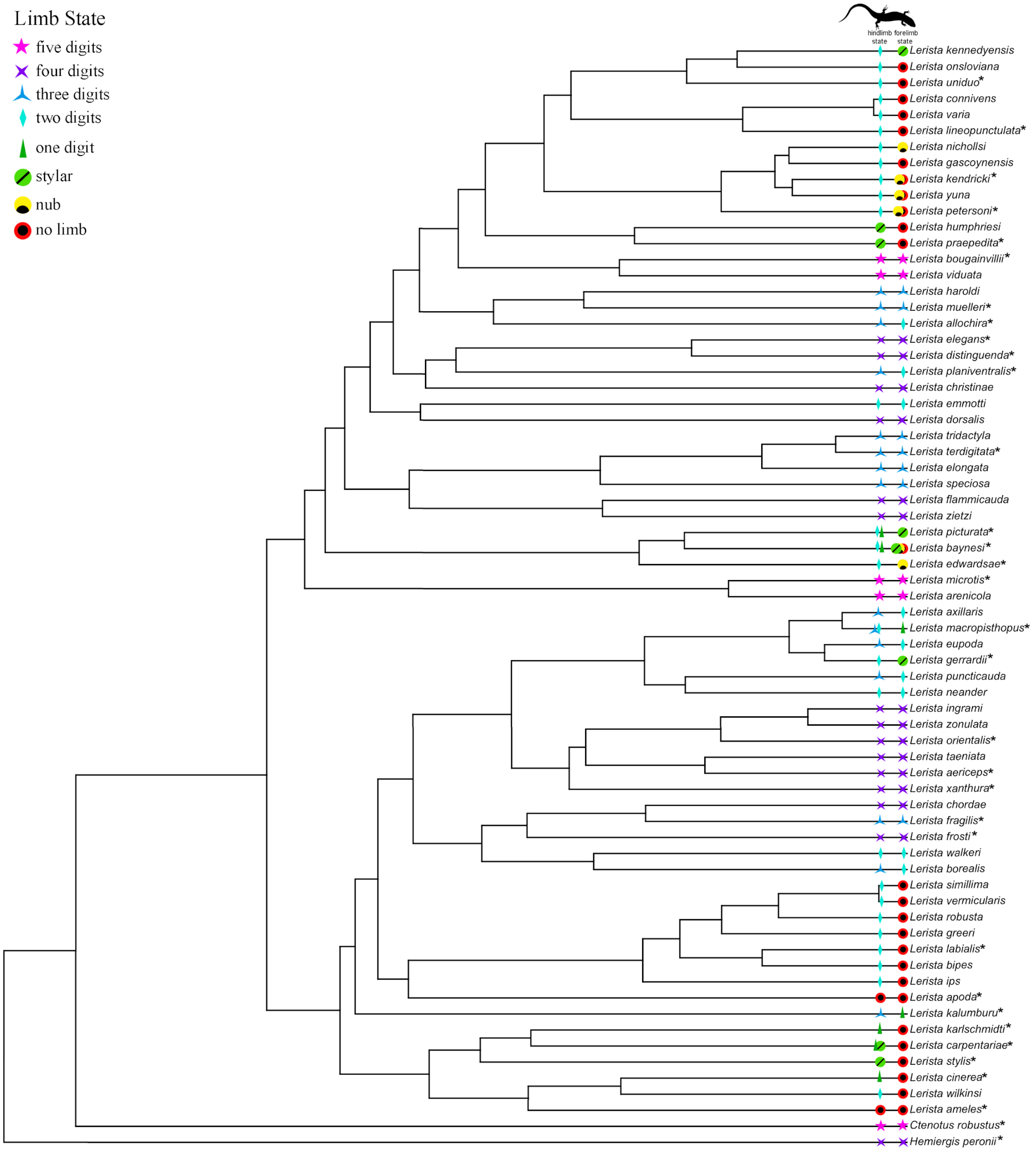

Limb State
five digits
four digits
three digits
two digits
one digit
stylar
nub
no limb
hindlimb state
forelimb state
Lerista kennedyensis
Lerista onsloviana
Lerista uniduo*
Lerista connivens
Lerista varia
Lerista lineopunctulata*
Lerista nichollsi
Lerista gascoynensis
Lerista kendricki*
Lerista yuna
Lerista petersoni*
Lerista humphriesi
Lerista praepedita*
Lerista bougainvillii*
Lerista viduata
Lerista haroldi
Lerista muelleri*
Lerista allochira*
Lerista elegans*
Lerista distinguenda*
Lerista planiventralis*
Lerista christinae
Lerista emmotti
Lerista dorsalis
Lerista tridactyla
Lerista terdigitata*
Lerista elongata
Lerista speciosa
Lerista flammicauda
Lerista zietzi
Lerista picturata*
Lerista baynesi*
Lerista edwardsae*
Lerista microtis*
Lerista arenicola
Lerista axillaris
Lerista macropisthopus*
Lerista eupoda
Lerista gerrardii*
Lerista puncticauda
Lerista neander
Lerista ingrami
Lerista zonulata
Lerista orientalis*
Lerista taeniata
Lerista aericeps*
Lerista xanthura*
Lerista chordae
Lerista fragilis*
Lerista frosti*
Lerista walkeri
Lerista borealis
Lerista simillima
Lerista vermicularis
Lerista robusta
Lerista greeri
Lerista labialis*
Lerista bipes
Lerista ips
Lerista apoda*
Lerista kalumburu*
Lerista karlschmidti*
Lerista carpentariae*
Lerista stylis*
Lerista cinerea*
Lerista wilkinsi
Lerista ameles*
Ctenotus robustus*
Hemiergis peronii*

Figure 4. A tree of Lerista, taken from (Zheng and Wiens, 2016) and trimmed to only include Lerista. The symbols near the tips of the tree represent limb morphologies that can be observed by examining external anatomy. The symbol on the right refers to the forelimb and the symbol on the left refers to the hindlimb. Where symbols overlap, multiple limb morphologies have been observed within species. Limb morphology descriptions taken from Cogger, 2014. Species marked with an asterisk are included in this study.

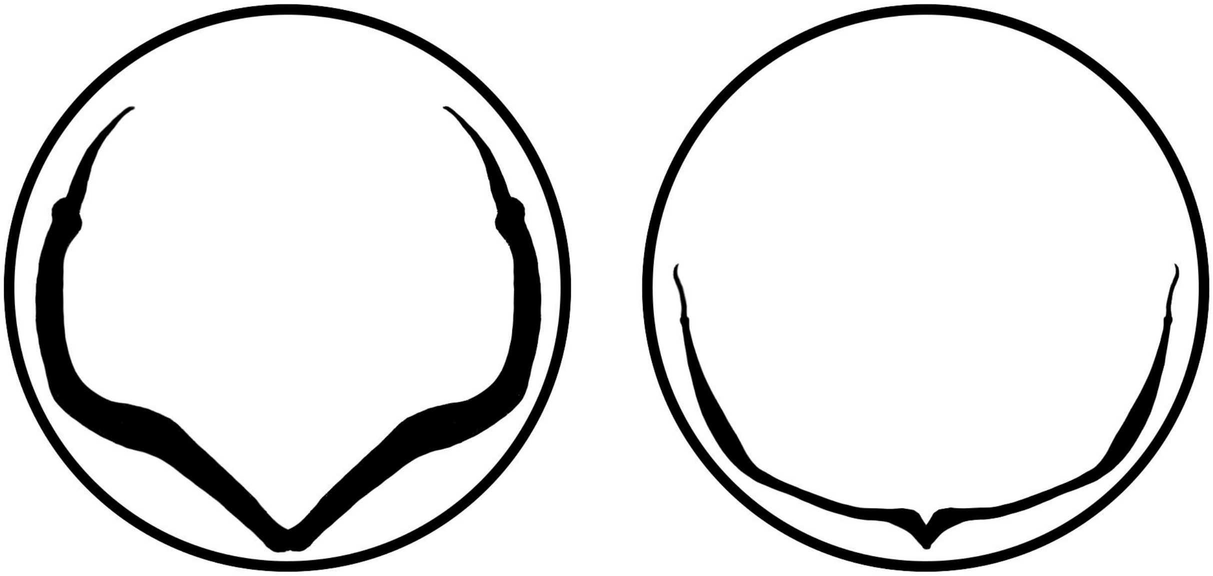


Figure 5. An illustrated cross section of two hypothetical Lerista species, one with a well developed pectoral girdle and one that is reduced. Because the pectoral girdle follows the curvature of the body wall, the width of the two specimens would be equal and would not reflect the degree of reduction in the specimen on the right. For this reason, I measured width by following the curve of the pectoral girdle rather than simple width.

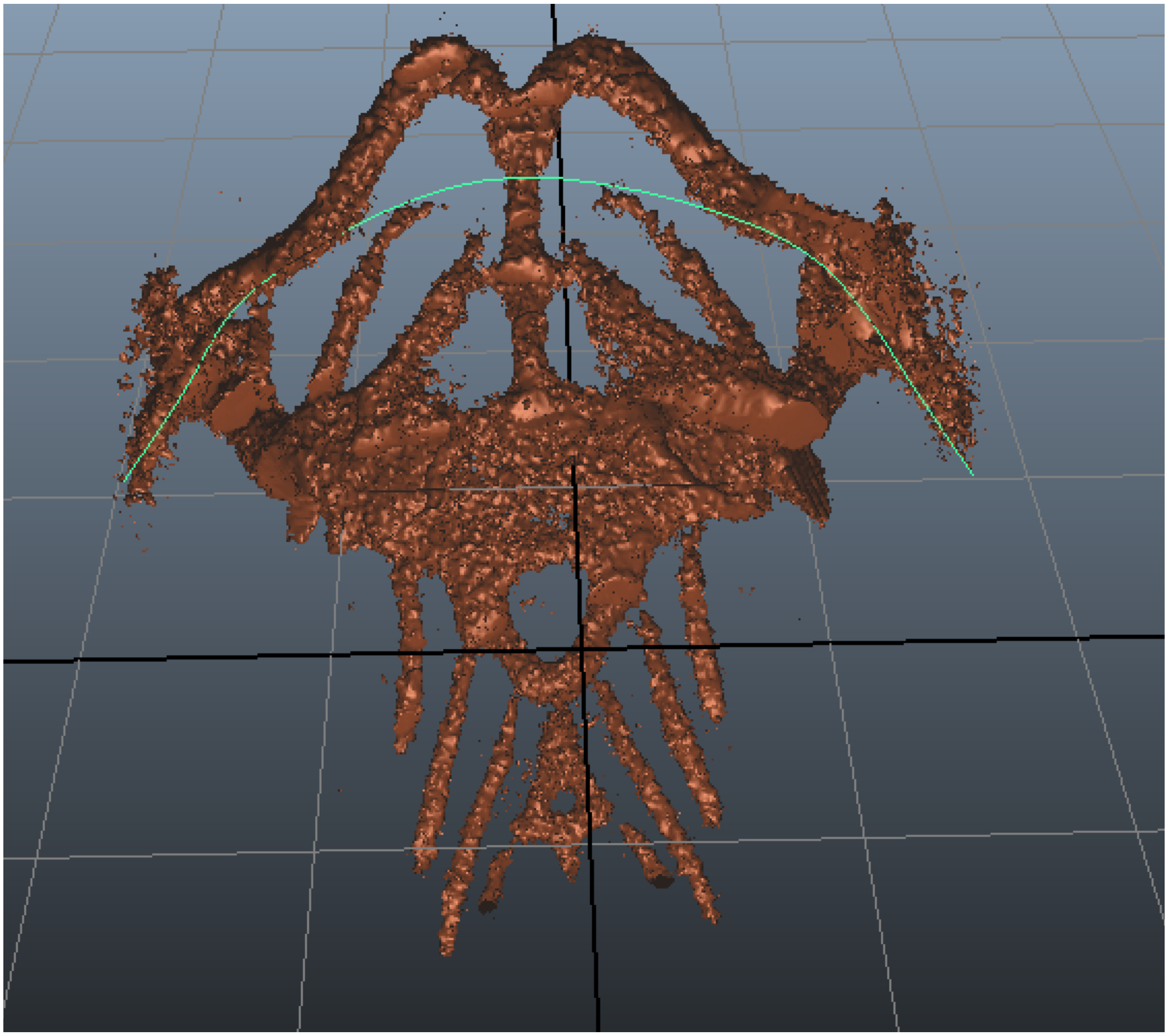


Figure 6. the measurement of a pectoral girdle in Maya (Autodesk, 2015) using the arc measurement tool.

Figure 7. *Lerista edwardsae* (YPM R-13679) has a small humerus, less than a millimeter long (highlighted in blue) that does not extend past the body wall, but is evident in the claw-like structures on the ventrolateral sides. When the CT scan of this specimen is superimposed on its photograph, it is clear the claw-like structure is in the same place as the end of the rudimentary humerus.

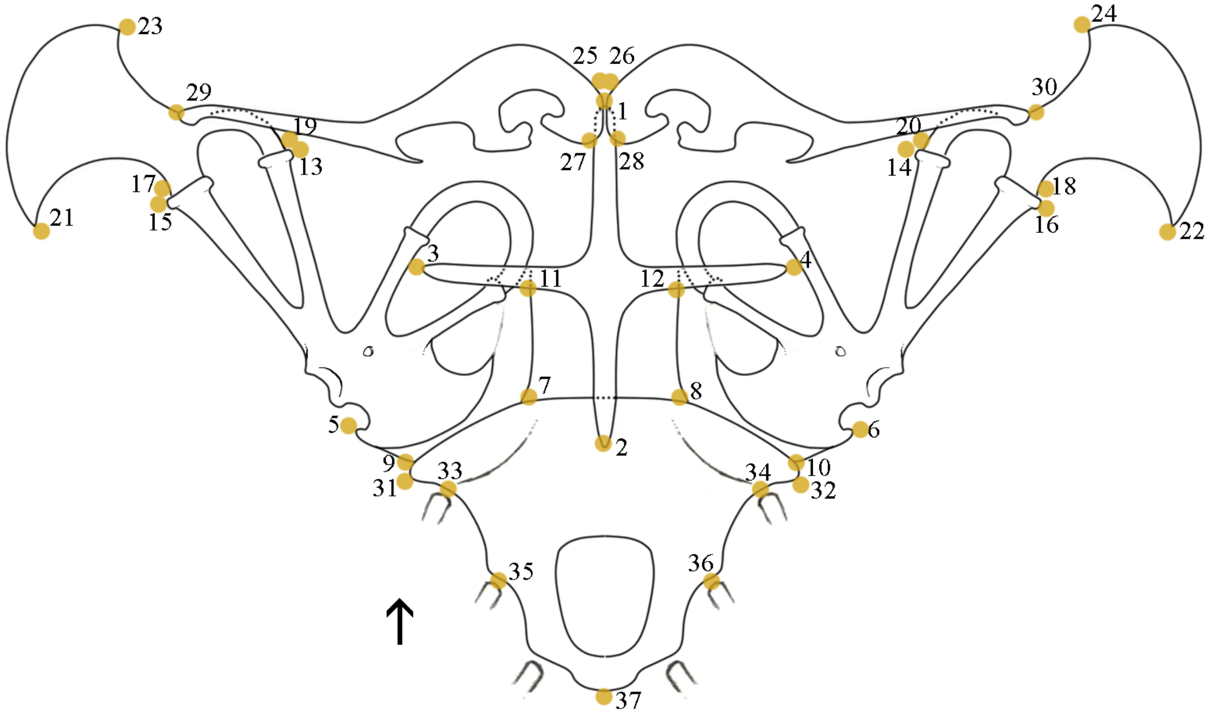

24
23
25
26
30
29
1
20
19
27
28
14
13
17
18
21
22
15
16
3
4
11
12
7
8
5
6
2
9
10
31
33
34
32
35
36
37

Figure 8. Fixed landmark points placed on a generalized pectoral girdle in ventral view(based primarily off of CAS 77518). Guidelines for placement may be found in Table 3. Each point represents a homologous point that may be found on all specimens included in the geometric morphometric analysis.

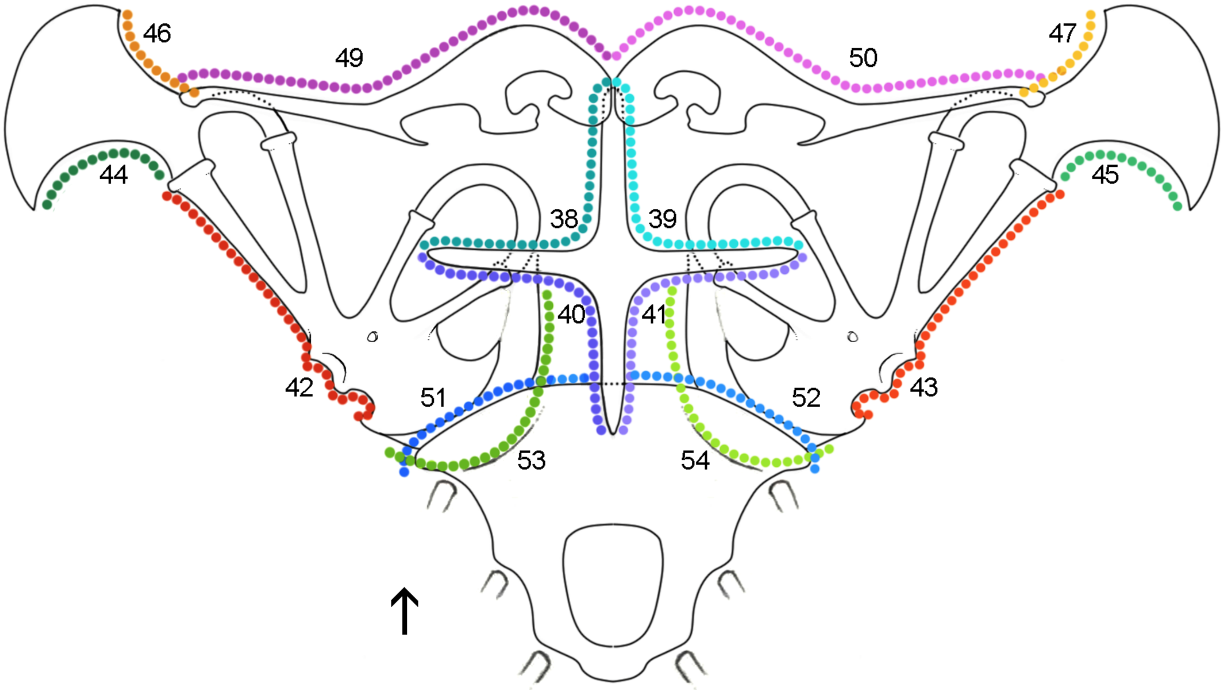

46
49
50
47
44
45
38
39
40
41
42
43
51
52
53
54
↑

Figure 9. Semilandmark points placed on a generalized pectoral girdle in ventral view(based primarily off of CAS 77518). Guidelines for placement may be found in Table 3. Each semilandmark may be comprised of multiple curves.

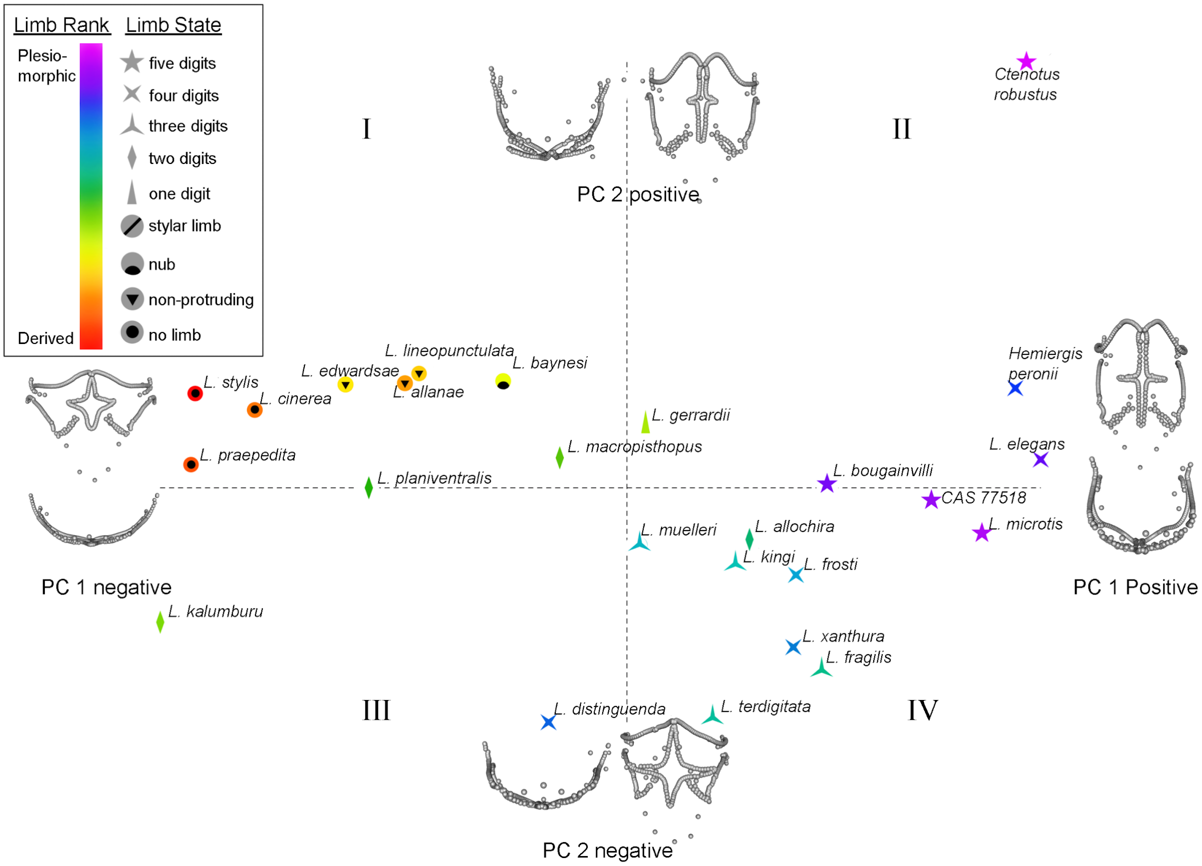

Limb Rank
Limb State
Plesio-
morphic
Derived
five digits
four digits
three digits
two digits
one digit
stylar limb
nub
non-protruding
no limb
I
II
III
IV
PC 2 positive
PC 2 negative
PC 1 negative
PC 1 Positive
Ctenotus
robustus
Hemiergis
peronii
L. lineopunctulata
L. baynesi
L. edwardsae
L. stylis
L. cinerea
L. allanae
L. gerrardii
L. macropisthopus
L. praepedita
L. planiventralis
L. elegans
L. bougainvilli
CAS 77518
L. microtis
L. muelleri
L. allochira
L. kingi
L. frosti
L. kalumburu
L. xanthura
L. fragilis
L. distinguenda
L. terdigitata

Figure 10. A PCA analysis generated from 3D landmarks placed on pectoral girdles. Landmark positions of representatives of the PC axis extremes can be seen at the ends of the axes. The data points are colored by limb rank and the symbols communicate the limb state of the forelimb.

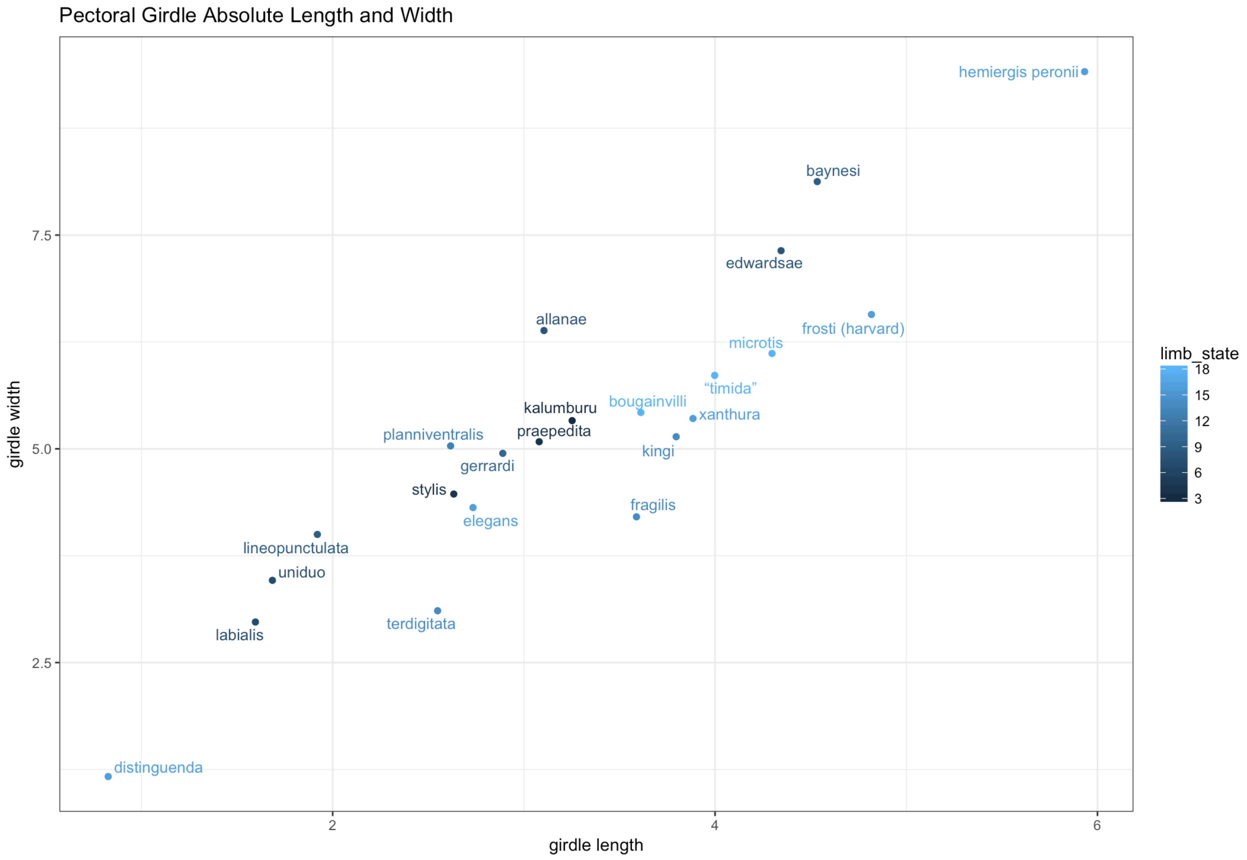

Pectoral Girdle Absolute Length and Width
girdle width
girdle length
7.5
5.0
2.5
2
4
6
limb_state
18
15
12
9
6
3
hemiergis peronii
baynesi
edwardsae
frosti (harvard)
allanae
microtis
"timida"
bougainvilli
xanthura
kalumburu
praepedita
planniventralis
kingi
gerrardi
stylis
elegans
fragilis
lineopunctulata
uniduo
labialis
terdigitata
distinguenda

Figure 11. There is a strong relationship between the circumferential width of the pectoral girdle and the anteroposterior length.

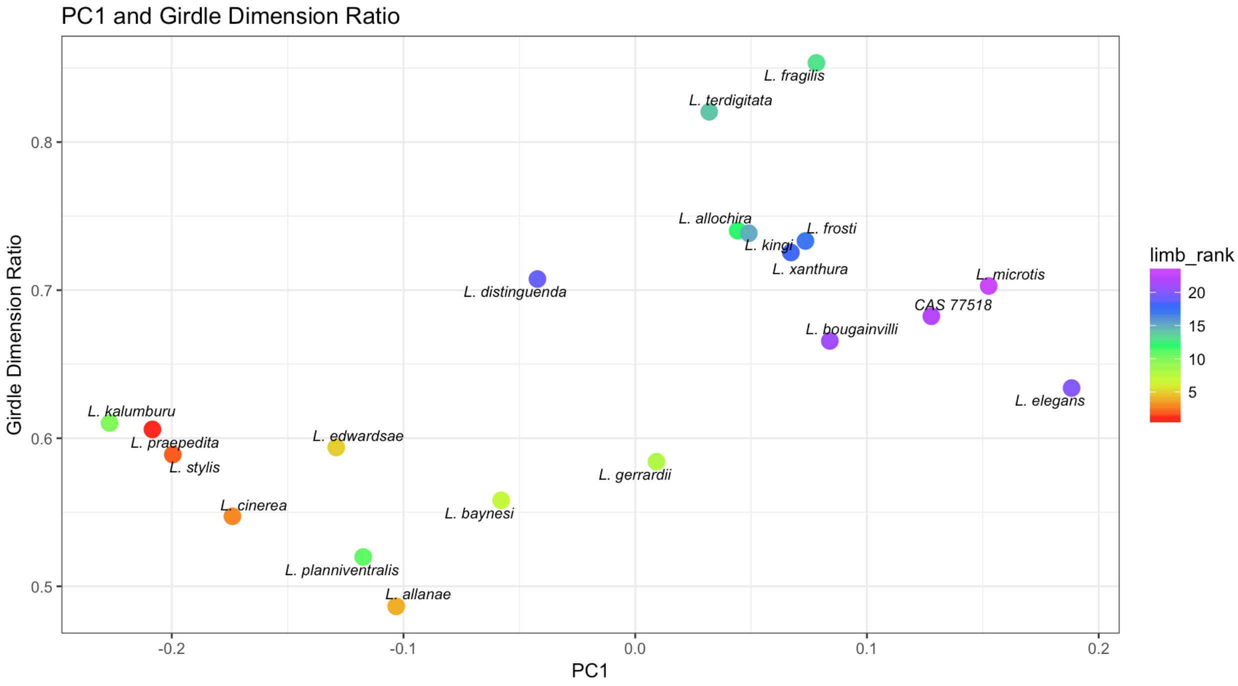

PC1 and Girdle Dimension Ratio
Girdle Dimension Ratio
PC1
0.8
0.7
0.6
0.5
-0.2
-0.1
0.0
0.1
0.2
limb_rank
20
15
10
5
L. fragilis
L. terdigitata
L. allochira
L. frosti
L. kingi
L. xanthura
L. distinguenda
L. microtis
CAS 77518
L. bougainvilli
L. elegans
L. kalumburu
L. praepedita
L. stylis
L. edwardsae
L. gerrardii
L. cinerea
L. baynesi
L. planniventralis
L. allanae

Figure 12. PC1 plotted against the length to width ratio of the pectoral girdle results in a relationship that appears similar to the PCA plot in Fig. 10

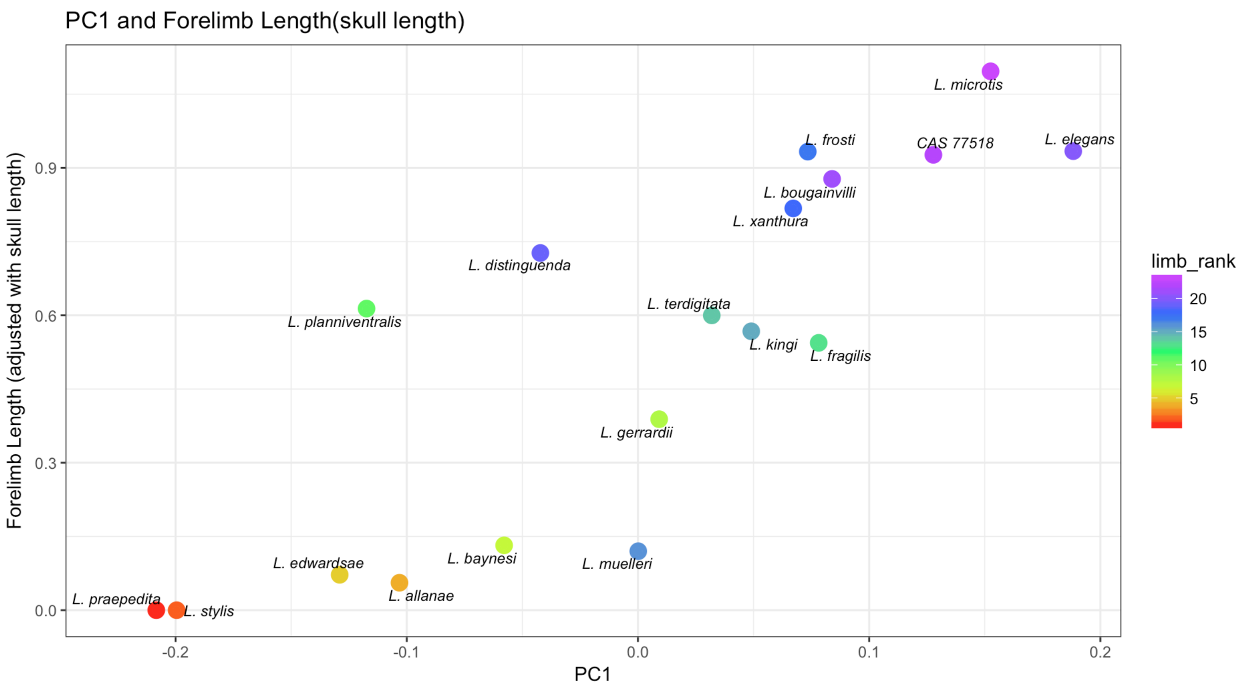

PC1 and Forelimb Length(skull length)
Forelimb Length (adjusted with skull length)
PC1
0.0
0.3
0.6
0.9
-0.2
-0.1
0.0
0.1
0.2
limb_rank
20
15
10
5
L. microtis
L. frosti
CAS 77518
L. elegans
L. bougainvilli
L. xanthura
L. distinguenda
L. planniventralis
L. terdigitata
L. kingi
L. fragilis
L. gerrardii
L. edwardsae
L. baynesi
L. muelleri
L. praepedita
L. stylis
L. allanae

Fig 13. PC1 scores plotted against forelimb length relative to skull length

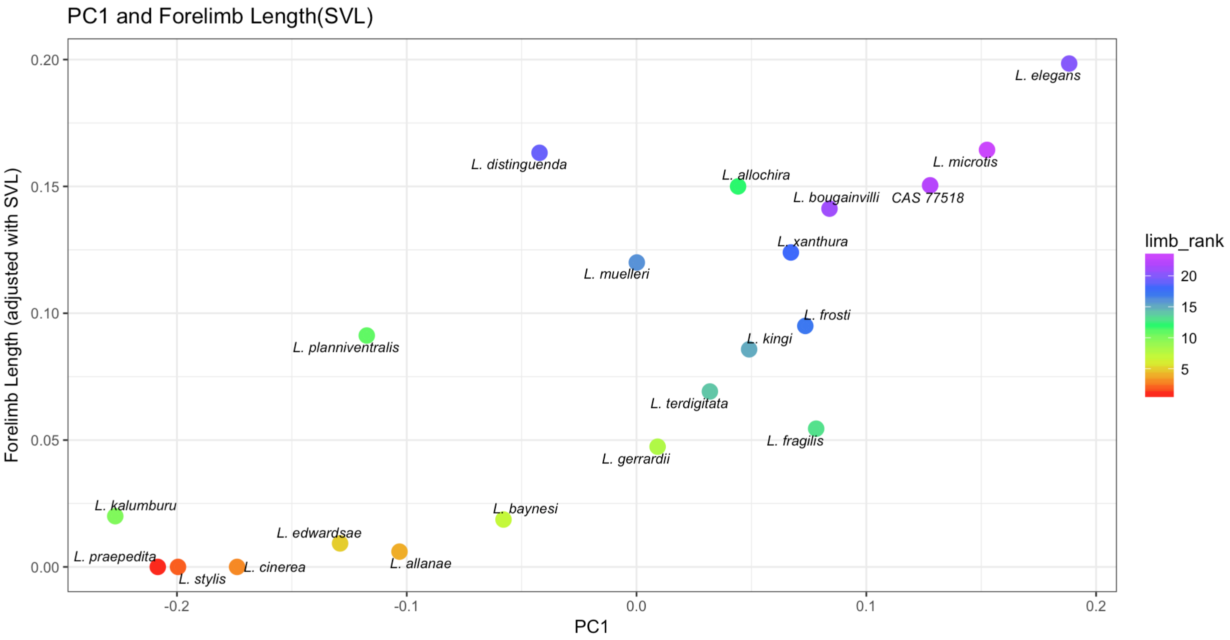

PC1 and Forelimb Length(SVL)
Forelimb Length (adjusted with SVL)
PC1
0.20
0.15
0.10
0.05
0.00
-0.2
-0.1
0.0
0.1
0.2
limb_rank
20
15
10
5
*L. elegans*
*L. distinguenda*
*L. microtis*
*L. allochira*
*L. bougainvilli*
*CAS 77518*
*L. xanthura*
*L. muelleri*
*L. frosti*
*L. kingi*
*L. planniventralis*
*L. terdigitata*
*L. fragilis*
*L. gerrardii*
*L. kalumburu*
*L. baynesi*
*L. edwardsae*
*L. praepedita*
*L. cinerea*
*L. allanae*
*L. stylis*

Figure 14. PC1 plotted against forelimb as a percent of SVL

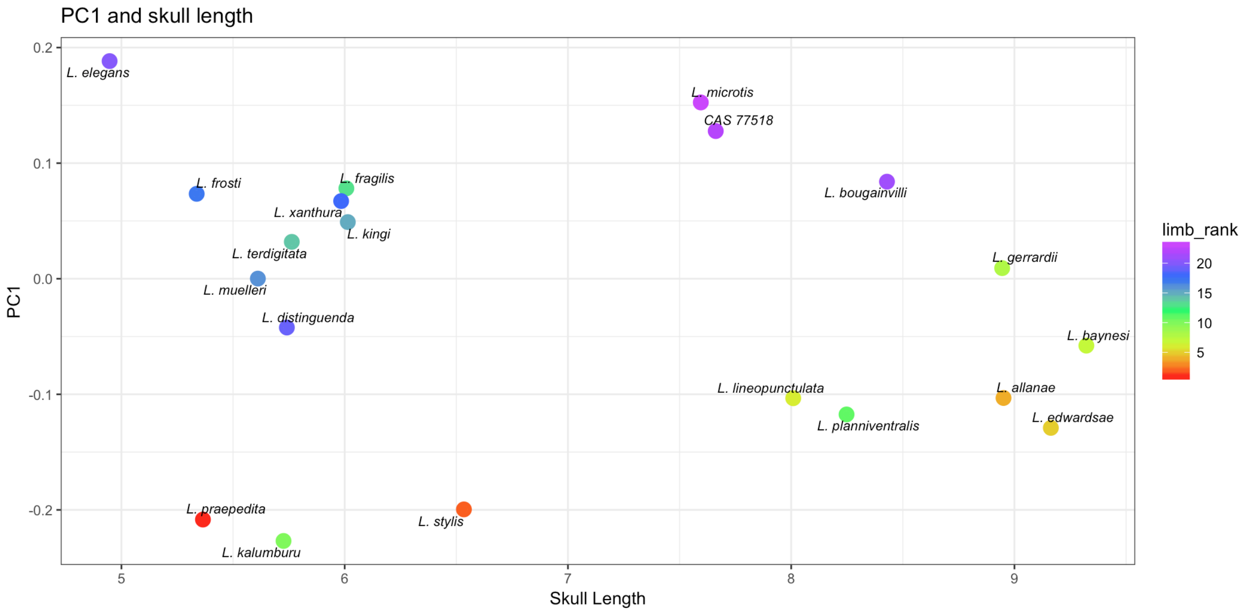

PC1 and skull length
PC1
Skull Length
0.2
0.1
0.0
-0.1
-0.2
5
6
7
8
9
limb_rank
20
15
10
5
L. elegans
L. microtis
CAS 77518
L. frosti
L. fragilis
L. xanthura
L. kingi
L. bougainvilli
L. terdigitata
L. muelleri
L. gerrardii
L. distinguenda
L. baynesi
L. lineopunctulata
L. allanae
L. planniventralis
L. edwardsae
L. praepedita
L. stylis
L. kalumburu

Figure 15. PC1 scores plotted against skull length

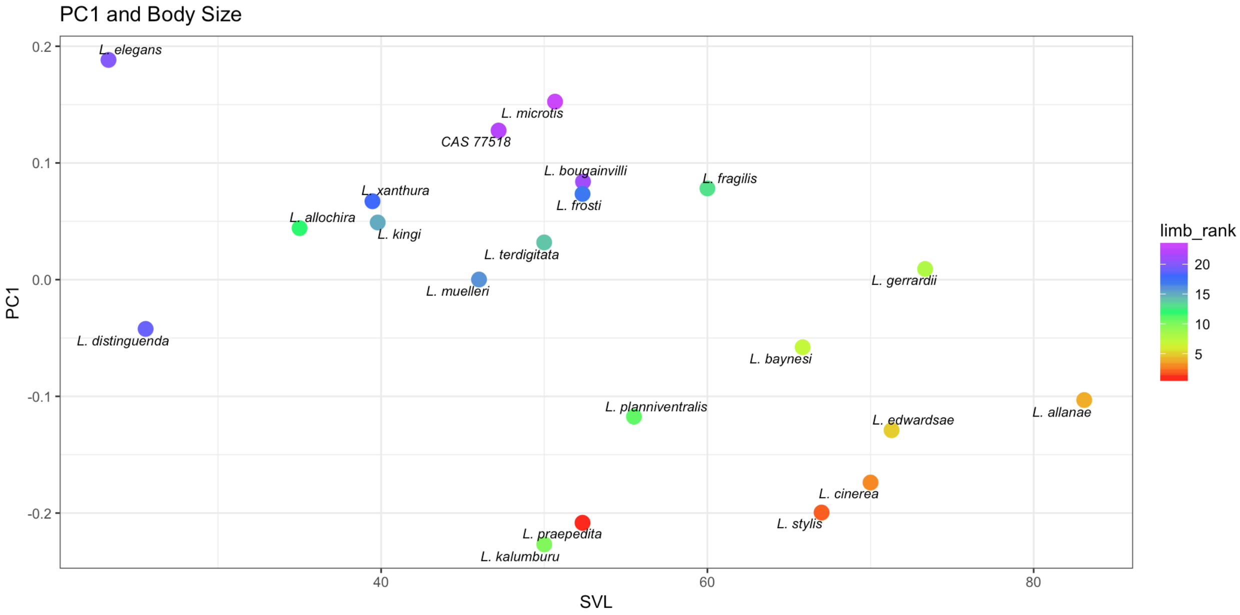
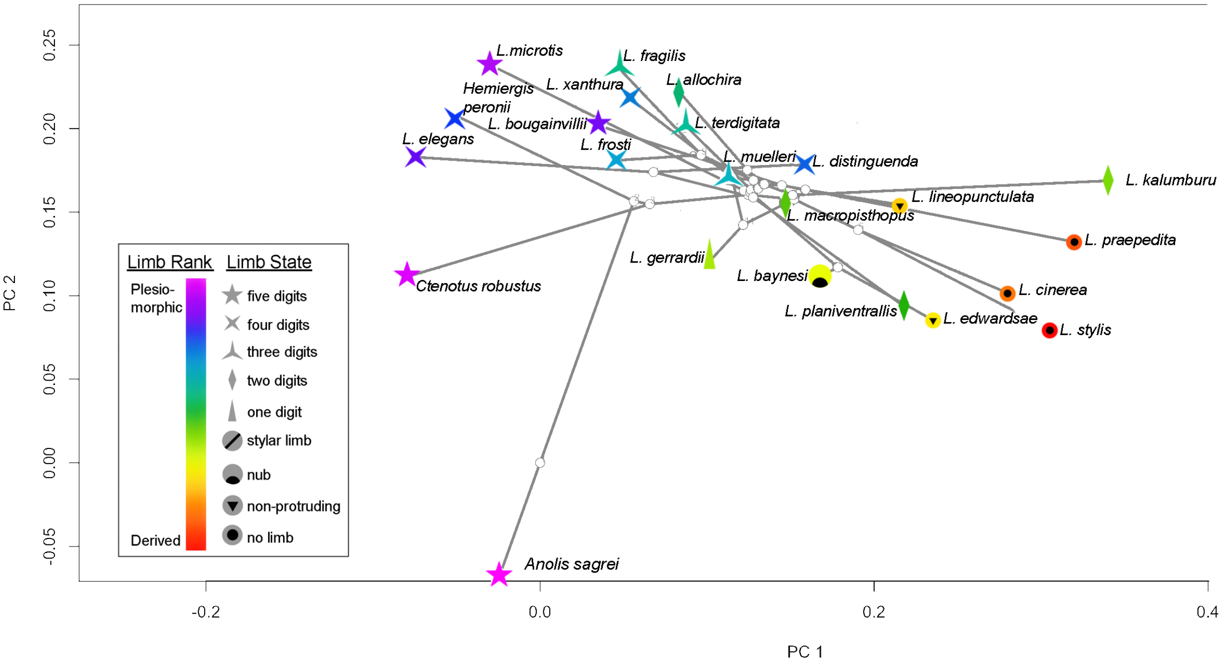

PC1 and Body Size
PC1
SVL
0.2
0.1
0.0
-0.1
-0.2
40
60
80
limb_rank
20
15
10
5
L. elegans
L. microtis
CAS 77518
L. bougainvilli
L. fragilis
L. xanthura
L. frosti
L. allochira
L. kingi
L. terdigitata
L. gerrardii
L. muelleri
L. distinguenda
L. baynesi
L. planniventralis
L. allanae
L. edwardsae
L. cinerea
L. stylis
L. praepedita
L. kalumburu

Figure 16. PC1 scores plotted against SVL



PC 2
PC 1
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.2
0.0
0.2
0.4
L.microtis
L. fragilis
Hemiergis peronii
L. xanthura
L. allochira
L. bougainvillii
L. terdigitata
L. elegans
L. frosti
L. muelleri
L. distinguenda
L. kalumburu
L. lineopunctulata
L. macropisthopus
L. praepedita
L. gerrardii
L. baynesi
Ctenotus robustus
L. cinerea
L. planiventrallis
L. edwardsae
L. stylis
Anolis sagrei
Limb Rank
Limb State
Plesio-morphic
Derived
five digits
four digits
three digits
two digits
one digit
stylar limb
nub
non-protruding
no limb

Figure 17. A phylogenetic PCA showing the relationship between phylogeny and the morphospace occupied by pectoral girdles of Lerista specimens of various limb states.

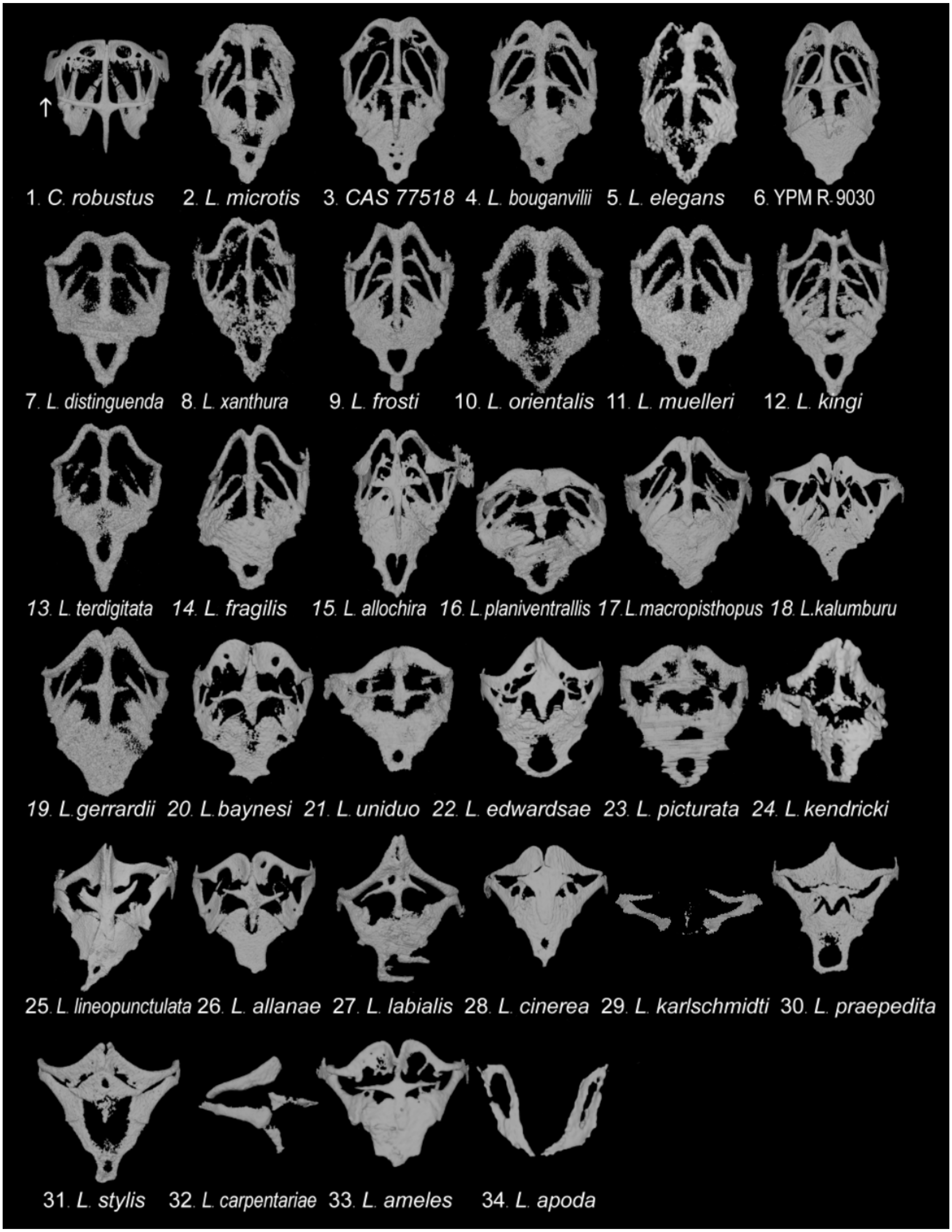

1. C. robustus
2. L. microtis
3. CAS 77518
4. L. bouganvilii
5. L. elegans
6. YPM R-9030
7. L. distinguenda
8. L. xanthura
9. L. frosti
10. L. orientalis
11. L. muelleri
12. L. kingi
13. L. terdigitata
14. L. fragilis
15. L. allochira
16. L. planiventrallis
17. L. macropisthopus
18. L. kalumburu
19. L. gerrardii
20. L. baynesi
21. L. uniduo
22. L. edwardsae
23. L. picturata
24. L. kendricki
25. L. lineopunctulata
26. L. allanae
27. L. labialis
28. L. cinerea
29. L. karlschmidti
30. L. praepedita
31. L. stylis
32. L. carpentariae
33. L. ameles
34. L. apoda

Figure 18. The pectoral girdles of all specimens in this study in ventral view including all *Lerista* specimens as well as *Ctenotus robustus* and *Hemiergis peronii*. They are arranged by limb rank, with C. robustus representing the most plesiomorphic and L. apoda, the most derived and snake-like.

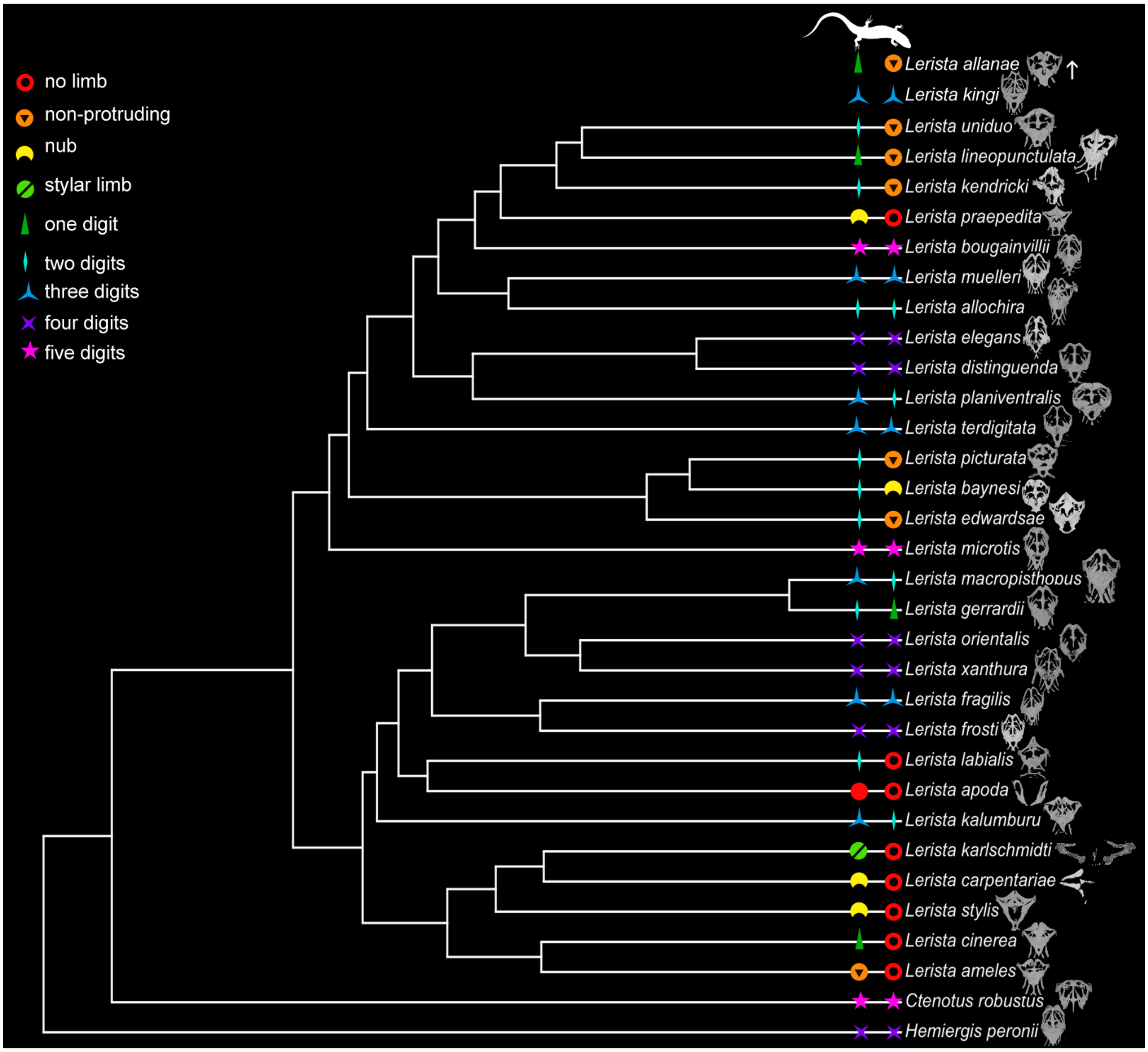

no limb
non-protruding
nub
stylar limb
one digit
two digits
three digits
four digits
five digits
Lerista allanae
Lerista kingi
Lerista uniduo
Lerista lineopunctulata
Lerista kendricki
Lerista praepedita
Lerista bougainvillii
Lerista muelleri
Lerista allochira
Lerista elegans
Lerista distinguenda
Lerista planiventralis
Lerista terdigitata
Lerista picturata
Lerista baynesi
Lerista edwardsae
Lerista microtis
Lerista macropisthopus
Lerista gerrardii
Lerista orientalis
Lerista xanthura
Lerista fragilis
Lerista frosti
Lerista labialis
Lerista apoda
Lerista kalumburu
Lerista karlschmidti
Lerista carpentariae
Lerista stylis
Lerista cinerea
Lerista ameles
Ctenotus robustus
Hemiergis peronii

Figure 19. The pectoral girdles of all specimens included in this study in ventral view placed on a phylogeny. Limb states are indicated with colors and symbols to the left of the species name. Forelimb states are indicated by the symbol on the right and hindlimb states are indicated by the symbol on the left.

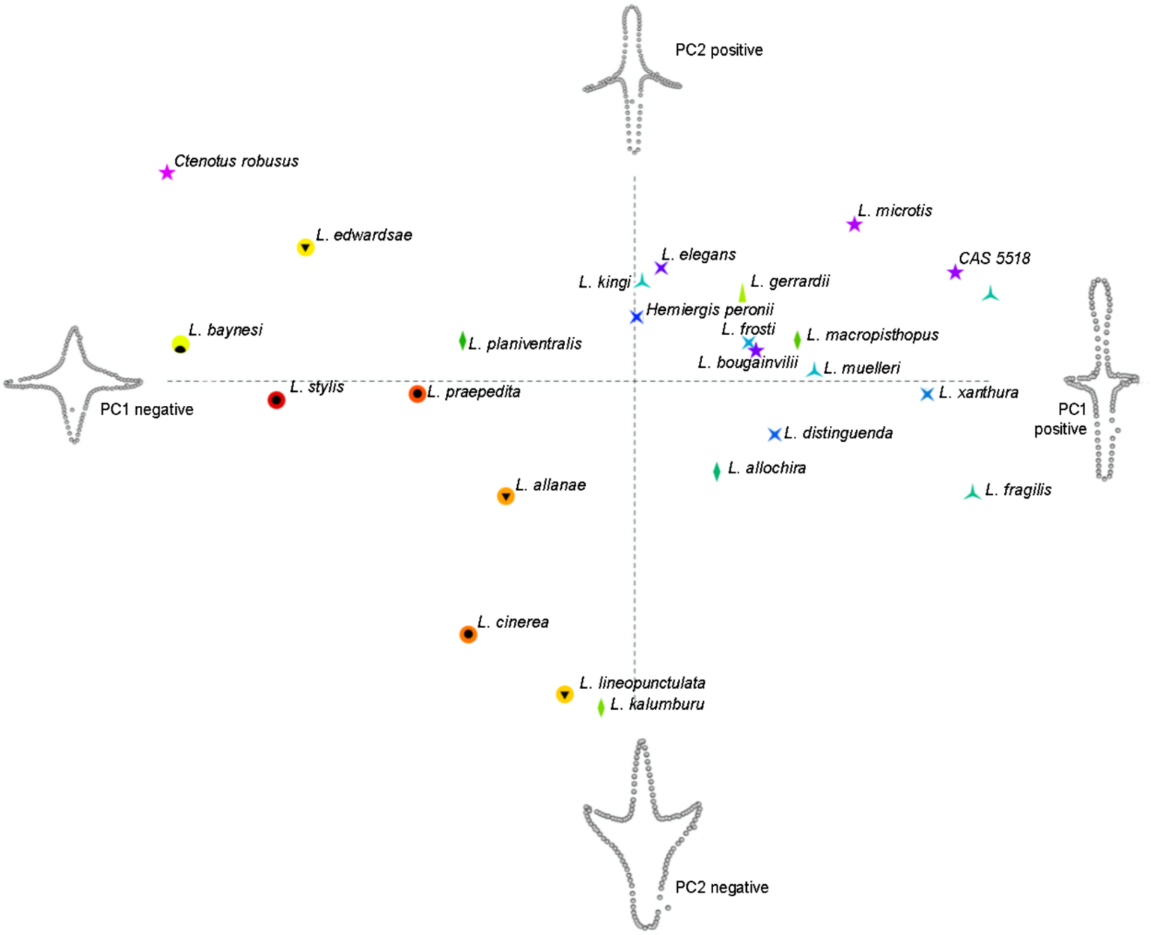

PC2 positive
Ctenotus robusus
L. microtis
L. edwardsae
L. elegans
CAS 5518
L. kingi
L. gerrardii
Hemiergis peronii
L. baynesi
L. frosti
L. macropisthopus
L. planiventralis
L. bougainvilii
L. muelleri
L. stylis
L. praepedita
L. xanthura
PC1 negative
PC1 positive
L. distinguenda
L. allochira
L. allanae
L. fragilis
L. cinerea
L. lineopunctulata
L. kalumburu
PC2 negative

Figure 20. A PCA plot with only landmarks on the interclavicle.

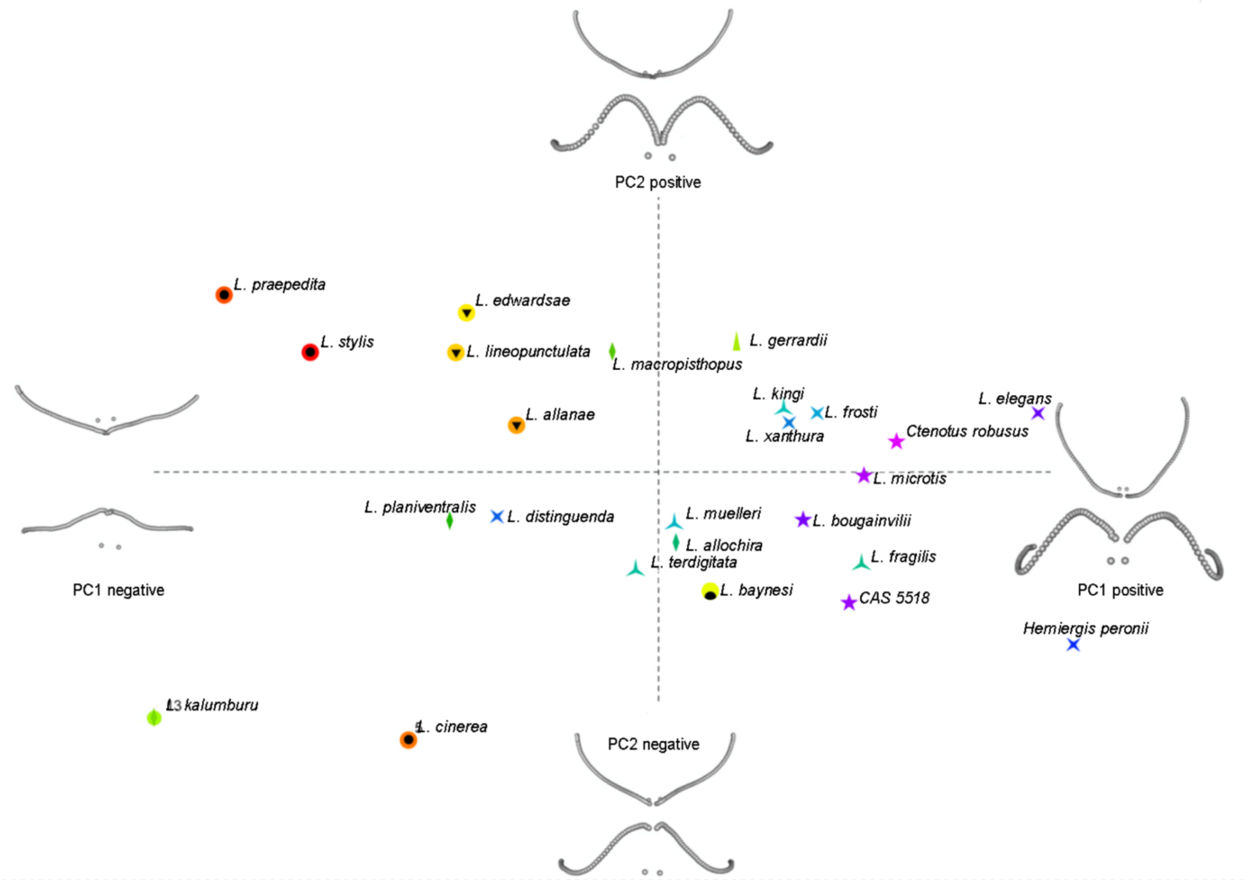

PC2 positive
L. praepedita
L. edwardsae
L. stylis
L. lineopunctulata
L. macropisthopus
L. gerrardii
L. kingi
L. frosti
L. elegans
L. xanthura
Ctenotus robusus
L. allanae
L. microtis
PC1 negative
PC1 positive
L. planiventralis
L. distinguenda
L. muelleri
L. bougainvilii
L. allochira
L. terdigitata
L. fragilis
L. baynesi
CAS 5518
Hemiergis peronii
L. kalumburu
L. cinerea
PC2 negative

Figure 21. A PCA plot with only landmark points on the clavicle.

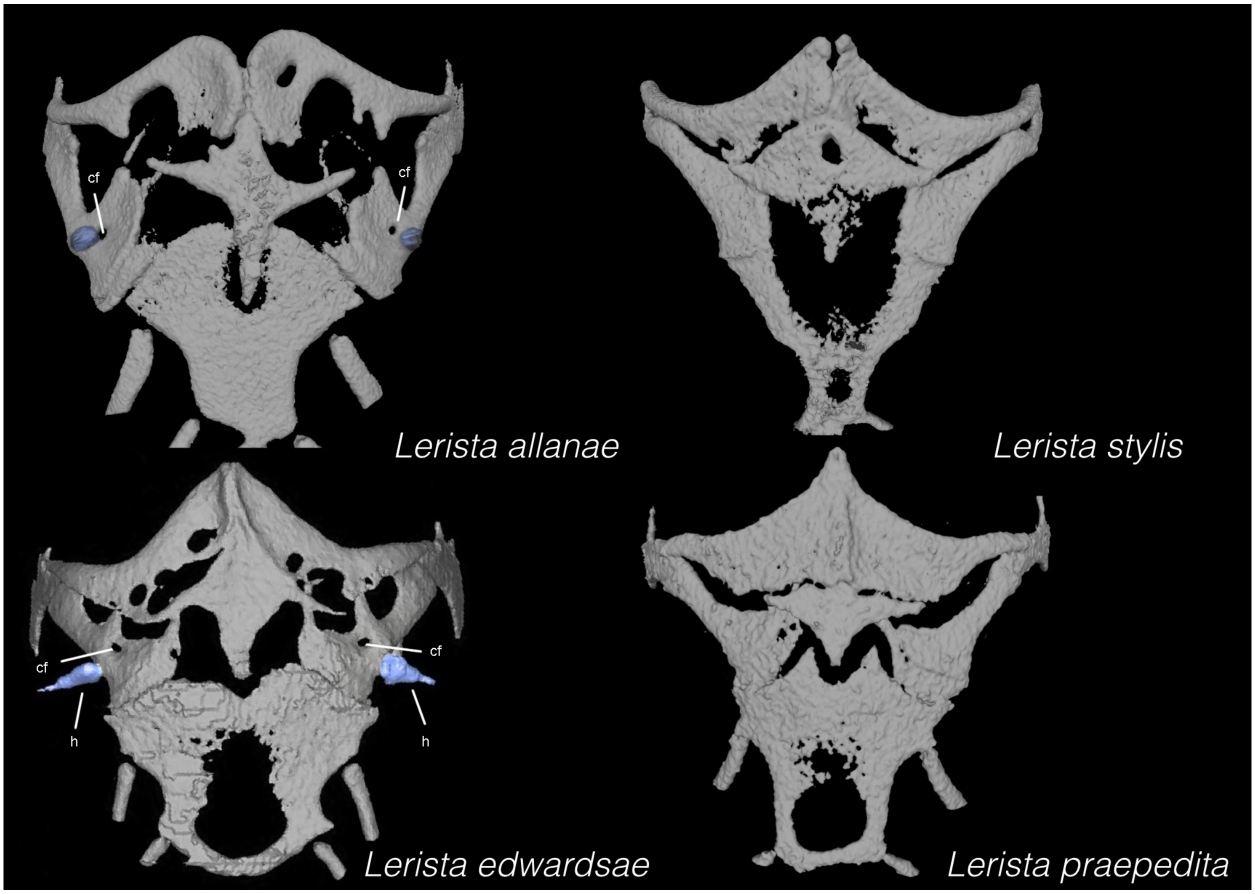

cf
cf
Lerista allanae
Lerista stylis
cf
cf
h
h
Lerista edwardsae
Lerista praepedita

Figure 22. Species with a small humeral remnant (colored blue) still have a coracoid foramen (cf), but species that have no humerus do not have a coracoid foramen.

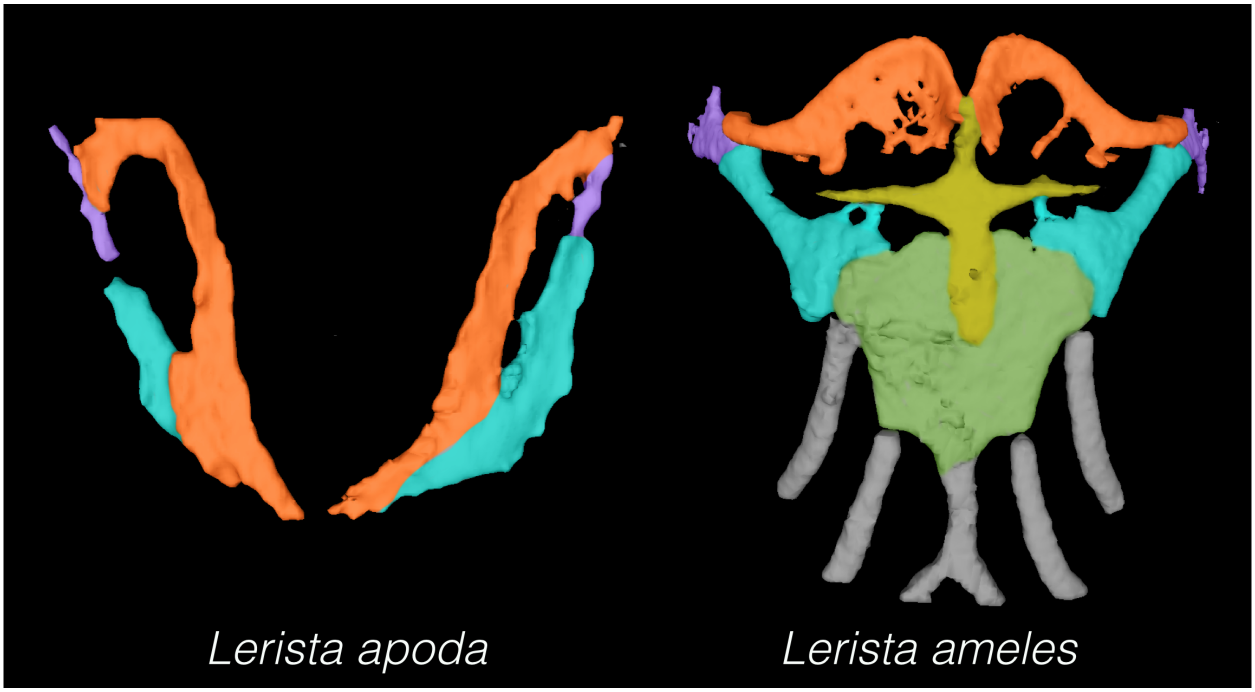

*Lerista apoda*
*Lerista ameles*

Figure 23. *Lerista apoda* and *Lerista ameles* both have no external evidence of limbs and no internal remnants of a forelimb but *L. apoda* has a much more dramatically reduced pectoral girdle than *L. ameles*.

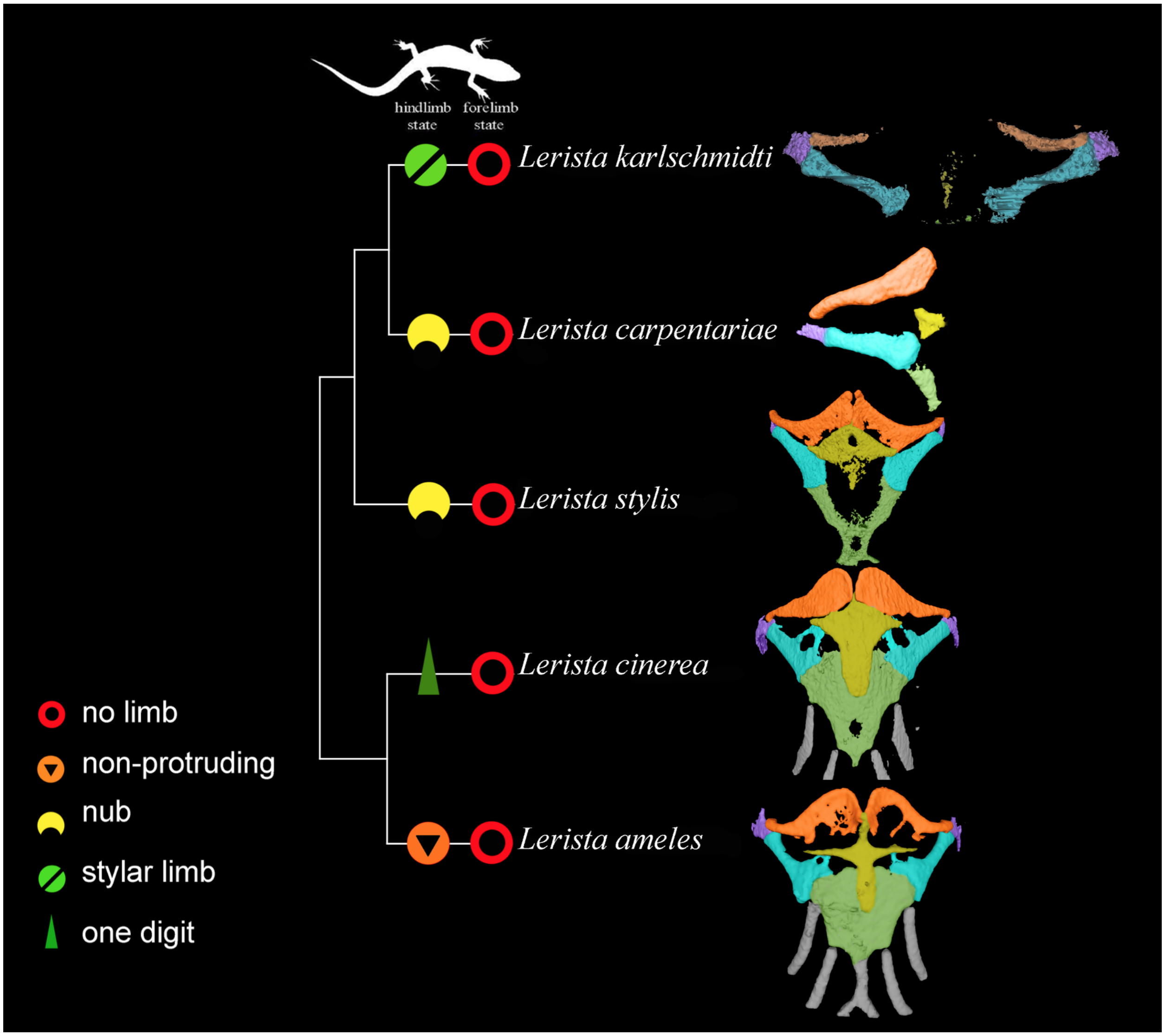
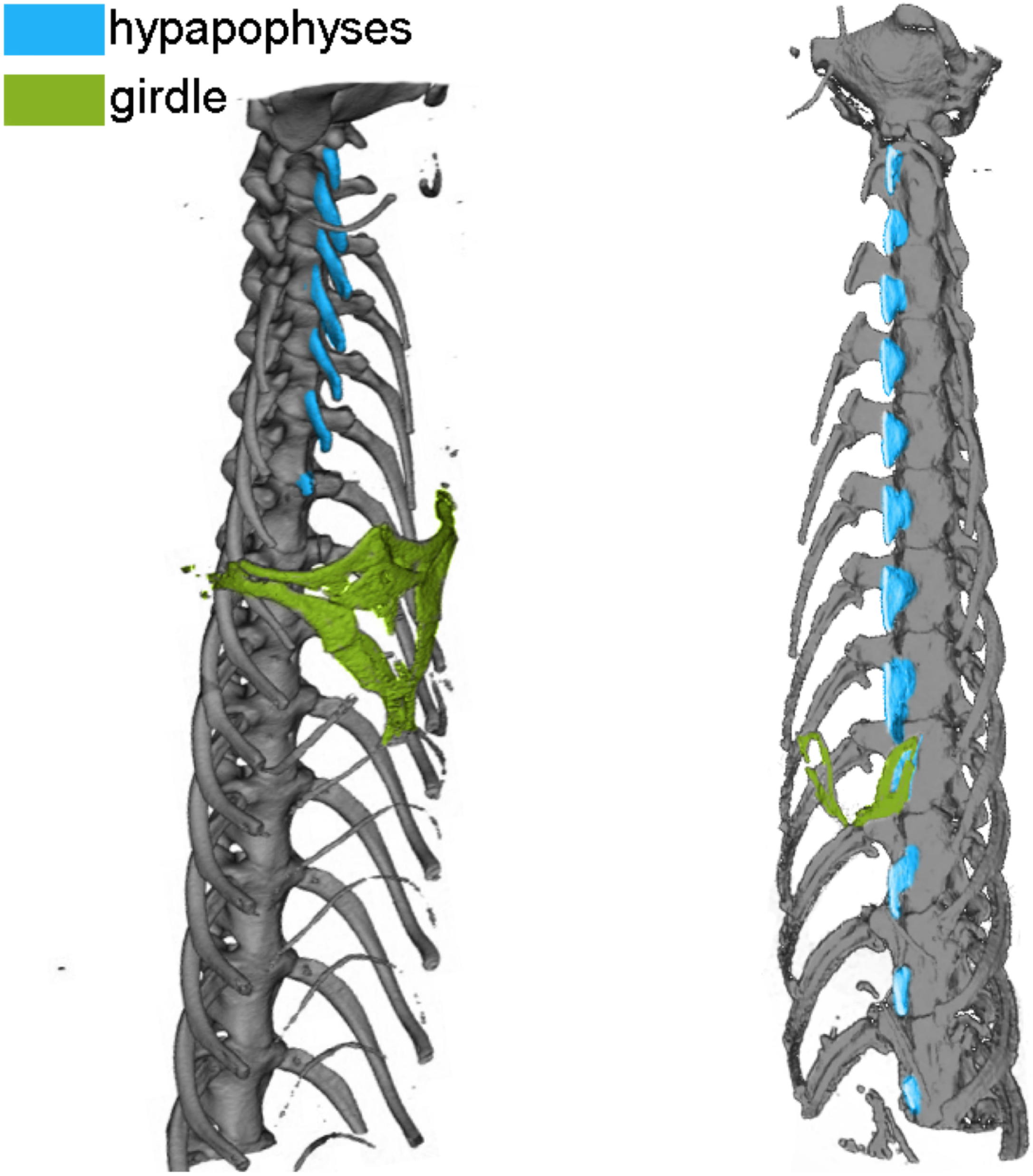

hindlimb state
forelimb state
Lerista karlschmidti
Lerista carpentariae
Lerista stylis
Lerista cinerea
Lerista ameles
no limb
non-protruding
nub
stylar limb
one digit

Figure 24. Two closely related clades appear to have aspects of their morphology that are unique to their subclades and are not merely due to their limb states.

Figure 25. A CT scan of *Lerista carpentariae* (USNM 128409), which only has pectoral girdle elements (highlighted in green) on its left side.



*Lerista stylis* *Lerista apoda*

Figure 26. *Lerista stylis*, with hypapophyses (highlighted in blue) extending posteriorly only to the pectoral girdle (highlighted in green). In *Lerista apoda*, the hypapophyses extend past the pectoral girdle.

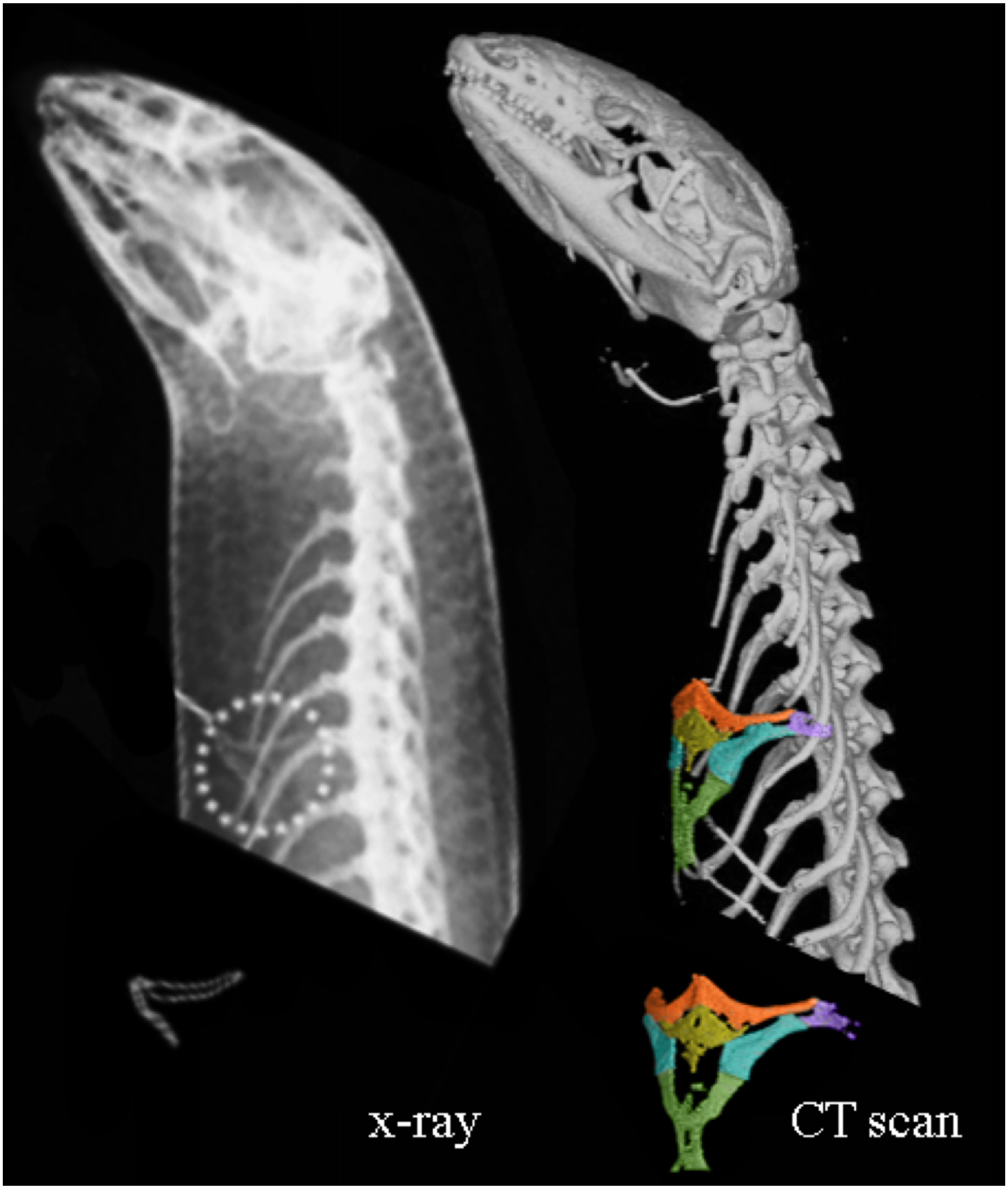

x-ray
CT scan

Figure 27. The image on the left is the x ray radiograph taken from Moch and Senter, 2011:pg.3, Fig. 30 c2 of *Lerista stylis* (USNM 128640). The image on the right is the CT scan that resulted from this study of the same specimen in the same orientation.

## 11. Tables

| **Species** | **Specimen number** | **Collection** | **notes on identification** |
|---|---|---|---|
| ***Lerista allanae*** | CAS 77099 | California Academy of Sciences | |
| ***Lerista allochira*** | WAM-R-132460 | West Australia Museum | |
| ***Lerista ameles*** | SAMA-R-55806 | South Australia Museum | |
| ***Lerista apoda*** | WAM-R-168495 | West Australia Museum | |
| ***Lerista baynesi*** | FMNH 75489 | Field Museum of Natural History | |
| ***Lerista bougainvillii*** | MCZ-R-129545 | Museum of Comparative Zoology, Harvard University | |
| ***Lerista carpentariae*** | USNM 128409 | National Museum of Natural History | |
| ***Lerista cinerea*** | SAM-R-54521 | South Australia Museum | |
| ***Lerista distinguenda*** | UMMZ-132393 | University of Michigan Museum of Zoology | |
| ***Lerista edwardsae*** | YPM R-13679 | Yale Peabody Museum | |
| ***Lerista elegans*** | CU R-0012147 | Cornell University Museum of Vertebrates | |
| ***Lerista fragilis*** | CAS 77102 | California Academy of Sciences | |
| ***Lerista frosti*** | MCZ-R-51893 | Museum of Comparative Zoology, Harvard University | |

| | | | |
|---|---|---|---|
| **Lerista gerrardii** | MCZ-R-33258 | Museum of Comparative Zoology, Harvard University | |
| **Lerista kalumburu** | WAM R-100188 | West Australia Museum | |
| **Lerista karlschmidti** | AMNH 120381 | American Museum of Natural History | |
| **Lerista kendricki** | CU R-0011970 | Cornell University Museum of Vertebrates | |
| **Lerista kingi** | CAS 254618 | California Academy of Sciences | |
| **Lerista labialis** | AMNH 86086 | American Museum of Natural History | |
| **Lerista lineopunctulata** | MCZ-R-33261 | Museum of Comparative Zoology, Harvard University | |
| **Lerista macropisthopus** | CU R-0014358 | Cornell University Museum of Vertebrates | |
| **Lerista microtis** | MCZ-R-24577 | Museum of Comparative Zoology, Harvard University | |
| **Lerista microtis** | CAS 77518 | California Academy of Sciences | originally identified as *Lerista timida* |
| **Lerista muelleri** | MCZ-R-86703 | Museum of Comparative Zoology, Harvard University | |
| **Lerista orientalis** | UMMZ-203363 | University of Michigan Museum of Zoology | |
| **Lerista picturata** | MCZ-R-130376 | Museum of Comparative Zoology, Harvard University | |
| **Lerista planiventralis** | UMMZ-241013 | University of Michigan Museum of Zoology | |

| | | | |
|---|---|---|---|
| ***Lerista praepedita*** | R-187806 | Museum of Comparative Zoology, Harvard University | |
| ***Lerista stylis*** | USNM 128640 | National Museum of Natural History | |
| ***Lerista terdigitata*** | MCZ-R-131081 | Museum of Comparative Zoology, Harvard University | |
| ***Lerista uniduo*** | CU R-11945 | Cornell University Museum of Vertebrates | |
| ***Lerista xanthura*** | ROM 15682 | Royal Ontario Museum | |
| ***Hemiergis peronii*** | YPM R-9030 | Yale Peabody Museum | originally identified as *Lerista frosti* |
| ***Ctenotus robustus*** | FMNH 125939 | Field Museum of Natural History | |

Table 1. Specimen numbers and sources

| **Species** | **Specimen number** | **number of forelimb digits** | **forelimb state** | **number of hindlimb digits** | **hindlimb state** | **forelimb length** | **hindlimb length** | **Snout-Vent length** | **head length** | **pectoral girdle length** | **pectoral girdle width** | **pectoral girdle dimension ratio** | **Limb rank** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **description of measurement** | | the number of digits on the forelimb | The category describing degree of limb loss: five digits, four digits, three digits, two digits, one digit, stylar limb (longer than wide), | | | average the length of the forelimbs; measured from the middle of the proximal end to the distal-most point (end of the claw of the longest digit, or the length of the distal | | the length from the anterior tip of the snout to the posterior-most edge of the scales covering the vent. some specimens were preserved in a way that made this measurement impossible with calipers. | heat lengths were obtained in meshlab from CT scans. the distance tool was used to measure from the | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | nub (about as long as it is wide), non-protruding (humerus present but does not extend past the body wall), no limb (no limb elements present) | | | ent of the limb in digit-less specimens). in specimens with malformed or amputated limbs, only the whole limb was included. measurements in millimeters. | | in some cases, measurements were taken digitally(indicated with an *) from photographs, but in some, measurements were not possible so estimates from Cogger, 2014 were used (indicated with **). | anterior-most part of the atlas visible in ventral view, to the anterior end of the snout. | | | | |
| ***Lerista allanae*** | CAS 77099 | 0 | non-protruding | 1 | one digit | 0 | 6.7 | 83.1* | 8.95 | 3.11 | 6.38 | 2.06 | 26 |
| ***Lerista allochir*** | WAM-R- | 2 | two digits | 3 | three digits | 5.25 | 8.75 | 35** | - | 3.51 | 4.75 | 1.35 | 15 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***a*** | 132460 | | | | | | | | | | | | |
| ***Lerista ameles*** | SAMA-R-55806 | 0 | no limb | 0 | non-protruding | 0 | 0 | 58** | - | - | - | - | 33 |
| ***Lerista apoda*** | WAM-R-168495 | 0 | no limb | 0 | no limb | 0 | 0 | 75** | - | 0.88 | 1.23 | 1.41 | 34 |
| ***Lerista baynesi*** | FMNH 75489 | 0 | nub | 2 | two digits | 1.23 | 6.51 | 65.84 | 9.32 | 4.53 | 8.13 | 1.79 | 20 |
| ***Lerista bougainvillii*** | MCZ-R-129545 | 5 | five digits | 5 | five digits | 7.40 | 13.09 | 52.375 | 8.43 | 3.61 | 5.43 | 1.50 | 4 |
| ***Lerista carpentariae*** | USNM 128409 | 0 | no limb | 0 | nub | 0 | 1.62 | 65.2 | 5.73 | 1.44 | 1.51 | 1.05 | 32 |
| ***Lerista cinerea*** | SAM-R-54521 | 0 | no limb | 1 | one digit | 0 | 3.5** | 70** | - | 2.72 | 4.96 | 1.83 | 28 |
| ***Lerista distinguenda*** | UMMZ-132393 | 4 | four digits | 4 | four digits | 4.17 | 7.3967 | 25.5 | 5.74 | 0.83 | 1.16 | 1.41 | 7 |
| ***Lerista edwardsae*** | YPM R-13679 | 0 | non-protruding | 2 | two digits | 0 | 11.8 | 71.29 | 9.16 | 4.35 | 7.32 | 1.68 | 22 |
| ***Lerista elegans*** | CU R-0012147 | 4 | four digits | 4 | four digits | 4.89 | 11.35 | 23.29 | 4.95 | 2.73 | 4.31 | 1.58 | 5 |
| ***Lerista fragilis*** | CAS 77102 | 3 | three digits | 3 | three digits | 3** | 10.2** | 60** | 6.01 | 3.59 | 4.21 | 1.17 | 14 |
| ***Lerista frosti*** | MCZ-R-51893 | 4 | four digits | 4 | four digits | 4.37 | 10.60 | 43.73 | 6.84 | 4.82 | 6.57 | 1.36 | 9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Lerista gerrardii*** | MCZ-R-33258 | 1 | one digit | 2 | two digits | 13.25 | 3.48 | 73.35 | 8.95 | 2.89 | 4.95 | 1.71 | 19 |
| ***Lerista kalumburu*** | WAM R-100188 | 2 | two digits | 3 | three digits | 1** | 6.5** | 50** | 5.73 | 3.25 | 5.33 | 1.64 | 18 |
| ***Lerista karlschmidti*** | AMNH 120381 | 0 | no limb | 0 | stylar | 0 | 2.29 | 46.0 | - | - | - | - | 29 |
| ***Lerista kendricki*** | CU R-0011970 | 0 | non-protruding | 2 | two digits | 0 | 8.2 | 65** | 5.97 | - | - | - | 24 |
| ***Lerista kingi*** | CAS 254618 | 3 | three digits | 3 | three digits | 3.413 | 8.54 | 39.79 | 6.01 | 3.80 | 35.14 | 1.35 | 12 |
| ***Lerista labialis*** | AMNH 86086 | 0 | no limb | 2 | two digits | 0 | 9.53 | 52 | - | 1.6 | 2.97 | 1.86 | 27 |
| ***Lerista lineopunctulata*** | MCZ-R-33261 | 0 | non-protruding | 1 | one digit | 0 | 10** | 100** | 8.01 | 4.38 | 4.0 | 1.65 | 25 |
| ***Lerista macropisthopus*** | CU R-0014358 | 2 | two digits | 3 | three digits | 3.6** | 13.5** | 90** | - | 4.05 | 6.12 | 1.64 | 17 |
| ***Lerista microtis*** | MCZ-R-24577 | 5 | five digits | 5 | five digits | 8.33 | 13.30 | 50.66 | 7.60 | 4.30 | 6.12 | 1.42 | 2 |
| ***Lerista microtis*** | CAS 77518 | 5 | five digits | 5 | five digits | 7.1 | 12.27 | 47.2 | 7.66 | 4.00 | 5.86 | 1.47 | 3 |
| ***Lerista muelleri*** | MCZ-R-86703 | 3 | three digits | 3 | three digits | 5.4** | 11.25** | 45** | 5.61 | - | - | - | 11 |
| ***Lerista orientali*** | UMM | 4 | four digits | 4 | four digits | 3.87 | 8.67 | 43.37 | 5.37 | 2.01 | 2.63 | 1.31 | 10 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***s*** | Z-203363 | | | | | | | | | | | | |
| ***Lerista picturata*** | MCZ-R-130376 | 0 | non-protruding | 2 | two digits | 0 | 12.3 | 74.9 | 9.19 | - | - | - | 23 |
| ***Lerista planiventralis*** | UMMZ-241013 | 3 | two digits | 3 | three digits | 5.3385 | 15.66 | 55.51 | 8.25 | 2.62 | 5.03 | 1.92 | 16 |
| ***Lerista praepedita*** | R-187806 | 0 | no limb | 0 | nub | 0 | 1.45 | 52.10 | 5.31 | 3.08 | 5.08 | 1.65 | 30 |
| ***Lerista stylis*** | USNM 128640 | 0 | no limb | 0 | nub | 0 | 1.29 | 67 | 6.53 | 2.63 | 4.47 | 1.70 | 31 |
| ***Lerista terdigitata*** | MCZ-R-131081 | 3 | three digits | 3 | three digits | 3.46 | 10.13 | 50.0 | 5.76 | 2.55 | 3.11 | 1.22 | 13 |
| ***Lerista uniduo*** | CU R-11945 | 0 | non-protruding | 2 | two digits | 0 | 9.35** | 55** | 5.79 | 1.68 | 3.46 | 2.05 | 21 |
| ***Lerista xanthura*** | ROM 15682 | 4 | four digits | 4 | four digits | 4.89 | 11.35 | 39.47 | 5.95 | 3.88 | 5.35 | 1.38 | 8 |
| ***Hemiergis peronii*** | YPM R-9030 | 4 | four digits | 4 | four digits | 7.26 | 11.33 | 40.70 | 9.00 | 5.93 | 9.41 | 1.59 | 6 |
| ***Ctenotus robustus*** | FMNH 125939 | 5 | five digits | 5 | five digits | - | - | - | 12.34 | - | - | - | 1 |

Table 2. Specimens and measurements

| **Landmark point** | **description** | **placement guidelines** | **Justification** |
|---|---|---|---|
| **1** | Anteriormost point of the anterior medial process of the interclavicle | The anterior medial process of the interclavicle is located dorsal to the clavicles. placement should be done in anterodorsal view. | This point marks the anterior extent of the interclavicle and can be used to show the relationship between the nexus of the clavicles and the interclavicle |
| **2** | Posteriormost point of the posterior process of the interclavicle | The posterior medial process is located just ventral to the sternum in most specimens. placement should be done in posteroventral view. | This point marks the posterior extent of the interclavicle. This and landmark 1 indicate how long the interclavicle is. this point can also be used to analyze the relationship between the anterior edge of the sternum and the interclavicle |
| **3** | Lateralmost point of the right lateral process of the interclavicle | The orientation of the lateral processes varies from specimen to specimen so placement should be done in a roughly lateroventral view. It is best to standardize placement orientation by the ease of placement at the distal tip of the lateral process, rather than viewing angle. The right lateral process refers to the right side of the specimen in dorsal view. | points 3 and 4 provide an understanding of how wide the interclavicle is and the relationship of the interclavicle to the scapulocoracoid. This and landmarks 11 and 12 can be used to represent and analyze the angle of the lateral arms of the interclavicle |
| **4** | Lateralmost point of the left lateral process of the interclavicle | see above | see above |

|  |  |  |  |
|---|---|---|---|
| **5** | Left ventrolateral corner of the coracoid | Below the glenoid fossa, the lateral edge of the coracoid curves to a point just anterior to the coracoid cartilage. This should be placed in dorsolateral view on curved pectoral girdles such as that of L. frosti (MCZ R-51893) or lateral view for specimens with less curved pectoral girdles such as L. praepedita (R-187806) | The coracoid is a structure without many homologois points that are shared by plesiomorphic specimens and specimens with highly reduced girdles. this point helps define the lateral and ventral extent of the coracoid. It can also be used to analyze the relationship between the sternum and coracoid. |
| **6** | Right ventrolateral corner of the coracoid | see above | see above |
| **7** | Right medial end of the coracosternal articulation | This point should be placed on the coracoid at the medial position in which the sternum comes in contact with the coracoid. In most cases, placement is easiest in a right medioventral view, however, due to scan quality or the level of reduction in the sternum, it may be easier in dorsal view or ventrolateral view. | In specimens with well developed pectoral girdles, the coracoid is nestled in the sternal groove. In species with reduced pectoral girdles, the coracoid is more loosely associated with the sternal groove. points 7, 8, 9, and 10 can be used to analyze the degree of association between the sternum and coracoid. |
| **8** | Left medial end of the coracosternal articulation | see above | see above |
| **9** | Right lateral end of the coracosternal articulation | The point should be placed on the coracoid at the lateral intersection between the sternum and coracoid. This is usually easiest in a lateroventral view but for some girdle-reduced specimens, lateral view may be best. | see above |

| | | | |
|---|---|---|---|
| **10** | Left lateral end of the coracosternal articulation | see above | see above |
| **11** | Intersection of second coracoid ray and lateral process of the interclavicle on the right side | because there is some distance between the lateral processes of the interclavicle and the coracoid rays, it is essential to place this landmark in a standard, perfectly ventral view. this landmark should be placed on the anterior surface of the coracoid ray where the ventral edge of the lateral process of the interclavicle overlaps with it. | This landmark, though a bit dubious in terms of placement, shows the relationship between the interclavicle and the coracoid and, with landmarks 3 and 4 can indicate the angle of the lateral processes of the interclavicle. |
| **12** | Intersection of second coracoid ray and lateral process of the interclavicle on the left side | see above | see above |
| **13** | mediodorsal point at the anterior end of the right scapular blade | This point should be placed in dorso-antero-medial view. in most specimens, the scapular blade ends in a flared fan shape. the point should go on the dorso-lateral point at the end of the fan. | This point defines the lateral extent of the scapular blade. this and point 15 describe the width of the scapula. and describes the relationship between the scapula an the suprascapula |
| **14** | mediodorsal point at the anterior end of the left scapular blade | see above | This point defines the lateral extent of the scapular blade. this and point 16 describe the width of the scapula. and can describes the relationship between the scapula an the suprascapula |

| | | | |
|---|---|---|---|
| **15** | laterodorsal point at the end of the right scapular blade | in some Lerista species, the scapula has an accessory ray with a fenestra formed by the fork in the scapula and the suprascapula. however most specimens do not. It appears as if the fenestra is lost in the latter group because the fenestra between the scapular ray and scapular blade closes up, rather than through the loss of the scapular ray(see L. distinguenda (132393) and L. muelleri (86703) which appear to show intermediate morphologies). This landmark should be placed on the medialmost point of the anterior edge of the scapular blade in most specimens and on the medialmost point of the scapular ray in specimens that have a scapular ray. | This point defines the medial extent of the scapular blade. this and point 13 define the width of the anterior edge of the scapula |
| **16** | laterodorsal point at the end of the left scapular blade | see above | This point defines the medial extent of the scapular blade. this and point 14 define the width of the anterior edge of the scapula |
| **17** | proximolateral corner of the right suprascapula | This landmark is best placed in lateral view. it should be placed on the suprascapula, where it intersects with the scapula | though points 13-16 are very close to points 17-20, they define different elements. The proximal end of the suprascapula appears to be narrower relative to the distal end of the scapula and these points will capture this relationship. |
| **18** | proximolateral corner of the left suprascapula | see above | see above |

| | | | |
|---|---|---|---|
| **19** | proximomedial corner of right suprascapula | This landmark is best placed in mediodorsal view. It should be placed on the suprascapula, where it intersects with the scapula | see above |
| **20** | proximomedial corner of left suprascapula | see above | see above |
| **21** | distoventral corner of the right suprascapula | This landmark should be placed in anterior view | landmarks 21-24 define the dorsal extent of the suprascapulae. they also define how wide or narrow the suprascapula is. |
| **22** | ventral corner of the distal end of the left suprascapula | This landmark should be placed in anterior view | see above |
| **23** | anterior corner of the distal side of the right suprascapula | This should be placed in posterior view | see above |
| **24** | anterior corner of the distal side of the left suprascapula | This should be placed in posterior view | see above |
| **25** | mediodorsal point of right clavicle | This should be placed in posterior view. | landmarks 25 and 26 define the nexus of the clavicles. with landmarks 27 and 28, they describe how wide the clavicles are. |
| **26** | mediodorsal point of left clavicle | see above | see above |

| | | | |
|---|---|---|---|
| **27** | posterior medial extent of the right clavicle | placement of landmarks 27 and 28 can be difficult in terms of interpretation. the posterior edge of some clavicles curves gradually from medial to lateral and findint a point to define the posterior medial corner may be difficult. Unless this corner was obvious, I placed these landmarks on the clavicle where the interclavicle becomes visible posterior to the nexus of the clavicles. | even though the placement of this landmark in terms of homology and consistency is a little dubious, it is important to define the thickness of the clavicles and I believe this is the best landmark for that. |
| **28** | posterior medial extent of the left clavicle | see above | see above |
| **29** | Lateral end of the right clavicle | this should be placed in ventrolateral view | this point defines the length of the clavicle |
| **30** | Lateral end of the left clavicle | see above | see above |
| **31** | right anterolateral corner of the sternum | this should be placed in lateral view | this landmark defines the lateral edge of the sternum. It also helps define the relationship between the sternum and the coracoid |
| **32** | left anterolateral corner of the sternum | see above | see above |

| | | | |
|---|---|---|---|
| **33** | first sternal ribs on the right | this should be applied in dorsolateral view. | The attachment points of the sternal ribs define the lateroventral edge of the sternum. some specimens have three on each side but some have just two. |
| **34** | first sternal ribs on the left | | |
| **35** | second sternal ribs on the right | | |
| **36** | second sternal ribs on the left | | |
| **37** | posterior extent of the pectoral girdle | this should be placed in anterior view. | this point defines the posterior extent of the sternum |
| **Semi-land-marks** | | | |

|  |  |  |  |
|---|---|---|---|
| **38** | right anterior edge of the interclavicle | made up of three curves. The first curve should be placed in ventral view beginning at the anterior apex of the interclavicleand ending before the medial curve where the anterior medial process meets the right lateral process.the second curve should be placed in anterolateral view. The second curve should begin where the last curve eft off, then include a point at the apex of the curve made by the meeting of the Anterior medial process and the right lateral process, and should end about halfway along the lateral process. The third curve should begin where the second curve left off and should include a point near the distal end of the lateral process, and end at the lateralmost extent of the lateral process | semilandmarks 38, 39, 40 and 41 define the shape of the interclavicle. they represent how broad or narrow the processes are and whether or not the lateral processes are angled. |
| **39** | left anterior edge of the interclavicle | as above, but on the left side | see above |
| **40** | right ventral edge of the interclavicle | semilandmarks 40 and 41 should be placed in a similar way to landmarks 38 and 39 but placement should begin at landmark points 3 and 4. | see above |
| **41** | left ventral edge of the interclavicle | see above | see above |

| | | | |
|---|---|---|---|
| **42** | ventral edge of the right scapulocoracoid | made up of five landmark curves from landmarks 5 and 6 to landmarks 16 and 17. These should follow the ventral side of the element and should be placed inthe glenoid fossa when one exists. | This semilandmark is what captures variation and the reduction in complexity of the scapulocoracoid. this element probably exhibits the most dramatic change in terms of feature loss of the pectoral girdle elements. This reduction means that few homologous landmarks are preserved and cannot be used included as landmark points, however semilandmarks around the glenoid can capture some of this loss of complexity. |
| **43** | ventral edge of the left scapulocoracoid | see above | see above |
| **44** | ventral edge of right suprascapula | This should be comprised of one landmark curve from landmarks 17 to 21 on the right side or 18 to 22 on the left side | This landmark captures the ventral side of the suprascapula, which fans out at the lateral edge in specimens with well developed pectoral girdles but not in those with more reduced girdles. this landmark as well as 46 and 47 capture variation in this bell shape. |
| **45** | ventral edge of left suprascapula | see above | see above |
| **46** | anterior edge of right suprascapula | This landmark is comprised of two landmark curves from landmark 19 to 23 on the right side or landmark 20 to 24 on the left side | This landmark captures the anterior edge of the suprascapula and gives an indication of how wide the suprascapula is and how much it flares out laterally |
| **47** | anterior edge of left suprascapula | see above | see above |

| | | | |
|---|---|---|---|
| **48** | anterior edge of the right clavicle | this is comprised of seven landmark curves from landmark 25 to 29 on the right side or 26 to 30 on the left side. | These landmarks capture the complex curve of the clavicles around the circumference of the body as well as the variation in anteroposterior position from medial to distal. |
| **49** | anterior edge of the left clavicle | see above | see above |
| **50** | anterior edge of the right side of the sternum. | This landmark should capture the anterior edge of the sternum. its beginning and endpoints are somewhat dubious but should be placed from medial to lateral and should be placed in ventral view. this semilandmark should consist of one curve from the point at which the sternum and interclavicle overlap to the point at which the sternum's anterior edge ands and at which the posterolateral edge begins. | this landmark represents the curve of the anterior part of the sternum. this portion of the sternum articulated with the scapulocoracoid and, as this articulation is heavily involved in limb-based locomotion, it is an important one to analyze in limbless species. |
| **51** | anterior edge of the left side of the sternum. | see above | see above |
| **52** | ventromedial edge of the right coracoid | this landmark should be placed in dorsal view and should be done from landmark 11 to the point at which the epicoracoid meets the coracoid on the posterior edge of the scapulocoracoid. it should be comprised of one curve. the sternum bulges out slightly along the sternal groove and the curve line should follow the line of the bulge and should be just posterior to it. | this landmark should capture the ventral edge of the epicoracoid cartilage. The epicoracoid is tucked in the sternal groove in most lizards, however, in limb reduced specimens, this articulation is much more loose and the coracoid is somewhat removed from this groove. this and landmark 53 capture the curve of the sternal groove and can be used to analyze how |

| | | | |
|---|---|---|---|
| | | | closely articulated the sternum and coracoid are |
| **53** | ventromedial edge of the left coracoid | see above though the origin of this line for the left side should be landmark 12 | see above |

Table 3. Descriptions and justifications for each landmark and semilandmark

| species | specimen number | PCA analysis | Phylogenetic PCA | phylogeny | qualitative analysis |
|---|---|---|---|---|---|
| ***Lerista allanae*** | CAS 77099 | yes | | | yes |
| ***Lerista allochira*** | WAM-R-132460 | yes | yes | yes | yes |
| ***Lerista ameles*** | SAMA-R-55806 | | | yes | yes |
| ***Lerista apoda*** | WAM-R-168495 | | | yes | yes |
| ***Lerista baynesi*** | FMNH 75489 | yes | yes | yes | yes |
| ***Lerista bougainvillii*** | MCZ-R-129545 | yes | yes | yes | yes |
| ***Lerista carpentariae*** | USNM 128409 | | | yes | yes |
| ***Lerista cinerea*** | SAM-R-54521 | yes | yes | yes | yes |
| ***Lerista distinguenda*** | UMMZ-132393 | yes | yes | yes | yes |
| ***Lerista*** | YPM R-13679 | yes | yes | yes | yes |

| | | | | | |
|---|---|---|---|---|---|
| ***edwardsae*** | | | | | |
| ***Lerista elegans*** | CU R-0012147 | yes | yes | yes | yes |
| ***Lerista fragilis*** | CAS 77102 | yes | yes | yes | yes |
| ***Lerista frosti*** | MCZ-R-51893 | yes | yes | yes | yes |
| ***Lerista gerrardii*** | MCZ-R-33258 | yes | yes | yes | yes |
| ***Lerista kalumburu*** | WAM R-100188 | yes | yes | yes | yes |
| ***Lerista karlschmidti*** | AMNH 120381 | | | yes | yes |
| ***Lerista kendricki*** | CU R-0011970 | yes | yes | yes | yes |
| ***Lerista kingi*** | CAS 254618 | yes | | | yes |
| ***Lerista labialis*** | AMNH 86086 | yes | yes | yes | yes |
| ***Lerista lineopunctulata*** | MCZ-R-33261 | yes | yes | yes | yes |

| | | | | | |
|---|---|---|---|---|---|
| ***Lerista macropisthopus*** | CU R-0014358 | yes | yes | yes | yes |
| ***Lerista microtis*** | MCZ-R-24577 | yes | yes | yes | yes |
| ***Lerista microtis*** | CAS 77518 | yes | | yes | yes |
| ***Lerista muelleri*** | MCZ-R-86703 | yes | yes | yes | yes |
| ***Lerista orientalis*** | UMMZ-203363 | yes | yes | yes | yes |
| ***Lerista picturata*** | MCZ-R-130376 | | | yes | yes |
| ***Lerista planiventralis*** | UMMZ-241013 | yes | yes | yes | yes |
| ***Lerista praepedita*** | R-187806 | yes | yes | yes | yes |
| ***Lerista stylis*** | USNM 128640 | yes | yes | yes | yes |
| ***Lerista terdigitata*** | MCZ-R-131081 | yes | yes | yes | yes |

| | | | | | |
|---|---|---|---|---|---|
| ***Lerista uniduo*** | CU R-11945 | yes | yes | yes | yes |
| ***Lerista xanthura*** | ROM 15682 | yes | yes | yes | yes |
| ***Hemiergis peronii*** | YPM R-9030 | yes | yes | yes | yes |
| ***Ctenotus robustus*** | FMNH 125939 | yes | yes | yes | yes |

Table 4. The specimens included in each analysis

## 12. Code

```
####Trimming the Tree####
# what you need:
#a previously made tree
#a .csv file with only the names of the species you want to include.
    #the .csv should consist of a single column with the header "Species"
    # and should have all of your species names exactly correct with an underscore between the genus and species names
library("rotl")
require(rotl)
library(phytools)
require(ape)
require(geiger)
library(caper)
library(phangorn)
library(ape)
library(geiger)

tree <- rotl::get_study(study_id="ot_1041")#I was given this link to Zheng and Wiens, 2016 tree file

plot(tree) # this will plot the tree but the names will be chaos and it will be a jumble.

lrdata<-read.csv("Lerista_thesis_23_Names_outgroups.csv", header=1) ### this is my .csv file of names

rownames(lrdata)<-lrdata$Species # make sure 'Species' is the row name for your species

lerspec<-row.names(lrdata) ## create an object containing a list of all the species in your data set

lerspec #if you run this and it spits out a bunch of nubers like "[1] 1" or something,
#that means you probably didn't put underscores between the genus and species names.
#"Lerista_allochira"

pruned_Lerista<-drop.tip(tree, setdiff(tree$tip.label, lerspec)) # this will prune the tree to include just the species in your data set

write.tree(pruned_Lerista, file = "lerspec.tre") #turns your tree into a .tre file,
#which you can edit using a text editor. for some reason when I used the tree straight from R, it didn't recognize it as a file type
```

```
#"phylo" so the solution was to make a .tre file and read that.

plotTree(pruned_Lerista, ftype="i") ## plot your pruned tree

library(zoom) #package for zooming in on the plot. Its so handy!

zm(tree)



#### PCA plot####
#What you need: an .nts file of landmarks. make sure to edit the heading.

july1allpointsnts <- readland.nts("Lerista_thesis_Dec23nooutgroupsfrostianchor.nts")#reading the .nts file with the landmark data from landmark editor that I then edited in a text editor and saved as an .nts file

gpa.lands <- gpagen(july1allpointsnts)# the generalized procrustes analysis. It aligns all your landmarks so size and orientation aren’t factors.

plot(gpa.lands) #This will show you all the landmark points superimposed upon eachother. It will look like a monstrosity but should also look like whatever structure you’re trying to compare.

PCA<-plotTangentSpace(gpa.lands$coords, label=TRUE, warpgrids = TRUE) #this is the PCA analysis. The numbers it spits out should correspond to your specimens. The gpa.lands$coords is just the coordinate data from the procrustes analysis. I added the “PCA<-” so I could create a named object I can use for getting a summary of the data or individual PC scores

PCA$pc.scores #I can use this to reference the PCA analysis and call up the individual PC scores for each specimen

PCA$pc.summary # a summary of the PCA. this will show you what relative contribution each PC makes to the variance. This (and probably some other tests if you’re doing it right) can allow you to make decisions about whether to use a 2D or 3D PCA


###Phylogenetic PCA####
#What you need:
# a .tre file that has been pruned to include just the species you want to include
```

```
# a .nts file with landmark data

lernts <- readland.nts("Lerista_thesis_Dec23phylo.nts")

lergpa <- gpagen(lernts)

lertree<-read.tree(file = "lerspec.tre")

plot(lertree)

physignal<-physignal(lergpa$coords, lertree, iter = 249, c("Kmult"))

summary(physignal)

lerphyloplot<-plotGMPhyloMorphoSpace(lertree, lergpa$coords)

library(zoom) #package for zooming in on the plot

zm(lerphyloplot)
```