Morphological Correlates of Locomotion in Anurans: Limb Length, Pelvic Anatomy and Contact Structures

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ABSTRACT

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Morphological Correlates of Locomotion in Anurans: Limb Length, Pelvic Anatomy and Contact Structures (87 pp.)

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This study examines morphological features of the postcranial skeleton in a sample of modern anurans of known locomotor style within the context of a recent phylogenetic hypothesis. Non-destructive methods for collecting skeletal morphometric data from alcohol preserved anuran specimens are herein proposed and tested. Analysis of covariance (ANCOVA) is used to compare morphological structures taking into account differences in body size (SVL) among taxa.

Results of this study differ from previous studies in that the patterns of morphological correlates in anurans may not span the entire anuran clade, but are identifiable within phylogenetically focused comparisons. Manus size was predicted to be larger in arboreal than non arboreal anurans, a pattern supported by comparisons within the family Hylidae, and in the derived group Natatanura. Arboreal bufonid species exhibit wider sacra and longer fore- and hind limbs relative to SVL than do terrestrial species. natatanurans capable of long distance jumping exhibit a larger pes (wider and longer) relative to SVL than do arboreal species. Sacral diapophyseal angle (SDA) provides a method of separating walking/running species from all other locomotor groups examined with the exception of members of the strictly aquatic family Pipidae.

Fossil anuran specimens recently discovered in Oligocene deposits in the Rukwa Rift Basin of Tanzania preserve pelvic anatomy associated with forceful jumping in modern forms. An estimated sacral diapophyseal angle (SDA) for RRBP 04101 of 21.409° is consistent with this locomotor assessment.

Approved:____

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To my family, thank you all for everything.

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INTRODUCTION

Anura is a very diverse clade of organisms represented by just over 5200 species from 32 families (Frost et al., 2006). Anurans, or frogs, inhabit a wide variety of environments around the world ranging from tropical rainforests of South America to deserts of Southern Africa. Anurans utilize several different methods to move throughout the environments that they inhabit. These different locomotor modes may be associated with differences in skeletal and contact structure morphology that can be quantified and studied. This study seeks to test the relationship between patterns of morphology and five locomotor behaviors commonly found within the clade anura: jump, hop, swim, arboreal, and walk/run (Figure 1). This examination of the modern range of variation in morphology and behavior in anurans is important for understanding the evolutionary history of these animals, and also encourages the development of methods to help infer how extinct fossil frogs may have moved.

Jumping is the most common locomotor mode among terrestrial anurans (Emerson, 1978; Dobrowlska, 1973). A jump can be briefly described as the paired extension of hind limbs that propels the animal off of the ground. Based on anatomical features preserved in the Jurassic species *Prosalirus bitis* from the Early Jurassic (~183-189 mya), jumping is hypothesized to have been the ancestral or plesiomorphic condition (Shubin and Jenkins, 1995; Jenkins and Shubin, 1998). One subtle distinction among anuran locomotor styles is between hopping (toad-like) and jumping (frog-like) movements. Both of these involve the synchronous bilateral extension of the hind limbs. The distance traveled during these otherwise similar movements defines the distinction between a jump and a hop. Jumping is defined as a leap of greater than 10 times SVL (snout-vent length), whereas hopping results in a travel distance less than 10 times SVL (Emerson, 1979). A typical hopping anuran is the Giant Toad (*Bufo marinus*).

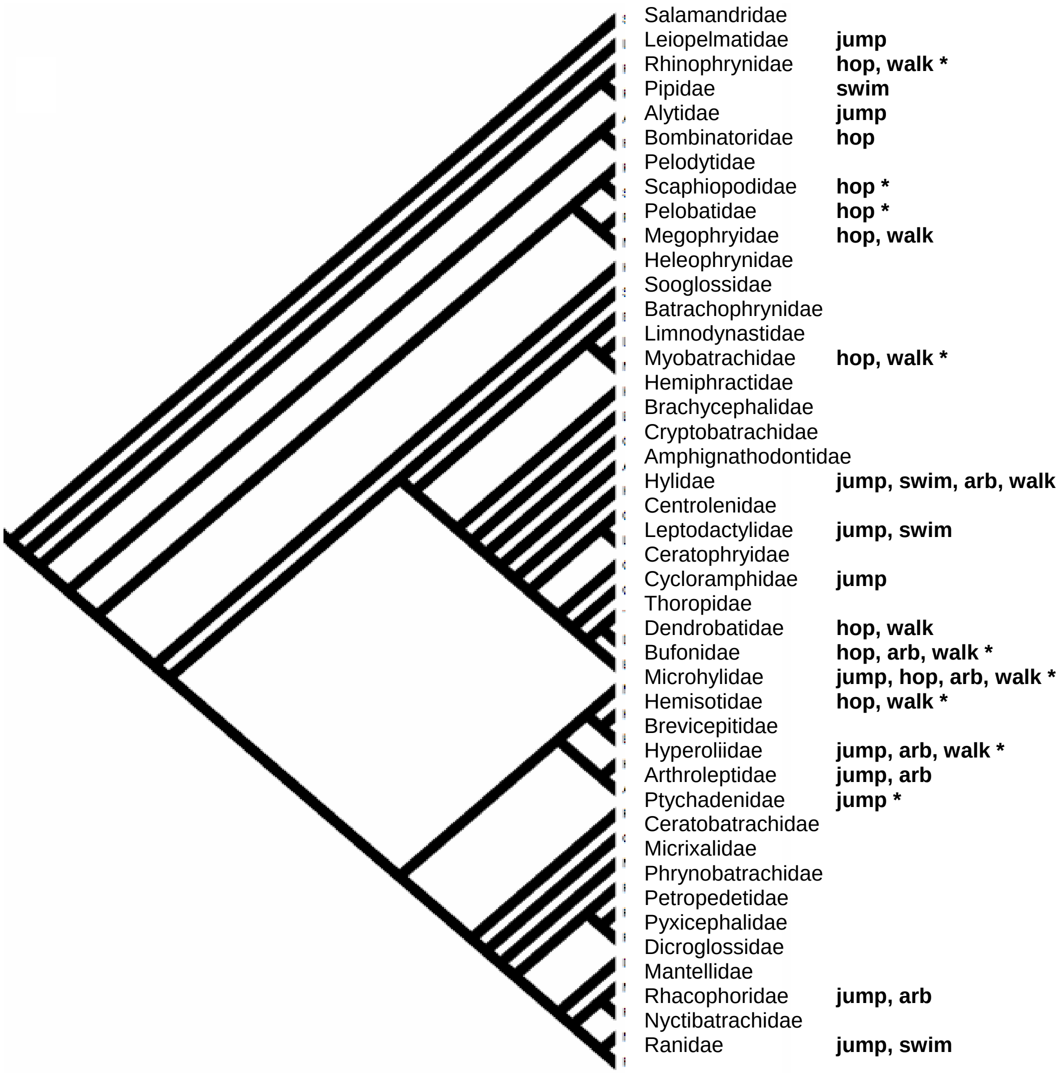

Figure 1. Locomotor categories mapped onto a recent family-level phylogenetic hypothesis of anura modified from Frost et al. (2006). Locomotor categories include jumpers (jump), hoppers (hop), swimmers (swim), arborealists (arb), and walkers (walk), fossorial species indicated by * (Emerson, 1979, and Table 1).

B. marinus is terrestrial and typically covers relatively short intervals with each bilateral hind limb extension. Jumping locomotion is exemplified by *Rana* (*Lithobates*) *pipiens* (Northern Leopard frog), which uses long-distance leaps to avoid predation (Duellman and Trueb, 1994).

Although hopping and jumping are the predominant locomotor style utilized by anurans, a few other locomotor styles are also of particular note. For example, during development, all anurans pass through an obligatory aquatic phase. Some frogs, such as the Surinam Toad (*Pipa pipa*), do not leave the aquatic environment even in adulthood and rarely use any mode of locomotion other than swimming. In contrast, the family Hylidae includes many arboreal species. Arboreal forms such as the Gray tree frog (*Hyla versicolor*) and other arboreal frogs exhibit characteristic expanded terminal toe discs or pads, which possess adhesive properties and increase the surface area of their toes for grasping (Green, 1981; Burton, 1998). Even more rare among anurans is cyclical movement of contralateral forelimb/hind limb pairs to produce walking or running locomotion similar to that observed in other tetrapods. Walking or running in anurans is exemplified buy *Kassina maculata* (Red-Legged Running Frog) (Ahn et al., 2004). Also some anurans (e.g., Microhylidae, *Glyphoglossus)* routinely use their limbs to engage in fossorial behaviors (burrowing into the mud) to avoid desiccation during seasonal dry periods.

Extant anurans truly exhibit a wealth of interesting locomotor and movement patterns that allow them to negotiate a broad range of terrains (e.g., land vs. water). Despite the fact that frogs exhibit a remarkably conserved Bauplan characterized by relatively long hind limbs and short forelimbs coupled to a stout body that lacks a tail, previous studies on extant frogs have linked morphological skeletal characters to particular locomotor modes and other behaviors (Zug, 1978, 1972; Dobrowolska, 1973: Emerson, 1978, 1979, 1983, 1985; Emerson and De Jongh, 1980, Duellman and Trueb, 1994; Zani, 2000; Channing and Broadley, 2002; Bain et al., 2003; Nauwelaerts et al., 2005, 2007). Indeed, differences in limb proportions, contact structures, and pelvic morphology have been suggested to enable anurans to navigate different environments (Zug, 1972, 1978; Dobrowolska, 1973; Emerson, 1979, 1982).

This thesis builds upon these themes by developing a morphometric and character-based method for assessing the locomotor capabilities of extant and fossil anurans. To do so, this study examines extant anurans of known locomotor habits, expanding on prior studies through careful selection of a broad taxonomic sample, employing statistical methods to take into account differences in body size and advances in our understanding of anuran phylogenetic systematics. Relationships between morphology and function can also be useful in interpreting the fossil record. For this reason, postcranial characters found to be informative in interpreting the locomotor behavior of modern anurans are applied in a functional analysis of fossil anuran specimens recently discovered in Oligocene deposits in the Rukwa Rift Basin of Tanzania.

Anuran Limb Proportions and Locomotor Style:

Relationships among relative limb length, limb heterotypy and locomotor behavior in anurans have been the focus of several studies (Gans and Parsons, 1966;

Dobrowolska, 1973; Zug, 1972, 1978; Emerson, 1978; Chadwell et al., 2002). Zug (1972) examined linear measurements to explore the relationship between limb length ratios and locomotor style and showed that in his study sample, long distance jumping frogs had relatively shorter forelimbs in relation to their hind limbs whereas more terrestrial species possessed relatively large feet. Work by Dobrowolska (1973) supported these observations and provided evidence of relatively short hind limbs for frogs that hop or walk, and relatively longer hind limbs for anurans that use jumping as their main mode of terrestrial locomotion. Emerson (1978) noted an association between the ratio of hind limb length (HLL) and snout-vent length (SVL) and the dominant type of locomotion. Combined, these studies suggest that predominantly walking frogs typically exhibit a low HLL/SVL ratio whereas predominantly jumping frogs typically exhibit a high HLL/SVL ratio. Intermediate ratios generally indicate frogs that incorporate a mix of walking, hopping, and burrowing in their locomotor repertoires. Some anurans also possess additional adaptations for burrowing that are reflected in the postcranial skeleton (Emerson, 1976; Duellman and Trueb, 1994; Channing and Broadley, 2002; Dutta et al., 2004). For example, modifications of the forelimb and pectoral girdle are found in frogs that habitually dig headfirst into the substrate (Channing and Broadley, 2002; Dutta et al., 2004). This type of digging is also associated with relatively short tibiofibulae (Emerson, 1976).

The Anuran Pectoral Girdle:

The highly derived pectoral girdles of anurans have also been examined in relation to locomotor style. Generally, two types of pectoral morphology have been identified in anurans (Figure 2), although there is some overlap between them (Kaplan 2004). The most common form is the arciferal, in which the right and left epicoracoid cartilages are free (i.e., not fused to one another) and overlapping (Emerson, 1983; Duellman and Trueb, 1994). In contrast, the firmisternal form is characterized by right and left epicoracoid cartilages that are fused on the midline (Duellman and Trueb, 1994; Emerson, 1983). The two types of pectoral girdles are distinguished from one another by the presence of epicoracoid horns, present in the arciferal, but not firmisternal conditions (Trueb, 1973; Duellman and Trueb, 1994; Emerson, 1983). The distinction between pectoral girdle types has been used as a phylogenetic character (Griffiths, 1963; Trueb, 1973; Emerson, 1983; Duellman and Trueb, 1994).

Functionally, it has been hypothesized that landing forces associated with anuran saltatory locomotion load the pectoral girdle with compressive forces, resulting in a medial translation of the overlapping epicoracoid cartilages. This hypothesis was tested and supported by observing a decrease in distance (viewed with cineradiography) between metallic implant markers placed in the epicoracoid cartilage of arciferal anurans (Emerson, 1983). Emerson (1983) postulated that arciferal pectoral morphology allows an animal to decelerate over a greater distance than that provided by the firmisternal condition. Yet, there seems to be little correlation between pectoral girdle type and locomotor variability in modern anurans. It would seem that the two forms would lead to vastly differing loading conditions and force dissipation patterns (Emerson, 1983). Perhaps the lack of a functional correlation between pectoral girdle type and functional use in locomotion reflects a phylogenetic pattern: the arciferal condition is thought to be ancestral, with the firmisternal girdle having arisen from an arciferal template several times within the anuran clade (Trueb, 1973; Emerson, 1983; Figure 2).

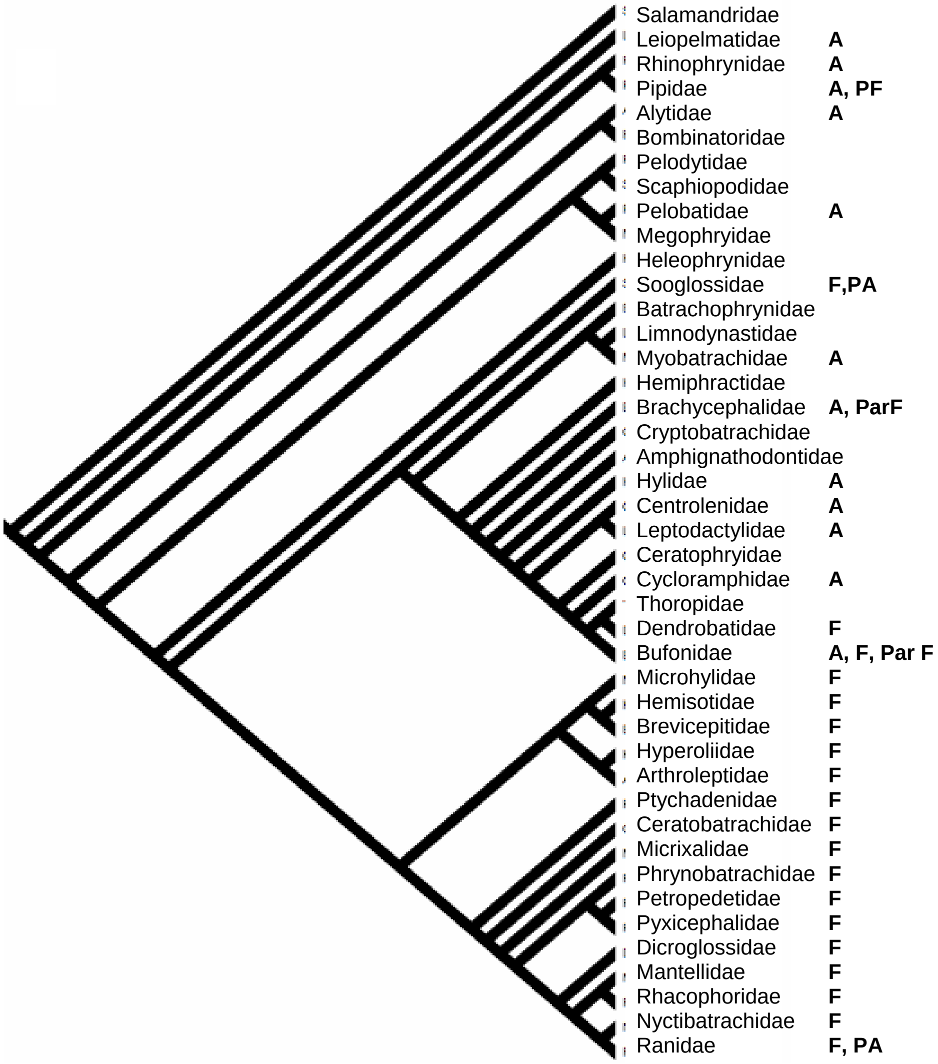

Figure 2. Pectoral girdle morphologies mapped onto a recent phylogenetic hypotheses from Frost et al. (2006). Morphologies are arciferal (A), firmisternal (F), other morphologies are intermediate or exhibit characters of both of the two main types; pseudoarciferal (PA), pseudofirmisternal (PF), partial firmisternal (Par F) (Trueb, 1973; Duellman and Trueb, 1994; Frost et al., 2006).

The Anuran Pelvic Girdle:

The structure and function of the anuran pelvic girdle has been a focus of study for many researchers (e.g., Emerson, 1979, 1982; Jenkins and Shubin, 1998; Videler and Jorna, 1985; Rocková and Roček, 2005; Kovalenko, 1999). The basic structure of an elongate ilium positioned ventral to the sacral diapophysis (Emerson, 1979, 1982), associated with a midline urostyle, is preserved in fossil forms known from the Early Jurassic (e.g., *Prosalirus bitis* Prosaliridae; Shubin and Jenkins 1995). Emerson (1979, 1982) sought to formalize links between locomotor style and pelvic morphology, recognizing three distinct morphologies of the ilio-sacral joint articulation (Types I, IIA, and IIB; Figures 3 and 4). These morphologies are diagnosed by the presence/absence and location of ligament scars on the dorsal surface of the sacral vertebra, the orientation of the sacral transverse process, and shape of the sacral diapophysis. Diapophyseal shape alone is insufficient for definitively discriminating among the pelvic types (Emerson, 1979), although it retains limited utility for interpreting incomplete fossils (e.g., Asher and Krause, 1998). Importantly, there is somewhat of a continuum of variation, with overlap among Emerson's three recognized pelvic types, which can add complexity to functional interpretations (Manzano and Barg, 2005).

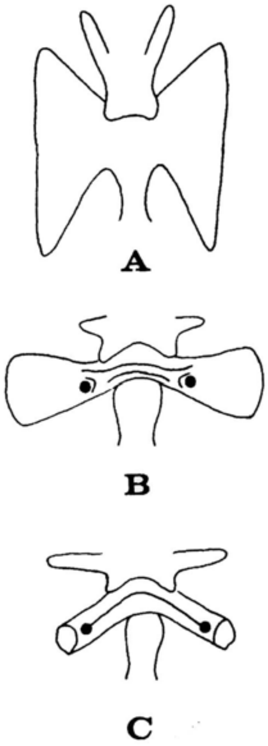

Figure 3. Sacral vertebral types (Emerson 1979) showing general morphology and location of ligament scars (lines connecting dark circles). (A) Type I, (B) Type IIA, and (C) Type IIB, all in dorsal view.

Type I

The Type I ilio-sacral form (Figure 3A) has a broadly extended (anteroposterior) sacral diapophysis and does not possess a ligament attaching between the sacral vertebra and ilium. As such, it does not have a ligamental scar on the dorsal surface of the sacral vertebra. Instead, a superficial ligament spans between the two ilia and passes over the dorsal aspect of the sacrum, thereby forming a “cuff like” ligament that permits sliding movements between the sacral diapophysis and ilium (Trueb, 1996; Emerson and De Jongh, 1980; Emerson, 1979).

The “sliding pelvis” has been hypothesized to enhance both swimming and jumping capabilities (Channing, 2001; Nauwelaerts et al., 2005) and can be classified as a Type I pelvis (Emerson, 1979). The long ilium and expanded sacral diapophysis do not allow the pelvis to rotate laterally or vertically (Kovanlenko, 1999), but rather permit an anterior-posterior sliding motion that results in functional body length changes as the hind limbs and ilia translate caudally. Such an adaptation may increase the distance over which acceleration can occur during aquatic locomotion, for example in pipids, because the extension of the hind limb is amplified as the pelvis slides from its cranial position caudad (Videler and Jordana, 1985). Type I pelves are neither phylogenetically nor functionally restricted, as they are found in anurans that engage in swimming, walking, hopping, and burrowing, across several families of frogs (Emerson, 1979).

Type II (A and B)

Type II ilio-sacral articulations (Figure 3B, 3C) are diagnosed by two ligamentous scars on the dorsal surface of the sacral vertebra. These scars reflect the attachment of ligaments that originate on the anterolateral aspect of each ilium, pass medially over the dorsal aspect of the element, and then attach to the dorsal surface of the sacral vertebra.

Type IIA ilio-sacral articulations (Figure 3B) are characterized by a distally expanded, laterally convex diapophysis that bears a ventrally cupped cartilaginous lip. A broad ligament attaches to the dorsal surface of the diapophysis near the middle of the sacrum and a sesamoid element lies between the ligament and the distal tip of the diapophysis. The ilium articulates with the ventral aspect of cartilaginous lip.

Type IIA ilio-sacral articulations are associated with lateral rotation, a trait that is an integral movement for anuran walking. As one ilium translates anteriorly below the end of the sacral diapophysis, the contralateral ilium slides posteriorly through this groove (Emerson, 1979). These movements are hypothesized to follow an arc along the distal margin of the sacral diapophysis, thereby functionally increasing the stride length. Type IIA morphology is found in anurans that habitually engage in walking, hopping, and burrowing behaviors and is widely distributed throughout the clade (Emerson, 1979). Anurans that are classified as having a type IIA ilio-sacral articulation also have relatively longer ilia and relatively narrow sacral widths to allow this lateral rotation during walking or climbing (Emerson, 1982).

Type IIB ilio-sacral articulations (Figure 3C) have a non-expanded diapophysis positioned at the end of a posterolaterally-orientated transverse process. A narrow internal ligament attaches near the distal tip of the diapophysis. Distally, the diapophysis has a cartilaginous tip that lacks the groove present in Type IIA. A sesamoid is also present (Emerson, 1979).

Emerson (1979) noted that Type IIB pelvic morphology is correlated with dorsoventral rotation of the ilio-sacral articulation in extant frogs. This behavior is associated with long-distance jumping, the primary locomotor behavior recorded for all but one of the frog species in Emerson’s sample exhibiting Type IIB morphology. The one exception noted by Emerson is the dendrobatid *Dendrobates tinctorius*, a species that utilizes both walking and hopping (1979). Several authors have suggested that during the initiation of a jump, such rotation permits elevation of the trunk (take-off angle) cranial to the ilio-sacral articulation, aligning it with the propulsive forces produced by the hind limbs (Emerson and De Jongh, 1980; Jenkins and Shubin, 1998; Whiting, 1961). Emerson (1982) considered the Type IIB morphology to represent a functional complex that includes (1) relatively short ilia (2) relatively long sacral transverse processes, and (3) the presence of a crest along the dorsal margin of the ilium. Emerson (1982) stressed the non-independence of these characters, and their association in a biomechanical complex associated with a preference for jumping, rather than walking. The presence of an ilial crest is correlated in jumping frogs with a large coccygeoiliacus muscle (Emerson, 1982). The relatively wide sacrum is hypothesized to help limit lateral rotation during a forceful bilateral extension of the hind limbs during a long distance jump.

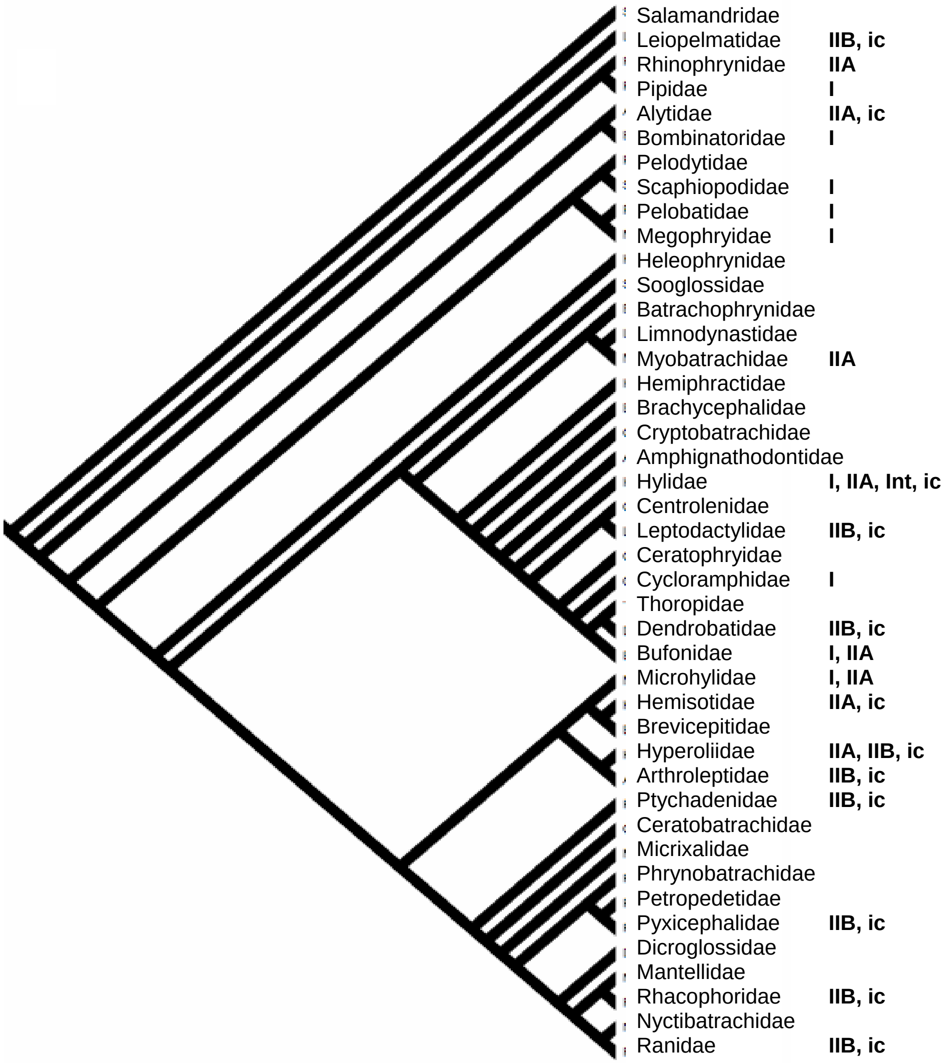

Figure 4. Ilio-sacral morphologies mapped onto a recent phylogenetic hypotheses from Frost et al. (2006). Morphologies are I, IIA, and IIB, and intermediate (Int) and dorsal crest of the ilia (ic) (Emerson, 1979, 1982, 1986; Manzano and Barg, 2005).

Contact Structures in Anruans

Few investigations of anuran morphology and locomotor behavior have included data on contact structure (manus and pes) length and none have included morphometrics on manus and pes width. Several structures of the manus and pes have been linked to certain behaviors. Spades or claws (e.g. *Xenopus laevis*, *Leptodactylus pentadactylus*) have been noted to enhance not only digging but also traction, as well as to aid in grasping during amplexus (Duellman and Trueb, 1994; Dutta et al., 2004). Digital webbing is commonly found on the pes of anurans and is occasionally seen on the manus as well. Digital webbing, found in many families of anurans, increases surface area of these structures to aid in propulsion through the water and as an airfoil in anurans that parachute or glide (Duellman and Trueb, 1994; Emerson and Koehl, 1990). Two mechanisms have been identified by which terrestrial and arboreal anurans use toe pads to adhere to various substrates. One mechanism features capillary action to adhere to smooth surfaces and the other features specialized structures on the base of the toe pad that interlock with rougher surfaces (Emerson and Diehl, 1980; Hanna and Barnes, 1991). Notably, relatively long digits can also improve the gasping ability of an animal's hands and feet, permitting them to exert pressure over a relatively broader area on a given substrate (Napier, 1993).

HYPOTHESES CONSIDERED IN THIS STUDY

Based on the previous research on anuran functional anatomy outlined above, it is possible to formulate a series of hypotheses concerning the relationships between morphology and locomotor behavior that may be examined, taking into account both allometric considerations, as well as recent advances in anuran phylogenetics. Although not exhaustive in nature, three primary hypotheses are considered in this thesis, relating to limb proportions, contact structure dimensions, and pelvic morphology. In each case, the hypotheses are tested against a null hypothesis predicting no relationship between structure and function.

$H1_o$ Anuran limb lengths (relative to snout vent length) do not vary as a function of locomotor affinity.

$H1_a$ Anuran limb lengths (relative to snout vent length) vary in a predictable way with respect to locomotor habits.

> Prediction 1: Anurans that regularly engage in long distance leaping will exhibit relatively longer hind limbs than those that do not habitually engage in long distance jumping. Longer hind limbs are associated with larger muscles as well as longer tendons that have been hypothesized to aid in energy storage for jumping (Emerson, 1978; Biewener, 2003). Longer hind limbs also allow for longer lever arms that permit jumping forces to be applied to the substrate for longer periods of time during launch (Emerson, 1988, Biewener, 2003; Chadwell et al., 2002).
>
> Prediction 2: Anurans that frequently engage in arboreal climbing will exhibit both relatively longer forelimbs and longer hind limbs in order to bridge spatial

gaps between substrates. This is proposed based on patterns found in larger gap-bridging animals such as primates and arboreal xenarthrans (e.g., Cartmill, 1985).

Prediction 3: Anurans that are mainly aquatic (swimmers) will exhibit relatively shorter forelimbs than those that swim less frequently. This is because the hind limb provides most or all of the propulsion during this type of locomotion whereas the forelimbs cause drag and are only used for fine directional adjustments and in feeding (Deuchar, 1975; Gray et al., 1997).

$H2_o$ Size of contact structures (manus and pes) in anurans will not vary among locomotor behaviors (arboreal, swimming, hopping, walking or jumping).

$H2_a$ Size of contact structures (manus and pes) in anurans will vary in a predictable manner among locomotor behaviors (arboreal, swimming, hopping, walking or jumping).

Prediction 1: Anurans that are habitual swimmers will exhibit relatively larger pes than frogs that swim less frequently in order to maximize the amount of thrust transferred to the water perpendicular to the direction of frog during swimming (Webb and Blake, 1985).

Prediction 2: Anurans that are capable of arboreal locomotion will exhibit both large manus and pes relative to SVL to better grasp the substrate on which they are climbing (Napier, 1993).

$H3_o$ Anuran pelvic morphology will not vary among taxa exhibiting different locomotor behaviors.

$H3_a$ Anuran pelvic morphology will vary in a predictable manner among taxa exhibiting different locomotor behaviors.

> Prediction 1: Anurans that regularly jump long distances relative to SVL will exhibit relatively narrower sacral diapophyses. This is hypothesized to aid in the pivoting of the ilio-sacral articulation during a jump (Emerson, 1982)
>
> Prediction 2: Anurans that habitually engage in hopping, walking, and swimming will exhibit relatively longer ilia than those capable of long distance jumping. Longer ilia are hypothesized to permit lateral rotation of the ilio-sacral articulation (type IIA) and or anterior-posterior sliding motion (type I) (Emerson 1979; Kovanlenko, 1999).
>
> Prediction 3: Anurans capable of jumping long distances relative to SVL will exhibit a relatively wider sacrum to resist any lateral rotation of the ilio-sacrla articulation during a jump (Emerson, 1982).

MATERIALS AND METHODS

Study Sample

The study sample includes anurans representing 22 species, 20 genera, and 10 anuran families spanning a range of habitual locomotor patterns (Figure 5, Table 1). Study specimens were measured at, or borrowed from the following institutions: Carnegie Museum of Natural History, Pittsburg; The Field Museum of Natural History, Chicago; Michigan State University Museum; Ohio University Vertebrate Collection; Museum of Zoology, University of Michigan.

For each specimen in this sample (n = 69), measurements of snout vent length (SVL) and contact structures were collected on wet specimens, and radiographic images were used to collect metric and angular data on pelvic structures and limb segment lengths following the methodology outlined below. The primary dataset was supplemented with (n = 40) skeletonized specimens for measurements relating to sacral and pelvic morphologies.

Table 1. Taxa observed with habitual locomotor category indicated and reference information.

Family	**Genus**	**Species**	**loco type**	**source**
Brevicipitidae	*Breviceps*	*mossambicus* (n = 6)	walker	Channing and Howell, 2006
Brevicipitidae	*Callulina*	*kreffti* (n = 1)	terrestrial but climbs well	Spawls et al., 2006; Channing and Howell, 2006
Bufonidae	*Anaxyrus (Bufo)*	*americanus* (n = 11)	hopper	Emerson and De Jongh, 1980; Conant and Collins, 1998
Bufonidae	*Epidalea(Bufo)*	*calimita* (n = 14)	walker	Blair, 1962
Bufonidae	*Pedostibes*	*hosii* (n = 6)	arboreal	Zug et al. 2001, Pough et al., 2004
Hylidae	*Agalychnis*	*callidryas* (n = 5)	arboreal/ glide	Savage, 2002; Duellman, 2001
Hylidae	*Agalychnis*	*morleti* (n = 2)	arboreal	Savage, 2002
Hylidae	*Hyla*	*vasta* (n = 9)	arboreal	Trueb and Tyler, 1974
Hylidae	*Hyla*	*versicolor* (n = 10)	arboreal	Hanna and Barnes, 1991
Hylidae	*Pseudis*	*paradoxa* (n = 12)	swimmer	Pough et al. 2,004
Leiopelmatidae	*Ascaphus*	*truei* (n = 14)	jumper	Emerson, 1979
Leptodactylidae	*Hydrolaetare*	sp. (n = 4)	swimmer	Rodriguez and Duellman, 1994; Jansen et al. 2007
Leptodactylidae	*Leptodactylus*	*pentadactylus* (n = 13)	jumper/ swimmer	Emerson, 1979
Leptodactylidae	*Telmatobius*	*coleus* (n = 3)	swimmer	Zug et al. 2001, Pough et al. 2004
Pipidae	*Pipa*	*pipa* (n = 6)	swimmer	Zug et al. 2001, Rodriguez and Duellman, 1994
Pipidae	*Xenopus*	*laevis* (n = 18)	swimmer	Zug et al. 2001
Ptychadenidae	*Ptychadena*	*mascareniensis* (n = 6)	jumper	Spawls et al., 2006; Channing and Howell, 2006
Ranidae	*Rana (Afrana, Amietia)*	*angolensis* (n = 6)	jumper	Spawls et al., 2006
Ranidae	*Lithobates (Rana)*	*clamitans* (n = 12)	jumper	Conant and Collins, 1998
Rhacophoridae	*Chiromantis*	*xerampelina* (n = 6)	arboreal	Spawls et al., 2006; Channing and Howell, 2006
Rhacophoridae	*Rhacophorus*	*nigropalmatus* (n = 6)	arboreal/ glide	Emerson and Koehl, 1990
Scaphiopodidae	*Scaphiopus*	*holbrookii* (n = 9)	hopper	Emerson, 1978

Figure 5. Study sample with sampled families in bold text and habitual locomotor categories indicated at right. Locomotor categories include jumpers (jump), hoppers (hop), swimmers (swim), arborealists (arb), and walkers (walk).

Measurement Types:

Skeletal:

Within vertebrates, the skeleton comprises the basic structure that determines the length of limb segments. Thus measuring dimensions of skeletal elements is a widely accepted method for collecting morphometrics data for studies such as this one. Several previous studies have used skeletal metrics to examine jump performance and locomotor capabilities of various anuran taxa (Zug, 1972, 1978; Emerson, 1978, 1982). Yet skeletal specimens often lack reliable records of important measurements such as SVL; moreover, age and sex information is also often missing. Difficulties also arise in reliably rearticulating complex structures to obtain composite measurements such as manus length/pes length, with informative data missing on intervening soft tissue structures that are no longer in position. Finally, most herpetological collections consist of wet specimens, and sufficient sample sizes of prepared anuran skeletons from a wide variety of species are not readily available for study.

Wet Specimens:

For many of these reasons, some studies of anuran limb morphometrics have relied on measurements of limb structures taken externally from alcohol preserved specimens (e.g., Dobrowolska, 1973). Wet specimens are widely available, even for rare species, making data collection for comparative studies more feasible. Soft tissue measurements lend themselves well to certain research questions. For example, the descriptive herpetological standard for anuran body size is the snout vent length (SVL). This measure, as well as other measurements such as those representing serial skeletal elements that represent the contact structures (manus and pes), are best collected on soft tissue specimens (Figure 6). In these cases, the presence of intervening soft tissues is biologically informative about the structure as a whole. Thus this study utilized wet specimen data for the following measurements: SVL, manus length and width and pes length and width.

Table 2. List of measurements obtained in this study.

Measurement	Description of measurement
SVL	Snout to vent length
PL	Length of pelvis –distance from cranial end of ilium to the caudal end of the ischium.
UL	Length of urostyle–distance from cranial end of urostyle to the caudal end of the urostyle
SVW	Width of the sacral vertebra –distance from the axial mid line of the sacral vertebra to the distal edge of the sacral diapophysis, perpendicular to the long axis of the axial skeleton.
PSD	Proximal sacral diapophysis width – the greatest length between the cranial and caudal edges of the sacral diapophysis adjacent to the vertebral centrum
DSD	Distal sacral diapophysis width -the greatest length between the cranial and caudal edges of the ilio-sacral articular surface of the sacral diapophysis.
SDA	Angle of sacral diapophysis -angle formed by two lines that start at the cranial most point of each of the sacral diapophysis and end at the most caudal edge of the other sacral diapophysis.
FEL	Femur length –maximum length measured from proximal end of the femoral head to the distal articulation facet.
TFL	Tibio-Fibula length –maximal length measured from the proximal end of the tibio-fibula to the distal end.
TARL	Tarsal length –maximal length measured from the proximal end of the fused tarsal elements (Fibulare and Tibiale) to the distal articulation facet.
FL	Length of pes (foot) –tarsal/metatarsal joint to tip of the longest toe.
FW	Width of pes (foot) –width of an out stretched pes from medial most phalanx to the most proximal phalanx.
HLLm	Length of the hind limb (FE+TF+TARL).
SL	Scapula length –distance from proximal, to distal edge along the scapular midline.
CLL	Clavicle length –distance from the proximal end of the clavicle (point of contact with the coracoid at the glenoid fossa) to its distal end along the ventral surface.
COL	Coracoid length -the distance between the distal and proximal edge along the coracoids midline from the ventral surface.
HL	Humerus length -maximal length of the humerus measured from the proximal end of the humerus to the distal articular facet.
RUL	Length of the radio-ulna -maximal length of radio-ulna measured from the proximal articulation facet to the distal articular facet.
ML	Manus length -length measured from the proximal end of the carpals to the tip of the longest finger.
MW	Width of manus (hand) –width of an out stretched pes from medial most phalanx to the most proximal phalanx.
FLLm	length of the fore limb (HL+RUL)

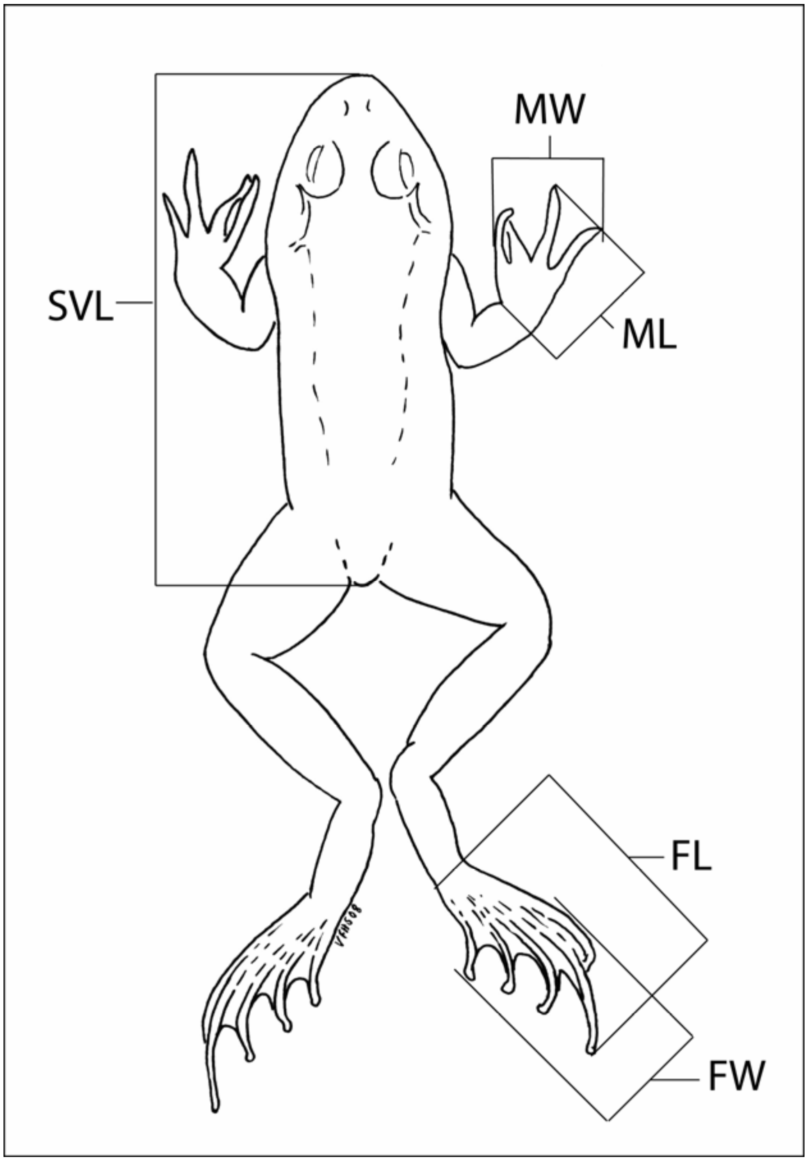

Figure 6. Measurements obtained from wet specimens. Snout-vent length (SVL), manus length (ML), manus width (MW), pes length (FL), and pes width (FW).

Yet measurements of individual skeletal elements are less easily defined in soft tissue specimens than they are in osteological specimens, and the presence of epithelia, fat, muscles, tendons, sesamoid bones, and articular cartilages may each obscure the dimensions of individual elements in an unpredictable manner. Measuring limb segment lengths on alcohol-preserved specimens relies on the examiner locating structural cues that outline the origin and terminus of the given limb segment. These cues are sometimes subject to skin movement and may be difficult to find especially in animals with large muscles or copious epithelial glands, such as in toads. For this reason, it was necessary to test whether wet specimens could provide an accurate estimation of individual limb segment lengths.

To do so, a fluid-preserved specimen of *Rana catesbeiana* was obtained from Wards Biological Supply. A series of external measurements of individual limb segments was obtained, including femur length (FEL), tibiofibula length (TFL), tarsal length (TARL), humerus length (HL) and radioulna length (RUL). Measurements were repeated (n = 10) at different times to enable an assessment of within-variable precision. Subsequently, the same specimen was skeletonized and the isolated skeletal elements were also measured ten times. The *precision within* each method (wet-tissue and skeletal) was well within acceptable limits (White, 2000), at less than 2.83% average measurement error (Table 3). However the *accuracy between* measurements taken on wet-tissue and skeletal specimens indicates alcohol-preserved specimens are not a reliable indicator of individual skeletal segment length. All variables were statistically different between the soft tissue and skeletal datasets (Table 3). Analysis of variance (ANOVA) was used to test among the means of measurements repeatedly taken externally from the wet specimen, the means of repeated measurements taken after skeletonization of the specimen, and the means collected for these measurements collected via the radiograph technique described in the next section (Appendix Table 1). ANOVA permits the experimenter to test for differences between sample means (Sokal and Rohlf, 1995). The Kruskal-Wallis test was used in cases for which assumptions of normality or homogeneity of variance were not met.

In addition, several vertebral and pelvic structures of interest in this study are also impossible to measure using wet specimens alone. For these reasons, a different methodology was required in order to obtain reliable skeletal dimensions from the available wet specimen study sample.

Table 3: Precision of wet and skeletal measurement methods showing mean, range, standard deviation (SD), coefficient of variation as a percentage of the mean (CV %), and measurement error as a percentage of the mean (ME %). Accuracy between measurement methods is represented by the results of ANOVA with p-values indicated.

Measure	Mean	Range	SD	CV (%)	ME %	p-value
FEL skeletal	40.044	0.450	0.150	0.373	0.302	$p < 0.001$
FEL wet	44.016	1.170	0.360	0.817	0.592	
TFL skeletal	43.345	0.830	0.224	0.516	0.316	$p < 0.001$
TFL wet	46.532	0.940	0.345	0.742	0.611	
TARL skeletal	22.564	0.320	0.104	0.459	0.342	$p < 0.001$
TARL wet	25.947	1.180	0.376	1.448	1.160	
HL skeletal	24.875	0.320	0.109	0.440	0.370	$p < 0.001$
HL wet	26.974	2.010	0.668	2.476	1.909	
RUL skeletal	15.671	0.380	0.110	0.703	0.503	$p < 0.001$
RUL wet	21.421	1.720	0.609	2.841	2.359	

Radiographs:

Plain film radiography permits visualization of bony structures and can be readily performed on alcohol-preserved specimens. Since radiographs are non-invasive and non-destructive they are suitable for the study of even rare museum specimens. However, radiographs also present methodological considerations, as they project a three-dimensional structure onto a two-dimensional film. This can cause limb elements to appear shorter than they truly are, unless the segment of interest is carefully aligned parallel and adjacent to the x-ray film and measurements are calibrated appropriately. The present study has modified techniques for collecting dimensional and character data from radiographs (Eastman, 1983) to develop a methodology for collecting morphometric data on alcohol preserved anurans. This method uses a Hewlett-Packard Faxitron soft X-ray machine (model 43805N) and Kodak Industrex M Film (M-5, 14” x 11”) following Eastman (1983). The machine was set to 30kVp and 2.75mA with a film to source distance of 122cm (Eastman, 1983). Anuran specimens were methodologically pinned to ½-inch polyethylene foam (13mm x 100mm x 360mm) in order to secure the limbs parallel to the x-ray film, achieving a dorsoventral view. A calibration object (coin) was then placed on top of the foam to act as a radio-opaque scale for subsequent measurement calibration. Specimens were then subjected to exposure times of 3.5-11.0 min depending on the size of the specimens. The specimen, still pinned to the foam, was rotated 90 degrees and x-rayed again in lateral view, along with the calibration object. X-ray film was developed by hand and radiographs were scanned using an Epson Expression 10000 XL flat bed scanner. Images were calibrated and measured in Image J (NIH image, version 1.38x).

To test the precision and accuracy of measurements obtained from radiographs; multiple radiographs were produced using the aforementioned fluid-preserved specimen of *Rana catesbeiana* from Wards Biological Supply (prior to skeletonization), following the above radiographic protocol. Each image was then measured to assemble a series of twelve metric variables. The measurements are as follows: pelvic length (PL), urostyle length (UL) sacral vertebral width (SVW), proximal sacral diapophysis width (PSD), distal sacral diapophysis width (DSD), sacral diapophysis angle (SDA), femur length

(FEL), tibio fibula length (TFL), tarsal length (TARL), humerus length (HL) and radio-ulna length (RUL) (Table 4 and figure 7A and 7B). As above, each measurement was repeated ten times. Table 4 outlines the mean, range standard deviation, coefficient of variation (as a percentage of the mean), and average measurement error as a percentage of the mean (White, 2000).

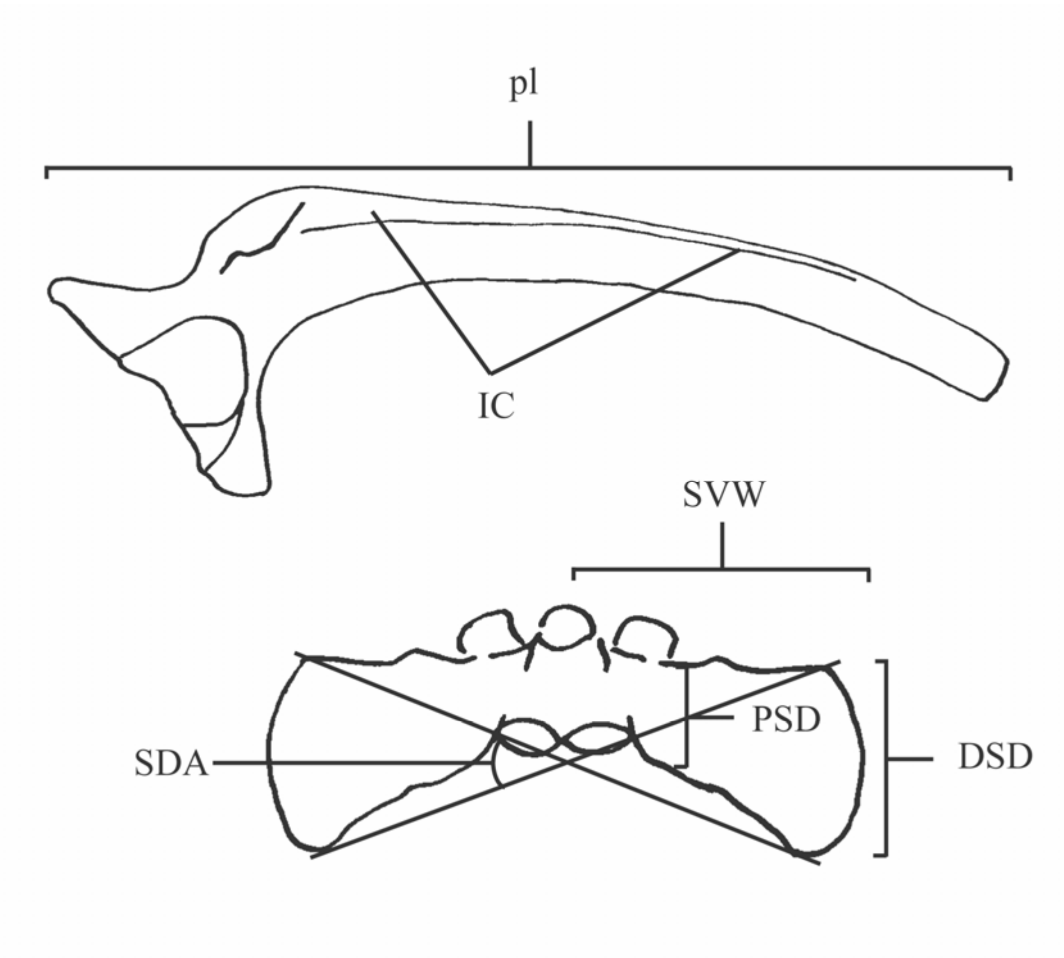

Figure 7A. Pelvic measurements obtained for this study. Pelvic length (PL), sacral vertebral width (SVW), sacral diapophyseal angle (SDA), proximal sacral diapophysis length (PSD), and distal sacral diapophysis length (DSD). (Drawings modified from Sanchiz, 1998)

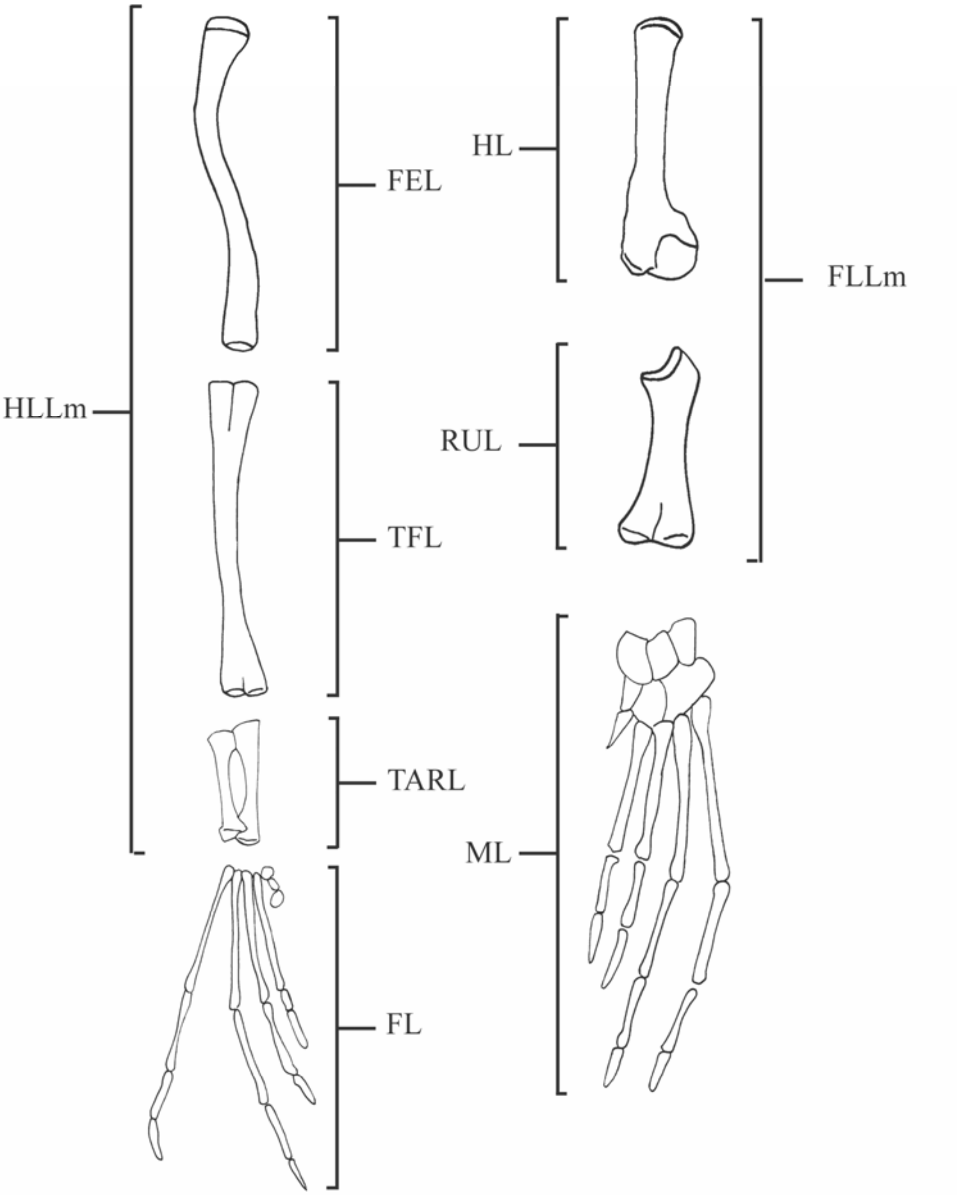

Figure 7B. Limb segment and contact structure lengths obtained for this study. Femur length (FEL), tibiofibula length (TFL), tarsal length (TARL) pes length (FL), hind-limb length minus pes length (HLLm), humerus length (HL), radio ulna length, (RUL), manus length (ML), and forelimb length minus manus (FLLm). (Drawings modified from Sanchiz, 1998)

Table 4. Mean, range, standard deviation (SD), coefficient of variation (CV) as a percentage of the mean, and average measurement error (as a percentage of the mean) for two randomly selected radiographs of an anuran specimen.

Measure	Mean	Range	SD	CV (%)	Ave. Meas. Error %
PL x-ray 1	40.559	1.547	0.513	1.264	1.096
PL x-ray 2	40.762	2.378	0.691	1.696	1.276
UL x-ray 1	31.609	0.326	0.103	0.325	0.258
UL x-ray 2	31.592	0.297	0.088	0.277	0.210
SVW x-ray 1	8.195	0.195	0.070	0.850	0.700
SVW x-ray 2	8.199	0.130	0.041	0.506	0.389
PSD x-ray 1	2.056	0.184	0.063	3.050	2.615
PSD x-ray 2	2.133	0.114	0.038	1.766	1.451
DSD x-ray 1	2.965	0.142	0.048	1.609	1.330
DSD x-ray 2	3.013	0.256	0.067	2.228	1.520
SDA x-ray 1	21.054	1.119	0.360	1.708	1.355
SDA x-ray 2	21.095	1.074	0.358	1.698	1.309
FEL x-ray 1	41.409	0.656	0.198	0.478	0.346
FEL x-ray 2	41.646	0.720	0.227	0.546	0.432
TFL x-ray 1	43.334	0.486	0.144	0.331	0.252
TFL x-ray 2	43.485	0.646	0.183	0.420	0.302
TARL x-ray 1	22.195	0.751	0.268	1.206	1.046
TARL x-ray 2	22.548	0.817	0.289	1.283	1.013
HL x-ray 1	25.249	0.481	0.135	0.536	0.383
HL x-ray 2	25.193	1.029	0.312	1.237	0.975
RUL x-ray 1	15.480	1.355	0.444	2.868	2.377
RUL x-ray 2	15.396	0.795	0.221	1.435	1.043

The accuracy of radiographic measurements was assessed using an analysis of variance (ANOVA, NCSS 2004). Only two of the 12 measurements are statistically different between different radiographs of the same specimen; these were proximal sacral diapophysis width (PSD), and tarsal length (TARL). Notably, in both cases the means of measurements still differs by less than 4% of the total segment length (< 0.3 mm, Table 4).

Measurements obtained from radiographs were also compared to measurements made on the specimen after skeletonization. Three measurements differ statistically between skeletal and radiographic data sets from the same specimen: pelvic length (PL), urostyle length (UL), and femur length (FEL), suggesting that radiographic measurements for these variables should be interpreted with caution. Nonetheless, means of these measurements differ by < 3.7% of the skeletal measurement (< 1.5 mm). The remaining eight measurements are not statistically different among the skeletal dataset and the two radiographic datasets, suggesting that radiographs provide an accurate measure of these variables when skeletal specimens are not available (Appendix Table 1).

Ilio-Sacral Types and Sacral Diapophyseal Angle

Despite endeavors to provide a basic functional framework for understanding the relationship between axial elements related to hind limb function, the classification of ilio-sacral articulations into Emerson's (1979) three types (I, IIA, and IIB) proves difficult for several reasons. First, work by Manzano and Barg (2005) has revealed that certain species of the family Hylidae exhibit ilio-sacral articulations that are morphologically intermediate between types IIA and IIB as originally described by Emerson (1979). Furthermore the classification of ilio-sacral articulation in fossil specimens (Asher and Krause, 1998) cannot rely on the shape of the sacral diapophysis alone (Emerson 1979). This is problematic in that unusual fossil preservation is necessary to reveal morphologies related to ligament scars on the sacral diapophysis. These issues also affect the accurate classification of ilio-sacral type in skeletonized specimens. Further complications arise during collection of morphometric or character data from radiographed anurans because ligament attachment location is not apparent on radiographs. One of the objectives of this study is to explore other ways of quantifying morphometric differences in the gross morphology of the ilio-sacral articulation. The use of angular measurements to characterize the morphology of sacral diapophyses offers promise for contributing new information for a few reasons. Angles are nonlinear measurements that permit comparisons among different species of anurans varying in body mass. Because angles are continuous variables, they can be easier to analyze statistically than categorical data such as ilio-sacral articulation types. This study includes an easily replicable sacral diapophyseal angle (SDA) that incorporates information about both the diapophyseal length and breadth. SDA is the angle between two straight lines, one passing through the most cranial corner of the right sacral diapophysis and extending through the most caudal corner of the left sacral diapophysis, the second passing through the most cranial corner of the left sacral diapophysis and extending it through the most caudal corner of the right sacral diapophysis (Figure 7A). This SDA measurement can be obtained from a photograph of a skeletonized anuran specimen, a fossil, or a dorsal radiograph of an alcohol-preserved anuran specimen as long as the frontal plane of the vertebral centrum is parallel to the lens of the camera or radiograph film.

Analysis of Covariance

Analyses of covariance (ANCOVA) test the means of a dependent variable for homogeneity while accounting for differences in these dependent variables caused by a covariate or independent variable (Sokal and Rohlf, 1995). Locomotor groups are first tested for equality of slopes. If this assumption is met, and slopes are found to be equal between groups, the y-intercepts are tested to see if they are significantly different (Sokal and Rohlf, 1995).

Data were examined in SPSS (version 13) using ANCOVA with SVL as the covariate in order to take into account differences in body size when seeking to determine whether relationships exist between locomotor mode and various morphometric measurements across this diverse sample (table 2, figures 6, 7A and 7B). Because not all variables were normally distributed, data sets were rank transformed prior to analysis. Rank transformation permits the use of parametric statistics for variables that are not normally distributed (Conover and Iman, 1982).

Within-family comparisons of morphometric data for species with different locomotor affinities were also conducted using ANCOVA. This was done in order to identify significant morphological correlates of locomotor mode within a more phylogenetically constrained context. Examinations explored differences in pelvic structural dimensions, hind limb and forelimb segment lengths, and contact structures in three species within the family Hylidae: *Hyla versicolor* (arboreal), *Hyla vasta* (arboreal) and *Pseudis paradoxa* (swimmer), and differences in hind limb and forelimb lengths for three species from the family Bufonidae: *Pedostibes hosii* (arboreal), *Anaxyrus americanus* (hopper), and *Epidalea calimita* (walk/runner).

Another comparison of closely related anurans was conducted using four species of the derived anuran clade Natatanura. Two species from the Family Rhacophoridae, *Chiromantis xerampelina* (arboreal) and *Rhacophorus nigropalmatus* (arboreal glider), one species from the family Ranidae, *Rana angolensis* (jumper), and one species from the family Ptychadenidae, *Ptychadena mascareniensis* (jumper) were compared. Finally, *Lithobates clamitans* (jumper) was compared with the above natatanurans to explore differences in contact structure among locomotor groups.

Analysis of Variance

Sacral diapophyseal angles (SDA, Figure 7A) from both skeletonized specimens radiographed specimens were statistically analyzed using a non-parametric analysis of variance by ranks (Kruskal-Wallis test). Data from both skeletonized specimens and radiographed specimens were used to increase sample size (n = 100) since this measurement were not significantly different between these two measurement types for the test specimen of *R. catesbeiana*.

Narrow Allometric Comparisons

A narrow allometric comparison controls for size by examining morphometric differences between species of similar size (Smith, 1980), here represented by snout vent length (SVL). If these species are closely related and represent two (or more) distinct locomotor types, differences between anatomical structures of the limbs and pelvis can more confidently be attributed to differences in locomotion. Two narrow allometric comparisons were made between two species of the Bufonidae and two species from the clade Natatanura. These species were chosen because of their close phylogenetic relationship and similarities in SVLs, and because they represent differing locomotor types. Hence it was possible to directly compare closely related anurans of differing locomotor types while controlling for differences in body length.

Reduced Major Axis Regression

Within the phylogenetically focused comparisons of the family Bufonidae and the clade Natatanura, the assumption of parallel slopes for ANCOVA is not always supported. In those cases, reduced major axis (RMA) regression analyses were employed to permit further examinations of the effects of differences in scaling for the structures examined (table 5). RMA regression permits measurement error in both variables (i.e. SVL and the variable of interest).

Table 5: Log10 Reduced major axis regressions of linear measurements on SVL for within clade comparisons where assumption of equal slopes was violated. Slope (b), r^2, and sample size (n) are reported for two clades: Bufonidae (Bufo), and Natatanura (Nata).

Bufo DSD	**b**	**r^2**	**n**
arb	1.62	0.98	5
hop	2.33	0.01	6
walk	-1.56	0.33	5
Nata PSD			
jump	2.27	0.73	12
arb	0.78	0.7	12
Nata DSD			
jump	2.08	0.61	12
arb	0.97	0.6	12
Nata FL			
jump	0.96	0.48	18
arb	1.55	0.98	12

RESULTS

The results of analysis of covariance (ANCOVA) performed on rank transformed dimensional data from the total anuran data sets of the pelvic area, limb segment lengths, summed limb lengths minus contact structure length for radiographed anurans, and contact structure dimensions for wet measured anurans (all using the wet measured snout vent length, SVL, as the covariate) yield significantly different slopes between locomotor types. Thus these parameters are scaling differently between the locomotor groups. Proximal sacral diapophyseal width (PSD) is the only measurement for which slopes are not significantly different between locomotor types for the total radiographed anuran data set. Results for this variable indicate significant differences ($p < 0.01$) in PSD width between locomotor types relative to SVL. PSD is significantly greater in hoppers, swimmers and walkers than in the arborealists relative to body length. Also arborealists, hoppers, swimmers, and walkers exhibit significantly greater values for PSDs relative to SVL than those measured in jumping anurans.

The ANCOVA analysis of pectoral girdle parameters with SVL as the covariate using skeletonized specimens reveals significant differences: coracoid length (COL), clavicle length (CLL), and scapula length (SL). However, sample sizes for these parameters are too low for confident interpretation of the results. Unfortunately these skeletal parameters proved too difficult to collect from radiographed specimens. Further investigations of the skeletal morphometrics of the anuran pectoral girdle are necessary, but outside of the scope of this study.

The results of the within family ANCOVA comparisons for the Hylidae (radiograph n = 13, wet n = 18) indicate differences for sacral vertebral width (SVW) and distal sacral diapophysis (DSD) relative to SVL. The swimmer (*P. paradoxa*) exhibits a significantly wider sacral vertebra SVW than the arborealists (*H. vercicolor, H, vasta*) (p = 0.036), whereas the arborealists exhibit a greater DSD value than the swimming hylids relative to body length (p < 0.001). Also, the relative pes width of the swimmers is significantly greater than that of the arboreal hylid (p = 0.01) although the manus length (ML) relative to SVL is significantly greater for arborealists when compared with that of the swimming hylids (p = 0.025).

Within Bufonidae (radiograph, n = 16; wet, n = 19) the ANCOVA reveals that slopes are different among three locomotor types for the comparison of DSD. However, slopes for the other variables are not significantly different between the three locomotor types represented in this comparison. The proximal portion of the sacral diapophysis (PSD) is significantly wider (p < 0.043) for the walking bufonids (*Epidalea calimita*) than for the hoppers (*Anaxyrus americanus*) and arborealists (*Pedostibes hosii*). Both the forelimb length minus the manus (FLLm) and the hind limb length minus the pes (HLLm) are significantly greater (p ≤ 0.027) relative to body length in the arboreal bufonids examined. This is driven by both the humerus (HL) and radio-ulna (RUL) of the arboreal species being significantly longer (p ≤ 0.028) than that of the walking bufonid relative to SVL. In the hind limb, the femur (FEL) and tarsal (TARL) segments are significantly longer in the arboreal taxon than in the walker relative to body length (p ≤ 0.009). The pes (FW) is significantly wider in arborealists than in either the walking or hopping species examined from this family relative to SVL (p ≤ 0.035).

Within the derived anuran clade Natatanura (radiograph, n = 24; wet, n = 30) ANCOVA results indicate no significant differences between pelvic length (PL), urostyle length (UL), and sacral vertebral width (SVW) in a comparison between jumpers: *Ptychadena mascareniensis* and *Rana angolensis*, and arborealists: *Chiromantis xerampelina* and *Rhacophorus nigropalmatus*. The slopes for PSD and DSD in these anurans are different between locomotor styles. Hind limb length minus the pes is significantly larger relative to SVL for the jumping anurans than in the arboreal natatanurans that were examined as part of this study. The femur is the segment that drives this difference in length, as this element is also significantly longer ($p = 0.018$) in jumpers than in arborealists. The manus of arborealists are significantly longer ($p = 0.001$) and wider ($p < 0.001$) than those of the jumping natatanurans.

Results of narrow allometric comparisons using Kruskal-Wallis non-parametric ANOVA for the Bufonidae and natatanurans exhibit similar results to those provided by the within-clade ANCOVAs above. Comparisons between morphometrics of two bufonid species, *P. hosii* (arboreal) and *E. calimita* (walker/runner) (n = 13) indicate SVLs are not significantly different.

Also PL, UL, and PSD do not differ significantly between bufonids of different locomotor types. Hind limb length minus pes length (HLLm) is significantly different ($p = 0.028$) between the two bufonid species with the arborealists having the longer hind-limb length. This length difference is driven by a statistically longer femur ($p = 0.009$) and statistically longer tarsal bone ($p = 0.028$) for the arboreal bufonid. Forelimb length minus the manus (FLLm) is also statistically longer ($p = 0.009$) in arborealists than in walking bufonids. This statistical difference is driven by both of the forelimb elements being statistically longer (humerus, p = 0.03; and radio-ulna, p = 0.009) for the arboreal species of the Bufonidae. In this narrow allometric comparison, all of the contact structure measurements (n = 11) are statistically larger (FL, p = 0.028, FW, p = 0.01, ML, p = 0.006, and MW, p = 0.028) in arborealists than walking species.

In narrow allometric comparison within the clade Natatanura, morphometrics data for species *P. mascareniensis* (jumper) is compared with that of the similarly sized arborealist *C. xerampelina*. These two species exhibit SVLs that are not significantly different from each other (n = 12). None of the pelvic measurements (PL, UL, SVW, PSD, and DSD) differ statistically between these two anurans. Hind limb length minus the pes (HLLm) is significantly longer (p = 0.006) for jumping species than arboreal species. This difference is due to statistically longer femur (p = 0.006) and tibiofibula (p = 0.006) in the jumping frog. Pes length is also statistically greater (p = 0.004) for the jumping natatanuran than the arborealist. The manus of the arboreal natatanuran is significantly wider (p = 0.025) than that of the jumping species.

Sacral Diapophyseal Angle

Non-parametric ANOVA (Kruskal-Wallis) results for sacral diapophyseal angle (SDA) (Figure 7A) indicate significant differences between locomotor groups (n = 100) (Table 6, Figure 8).

Table 6: Sacral diapophyseal angle (SDA) means and range (in degrees) for anurans examined herein.

Locomotor type	Group		
	Total		
	mean	Largest	Smallest
Arboreal	36.06	68.61	12.22
Hopper	39.31	65.65	27.92
Jumper	21	35.32	15.9
Swimmer	57.81	110.4	15.31
Walker	69.8	80.81	64.12
	Total minus Pipidae		
Arboreal	36.06	68.61	12.22
Hopper	39.31	65.65	27.92
Jumper	21	35.32	15.9
Swimmer	22.45	33.51	15.31
Walker	69.8	80.81	64.12
	Bufonidae		
Arboreal	45.23	49.11	39.12
Hopper	33.18	39.48	29.92
Walker	65.4	67.45	64.12
	Hylidae		
Arboreal	44.7	68.61	29.64
Swimmer	18.14	20.48	15.31
	Leptodactylidae		
Jumper	21.08	24.54	18.13
Swimmer	28.91	33.51	25.01
	Natatanura		
Arboreal	16.98	22.31	12.22
Jumper	18.23	23.1	15.9

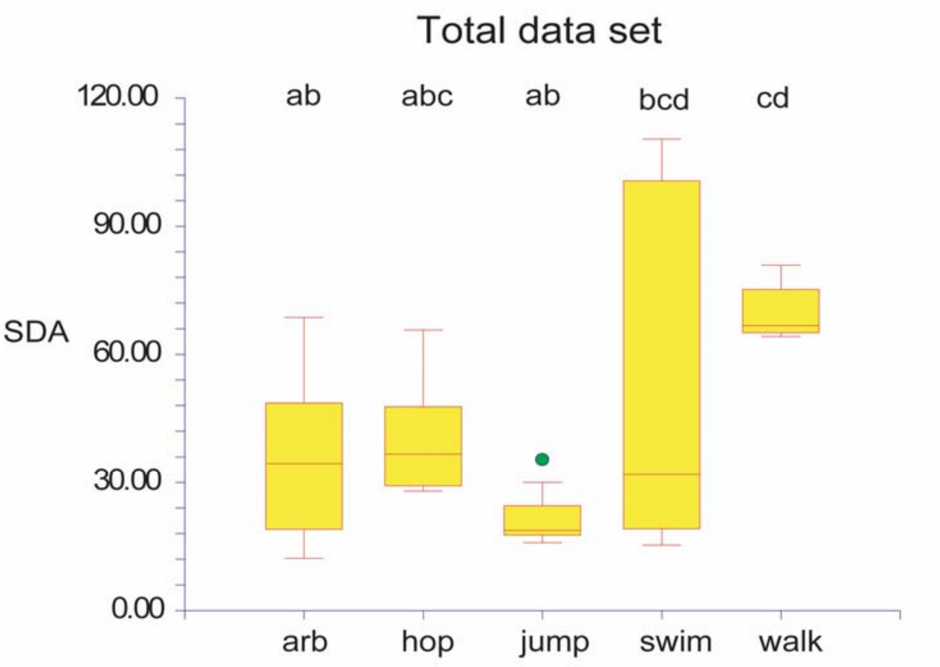

Figure 8: Sacral diapophyseal angle (SDA) (in degrees) by locomotor habit for the total anuran data set. Alphabetic groupings at top indicate groups that are not significantly different from one another (Scheffe's multiple comparison test).

A post hoc Scheffe's multiple comparison test shows that mean SDA in arboreal anurans is significantly larger than the swimmers and significantly smaller than in the walking anurans. The SDA for hoppers is significantly smaller than that of walkers. Jumping anurans have SDA values that are significantly smaller than both swimming anurans and walking anurans. ANOVA tests reveal that the swimming group is bimodal with specimens from the family Pipidae, a family known for its highly aquatic species, exhibiting extremely high SDA values. Removal of this family from the ANOVA (n = 92) reveals similar results (Table 6 and Figure 9). Walkers have an SDA greater than any of the other four locomotor groups. Arborealists have a significantly greater SDA than jumpers and swimmers, and have significantly smaller SDA than only walkers. Hoppers have a significantly greater SDA than jumpers and an SDA significantly smaller than that of walkers. Jumpers have an SDA significantly smaller than arborealists, hoppers and walking anurans. The swimmers also have an SDA smaller than arborealists and walkers.

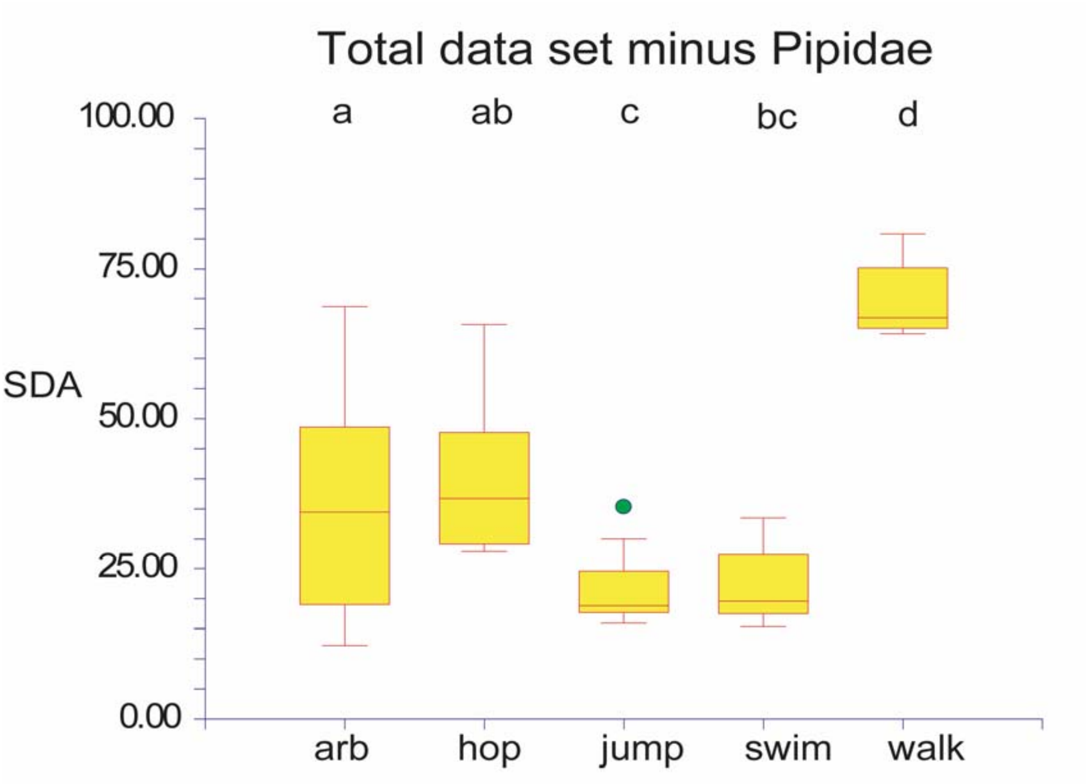

Figure 9: Graphical representation of sacral diapophyseal angle (SDA) (in degrees) by locomotor habit for the total anuran data set minus the members of the Pipidae. Alphabetic groupings at top indicate groups that are not significantly different from one another (Scheffe's multiple comparison test).

Within the family Bufonidae (n = 17) significant differences in SDA were detected for the three species examined (Table 6, Figure 10). These species are *E. calimita* (walker), *A. americanus* (hopper), and *P. hosii* (arborealists). Walking bufonids have an SDA that is significantly greater than the other two species. Arborealists have a significantly greater SDA than do hoppers, and significantly smaller value than the walking bufonids.

The hopping bufonids have an SDA that is significantly smaller than either of the other two species.

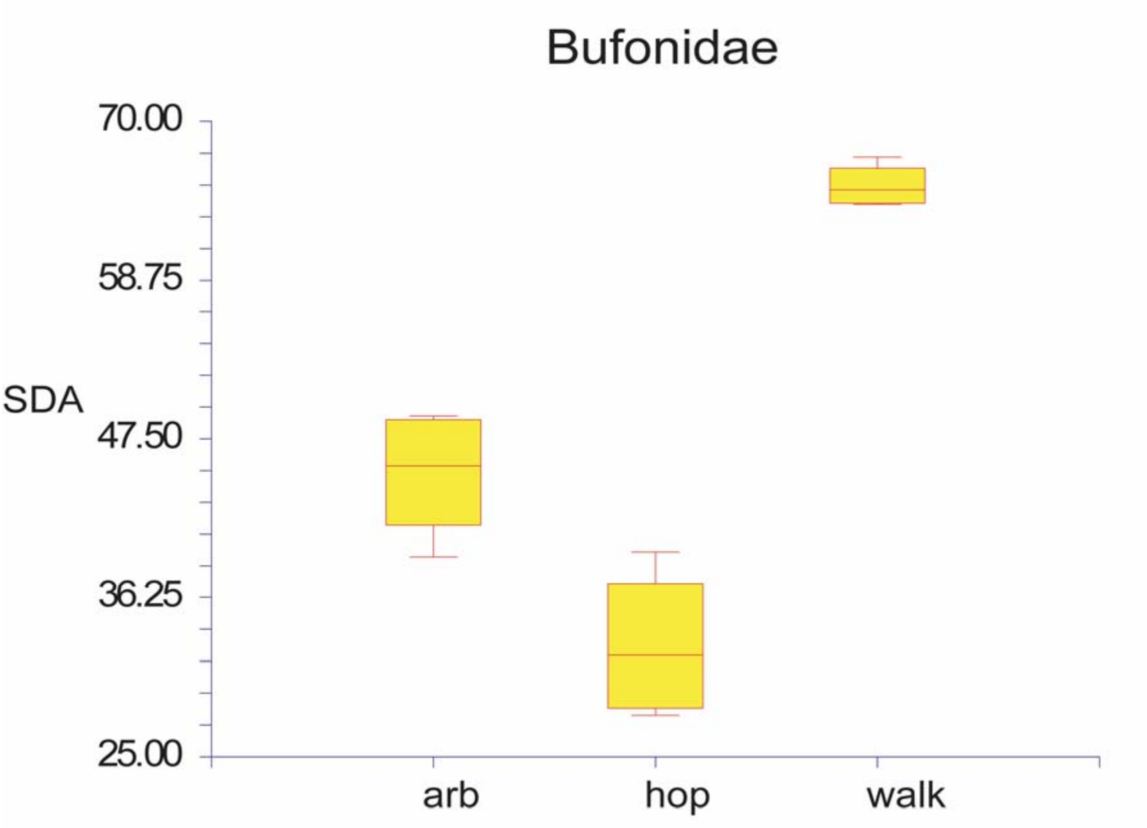

Figure 10: Graphical representation of sacral diapophyseal angle (SDA) for the family Bufonidae. SDA values are significantly different among all three locomotor groups (Scheffe's multiple comparison test).

The SDA values were also explored for four species within Hylidae (n = 24), *P. paradoxa* (swimmer), *H. vercicolor, H. vasta,* and *Agalychnis callidryas* (all arboreal) (Table 6, Figure 11). Arborealists exhibit significantly greater SDA than do swimming species within this family.

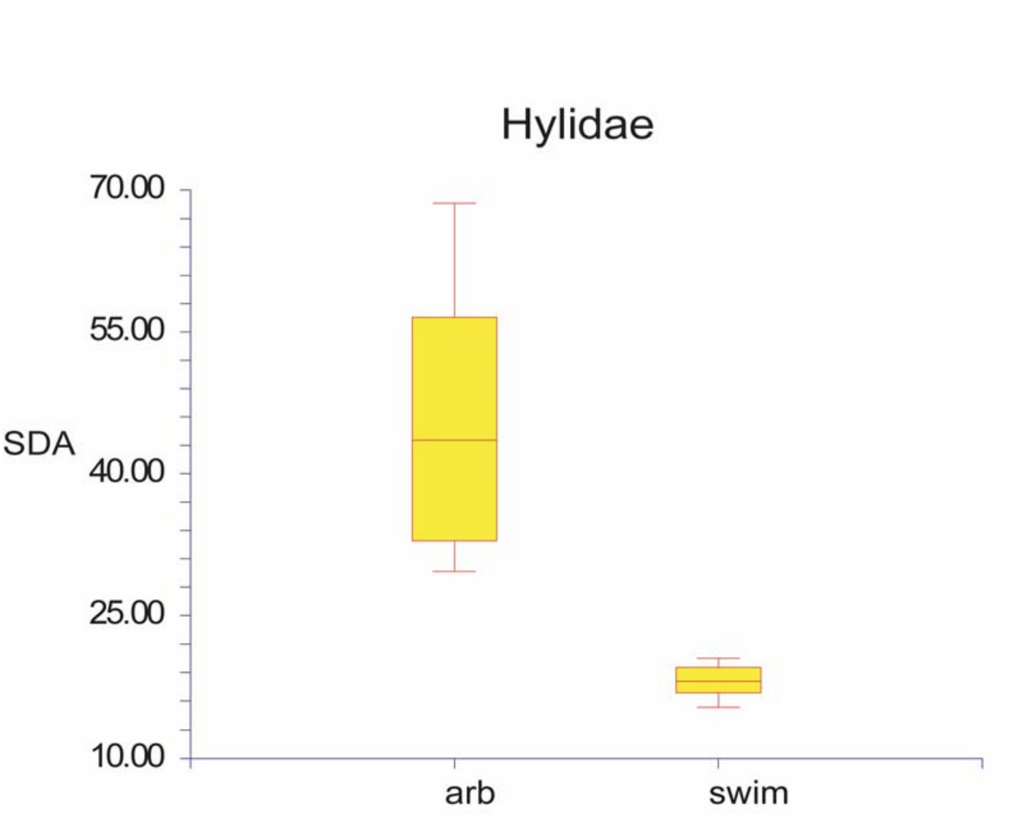

Figure 11: Graphical representation of sacral diapophyseal angle (SDA) for the family Hylidae. Arboreal species exhibit significantly larger SDAs than swimming species (Kruskal-Wallis non-parametric ANOVA).

Within the family Leptodactylidae (n = 9), swimming species (*Hydrolaetare* sp.) have significantly greater SDA than do jumping species (*Leptodactylus pentadactylus*) (Table 6 and Figure 12).

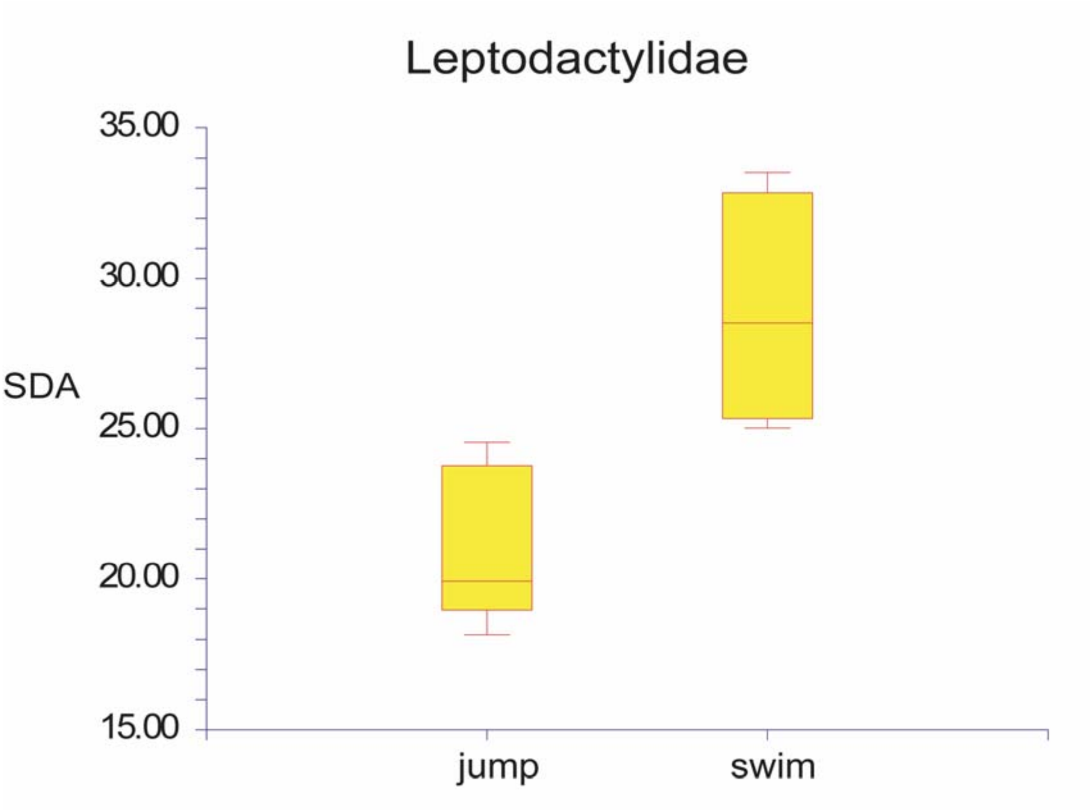

Figure 12: Graphical representation of sacral diapophyseal angle (SDA) for the family Leptodactylidae. Swimming species exhibit significantly larger SDAs than jumping species (Kruskal-Wallis non-parametric ANOVA).

Within the clade Natatanura (n = 28) SDA is not significantly different between the two locomotor types examined as part of this study (Table 6 and Figure 13). Species examined in this clade include *Lithobates* (*Rana*) *clamitans*, *P. mascareniensis*, and *R. angolensis*, all jumping anurans, and arborealists: *C. xerampelina* and *R. nigropalmatus*.

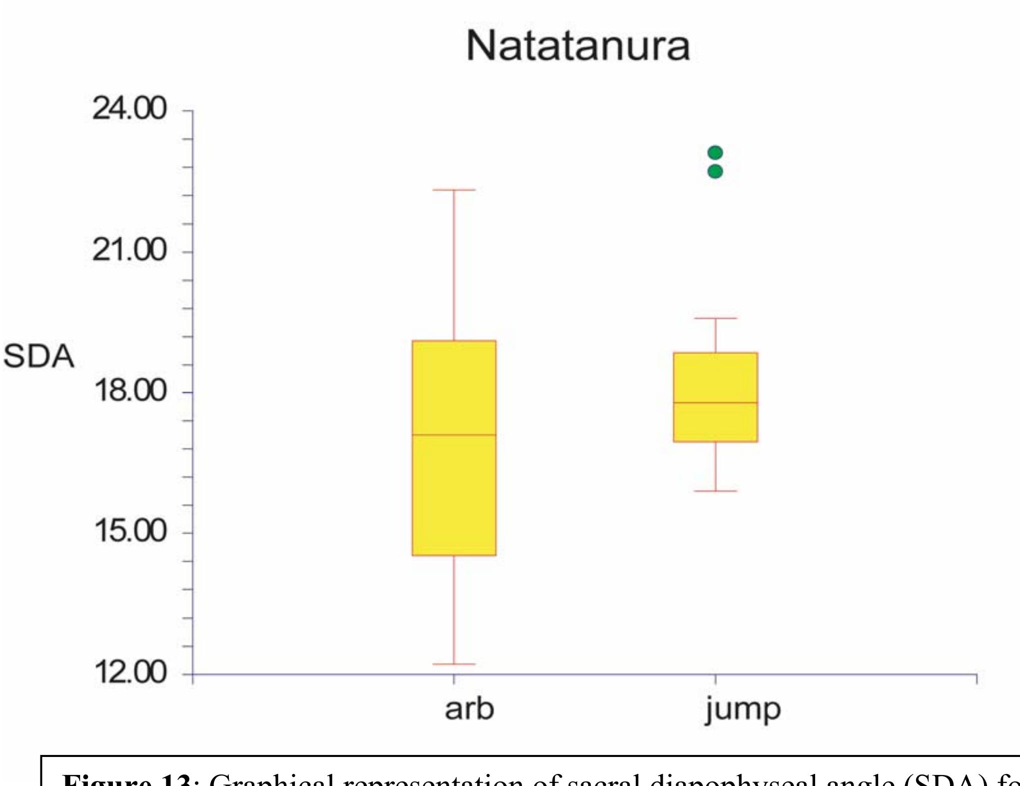

Figure 13: Graphical representation of sacral diapophyseal angle (SDA) for the clade Natatanura. SDAs are not significantly different between locomotor groups (Kruskal-Wallis non-parametric ANOVA).

DISCUSSION

Limb Lengths Relative to Locomotor Mode- Within Clade Comparisons

It is now convenient to return to the initial hypotheses of this project. It is hypothesized that anuran limb length varies in a predictable way with respect to locomotor behaviors. Predictions related to this hypothesis include the following; that anurans that are capable of long distance jumping possess long hind limbs, anurans capable of arboreal locomotion possess relatively long fore- and hind limbs, and swimmers possess a combination of relatively short forelimbs and long hind limbs.

Results of within clade ANCOVA comparisons permit examination of limb length as it relates to locomotor type.

Natatanura

ANCOVAs focused on related species from clade Natatanura to explore relative limb length between two different locomotor groups (jumping and arboreal). As predicted, jumping species (*P. mascareniensis*, *R. angolensis*) exhibit significantly longer hind limbs minus pes length (HLLm) than those species that are classified as arborealists (*C. xerampelina*, *R. nigropalmatus*). Similarly, jumping species (*P. mascareniensis*) exhibit significantly longer HLLm than do similarly sized arboreal species (*C. xerampelina*) in narrow allometric comparisons conducted using a Kruskal-Wallis ANOVA. Both of these results indicate that anurans within the clade Natatanura capable of long distance jumping exhibit longer hind limbs than do their arboreal relatives. This result suggests that in natatanurans, jumping may drive hind limb length more than use of the arboreal habitat.

No statistical difference in forelimb length is found between long distance jumpers and arborealists in this clade.

Bufonidae

Locomotor variation in the family Bufonidae permits testing of the prediction that arboreal anurans have longer fore- and hind limbs relative to frogs that primarily engage in hopping and walking locomotion on the ground. As predicted, within bufonids, the arboreal *P. hosii* exhibits significantly longer forelimb length minus manus (FLLm) and significantly longer hind limb length minus pes (HLLm) than do the hopping species (*A. americanus*) and the walking species (*E. calimita*). Longer limbs may enable arboreal species to span gaps between arboreal substrates during climbing. Longer hind limbs in arboreal species may also relate to a stronger leaping ability than is demanded of habitual hoppers and walkers, a pattern observed in terrestrial long distance jumpers of other families. Longer forelimbs in arboreal species reflect that both forelimb skeletal elements (humerus, and radio-ulna) in these taxa are statistically longer than in the two species of terrestrial bufonids (hoppers and walkers). Longer hind limbs likely relate to both statistically longer femora and tarsal bones relative to body size. This should, in the flexed position, work to move the pes of the animal more cranially and presumably closer to the center of mass of the anuran. Perhaps this assists balance as forelimbs reach out ahead of the animal during arboreal movements.

This pattern of results is repeated in the narrow allometric comparison between similar sized *P. hosii* (arboreal) and *E. calimita* (walker). As predicted, FLLm and HLLm are both significantly longer in the arboreal species than in the walking taxon.

Hylidae

The generally arboreal family Hylidae offers another opportunity to investigate difference in limb lengths between frogs exhibiting two different common anuran locomotor patterns. The North American species *H. vercicolor* and the Neotropical *H. vasta* are arboreal, and the species *P. paradoxa* are mostly aquatic swimmers. Counter to predictions, ANCOVA comparisons within this family did not provide any significant difference in limb length between the arborealists and the swimmer. This may indicate that the phylogenetic signal is stronger than the functional signal in this clade as it relates to limb length.

Contact Structure Size Relative Locomotor Mode

To revisit another of the hypotheses of this study, it was suggested that contact structure size (manus length (ML), manus width (MW), pes length (FL), and pes width (FW)) should vary in a predictable manner as it relates to habitual locomotor styles. It was predicted that swimming anurans exhibit larger pes (FL, and FW) to aid in propulsion, whereas anurans that habitually climb in trees exhibit large contact structures to permit grasping of arboreal substrates. Sampling natatanuran, bufonid and hylid clades permits further investigation of the relationship between contact structure size and locomotion.

Natatanura

ANCOVAs comparing the size of contact structures of taxa in the clade Natatanura permit us to explore the relationship of contact structure size and habitual locomotor pattern between arboreal species (*C. xerampelina*, *R. nigropalmatus*) and strong jumping species (*P. mascareniensis*, *R. angolensis*) taking into consideration the effects of body size (SVL). Slopes of pes length (FL) relative to body size (SVL) were different between taxa representing these two locomotor styles (Figure 14). Reduced major axis (RMA) regression of log10 transformed data are: arboreal slope = 1.560 and jumper slope = 0.689 (Table 5).

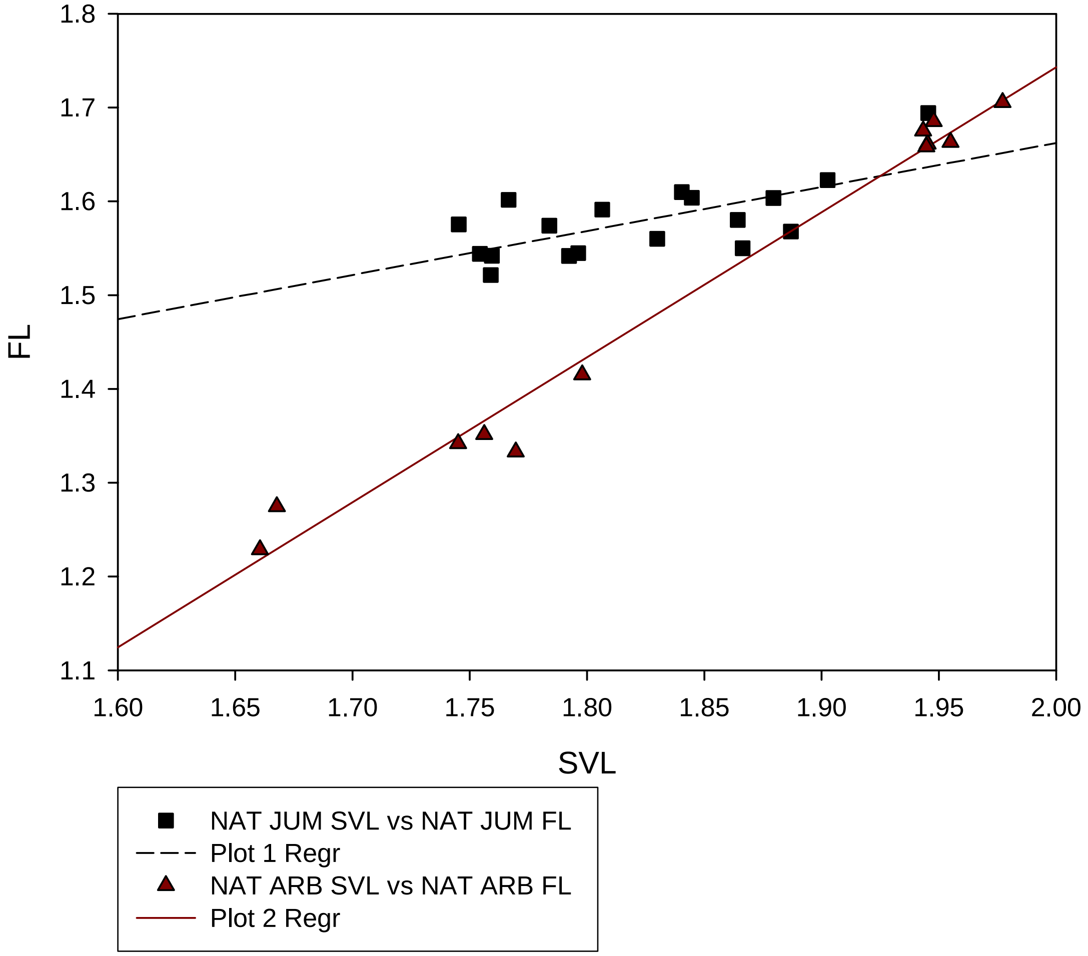

Figure 14: Log-log RMA regression plot for pes lengths (FL) relative to body length (SVL) for jumping and arboreal natatanuran species. RMA slope values, arboreal (ARB): 1.560 and jumper (JUM): 0.689(Table 5). Data points indicate individual specimens.

Arboreal species exhibit greater increase in pes length (FL) relative to body length than do jumping species of this clade. This may be an artifact of including gliding species from the family Rhacophoridae, *R. nigropalmatus*, the largest natatanurans examined in this study. This large natatanuran is arboreal, but also glides using hands and feet as its largest airfoils, so large feet would greatly benefit its gliding ability by increasing the surface area for lift to act upon. Upon removal of this species from the comparison, remaining natatanurans classified as jumpers exhibit statistically longer values for FL. This is counter to the prediction that arboreal anurans possess larger contact structures than non-arboreal forms. Many strong jumpers will intentionally jump into a body of water to avoid predation. Perhaps these species exhibit large feet as a compromise to a partially aquatic locomotor repertoire. Pes width (FW) does not differ between locomotor groupings.

Narrow allometric comparison of the similarly sized *C. xerampelina* (arboreal) vs. *P. mascareniensis* (jumper) yields significantly longer pes length (FL) for the jumping species, also counter to the prediction of this study. Neither narrow allometric comparisons nor whole clade ANCOVAs detect a significant difference in pes width (FW). A long pes may aid arboreal anurans in grasping arboreal substrates, whereas swimmers and terrestrial long distance jumpers may be advantaged in propulsion by possessing broader hind limb contact structures.

Manus width and length are significantly different among the species examined from this clade (Figure 15). Within narrow allometric examinations between the similarly sized *P. mascareniensis* and *C. xerampelina* manus width is greater for the arboreal species. This fits the predictions that long and wide forelimb contact structures will aid an arboreal anuran's ability to grasp arboreal substrates.

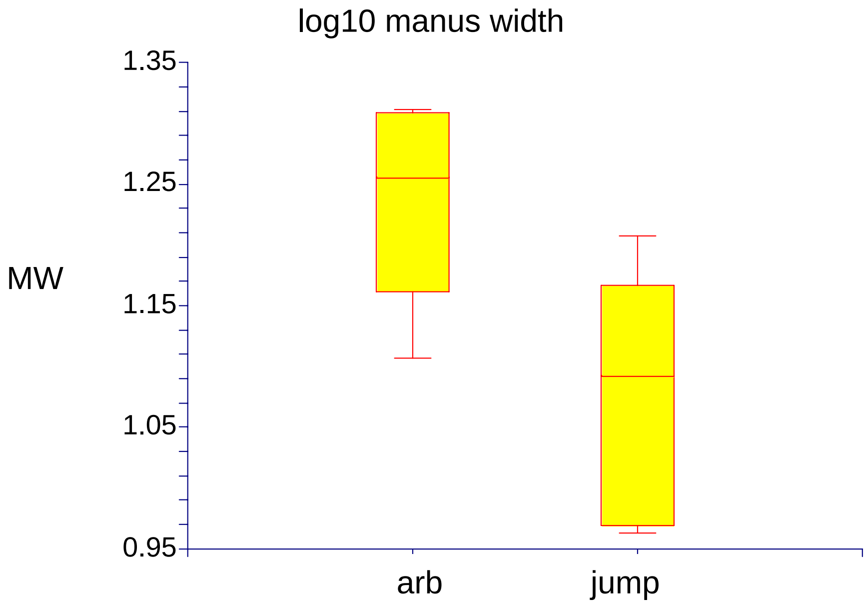

Figure 15: Graphical representation of manus width for arboreal (arb) and jumping (jump) natatanurans using the narrow allometric comparison (ANOVA). log10 Manus width (MW), arboreal (arb), and jumper (jump) ($p < 0.014$).

Bufonidae

An ANCOVA, with size (SVL) as the covariate, permits exploration of contact structure dimensions among three locomotor groups represented by three species from the family Bufonidae (*P. hosii*; arboreal, *A. americanus*; hopper, and *E. calimita*: walker). Here pes length (FL), pes width (FW), manus length (ML) and manus width (MW) relative to body length (SVL) is examined among three species. Manus width is the only parameter found to differ significantly with locomotor group in this family, with a wider (MW) value for the arboreal species. Narrow allometric comparisons of similarly sized *P. hosii* (arboreal) and *E. calimita* (walker) reveals that all metrics relating to contact structures (FL, FW, ML, and MW) are significantly larger in arboreal species, supporting predictions of this study (Figure 16). These results support the hypothesis that arboreal anurans rely on larger contact structures to assist in grasping arboreal substrates.

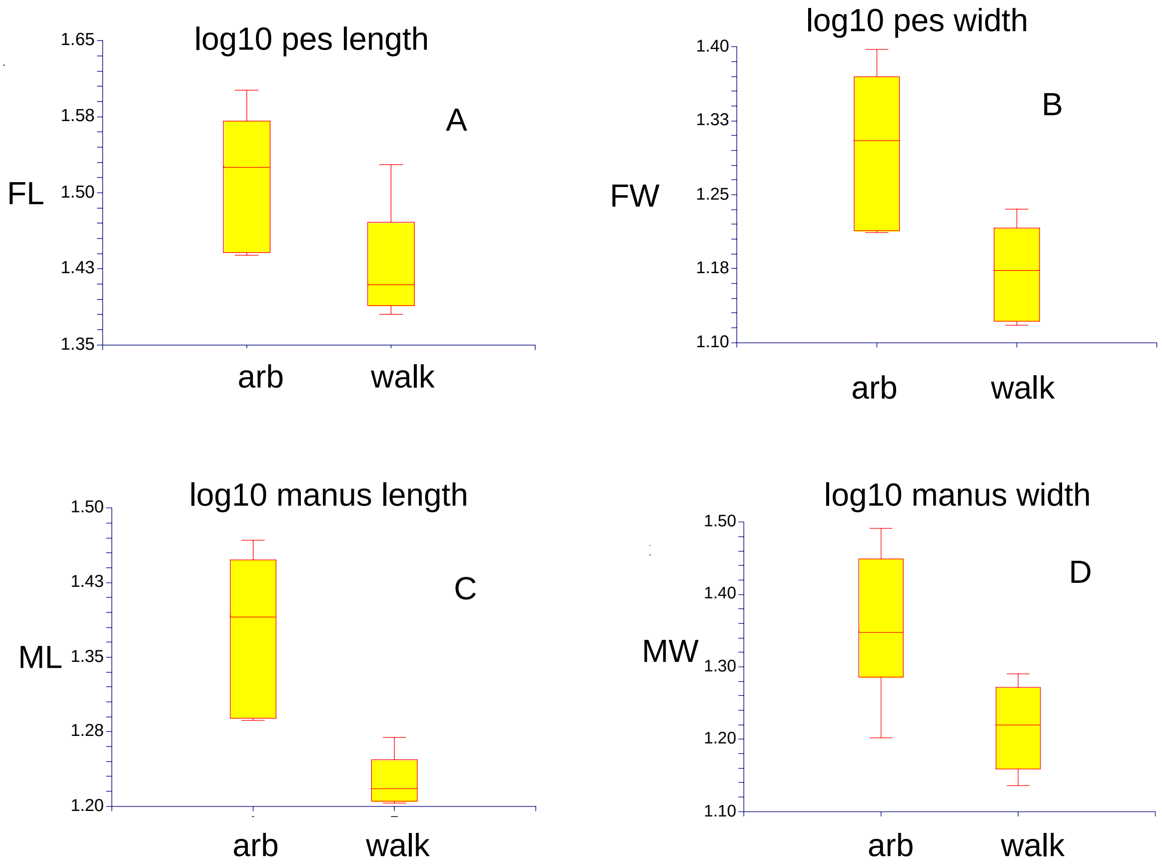

Figure 16: Graphical representation of contact structure variables for arboreal (arb) and walking (walk) bufonids using the narrow allometric comparison (ANOVA, and Kruskal-Wallis). All variables are significantly greater in arboreal species: (A) log10 pes length ($p < 0.042$), (B) log10 pes width ($p < 0.01$), (C) log10 manus length ($p < 0.006$), and (D) log10 manus width ($p < 0.025$).

Hylidae

ANCOVA, with body size (SVL) serving as the covariate, permits another exploration of the relationship of contact structure size and locomotor type via comparisons of two species representing two locomotor types from the family Hylidae. In these taxa, pes width (FW) is statistically greater relative to SVL for the swimming species *P. paradoxa*, and manus width (MW) is statistically greater relative to SVL for the arboreal species examined, *H. vercicolor*, and *H. vasta*. Interestingly, contact structure lengths (FL and ML) are not statistically different between locomotor types. These results suggest that highly aquatic species *P. paradoxa* uses relatively broad hind limb contact structures (pes) to assist in propelling itself through water. Manus structures may be reduced in width in these forms to avoid interference with the hydrodynamics of underwater locomotion. In contrast, a wide manus is likely to assist arboreal species with grasping arboreal substrates. For similar reasons the pes of arboreal animals might be expected to also be large, relative to an animal that does not place as much emphasis on the hind limb contact structures for grasping or propulsion. Unfortunately, there are no terrestrial hopping frogs in this family for comparison with either the arborealists or the strong swimmers. It is perhaps not surprising, then, that pes width is not significantly greater in arborealists and that differences are not apparent in pes lengths, as swimmers are also likely to benefit by greater pes dimensions.

Pelvic Dimensions Relative to Locomotor Mode

It has been hypothesized that dimensions and characters of the anuran pelvis will vary in a predictable manner between different locomotor patterns. For example, strong jumping anurans were predicted to exhibit low sacral diapophyseal angle (SDA), relatively low values for distal sacral diapophysis length (DSD) and proximal sacral diapophyseal length (PSD), short ilia or pelvic length (PL), and a relatively wide sacral vertebral width (SVW). These hypotheses and predictions were based on previous studies of the anuran pelvic girdle (Emerson, 1979, 1982). Such investigations sought to classify pelvic morphology into three “functional complexes” or ilio-sacral articulation types. Two of these three categories do not correspond to locomotor categories. Recent studies have also shown that certain taxa form the family Hylidae exhibit morphologies that are intermediate between these categories (Manzano and Barg, 2005). Thus this study explores some of the linear parameters proposed in earlier investigations while avoiding problematic morphological categories.

Urostyle length (UL) and pelvic length (PL) were compared among locomotor groups for the broad sample of anurans in this study. Emerson (1982) contrasted animals that exhibit relatively short ilia, small, cylindrical diapophyses, and a wide sacral vertebra, associating these features with a vertical rotation (as in strong jumpers), from animals that have long ilia, craniocaudally elongate sacral diapophyses, and relatively narrow sacral vertebrae, associating these features with rotation in the horizontal plane (as in swimmers, walkers, and hoppers). A more expanded study sample reveals no significant relationships between locomotor style and either pelvic length or urostyle length.

Natatanura

Within the derived clade Natatanura, ANCOVA results demonstrate that both sacral diapophyseal measurements, distal sacral diapophysis (DSD) and proximal sacral diapophysis (PSD), scale differently between arboreal species (*C. xerampelina*, *R. nigropalmatus)* and jumping species (*P. mascareniensis*, *R. angolensis)*. Sacral parameters in jumpers exhibit a greater increase relative to overall size than those of arboreal natatanurans (Figure 17A and B). Model II RMA slopes are: PSD slope = 0.777 and DSD slope = 0.970 for arboreal species, and PSD slope = 2.273 and DSD slope = 2.082 for jumping species from this clade (Table 5). Because the largest arboreal species examined in this study (*R. nigropalmatus*) is capable of gliding jumps, these anurans may exhibit specialization for gracile skeletal structures in order to reduce body mass to enhance gliding distance. Analyses omitting *R. nigropalmatus* yield similar PDS slope results (arboreal = 1.257 and jumper = 2.273 Figures 17A, B, and C).

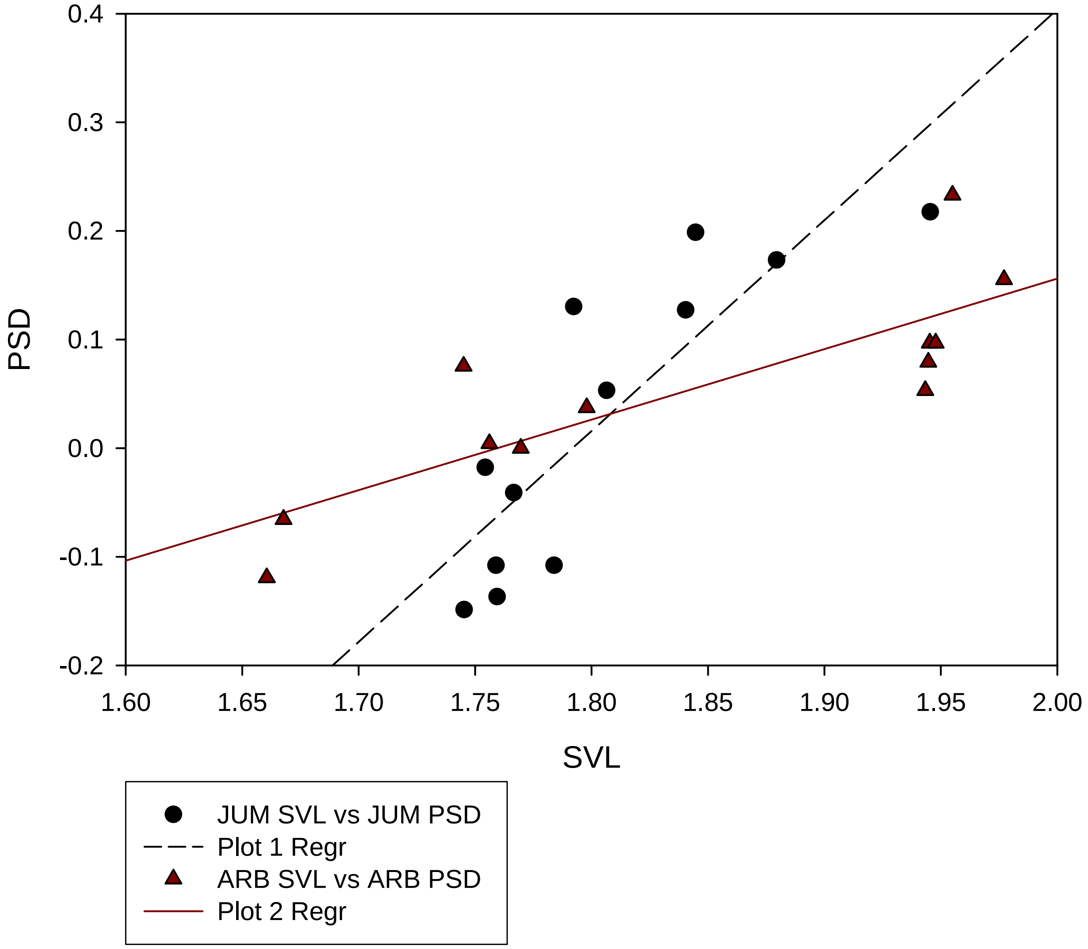

Figure 17A: Log-log RMA regression plot of proximal sacral diapophyseal width (PSD) relative to body length (SVL) for jumping and arboreal Natatanuran species. RMA slope values, arboreal (ARB): 0.777 and jumper (JUM): 2.273 (Table 5). Data points indicate individual specimens.

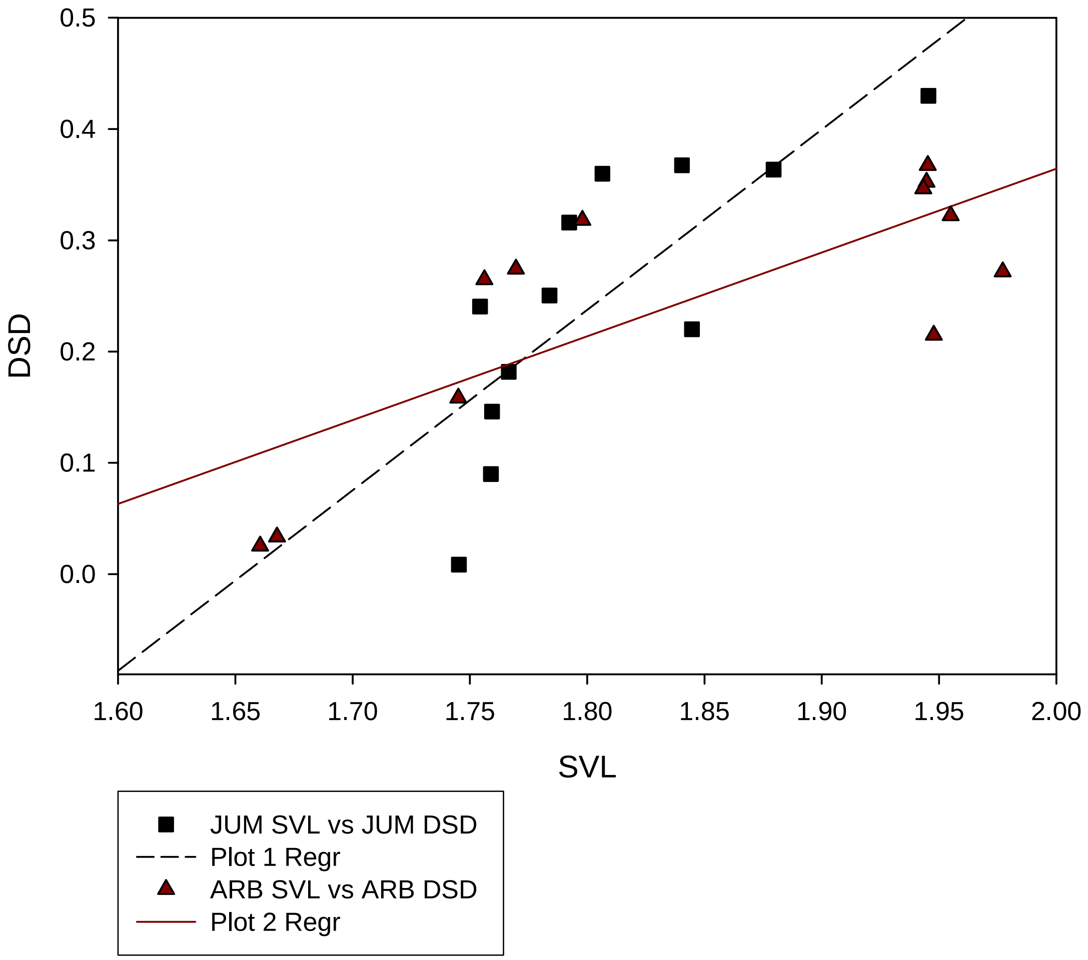

Figure 17B: Log-log RMA regression plot of distal sacral diapophyseal width (DSD) relative to body length (SVL) for jumping and arboreal Natatanuran species. RMA slope values, arboreal (ARB): 0.970 and jumper (JUM): 2.082 (Table 5). Data points indicate individual specimens.

A wide PSD may coincide with a larger attachment point of the coccygeo-sacralis muscle that is reportedly large in hopping anurans (Emerson and De Jongh, 1980). This muscle attaches on the caudal margin of the sacrum and then attaches to the most anterior portion of the lateral surface of the urostyle (Emerson and De Jongh, 1980). Another possibility is that an increase in bony material in this area may be related to a strengthening of the proximal sacral diapophysis for anurans that are capable of jumping long distances. This area could be the weakest point between the most cranial articulation of the two hind limbs and their attachment to the spinal column since this area exhibits a small amount of bone material and is very close to where forces from the hind limb pass onto the axial skeleton cranial to the pelvis.

Distal sacral diapophysis (DSD) width is statistically greater in jumping natatanurans after removal of the arboreal species *R. nigropalmatus*. Narrow distal sacral diapophyses have been correlated with strong jumping in anurans by permitting dorsoventral rotation between the ilium and sacrum during locomotion (Emerson, 1982). Both arboreal and terrestrial jumpers exhibit very small DSD values, relative to those found in other anurans in this sample. This may reflect either phylogenetic constraint, or functional similarity in that both species are capable of long distance jumping either on the ground or in the trees.

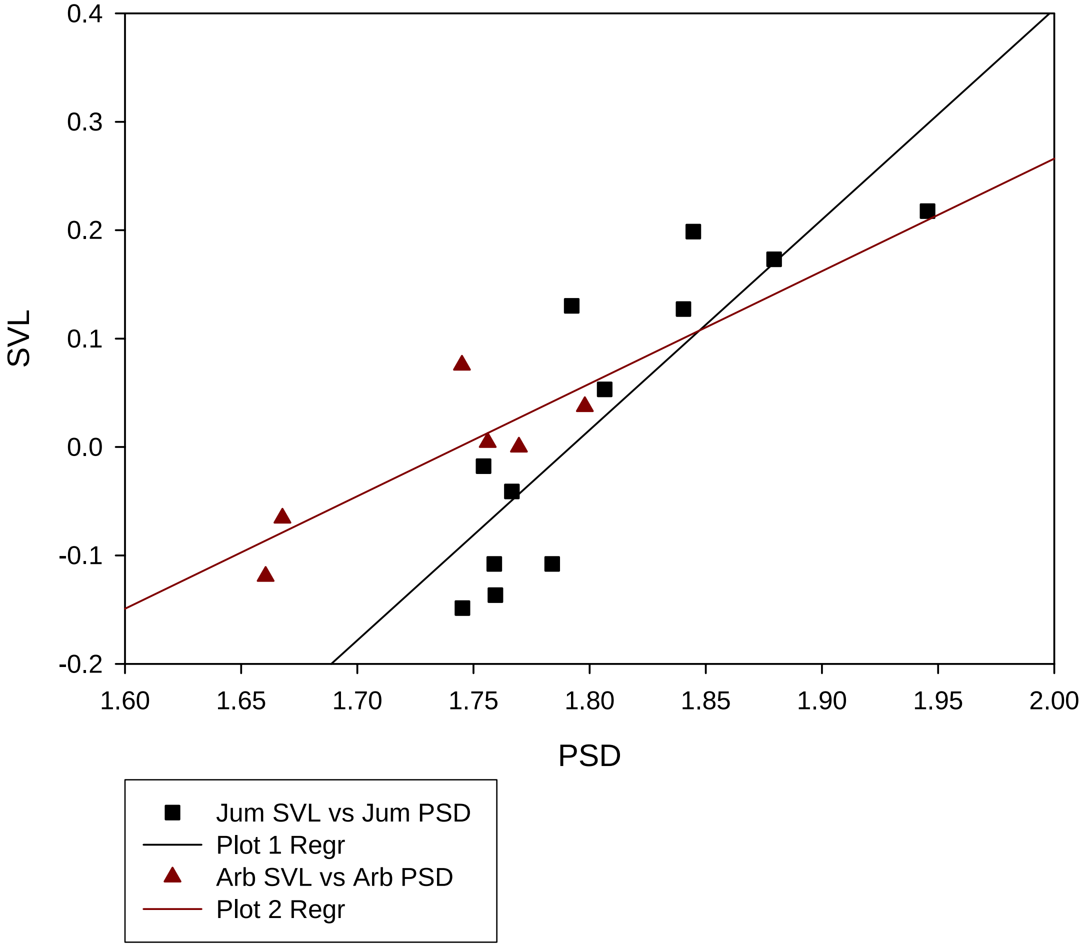

Figure 17C: Log-log RMA regression plot of proximal sacral diapophyseal widths (PSD) relative to body length (SVL) for jumping and arboreal natatanuran species minus the gliding species *R. nigropalmatus*. RMA slope values, arboreal (ARB): 1.257 and jumper (JUM): 2.273(Table 5). Data points indicate individual specimens.

The sacral diapophyseal angle (SDA) is not significantly different between anurans of these two locomotor types although the arboreal species mean (16.98º) SDA is slightly lower than the jumping species mean (18.23º) which corresponds to the larger distal diapophyseal measurement in the latter group.

Narrow allometric comparisons within this clade does not indicate significant differences between the two similarly sized species (*P. mascareniensis* and *C. xerampelina*) that habitually utilize different locomotor types.

Bufonidae

Results of the ANCOVAs derived from data collected on pelvic parameters for three bufonid species - *P. hosii* (arboreal), *A. americanus* (hopper), and *E. calimita* (walker) - permit investigation of pelvic parameters relative to locomotor type. These comparisons indicate different slopes among arborealists, walkers, and hoppers for distal sacral diapophysis (DSD), and significant differences in relative size between locomotor types for sacral vertebral width (SVW), and proximal sacral diapophysis (PSD) width. Among the three locomotor types the slopes of DSD relative to body size (SVL) from RMA Model II regressions vary greatly (hopper = 2.332, walker = -1.562, and arboreal = 1.62) (Table 5). This variability in slope must be viewed with caution and seems to be caused by small clusters of data points for two of the three locomotor groups (walker, and hoppers) defining the slope of the line (Figure 18). This cluster of measurements may result from small sample size and indeed the r^2 value for the hopper and walker RMA regressions are very low (0.01 and 0.33 respectively, Table 5).

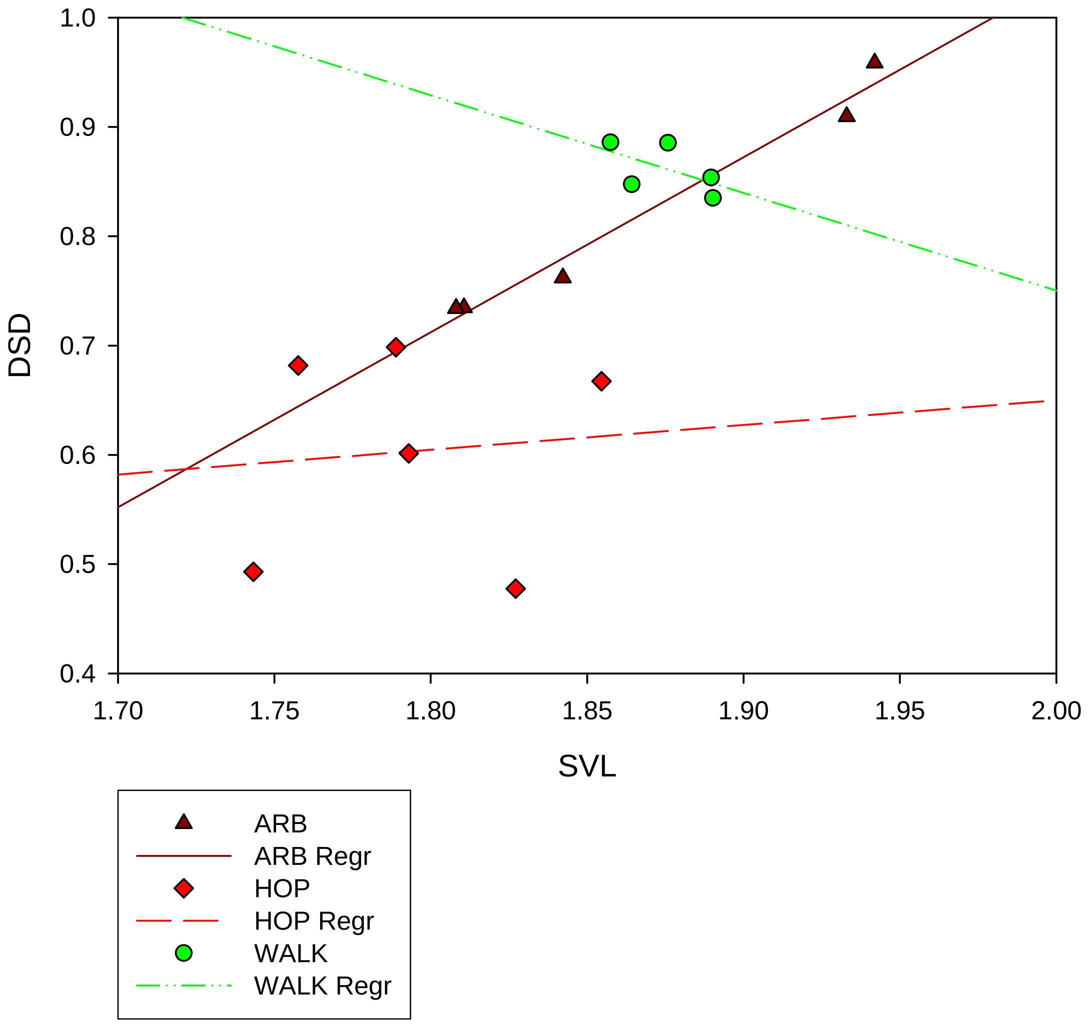

Figure 18: Log-log RMA regression plot of distal sacral diapophyseal widths (DSD) relative to body length (SVL) for arboreal, hopping, and walking bufonid species. RMA slope values, arboreal (ARB): 1.620, hopper (HOP): 2.332, walker (WALK): -1.562 (Table 5). Data points indicate individual specimens.

Sacral vertebra width (SVW) is significantly greater in arboreal bufonids than in walking species of this clade. Wider sacral vertebrae have been linked to an ability to engage in strong jumping, whereas the sacra of walkers, hoppers and burrowers exhibit relatively short transverse processes (i.e. narrow SVW, Emerson, 1982). Relatively narrow SVW permits easier lateral, or side-to-side, rotation at the ilio-sacral articulation during walking, whereas wide SVW can limit this motion and permit vertical rotation during a long-distance jump (Emerson, 1982). Similar patterns in SVW are detected between the similarly sized arboreal species (*P. hosii*) and walking species (*E. calimita*) in a narrow allometric comparison using Kruskal-Wallis ANOVA.

Significant differences in relative size of PSD are also detected by ANCOVA comparisons among the three locomotor groups of Bufonidae. PSD is significantly wider in walking bufonids than in either hoppers or arborealists from this family. This greater size may provide attachment for a larger coccygeo-sacralis muscle (Emerson and De Jongh, 1980). This muscle is likely to also be important in walking/running anurans as well as in those that regularly engage in burrowing behaviors. Further investigation into the size and attachment of this muscle in other anuran families, and among species that habitually utilize other forms of locomotion is beyond the scope of this study, but seems to be a natural next step for understanding locomotor function in the group.

Interestingly distal sacral diapophyses (DSD) size is not significantly different among locomotor modes despite the fact that the sacral diapophyseal angle (SDA) is significantly different among all three locomotor groups. SDA is the greatest in the walking species of this clade, intermediate in arborealists, and smallest in hopping species. This pattern of large SDA in walking species is reflected in the total data set when the anurans from the highly derived Pipidae are removed from the comparison. A large SDA is found in individuals bearing a narrow sacrum (low SVW) with distally expanded sacral diapophyses (wide PSD and DSD). This anatomy permits lateral rotation at the ilio-sacral articulation, a motion consistent with walking in anurans (Emerson, 1979; 1982). Walking and running are the only locomotor patterns that are truly distinct from the general anuran habit of using the paired hind limb extension observed in hopping, long distance jumping, and swimming. This might account for the difference in sacral platform revealed by patterns in SDA. It is also possible that arboreal bufonids which are the largest bufonids examined in this study may exhibit anatomy that is converging with the walking bufonids. This is indicated by the SDA angle, intermediate between low values for hoppers and high values for walkers. As these anurans increase in size the risks of falling become greater. Perhaps larger arboreal anurans shift towards a locomotor pattern and anatomy that allows climbing and alternating hind limb extensions over arboreal jumping where risks of falling may be higher as individuals increase in size.

Hylidae

To elucidate relationships between pelvic parameters and locomotor type comparisons were made among three species from the family Hylidae, *H. vercicolor* and *H. vasta* (arboreal), and *P. paradoxa* (swimmer). Swimming hylids exhibit a significantly wider sacral vertebra (SVW) than that of arboreal hylids. DSD is significantly smaller relative to body length in the swimming hylid species. Both of these anatomical differences, a wide sacrum and distally narrow sacral diapophyses are predicted to be found in species that are capable of jumping long distances (Emerson, 1982). Several long distance jumping anurans (*Lithobates (Rana) clamitans*, *Leptodactylus pentadactylus*) are also proficient swimmers and often jump into bodies of water to escape predation. It is therefore possible that similar constraints have formed the anatomy of this highly aquatic hylid because it is belongs to a clade of animals characterized by the habitual use of long distance jumping.

Leptodactylidae

Two species representing different locomotor types within Leptodactylidae permit further exploration of the relationship between SDA and locomotor type. The rare nocturnal aquatic species from the genus *Hydrolaetare* (Jansen et al., 2007) has significantly greater SDA than the jumping species *Leptodactylus pentadactylus*. This suggests that perhaps the pelvic morphology of aquatic Leptodactylids may be converging on the pattern seen in the highly aquatic and unrelated family Pipidae that possess a very high SDA.

Applying Morphological Correlates of Locomotion to Fossil Anurans

The above analysis permits a functional framework for interpreting fossil anuran specimens recently discovered in Oligocene deposits in the Rukwa Rift Basin of Tanzania. Representing the largest sample of vertebrates recovered from these localities to date, anuran specimens consist of postcranial elements, some of which have been found in articulation. Several ilia have been recovered along with at least two sacral vertebrae. One articulated specimen (RRBP 04101) preserves a nearly complete sacral vertebra, urostyle (partially obscured), fragmentary left ilium and puboischium as well as several presacral vertebra and numerous elements from both hind limbs. All recovered ilia (RRBP 04101, RRBP 05757, RRBP 06150, RRBP 06176) show evidence of a dorsal iliac crest and dorsal prominence. At least three of the ilia (RRBP 04101, RRBP 05757, RRBP 06176) exhibit a dorsal acetabular expansion, and of these, the latter two show evidence of a prominent ventral acetabular expansion. RRBP 05757 also bears a distinct preacetabular fossa. An SVL between 40mm and 80 mm has been estimated for this specimen based on femur length and ilio-sacral dimensions using a regression equation developed by Esteban et al. (1995), bearing in mind that such results should be interpreted with caution for species other than *Rana* (e.g., Blob et al., 2001). Nonetheless, partial specimens indicate that this anuran possesses a diplasiocoelous vertebral column exhibiting a biconcave eighth presacral vertebra. This character seems to be limited to the Ranoides, a large neobatrachian group that includes the modern *Rana* (Trueb, 1973; Frost et al., 2006).

Of the two sacral vertebrae recovered to date (RRBP 04101, RRBP 05757), RRBP 04101 is better preserved and is situated in the matrix so that it approximates anatomical position in relation to four presacral vertebrae. This specimen also preserves several characters consistent with extant anurans capable of long distance jumping relative to SVL. A composite photograph (Figure 19), created by reflecting the better preserved sacral diapophysis and projecting it on the more fragmented side of the specimen permits an estimation of SDA of about 21.409°. This low SDA value falls within the range of that seen in jumpers, swimmers, and in the lower end of arboreal species.

The ilium of RRBP 04101 also exhibits a prominent dorsal crest, a feature that in strong jumping frogs has been correlated with a large ilio-coccygeus muscle (Emerson, 1982). A fragmentary sacral vertebra (RRBP 05757) was found in association with a partial ilium and each of these elements exhibits characters consistent with those described above for RRBP 04101.

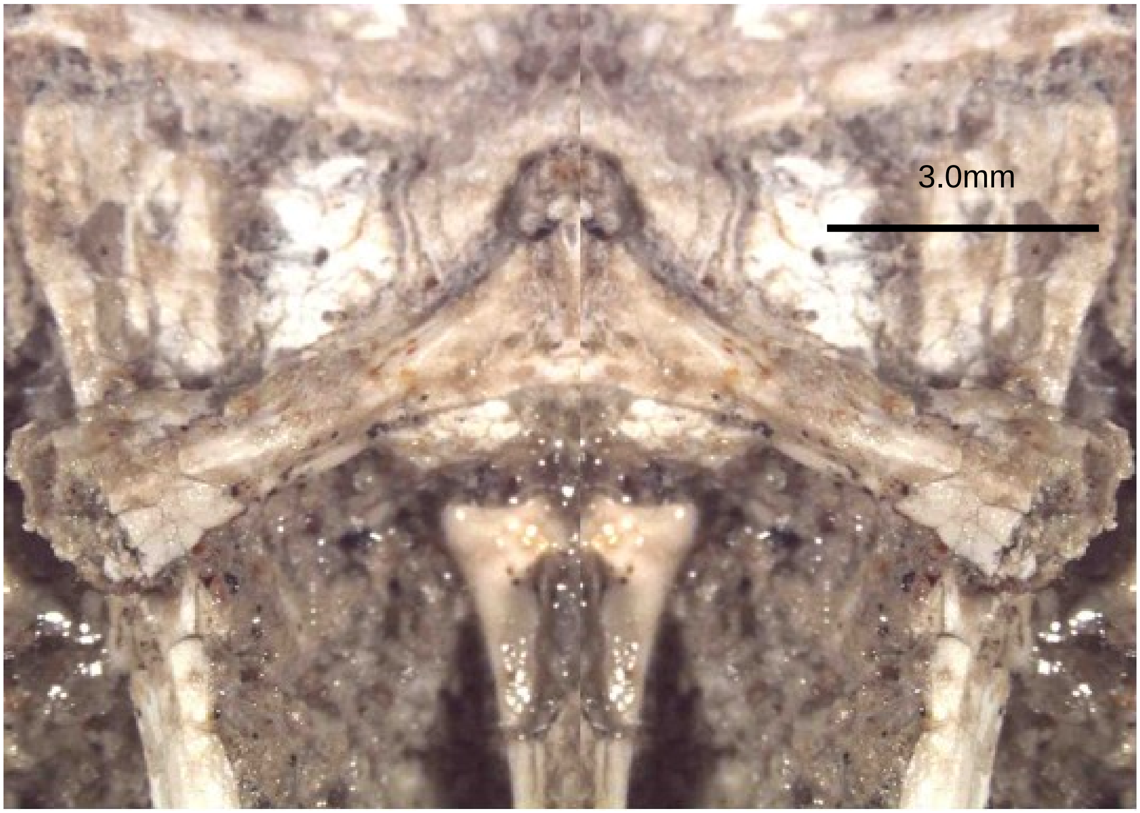

Figure 19: RRBP 04101 in dorsal view with right diapophysis reflected onto left more fragmentary diapophysis. This image allows measurement of the sacral diapophyseal angle (SDA) of about 21.409°, which is within the range of values exhibited by jumping and swimming anurans examined in this study.

This study provides a framework for inferring behavior of fossil anurans, suggesting that functional interpretations of fossil frogs may require more specific taxonomic resolution than previously recognized. It is also important to consider the completeness of individual specimens for obtaining valid estimates of SVL and other linear measurements for a given specimen. Exceptionally well preserved fossil anurans are frequently discovered (e.g. Baez and Harison, 2005) and several studies of anuran fossils report limb length estimates as well as estimates of SVL (e.g. Špinar, 1972).

CONCLUSIONS

Studies of extant frogs have documented a number of morphological features linked with particular locomotor modes. This permits the development of hypotheses about the relationship between skeletal morphology and locomotor capabilities of anurans. This study tests the utility of morphological features of the postcranial skeleton in a sample of modern species of known locomotor style and expands on earlier studies by including a large taxonomic sample and species that have not previously been included in locomotor functional analyses. Interpretations are discussed in the context of a recent phylogenetic hypothesis (Frost et al., 2006; Frost, 2007). Importantly, the project evaluates various methods for quantifying pelvic morphology as it relates to known locomotor capabilities. Non-destructive methods for collecting skeletal morphometric data on alcohol preserved anuran specimens, using direct measurements and plain film radiography are proposed and tested herein. Such methodologies can easily expand the collection of skeletal morphometric data by including specimens that are uncommon in osteological collections. Analysis of covariance (ANCOVA) is used to compare morphological structures among taxa in the context of body size differences.

As predicted, manus size is larger relative to SVL in arboreal hylids and natatanurans. Arboreal bufonids exhibit wider sacra and longer fore- and hind limbs relative to SVL than in terrestrial species examined. Among natatanurans, those species capable of long distance jumping exhibit larger pes relative to SVL than do arboreal species.

Despite the extensive work by Emerson to outline in detail the morphology and function of the anuran pelvic girdle into three ilio-sacral articulation types and associated characters, this study as well as others (Manzano and Barg, 2005) have shown that anuran morphology is difficult to classify across the entire clade of frogs. Two of these types or categories do not correlate well to locomotor habits, as morphologies often prove to defy the constraints of the categories to which we assign them. This study attempts to quantify one aspect of the anuran pelvic girdle using the sacral diapophyseal angle (SDA).
SDA provides a method of separating walking/running species from all of the other locomotor groups examined with the exception of members of the highly aquatic family Pipidae, a group that can be differentiated by their highly derived pelvic morphology featuring a urostyle fused to the sacrum. Functionally significant morphological patterns in extant frogs provide a context for locomotor interpretations of fossil anuran specimens, as illustrated using specimens recently discovered in Oligocene deposits in the Rukwa Rift Basin (RRB) of Tanzania.

Results of this study differ from previous studies in that the patterns of functional morphological correlates in anurans do not span the entire clade of anurans, but are identifiable primarily within phylogenetically focused groups. Within this study morphologies are identified across a wide assemblage of living anuran taxa. Inclusion of data about locomotor behavior for these extant taxa permits consideration of morphology in the context of locomotor function. Identification of morphological patterns in the skeletons of these functional groups allows correlation of morphology to function and behavior (Reilly and Wainwright, 1994). Behavior links morphology to the ecology of an organism; i.e. where phenotype meets the world (Ricklefs and Miles, 1994). Identifying morphology and tying it to biological function represent the first two levels of ecomorphological study (Reilly and Wainwright, 1994). For example, a given anuran morphology will allow extension of the hind limb during locomotion. The details of this morphology may differ slightly among animals that use extension of the hind limb for different observable behaviors such as swimming, walking, long-distance jumping and hopping. This difference in morphology is related to differences in the capacity of the hind limb to extend, vary direction relative to the animal's body, and produce different amounts of force, all to alter range of motion among different locomotor patterns. This involves interplay of total performance capacity and actual limb use in the environment (Reilly and Wainwright, 1994). Assuming that an animal is optimized for its most commonly used locomotor pattern it follows that long-distance jumping anurans would exhibit morphology optimized for hind limb extension that results in traveling long distance whereas hoppers using similar hind limb extension are morphologically optimized for endurance via multiple short hops. At the same time mostly aquatic species are morphologically optimized for swimming, whereas walking anurans exhibit morphology that is optimized for efficient alternate leg extensions.

It is possible to separately identify differences in behavior and differences in morphology, but connecting these two is only possible when patterns are consistent and predictable. This permits scientific testing of the relationships between form and function. Testing this relationship establishes some of the fundamental components to connect an animal's morphology to its fitness (Reilly and Wainwright, 1994) via adaptations of the skeleton. This study tests assumptions about how modern anuran form relates to function, and has direct implications for the interpretation of the behavior of extinct anuran taxa.

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APPENDIX

Appendix: Precision of x-ray, wet, and skeletal measurement methods showing mean, range, standard deviation (SD), coefficient of variation as a percentage of the mean (CV %), and measurement error as a percentage of the mean (ME %). Accuracy between measurement methods represented by the results of ANOVA where bold text measurements are statistically different than the same measurements collected via other measurement methods. P-values indicated at right.

Measure	Mean	Range	SD	CV (%)	ME %	p-value
PL x-ray	**40.661**	**2.378**	**0.601**	**1.479**	**1.211**	**p = 0.005**
PL skeletal	**39.936**	**1.020**	**0.359**	**0.900**	**0.738**	
PL wet	n/a	n/a	n/a	n/a	n/a	
UL x-ray	**31.601**	**0.326**	**0.093**	**0.295**	**0.239**	**p = 0.025**
UL skeletal	**31.904**	**1.120**	**0.355**	**1.111**	**0.781**	
UL wet	n/a	n/a	n/a	n/a	n/a	
SVW x-ray	8.197	0.203	0.056	0.681	0.550	
SVW skeletal	8.174	0.380	0.115	1.405	1.006	
SVW wet	n/a	n/a	n/a	n/a	n/a	
PSD x-ray	2.095	0.237	0.064	3.064	2.301	
PSD skeletal	2.074	0.190	0.056	2.682	2.025	
PSD wet	n/a	n/a	n/a	n/a	n/a	
DSD x-ray	2.989	0.256	0.062	2.072	1.611	
DSD skeletal	3.041	0.070	0.027	0.883	0.756	
DSD wet	n/a	n/a	n/a	n/a	n/a	
SDA x-ray	21.075	1.129	0.350	1.661	1.322	
SDA skeletal	21.468	1.847	0.687	3.202	2.831	
SDA wet	n/a	n/a	n/a	n/a	n/a	
FEL x-ray	**41.528**	**1.043**	**0.240**	**0.579**	**0.440**	**p < 0.001**
FEL skeletal	**40.044**	**0.450**	**0.150**	**0.373**	**0.302**	
FEL wet	**44.016**	**1.170**	**0.360**	**0.817**	**0.592**	
TFL x-ray	43.410	0.714	0.178	0.410	0.300	
TFL skeletal	43.345	0.830	0.224	0.516	0.316	
TFL wet	**46.532**	**0.940**	**0.345**	**0.742**	**0.611**	**p < 0.001**
TARL x-ray	22.371	1.116	0.326	1.459	1.187	
TARL skeletal	22.564	0.320	0.104	0.459	0.342	
TARL wet	**25.947**	**1.180**	**0.376**	**1.448**	**1.160**	**p < 0.001**
HL x-ray	25.221	1.029	0.236	0.934	0.701	
HL skeletal	24.875	0.320	0.109	0.440	0.370	
HL wet	**26.974**	**2.010**	**0.668**	**2.476**	**1.909**	**p < 0.001**
RUL x-ray	15.438	1.355	0.344	2.229	1.757	
RUL skeletal	15.671	0.380	0.110	0.703	0.503	
RUL wet	**21.421**	**1.720**	**0.609**	**2.841**	**2.359**	**p < 0.001**