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THE ILIOSACRAL ARTICULATION IN PSEUDINAE (ANURA: HYLIDAE)

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We analyzed the iliosacral joint and iliolumbaris muscle anatomy of the species of Pseudinae, based on the two types of articulations defined by Emerson. Unusual characters should be expected in the pseudine iliosacral articulation structure, given their aquatic habits and evolutionary history. In fact, the presence of a ligamentous cap joining the ilium with the sacral diapophysis has not been described previously, and may be unique to pseudines. This particular group lacks a single pattern of iliosacral articulation for the whole subfamily, and the articulations are not strictly referable to any of the types or subtypes described by Emerson. The iliosacral articulation is interspecifically variable within Pseudinae and is intermediate between the articulations of Type IIA and IIB.

Key words: Anura; Hylidae; Iliosacral articulation; Ligamentous cap; Pseudinae

THE PELVIS of anurans generally is formed by three pairs of elements (ilium, ischium and

pubis) that show some modifications within modern anurans. Within Neobatrachia, the pelvis has been described as a stable structure formed by the ilium and the ischium, leaving the pubis as a reduced, nonossified cartilage,

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with different degrees of mineralization (Trueb, 1973) in various taxa. There are some variations in the anuran pelvis that have been described before; for example, the presence of pre-pubic bones in Leiopelmatids, some Pipids (Cannatella and Trueb, 1988; Trueb, 1973), and *Discoglossus sardus* (Clarke, 1988; Pügener and Maglia, 1997), and post-pubic elements in *Ascaplus truei*. Additional elements can be mineralized or remain cartilaginous. These variations, and some related to the shape of pelvic bones, have been used in phylogenetic analyses (e.g., by Heyer, 1975; Inger, 1972; Lynch, 1973).

The expansion of the sacral diapophyses and their ventral joint with the ilium is a characteristic that makes modern anurans unique among the existing vertebrates (Jenkins and Shubin, 1998; Trueb, 1973). The arrangement of the iliosacral joint elements were categorized by Emerson (1979), who demonstrated that the type of articulation and its possible movements are related to the different forms of anuran locomotion. Emerson defined two types of articulations (I and II) based on the extension of the sacral diapophysis, the position and shape of the sesamoids, and the origin and insertion of the articular ligaments in extant anurans. The iliosacral articulation forms a functional complex with the muscle Iliolumbaris that is responsible for the movement of the pelvis during locomotion (Emerson, 1979; Whiting, 1961).

Herein, we describe and analyze the iliosacral functional complex within some species of the two genera of the hylid subfamily Pseudinae (Duellman, 2001). Pseudinae is an essentially aquatic group of South American anurans with characters that lead to controversial interpretations, such as the presence of intercalary elements and an opposable thumb of the manus (Ceï, 1980; Manzano, 1996). Functionally, opposable thumbs are associated more closely with an arboreal than an aquatic lifestyle, and are characters that are shared with other anuran groups, such as some Phyllomedusinae, Rhacophoridae, and Mantellidae.

Although Pseudinae seem to be a monophyletic group, it is difficult to separate pseudids from the Hylidae and Centrolenidae (da Silva, 1998; Ford and Cannatella, 1993). Da Silva (1998) undertook a phylogenetic analysis

including Allophryne, Centrolenidae, Hylidae, and Pseudidae, and presented evidence that Pseudidae belongs within Hylidae, as a sister group of Hylinae. Subsequently, Duellman (2001) included Pseudinae as a subfamily of Hylidae.

We accept Duellman's (2001) classification of Pseudinae. Nevertheless, there is still much to understand about the morphology and evolutionary relationships of this group, which includes variations of morphological novelties, (e.g., those of the iliosacral joint).

In this study, we focus our interest on the analysis of the iliosacral anatomy of the species of Pseudinae, based on the two types of articulations defined by Emerson (1979, 1982). Given their aquatic habits and shared evolutionary history, unusual characters could be expected in the pseudine iliosacral articulation structure.

MATERIALS AND METHODS

We dissected and observed the iliosacral articulation and associated musculature of 25 specimens of Pseudinae, five specimens of Centrolenidae, and six specimens of other Hylidae (see Appendix I). The specimens were subjected to differential staining methods to study musculature based on a modification of Wassersug's (1976) technique. The modification involves almost the same steps required for staining bones and cartilages, with differences in the time that the specimens remain in the solutions, depending on their size. Specimens previously skinned and preserved in 10% formaldehyde, were washed in tap water for a period of two hours before introducing them in a solution of Alcian Blue in Absolute Alcohol and Acetic Acid. After 1–2 days in the dye solution, the cartilage in a medium size frog, would take on a blue color; the specimens were then fixed in Absolute Alcohol (3 h). In the next step, the material was introduced into a solution of Potassium Hydroxide for approximately 2 minutes to neutralize the acid of the Alcian Blue solution. After that, specimens were kept for 1–2 days in a solution of Red alizarin in Potassium Hydroxide. Once the specimen bones were dyed red, the specimen was preserved in 70% ethanol, avoiding muscle maceration. With this technique, bones stain red, cartilage stains blue, and muscles remain unstained and can be observed intact.

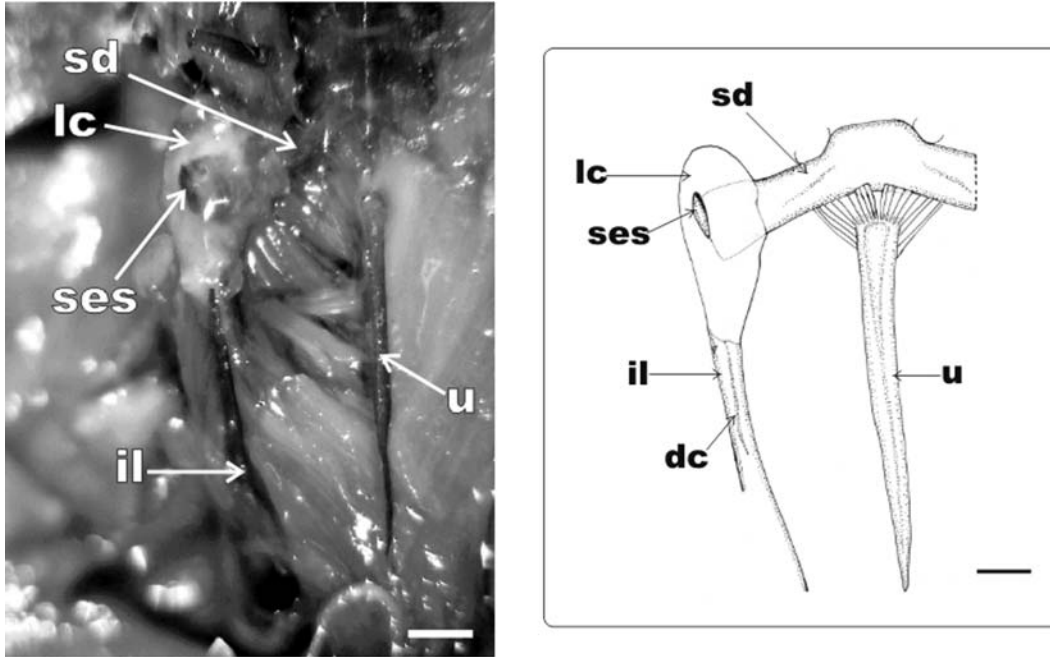


FIG. 1.—*Pseudis minuta* (FML:03676-1). (A) Photograph of dorsal view of the iliosacral articulation; (B) drawing of Figure 1A, dorsal view of the iliosacral articulation. dc, dorsal crest; il, iliac shaft; lc, ligamentous cap; sd, sacral diapophysis; ses, sesamoid; u, urostyle. Scales = 1 mm.

To study the articulation in detail, we embedded two specimens in paraffin for histological sections (Anderson and Bancroft, 2002), one of *Pseudis minuta* (FML03676) and one of *Lysapsus limellus* (DIAM 019), and stained them with hematoxylin-eosin according to the standard staining procedure for paraffin sections (Wilson and Gamble, 2002). The specimens were examined with the aid of a Olympus stereo microscope equipped with a camera lucida and photographs were taken with a camera attached to the microscope.

Pseudis minuta was used as a reference species, with descriptions of the articulation and morphology of its iliolumbaris muscle. The remaining species of Pseudinae and its subspecies were compared to *P. minuta*. We follow the terminology of Trueb (1973) for osteology and Gaupp (1896) for musculature.

Two specimens of *Pseudis minuta* were video-recorded with a GR-VL505 JVC camera to allow analysis of the movements of the pelvic girdle during swimming and resting. A specimen of *Xenopus laevis* also was video-recorded for comparison.

RESULTS

The Iliosacral Articulation

Pseudis minuta (Fig. 1).—The sacral diapophyses are narrow, dorsoventrally compressed (with an elliptic transversal section) and oriented posterolaterally. The distal margin of the sacral diapophysis is almost completely mineralized and not expanded. An oval, well-developed sesamoid is present on the lateral edge of the sacral diapophysis. The shaft of each ilium extends anteriorly well beyond the sacral diapophysis, reaching the mid-level of the Presacral Vertebra VIII. An expanded ligament arises from the anterior end on both margins of the iliac crest, and covers dorsally the anterior end of the iliac shaft. Laterally, the ligament encloses the terminus of each sacral diapophysis, forming a ligamentous cap and incorporating the sesamoid to this structure. This ligamentous cap inserts on the dorsal surface of the sacral diapophysis, including the anterior and posterior edges of the lateral terminus of this bony structure. The *M. iliolumbaris* originates as

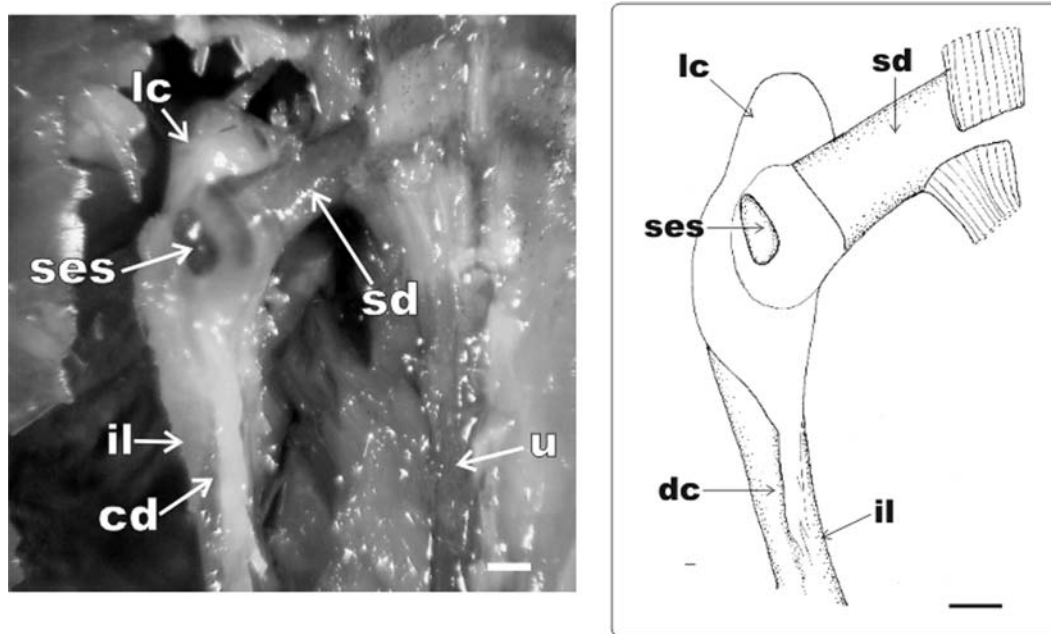


FIG. 2.—*Pseudis paradoxa platensis* (FML: 004661-1). (A) Photograph of dorsal view of the iliosacral articulation; (B) drawing of Fig. 2A, dorsal view of the iliosacral articulation. dc, dorsal crest; il, iliac shaft; lc, ligamentous cap; sd, sacral diapophysis; ses, sesamoid; u, urostyle. Scales = 1 mm.

a tendon on the ligamentous cap, from the anterior region of the iliac crest. Lateral processes of presacral Vertebrae VII and VIII are oriented anterolaterally and as long as the sacral diapophysis. The lateral processes of the resting presacral vertebrae are oriented posterolaterally.

Other species of Pseudis.—The subspecies of *P. paradoxa* (*P. p. paradoxa*, *P. p. occidentalis* and *P. p. platensis*) that we describe show an arrangement of the elements that conforms to the articulation described for *P. minuta* (Fig. 1). Nevertheless, the form and size of the sesamoid within *P. paradoxa* is more rounded than oval and relatively larger, being almost as long as the width of the lateral end of the sacral diapophysis (Fig. 2). In this species of *Pseudis*, the distal tips of each sacral diapophysis is at least partially mineralized. Within the subspecies of *P. paradoxa*, the articular ligament originally described by Emerson (1979, 1982) is not evident, but a ligamentous cap is present. The articular ligament of *P. cardosoi* and *P. bolbodactyla*, like that of *P. minuta*, has an insertion on the mid-dorsal region of each sacral diapophysis and is included in the ligamentous cap. Except for *P. bolbodactyla*, the

length of the iliac shafts of *Pseudis* species is very homogeneous, extending anteriorly to the sacral diapophysis and reaching the mid-level of presacral vertebra VIII. In *P. bolbodactyla*, the iliac shafts extend to the anterolateral border of the sacral diapophysis. There is an iliac crest present in all species studied, but with variation in size and length.

In the histological sections of the iliosacral joint, we observed a combination between Type IIA and IIB articulation for the species of *Pseudis* (Fig. 3). The tip of the sacral diapophysis is curved ventrally, with a wedge-like sesamoid, located laterally (as in a Type IIA articulation). Although, in the histological transversal section, the form of the ilium and the diapophysis with its small cartilaginous tip, correspond to Type IIB articulation. The ligamentous cap envelopes the ilium ventrolaterally and the sesamoid laterally, and inserts on the mid-dorsal surface of the sacral diapophysis.

Lysapsus limellus.—The condition of *Lysapsus limellus* is intermediate between Types IIA and IIB (Emerson 1979, 1982). The sacral diapophyses are expanded laterally with a cartilaginous distal border that is larger

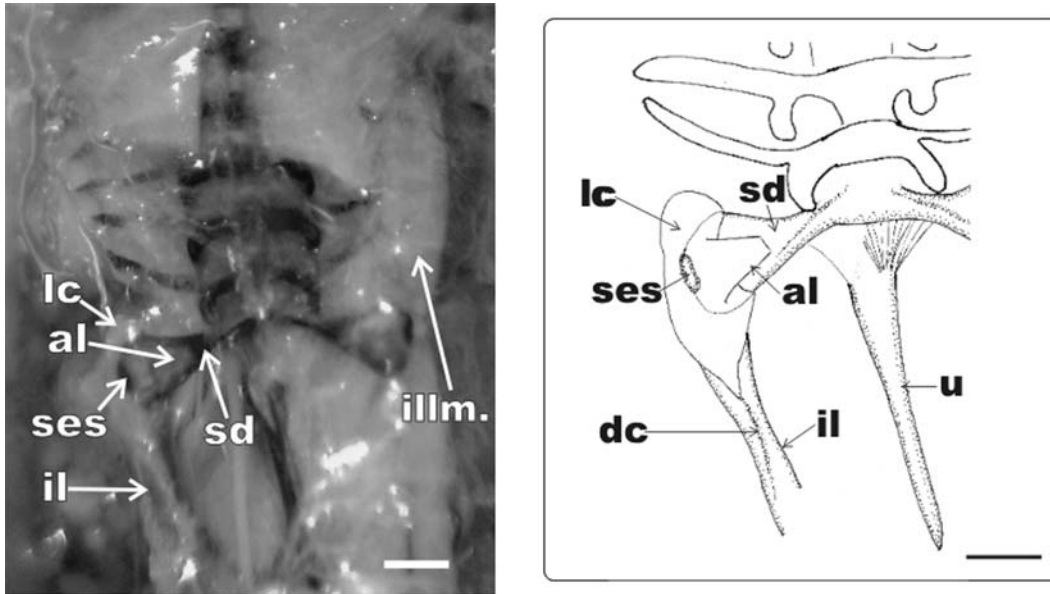


FIG. 3.—*Lysapsus limellus* (FML: 00725-1). (A) Photograph of dorsal view of the iliosacral articulation; (B) drawing of Fig. 3A, dorsal view of the iliosacral articulation. dc, dorsal crest; il, iliac shaft; illm, M. Iliolumbaris; la, articular ligament; lc, ligamentous cap; sd, sacral diapophysis; ses, sesamoid; u, urostyle. Scales = 1 mm.

than that of *Pseudis* (see Figs. 3, 5). The ligamentous cap envelops the lateral border of each sacral diapophysis, the anterior extreme of the iliac shaft, incorporating the sesamoid, and a very differentiated internal articular ligament. The articular ligament inserts on the mid-dorsal region of the sacral diapophysis and the insertion is not close to the vertebral body as in Emerson's Type IIA iliosacral articulation. The lateral processes of

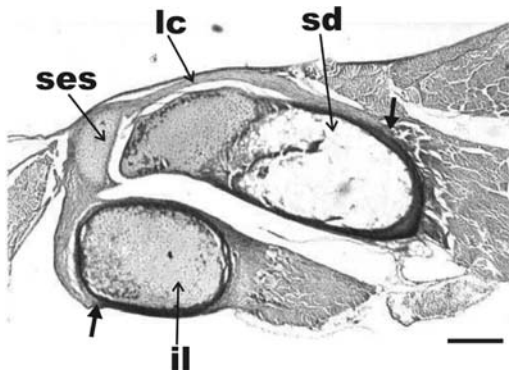


FIG. 4.—*Pseudis minuta* (FML03676-2). Histological section of the right iliosacral articulation, transversal view. il, ilium; lc, ligamentous cap; sd, sacral diapophysis; ses, sesamoid; arrows show the insertion point of the ligamentous cap. Scale = 0.5 mm.

Presacral Vertebrae VIII is as long as the sacral diapophysis (as in Emerson's Type IIB articulation) and is oriented strongly anterolaterally. The oval sesamoids are smaller than those of *Pseudis*, and cover only a third of the anterolateral edge of the sacral diapophysis (Fig. 4). The iliac shaft extends anteriorly as far as the posterior border of the Presacral Vertebra VII and has a small, short longitudinal crest. Histological sections of the pelvic girdle in *Lysapsus*, show a Type IIA iliosacral articulation (Fig. 5), and like *Pseudis minuta*, the tip of the sacral diapophysis is curved ventrally and the sesamoid is positioned laterally. The form and position of each sesamoid with respect to the ilium and the insertion of the ligament also is similar to the condition seen in *Pseudis minuta* (Figs. 3, 5). Ilium tips are completely enveloped by the ligamentous cap.

The Muscle Iliolumbaris

Pseudis minuta.—In *Pseudis minuta* (Fig. 6), the Iliolumbaris muscle has a wide origin by a tendon on the ligamentous cap, positioned on the anterolateral region of the shaft of the ilium and the anterolateral portion of the sacral diapophysis. This muscle inserts on the lateral processes of Presacral Vertebrae V–VIII, and

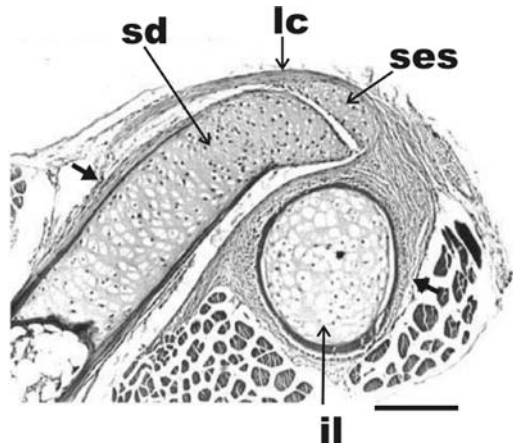


FIG. 5.—*Lysapsus limellus* (DIAM 019-2). Histological section of the left iliosacral articulation, transversal view. il, ilium; lc, ligamentous cap; sd, sacral diapophysis; ses, sesamoid; arrows show the insertion point of the ligamentous cap. Scale = 0.5 mm.

lies parallel to the Longissimus Dorsi muscle, covering most of the external surface of the lateral processes of Presacral Vertebrae VI–VIII. It is a thick, wide muscle with transverse tendinous inscriptions by which it inserts to the tip of each of the lateral processes of Presacral Vertebrae V–VIII. An incipient division of the muscle into two branches at the origin was observed, with the lateral branch being more developed and thicker than the medial one.

Other Pseudines.—In *Pseudis paradoxa platensis* and *P. p. occidentalis*, the Iliolumbaris muscle originates on the ligamentous cap by a short tendon, as was observed in *P. minuta*. It is present as single muscle and, although two bundles of muscle fibers are distinguishable, no division is observed. This muscle inserts on the lateral process of the Presacral Vertebrae IV–VIII.

Lysapsus limellus.—In *Lysapsus limellus*, the Iliolumbaris muscle is undivided and has a narrow origin on the anterolateral portion of the iliac shaft. It inserts on the tips of each lateral process of Presacral Vertebrae IV–VIII.

Monitoring Locomotion in *Pseudis minuta*

In the video-recording, we observed that *Pseudis minuta* swims by moving the posterior limbs without evident rotation of the iliosacral articulation. The leg motion is practically limited to flexions and extensions of the tibiofibula and

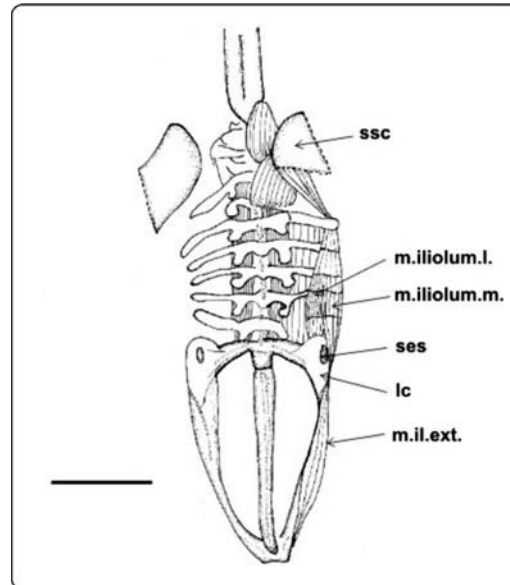


FIG. 6.—*Pseudis minuta*. Dorsal view of the iliosacral articulation. m.il.ext., M. Iliacus Externus; m. iliolum.l., M. Iliolumbaris Lateralis; m. iliolum.m., M. Iliolumbaris Medialis; lc, ligamentous cap; ses, sesamoid; ssc, supra-scapulae. Scale = 2 mm.

foot (i.e., tarsometatarsus). When the animal's foot is in contact with the substrate (a submerged rock or the aquarium floor), the swimming movement is propelled by a kick.

Most of the time specimens of this species remain floating with their heads out of the water and their webbed feet expanded at the same level as the head, partially out of the water. The back is curved dorsally in a concave arc and the iliosacral articulation appears to form an angle smaller than 170° , compared with the iliosacral articulation of *Rana catesbiana* in a resting position (shown in Jenkins and Shubin, 1998: Figs. 9A, 10A).

DISCUSSION

The morphology of the iliosacral articulation in pseudines does not coincide completely with any of the types described by Emerson (1979, 1982). Although some characteristics would indicate a Type IIB iliosacral articulation in some pseudines, important variations make it inappropriate to place them in this category. Also, in some species, there is a combination of characteristics from both types of articulation (IIA and IIB).

The ligamentous cap described herein has not been described previously, and may be unique to pseudines. In the case of *Pseudis*, the non expanded, cylindrical shape of the sacral diapophysis, the lateral processes of the presacral vertebrae that are as long as the sacral diapophysis, and the presence of an iliac crest would indicate a Type IIB iliosacral articulation (Emerson, 1979). However, the insertion of the ligamentous cap displaced from the lateral edges of the sacral diapophysis, as well as the absence of a well-defined ligament (Emerson, 1982), suggest variations in this pre-established pattern. Only in *Lysapsus limellus* was a well-defined ligament clearly differentiated from the ligamentous cap that generally is observed; the articular ligament was difficult to identify in *Pseudis* species, where it is included as a part of the ligamentous cap. In *Pseudis paradoxa* there is no evidence of the presence of the articular ligament.

The insertion of the M. Iliolumbaris on the lateral processes of Presacral Vertebrae IV–VIII only, instead of all the pre-sacral vertebrae, and their wide origins on the ligamentous cap (except in *Lysapsus*), at the same level as the lateral extremes of the sacral diapophysis, are other observed variations. These differences are more evident in *Lysapsus limellus*, where the sacral diapophysis are expanded, the sesamoid is small, and the insertion of the ligamentous cap is closer to the mid-region of the sacral vertebra than in *Pseudis*. This would suggest a Type IIA articulation, but the presence of the iliac crest and the long lateral presacral processes (such as in *Pseudis*) are characteristic of Type IIB articulation.

Nevertheless, except for the presence of a defined ligament in *Pseudis cardosoi*, little variation in the morphology of the iliosacral articulation in *Pseudis* was observed, especially among the subspecies of *Pseudis paradoxa*.

Bigalke (1927) mentioned a proximal bifurcation of the M. Iliolumbaris in *Rana* (*R. esculenta*, *R. fusca*, and *R. arvalis*) and describes an almost complete separation in two branches in *Bufo bufo* (as *Bufo vulgaris*) with or without tendinous origins. In pseudines, the branches of the M. Iliolumbaris have various degrees of differentiation; the medial branch is present only in species of *Pseudis*, and although in *P. paradoxa* the

medial branch is not separated from the lateral branch, it is identified easily. In *Lysapsus limellus* the muscle shows no branch separation. In all pseudines observed, the origin of the muscle is on the ligamentous cap.

In the remaining hylids analyzed, the division of the muscle varies from an incipient bifurcation (*Scinax acuminata*) to a double muscle (*Hyla pulchella* and *H. andina*). In Centrolenidae the muscle is single and originates from the iliac shaft. All centrolenids exhibit a Type IIA articulation, but also with some variations. The sacral diapophysis are expanded, there is no crest in the iliac shaft, they have long ovate-shaped sesamoids (widest at the base), and the articular ligament inserts on the distal edge of each sacral diapophysis (as in Type IIB). The ilium reaches the diapophysis anteriorly, not overlapping it.

Emerson (1979) considers that the pattern of iliosacral articulation is “family specific”, except in Hylidae, Microhylidae, and Discoglossidae. Additionally, she recognize the existence of exceptions within *Atelopus*, *Dendrobates*, and *Leptopelis*, and associated them to the differences in locomotion of those species (Emerson, 1982, 1988). Similarly, the subfamily Pseudinae, recently classified within Hylidae (Duellman, 2001), not only lacks a single pattern for the whole subfamily, but also the articulations are not strictly referable to any of those types and subtypes described by Emerson. The iliosacral articulation in Pseudinae is intermediate between the articulation Types IIA and IIB, and shows interspecific variations.

The ability to jump is present in some form in all anurans (Duellman and Trueb, 1986), but is limited by the flexibility of the trunk and iliosacral articulation (Jenkins and Shubin, 1998; Trueb, 1996). The anurans that are good jumpers have a very flexible iliosacral articulation (Green, 1931) with anteroposterior and rotational movements that, together with the sacral-urostylic articulation, align the vertebral column with the pelvic girdle during jumping (Jenkins and Shubin, 1998; Kargo et al., 2002). Some authors consider that aquatic anurans have a movement and design similar to those of saltatorial specialist jumpers, propelling themselves with a kick (Emerson, 1982; Gans and Parsons, 1965); although, others (Abourachid and Green, 1999) state that these could

be independently derived locomotory trends. Kargo et al. (2002) demonstrated that the iliosacral articulation is a gliding joint, in which the trunk translation and rotation are independent of one another. Emerson and de Jongh (1980) hypothesized that this translation of the trunk could be important during swimming, and is observed in frogs that are specialized for swimming.

In pseudines, the movement during swimming is propelled mainly by the flexion of the tibiofibula and the foot (tarsometatarsus), and flexion of the knee seems to be minimal or nonexistent. Except when the animal rests on the water's surface, it swims by a jumping-style movement of the legs. This kind of movement, which mainly involves the distal elements of the legs (tibiafibula and tarsometatarsus), was described previously for *Hymenochirus boettgeri* (Gal and Blake, 1988), but for the adjustment of direction rather than propulsion. In a common jumping frog like *Rana pipiens*, the involvement of the distal elements of the hind limbs could increase the jump distance (Kargo et al., 2002). Interestingly, *Pseudis* also is a very good jumper during an explosive escape response (A.S. Manzano and M. Barg, personal observation).

The iliosacral articulation acts as the main mechanical axis during jumping, transmitting the impulse from the legs towards the vertebral column. In this movement, the vertebral column aligns with the pelvic girdle and the angle of the iliosacral articulation rotates, from 120° (in resting position), to 170° (in jumping positions) (Callow and Alexander, 1973). During swimming, the iliosacral articulation seems to be less important comparing with the movements of the legs.

In specimens of *Pseudis* that are resting and floating, the angle formed by the iliosacral joint seems to increase from the 170° angle during swimming as a consequence of the dorsally curved, concave position of the back. In other resting, aquatic anurans, the vertebral column remains aligned with the pelvic girdle (e.g., *Xenopus laevis*, A.S. Manzano and M. Barg, personal observation). It is known that in *Xenopus laevis* the iliosacral joint is fused and akinetic (Jenkins and Shubin, 1998).

Although, a broad, cuff-like ligament joining the ilium with the sacral diapophysis has been described in pipids (Trueb, 1996), the mor-

phology of iliosacral articulation in pseudines is quite different. In pipids cuff-like ligament covers all the sacral vertebrae between the ilia. Among pipids, the greatly expanded sacral diapophyses and cuff-like ligaments restrict the movements of the iliosacral articulation to an anterior to posterior sliding movement.

A combination of characters of Type IIA and IIB iliosacral articulation in *Lysapsus limellus*, like the expanded sacral diapophyses and long lateral processes of the Presacral Vertebrae could reduce considerably the lateral rotating movements of the iliosacral articulation, but in this case (*Lysapsus limellus*), the lateral processes of the Presacral Vertebrae are strongly anterolaterally oriented thus the lateral rotating movement are not limited.

The presence of the ligamentous cap in the iliosacral articulation of the species of *Pseudis* could increase the range and angle articulation during the transition from resting to swimming positions, acting as a hinge and adding freedom to the movement of rotation. The variations in the morphology of the iliosacral articulation may or may not imply mechanical variations in its movement, but can contribute to the hyperextension of the back during rest.

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APPENDIX I

Specimens Examined

Specimens belong to the Herpetological Collections of Museu de Historia Natural, Campinas, Sao Paulo, UNICAMP (ZUEC); Museu de Ciencias e Tecnologia da PUCRS, Brazil (MCP); Carnegie Museum (Carn. Museum); Museo Argentino de Ciencias Naturales (MACN); Fundación Miguel Lillo (FML); Universidad de Corrientes (UNNEC); CICyTTP-CONICET Diamante, Entre Ríos, Argentina (DIAM); Fundación La Salle de Ciencias Naturales, Venezuela (EF).

Family Hylidae: Subfamily Pseudinae—*Pseudis bolbodactylus* ZUEC 11800, ZUEC 11801; *P. caribensis* EF-112, 13554; *P. cardosoi* MCP 3375, MCP 3775; *P. paradoxa paradoxa* Carn. Museum 49512; *P. paradoxa occidentalis* MACN 37698, MACN 37699, FML00708 (2 specimens); *P. minuta* MACN 37700, MACN 37701, MACN 37702, FML03676 (1 specimen for histological sections, 1 for photograph); *P. paradoxa platensis* UNNEC 03455, FML00936 (2 specimens); *P. paradoxa* FML04661 (2 specimens, photographs); *Lysapsus limellus* FML00791 (photograph), FML 00725, DIAM 019 (2 specimens, one for histological sections). Two living specimens of *Pseudis minuta*, male and female, were video-recorded and photographed; they remain in an aquarium in M. Barg possession at the University of Mar del Plata, Argentina.

Subfamily Phyllomedusinae—*Phyllomedusa hypochondrialis* FML 04286. **Subfamily Hylinae**—*Scinax nasicum* DIAM 023; *S. squalirostris* DIAM021; *Phrynohyas venulosa* DIAM 024; *Hyla andina* DIAM 022; *H. pulchella* DIAM 038.

Family Centrolenidae—*Hyalinobatrachium aureoguttatum* DIAM 055; *Centrolene robledoi* DIAM 056; *C. geckoideum* DIAM 075; *C. grandisone* DIAM 076; *Cochranella ignota* DIAM 057.