

OVIDUCT MODIFICATIONS IN FOAM-NESTING FROGS, WITH EMPHASIS ON THE GENUS *LEPTODACTYLUS* (AMPHIBIA, LEPTODACTYLIDAE)

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ABSTRACT. Various species of frogs produce foam nests that hold their eggs during development. We examined the external morphology and histology of structures associated with foam nest production in frogs of the genus *Leptodactylus* and a few other taxa. We found that the posterior convolutions of the oviducts in all mature female foam-nesting frogs that we examined were enlarged and compressed into globular structures. This organ-like portion of the oviduct has been called a “foam gland” and these structures almost certainly produce the secretion that is beaten by rhythmic limb movements into foam that forms the nest. However, the label “foam gland” is a misnomer because the structures are simply enlarged and tightly folded regions of the *pars convoluta* of the oviduct, rather than a separate structure; we suggest the name *pars convoluta dilata* (PCD) for this feature. Although all the foam-nesters we examined had a *pars convoluta dilata*, its size and shape showed considerable interspecific variation. Some of this variation likely reflects differences in the breeding behaviors among species and in the size, type, and placement of their foam nests. Other variation, particularly in size, may be associated with the physiological periodicity and reproductive state of the female, her age, and/or the number of times she has laid eggs.

KEYWORDS. Anura, Leiuperidae, Leptodactylidae, oviduct morphology, foam nest, reproduction

INTRODUCTION

Various morphological, physiological, ecological, and behavioral aspects of reproduction define the three clades of living amphibians (frogs, salamanders, and caecilians); these traits figure prominently in the evolutionary history of amphibians, more so than in any other group of vertebrates. The ancestral amphibian is thought to have been an aquatic breeder with free swimming larvae (Duellman and Trueb, 1986; Wake and Dickie, 1998). As amphibians shifted from an entirely aquatic to a more terrestrial life style, this basic pattern was modified. Numerous and varied modes of reproduction evolved within each of the three amphibian groups but especially among the Anura (Duellman, 2003; Haddad and Prado, 2005). One breeding mode that evolved independently at least three times within frogs is the production of a foam nest, that is, a frothy mass into which the eggs are incorporated (Altig and McDiarmid, 2007; Duellman and Trueb, 1986).

The first indication of foam-nesting as a reproductive mode in frogs likely is an illustration by Merian (1719: plate 71) in which she depicted a clutch of eggs, a foam nest, a developmental series of tadpoles, and an adult frog. This watercolor was almost

assuredly that of *Physalaemus ephippifer*, a species common around Paramaribo in Suriname where she spent nearly two years painting insects and their host plants. Nearly three centuries later, Hödl (1990) described the mating behavior and nest construction of *Physalaemus ephippifer* from observations made in the field and laboratory; he noted (p. 549) that “In the initial phases of nest building, the female deposited only transparent, probably oviductal, fluid, that was stirred up into a platform of foam by the beating and rotating movements of the male’s hind legs.”

The behavioral aspects of foam nest construction in various taxonomic groups have been documented and appear relatively consistent across different families. Published reports of foam-nesting species describe the extrusion of a clear, or at least translucent, gelatinous substance from the female’s cloaca prior to, and sometimes during, egg release. This gelatinous substance is beaten into foam that contains the fertilized eggs; together, the eggs and foam make up the foam nest. Which parent beats the substance into foam, whether the arms or the legs are used, and the type of motion employed (kicking, swimming, paddling, etc.) vary considerably among taxa (Hödl, 1990). In Rhacophoridae the female beats the cloacal fluid into foam and forms the nest with her hind legs

(Coe, 1974; Hödl, 1990), whereas in Leiuperidae and Leptodactylidae the male beats the liquid into foam using his hind legs (Gibson and Buley, 2004; Heyer and Rand, 1977; Hödl, 1990; Prado *et al.*, 2005; Silva *et al.*, 2005). In Australian Limnodynastidae, foam is created by a "paddling" motion of the female's arms that pushes a stream of bubbles posteriorly beneath her body; these bubbles become trapped in mucus that accompanies egg extrusion and form a bubble nest (Martin, 1970). Select aspects of nest construction are detailed below.

Coe (1974:19) reported that female *Chiromantis rufescens* used a swimming motion to beat a "pale fawn translucent viscous fluid" into stiff white foam. Liu (1950:366) described a similar behavior in *Rhacophorus leucomystax*: "Before the eggs appear she produces a small amount of fluid, and she beats this by moving her feet medially and laterally and turning them as they cross at the mid-line. When the primary foam for holding the eggs has been prepared, eggs and fluid come out together." In *Leptodactylus labyrinthicus*, Prado *et al.* (2005:280) commented that following amplexus in a hollow next to a water body "the female arched its body down and discharged a jelly-like secretion...the male started the foam production by stirring the secretion with its hind limbs."

Despite the detailed behavioral observations describing how a foam nest is constructed, only a few investigators have considered where the gelatinous substance that is beaten into foam is produced and whether the morphology of the female reproductive tract is modified for its production (Table 1). The size and morphology of the oviducts, the symmetrical tube-like structures by which ovulated eggs are gathered and transported out of the body during egg deposition, vary with reproductive mode (Bhaduri, 1953; Ehmccke *et al.*, 2003; Horton, 1984; Wake and Dickie, 1998). Cope (1889:237) was likely the first to mention oviduct modification in foam-nesting species. He noted that during certain seasons, the posterior portion of each oviduct in *Leptodactylus ocellatus* is greatly enlarged and filled with albuminous gelatin. Bhaduri (1953) studied the urinogenital system of 34 species of frogs and devoted a substantial portion of his monograph to describing modifications of the oviducts in species with unusual reproductive modes, including those that construct foam nests. He noted (Bhaduri, 1953:61) that "The *pars convoluta* is differently modified in *Leptodactylus* (three species) and *Pleurodema* among the leptodactylids, and in *Phyllomedusa* among the hylids. The posterior portion of

the *pars convoluta* increases enormously in diameter and is markedly evident in *Leptodactylus pentadactylus* and *Phyllomedusa dacnicolor*." He concluded (Bhaduri, 1953:70) "... that in all the species of *Leptodactylus* the terminal portion of the *pars convoluta* is enormously expanded and much distended with secretion ... it may safely be assumed that the oviduct is modified in consequence of this particular mode of breeding, and thus a correlation between structure and function is a reasonable deduction from the facts."

In his study of the genus *Chiromantis*, Coe (1974:23-25, fig. 3) described and illustrated a structure in females of *C. rufescens* that he called a "foam gland." He initially characterized it as a pad of tissue that turned out to be "three large and swollen oviducal folds, held together by connective tissue and mesentery." A transverse histological section (Coe, 1974: plate 4a) through the area showed that the lumen of the oviduct was filled with secretory material. Kabisch *et al.* (1998) adopted the term "foam gland" in their description of the posterior regions of the oviducts of female *Polypedates leucomystax*, a foam-nesting species. They described the foam glands as spherical, about 1 cm in diameter, and present on both sides of the body. These authors noted that this region of the oviduct was glandular, deeply indented, and had a diameter ten times as large as that of the anterior section. Horton (1984) examined the reproductive morphology of numerous frog species, including a number of foam-nesting species, mainly from Australia. She noticed the enlarged posterior oviduct in all species with this reproductive mode and provided valuable insights into its structure and histology.

We examine the gross morphology and histology of the posterior portion of the oviduct in a series of *Leptodactylus* species and related taxa to determine the extent of interspecific variation. We also attempt to explain this variation in light of differences in the reproductive behaviors of foam-nesting species and/or the characteristics of their foam nests. All members of the South American neotropical frog genus *Leptodactylus* produce foam nests. Furthermore, the foam-nesting behaviors of the species in this genus progress from an aquatic to a more terrestrial mode of reproduction, suggesting an adaptive trend (Heyer, 1969).

MATERIALS AND METHODS

We describe the oviductal morphology of 21 females from 13 foam-nesting species of *Leptodactylus*

TABLE 1. Species of frogs reported to have an enlarged posterior region of the oviduct.

Family and species	Reference
Hylidae	* <i>Pachymedusa dacnicolor</i>
Leiuperidae	<i>Engystomops pustulosus</i>
	<i>Physalaemus biligonigerus</i>
	<i>Pleurodema borellii</i>
	<i>Pleurodema brachyops</i>
	<i>Pleurodema cinereum</i>
Leptodactylidae	<i>Adenomera hylaedactyla</i>
	<i>Leptodactylus chaquensis</i>
	<i>Leptodactylus fragilis</i>
	<i>Leptodactylus fuscus</i>
	<i>Leptodactylus insularum</i>
	<i>Leptodactylus leptodactyloides</i>
	<i>Leptodactylus melanonotus</i>
	<i>Leptodactylus mystaceus</i>
	<i>Leptodactylus ocellatus</i>
	<i>Leptodactylus pentadactylus</i>
	<i>Leptodactylus podicipinus</i>
	<i>Leptodactylus savagei</i>
	<i>Lithodytes lineatus</i>
Limnodynastidae	<i>Adelotus brevis</i>
	<i>Heleioporus eyrei</i>
	<i>Lechriodus melanopyga</i>
	<i>Limnodynastes dorsalis</i>
	<i>Limnodynastes dumerilii</i>
	<i>Limnodynastes lignarius</i>
	<i>Limnodynastes peronii</i>
	<i>Limnodynastes tasmaniensis</i>
	<i>Platyplectrum ornatum</i>
Rhacophoridae	<i>Chiromantis petersii</i>
	<i>Chiromantis rufescens</i>
	<i>Chiromantis xerampelina</i>
	<i>Polypedates leucomystax</i>

* *Pachymedusa dacnicolor*: not a foam-nester, but has an unusual reproductive mode (see discussion).

and other genera. Our recognition of certain genera deserves comment. Frost *et al.* (2006) re-evaluated phylogenetic relationships of amphibians based primarily on molecular data. Their most parsimonious cladogram indicated that *Adenomera* and *Lithodytes* were sister taxa and in turn the clade *Adenomera* + *Lithodytes* was a sister group to the genus *Leptodactylus*. Based on these and other data (e.g., Heyer, 1998; Kokubum and Giaretta, 2005) they placed *Adenomera* in the synonymy of *Lithodytes* and treated *Lithodytes* as a subgenus of *Leptodactylus*. Interestingly, the branch lengths involved for *Adenomera*, *Lithodytes*, and *Leptodactylus* are similar to other branch lengths that Frost *et al.* (2006) used to support recognition of other clades as distinct genera (e.g., *Duellmanohyla* and *Ptychohyla*). Given the scope of the Frost *et al.* (2006) publication, the sampling of

species of *Adenomera*, *Leptodactylus*, and *Lithodytes* was minimal. Accordingly, we prefer to recognize *Adenomera*, *Leptodactylus*, and *Lithodytes* as distinct genera until a more taxon-rich analysis is available.

We include representatives from each species group within the genus *Leptodactylus* (Heyer, 1969) and from *Adenomera*, *Engystomops*, *Lithodytes*, and *Pleurodema*. To evaluate intraspecific variation, we examine the oviducts of six female *Leptodactylus podicipinus*. In addition, we examine one male each of two foam-nesting species (*L. podicipinus* and *L. mystaceus*) for evidence of a foam-secreting gland or duct. For comparative purposes we include a female of one non-foam-nesting species, *Pleurodema bufonina* (Leiuperidae) (three species of *Pleurodema* have been reported not to produce foam nests and 10 species to produce foam nests, Duellman and Veloso,

TABLE 2. Morphological data for foam-nesting species examined in this study showing reproductive state and measurements (widths on left [L] and right [R] sides) at three points along the oviducts. All measurements are in millimeters.

Species name	Reproductive state	Oocyte/ovum color	Anterior region of PC near juncture with <i>pars recta</i>		Middle region of <i>pars convoluta</i> anterior to PCD		PCD at an externally visible convolution	
			L	R	L	R	L	R
<i>Adenomera hylaedactyla</i>	large oocytes	yellow	0.6	0.6	1.1	0.9	1.8	1.8
<i>Engystomops pustulosus</i>	large oocytes	pale yellow	0.6	0.6	0.8	0.8	—	3.5
<i>Leptodactylus fragilis</i>	large oocytes	yellow	0.9	0.7	0.6	0.6	1.7	1.9
<i>L. fuscus</i>	medium oocytes	yellow	0.8	0.7	0.5	0.5	1.5	1.8
<i>L. insularum</i>	large oocytes	bicolor: pale grey to black	—	1.2	0.8	0.8	3.0	3.0
<i>L. leptodactyloides</i>	large oocytes	bicolor: pale grey to whitish tan	1.1	1.2	0.9	0.8	4.3	4.2
<i>L. mystaceus</i>	small oocytes	yellowish tan	0.8	0.6	0.6	0.5	1.5	1.8
<i>L. ocellatus</i>	small oocytes	dark grey	0.6	0.6	0.5	0.5	3.3	3.8
<i>L. petersii</i>	immature, ovaries small	—	0.2	0.2	0.2	0.2	0.5	0.7
<i>L. podicipinus</i>	large oocytes	bicolor: brown to black	0.5	0.6	0.6	0.8	3.3	3.0
<i>L. podicipinus</i>	large oocytes	bicolor: brown to black	0.7	0.7	0.5	0.5	—	—
<i>L. podicipinus</i>	large oocytes	bicolor: brown to black	0.8	0.9	0.7	0.7	3.6	3.0
<i>L. podicipinus</i>	large oocytes	bicolor: brown to black	0.8	0.8	0.7	0.8	3.6	3.5
<i>L. podicipinus</i>	large oocytes	bicolor: brown to black	0.8	0.7	0.8	0.8	3.6	3.2
<i>L. podicipinus</i>	no visible oocytes	—	0.7	0.7	0.5	0.6	3.0	2.7
<i>L. savagei</i>	small oocytes	bicolor: black and brown	1.1	1.4	0.9	1.1	10.9	11.0
<i>L. savagei</i>	immature, ovaries small	—	0.7	0.6	0.3	0.4	0.9	1.1
<i>Lithodytes lineatus</i>	large oocytes	yellow	0.7	0.7	0.9	—	4.8	5.1
<i>Pleurodema brachyops</i>	large oocytes; some ova in oviducts	bicolor: pale to dark brown	1.1	0.9	1.2	1.4	3.0	3.9
<i>P. brachyops</i>	large oocytes	bicolor: pale to dark brown	1.2	0.9	1.4	1.5	1.8	1.8
<i>P. bufonina</i> ¹	large oocytes	bicolor: brown to black	0.9	0.9	1.2	1.2	2.3	1.7

¹ *Pleurodema bufonina* is not a foam-nesting species, but was included for comparison.

1977). All specimens used in this study were taken from the National Collection of Amphibians and Reptiles (USNM) at the National Museum of Natural History in Washington, D. C. (Table 2). All specimens were preserved in 10% formalin and later transferred to 70% ethanol where they have been stored from about 5 to more than 25 years. Neither the condition of a specimen when originally fixed nor the length of

the period stored had any obvious effect on the gross external morphology of the oviducts.

Oviductal terminology follows Bhaduri (1953) and Horton (1984). The anuran oviduct consists of four sections (Fig. 1). The funnel-shaped, anterior most part of the oviduct that collects ova from the body cavity through ciliary action is the *ostium*. Following the *ostium* is a short, more or less straight,

TABLE 3. Species examined and sites in the region of the *pars convoluta dilata* where tissues were removed for histological examination. Abbreviations: PC, *pars convoluta* immediately anterior to PCD; aPCD, anterior fifth of PCD; mPCD, middle fifth of PCD; pPCD, posterior fifth of PCD.

<i>Adenomera marmorata</i> (USNM 209112) foam-nesting species: aPCD.
<i>Engystomops pustulosus</i> (USNM 216820) foam-nesting species: PC, pPCD.
<i>Leptodactylus mystaceus</i> (USNM 531535) foam-nesting species: PCD.
<i>Leptodactylus ocellatus</i> (USNM 209231) foam-nesting species: PC, aPCD, mPCD, pPCD.
<i>Leptodactylus podicipinus</i> (USNM 280688) foam-nesting species: PC, aPCD, mPCD, pPCD.
<i>Leptodactylus savagei</i> (USNM 338121) foam-nesting species: PC, aPCD, mPCD, pPCD.
<i>Lithodytes lineatus</i> (USNM 525788) foam-nesting species: PC
<i>Pleurodema brachyops</i> (USNM 302128) foam-nesting species: PC, aPCD, mPCD.
<i>Pleurodema bufonina</i> (USNM 36879) non-foam-nesting species: PC, pPCD.

thin walled tube called the *pars recta*. Posteriorly, the longest portion of the oviduct is the highly folded *pars convoluta* (PC), which ends in a broadly expanded portion called the *ovisac* just anterior to its exit into the cloaca. Some authors (*e.g.*, Bhaduri, 1953) have referred to the ovisac as the uterus. Alcaide *et al.* (2009) published detailed information on the histology and histochemistry of oviducts of *Leptodactylus chaquensis*, *Physalaemus biligonigerus*, and *Pleurodema borellii* that generally complements our data but they used a slightly different terminology and referred to the glandular area of the PC as albumin glands. Rather than adopting new terminology, we prefer to use that of Bhaduri and Horton with one modification. The greatly dilated posterior portion of the *pars convoluta* that forms the so-called “foam gland” or “albumin gland” we call the *pars convoluta dilata* (PCD). We agree with Horton (1984) that the term “foam gland” is misleading. The PCD is not a gland, and it does not directly produce foam. For these and other reasons we refrain from using ‘foam gland’ for anything associated with amphibian eggs.

With an ocular micrometer in a dissecting microscope we measured the widths of both oviducts at

three standard points (Fig. 1). Widths in the enlarged posterior region (PCD) were measured at an externally visible and centrally located posterior fold. Dimensions of the PCD are not necessarily good indicators of size, because the region was often flattened against the posterior wall of the body cavity by the distended and enlarged ovaries. Snout-vent lengths were measured with dial calipers. Apart from measurements, other morphological variation was difficult to quantify, in part, because oviducts consist of soft tissues and can easily be distorted. Because of these limitations, we provide brief descriptions and photographs (Fig. 2) of the relevant oviductal features of several species.

Oviductal tissues were taken from the right oviduct of nine species of leiuperid and leptodactylid frogs, eight of which are foam-nesters (Table 3). Tissues were removed from four points (Fig. 1) in the region of the *pars convoluta dilata*: 1) *pars convoluta* (PC) immediately anterior to the PCD; 2) anterior fifth of the *pars convoluta dilata* (= aPCD); 3) middle fifth of *pars convoluta dilata* (= mPCD); and 4) posterior fifth of *pars convoluta dilata* (= pPCD). In many species the PCD is quite large, and only a wedge of tissue could be removed for histological preparation. Tissues were prepared for light microscopy by standard plastic embedding in glycol methacrylate, sectioned at 5–6 μm , and stained with PASMY (periodic acid Schiff metanil yellow) and H & E (hematoxylin and eosin). The state of preservation of the oviduct varied among samples, so that we were not able to prepare useful histological slides from all four regions of the *pars convoluta* in the nine species examined (Table 3).

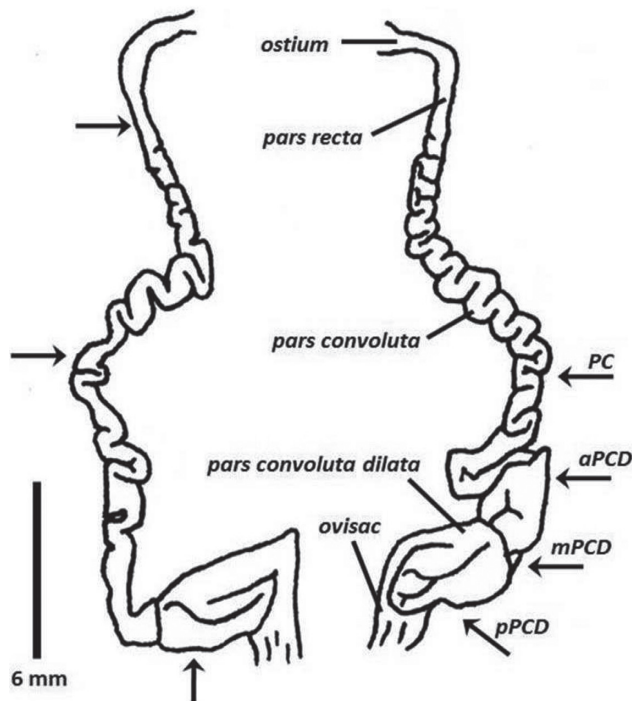


FIGURE 1. The oviducts of *Leptodactylus fuscus*, USNM 162881. Arrows denote three points at which oviductal widths were measured (left) and four regions where histological sections were taken (right). Abbreviations: PC: *pars convoluta*, aPCD: anterior *pars convoluta dilata*, mPCD: middle *pars convoluta dilata*, pPCD: posterior *pars convoluta dilata*.

RESULTS

In the following descriptive section we document the morphological variation in the reproductive tracts of a variety of species of *Leptodactylus* and other select forms. In all cases the left and right oviducts within a female were nearly symmetrical, so we provide composite descriptions.

Gross morphology – female

Family Leiuperidae. Engystomops pustulosus. The *pars convoluta* portion of the oviduct gradually enlarges into the *pars convoluta dilata*, which is large and occupies nearly the entire posteroventral quarter

of the abdominal cavity, where it has been pushed by the enlarged ovaries. The PCD is flattened, crescent-shaped, flexible, and tightly folded. The ovarian follicles are large and yellow (Fig. 2A).

Pleurodema brachyops. The two females examined are gravid; one (USNM 302128, Fig. 3B) has large oocytes filling the ovaries and some ova in the anterior section of the *pars convoluta*, and the other (USNM 302129, Fig. 3A) has ova in the *pars recta*,

the anterior portions of the *pars convoluta*, and filling the ovisac. A few ova are also present in the anterior two loops of the PCD in this latter specimen. Passage of ova through the PCD apparently affected the structure of its walls because they are flaccid and gray compared to the white, firmer walls of the PCD in the female that has just begun to ovulate. The *pars convoluta dilata* is relatively small, although distinct from the anterior region of the *pars convoluta*. The PCD consists of seven or eight loops and is

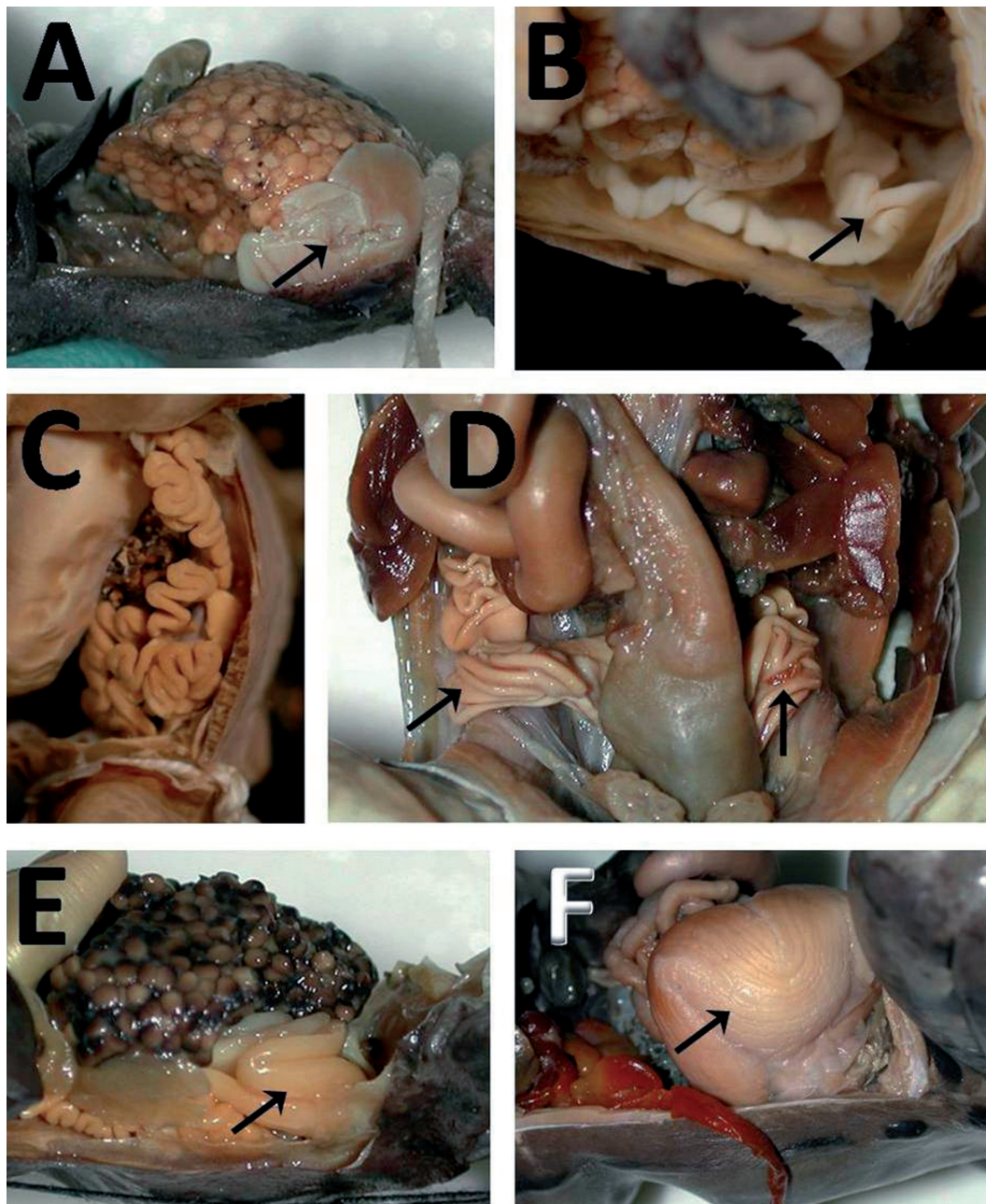


FIGURE 2. Photographs of *in situ* oviducts of six species of frogs. A: *Engystomops pustulosus*, USNM 216820. B: *Leptodactylus fuscus*, USNM 162881. C: *Pleurodema bufonina*, USNM 36879. D: *Leptodactylus ocellatus*, USNM 209231. E: *Leptodactylus podicipinus*, 260688. F: *Leptodactylus savagei*, USNM 338121. Arrows indicate the PCDs in five species.

compressed into a flattened ellipsoidal structure by the large eggs in the ovisac.

Pleurodema bufonina. The ovaries in this specimen are enlarged and contain well developed oocytes. The *pars convoluta* is slightly wider and considerably more convoluted posteriorly than anteriorly, but it lacks any enlarged and defined structure. This species does not make foam nests, and its oviductal morphology reflects this fact; no enlarged region corresponding to the PCD of the foam-nesting species is present (Fig. 2C).

Family Leptodactylidae. Adenomera hylaedactyla. Unlike most of the other foam-nesting species we examined, the oviductal convolutions are few, widely spaced, and easily counted (N = 10). The PCD consists of five convolutions and five others occur anterior to it. The PCD is ill-defined although the *pars convoluta* is wider in the posterior third than anteriorly. The ovaries of this female contain a few large, yellow vitellogenic follicles.

Leptodactylus fragilis. As in *Adenomera hylaedactyla*, this individual lacks a well defined *pars convoluta dilata* although the posterior most section of the *pars convoluta* is distinctly wider than the anterior part. Unlike the condition observed in many of the other species, the oviductal loops of the PCD are not pressed together to form a well defined structure. The ovaries in this specimen contain large, vitellogenic follicles.

Leptodactylus fuscus. The oviduct is nearly straight with noticeably fewer convolutions (Figs. 1, 4B) than in most other species (except *Adenomera hylaedactyla*). The PCD is relatively small and flexible. The ovaries contain numerous yellow follicles (Fig. 2B).

Leptodactylus insularum. The PCD is similar to that described for *Leptodactylus podicipinus*. The convolutions in the enlarged region are tightly packed. The ovaries are filled with mature oocytes that flatten the enlarged PCD against the back of the body cavity. The anterior portion of the *pars convoluta* is very narrow and tightly coiled.

Leptodactylus leptodactyloides. The structure of the PCD is very similar to that of *L. podicipinus*. The greatly enlarged ovaries and their large oocytes fill the entire body cavity pressing the oviducts against the dorsal body wall. Anterior to the PCD, the oviducts

have typical anuran morphology (Fig. 1); within the PCD the convolutions become increasingly wider and compressed with deep clefts between them.

Leptodactylus mystaceus. The coiled, anterior portion of the *pars convoluta* is narrow but gradually enlarges posteriorly. The PCD region is flattened and the loops highly flexible; it is not strikingly distinct and occupies little space (Fig. 4A).

Leptodactylus ocellatus. The *pars convoluta* is narrow and tightly coiled. Posteriorly, it widens gradually into the PCD. The PCD is oval shaped and consists of wide convolutions separated by deep clefts (Figs. 2D, 4C, 6A). The ovaries contain small granular follicles.

Leptodactylus petersii. The oviduct in this immature female is small and extremely narrow, with almost no signs of coiling; it is almost a straight tube from the *ostium* to the ovisac with a slight enlargement in the area where the PCD is likely to appear. The very small, highly lobed ovaries have tiny pale tan oocytes. Based on these observations, we conclude that it would be difficult to tell if a species were a foam-nesting form based on an immature specimen.

Leptodactylus podicipinus. We examined six female *Leptodactylus podicipinus* of comparable size (SVL 35.7–44.2) to assess intraspecific variation in oviductal shape. Because the coils are densely packed into a solid mass, we were unable to obtain an accurate count of the oviductal convolutions; uncoiling the oviducts of these preserved specimens would have caused tearing. Despite these obstacles, the oviducts appear nearly symmetrical in each specimen. While the coiling patterns of the oviducts are highly conserved, minor differences among specimens are apparent. One of the females (SVL = 39.6) has tiny ovaries with no visible oocytes. In this specimen the *pars recta* is short and nearly straight, and the *pars convoluta* (anterior to the PCD) straighter and less folded than in the other five specimens. The PCDs in the other five females, all of which were mature and have visible oocytes, are more enlarged and highly folded. We suggest that the difference in oviduct coiling sometimes indicates immaturity.

The shape of the PCD in the mature *Leptodactylus podicipinus* specimens we examined reflects the reproductive state of the female. When the ovaries contain small but visible oocytes, the PCD is a well-formed, oval-shaped structure. When the ovaries are

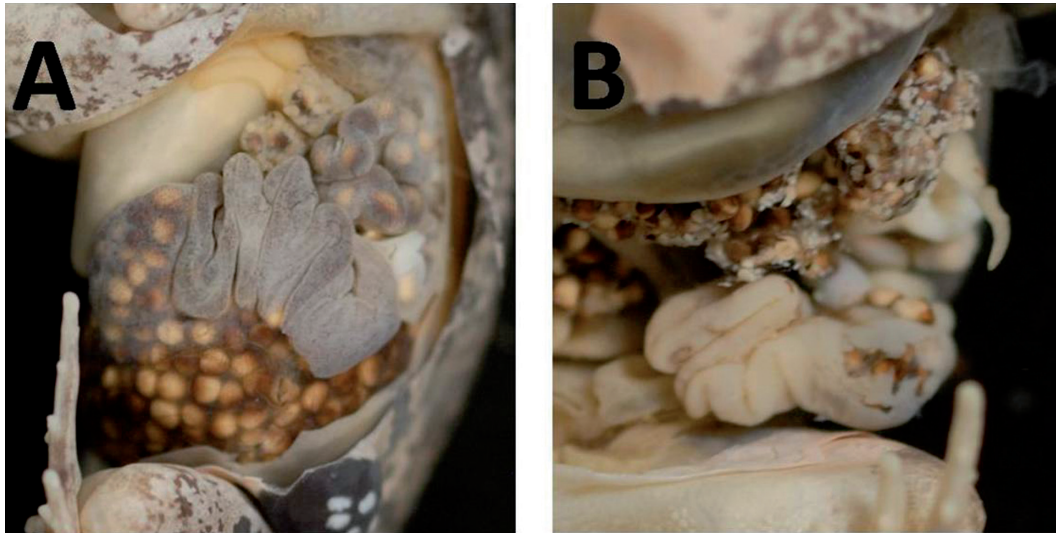


FIGURE 3. Photographs of *in situ* oviducts of two individuals of *Pleurodema brachyops*. A: USNM 302129, ova in ovisacs. B: USNM 302128, ova in oviducts.

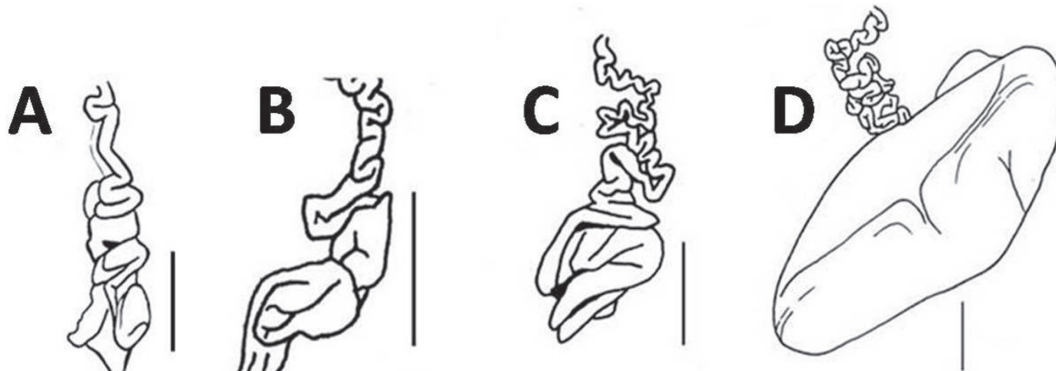


FIGURE 4. Variation in size and morphology of the PCD part of the left oviduct in four foam-nesting species of *Leptodactylus*. A: *Leptodactylus mystaceus*, USNM 531535. B: *Leptodactylus fuscus*, USNM 162881. C: *Leptodactylus ocellatus*, USNM 209231. D: *Leptodactylus savagei*, USNM 338121. Scale bars = 6 mm.

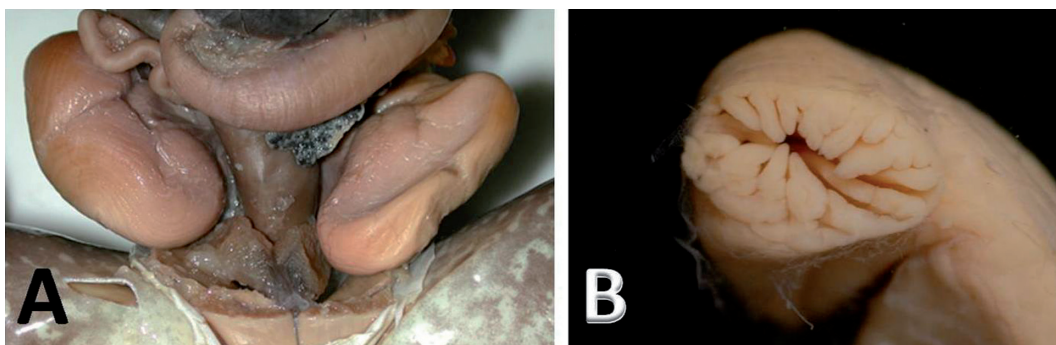


FIGURE 5. Photographs of the *pars convoluta dilata* of *Leptodactylus savagei*, USNM 338121. A: *In situ* ventral view of the oval-shaped, left and right *pars convoluta dilata*. B: Cross section of *pars convoluta dilata*.

distended with large oocytes, they completely fill the body cavity, often deforming the PCDs and pressing them against the posterior wall of the body cavity. This suggests that the PCD is malleable (not rigid as in *Leptodactylus savagei*, see next account) and that its shape can be highly variable (Fig. 2E).

Leptodactylus savagei. The visceral anatomy of an adult *L. savagei* (USNM 338121, SVL 124.2) is dominated by extremely large, nearly symmetrical, left and right PCDs (Figs. 2F, 4D, 5). Anterior to the PCD, the *pars convoluta* is tightly coiled. The *pars convoluta* abruptly enlarges into a PCD that consists of a series of five to seven large oviductal loops compacted into an oval mass and surrounded by the peritoneum. The PCD leads directly into the ovisac with only a slight narrowing of the diameter. At its widest point, the left oviduct measures about 10.9 mm. On the left side, the enlarged PCD structure is about 30 mm long, 13 mm wide, and 23 mm thick. The other *L. savagei* specimen (USNM 534218, SVL 112.7) is immature and its PCD is only about twice the thickness of the anterior portion of the *pars convoluta*, contrasting with the 10-fold thickness of the PCD in the mature specimen.

Lithodytes lineatus. This specimen is poorly preserved, and it is difficult to tell if the *pars convoluta* enlarges gradually or abruptly. The PCD is flattened against the back of the body cavity by the greatly distended stomach. The surface texture of the PCD is smooth, with long, wide, flattened convolutions.

Gross morphology – male

The two males (*Leptodactylus mystaceus*, *Leptodactylus podicipinus*; Table 2) examined have no secretion storing glands or other reproductive system modifications that would indicate that there was any secretion being produced that would contribute to the construction of a foam nest.

Histology of the *pars convoluta*

The histology of the oviduct is relatively simple, consisting of a thin external wall or *serosa*, a few layers of *lamina propria*, and a thick, interior lining or *mucosa* made up of columns of secretory cells usually capped with ciliated epithelium (Alcaide de Pucci, 1991b; Alcaide *et al.*, 2009; Horton, 1984). The following observations of oviductal histology address

only the morphology within the mucosa. The general description for each region is based on the morphology of the oviduct of *Leptodactylus savagei*, one of three species for which histological preparations of all four regions of the *pars convoluta* were available, together with supporting figures for *Leptodactylus ocellatus* (Figs. 6, 7, Table 3).

Pars convoluta (anterior to PCD). The mucosal layer from the *lamina propria* base to the oviductal lumen ranges from about 16 to 24 secretory cells thick. In cross-section, the mucosal wall appears as tightly compacted lobes. Each lobe, in turn, consists of 4-12 lobules, and each lobule in cross-section is formed from 3 to 6 secretory cells encased in a thin membrane that may be continuous with the outer wall between adjacent folds formed by the external most secretory cells. This ‘membrane’ has scattered secretory cell nuclei that appear flattened by the cell’s content. The cells are packed with small, circular secretory granules.

The lobes presumably are capped along the margin of the oviductal lumen with cuboidal ciliated epithelium. We use “presumably” to reflect the disassociated appearance of the ciliated epithelium from the tops (lumen-facing surface) of the lobes. Rather, the ciliated epithelium most often occurs as tear-shaped clusters of ciliated cells projecting from inter-lobe crevices.

In addition to *Leptodactylus savagei*, we prepared histological slides of this region for six other species: *Engystomops pustulosus*, *Leptodactylus ocellatus*, *L. podicipinus*, *Lithodytes lineatus*, *Pleurodema brachyops*, and *P. bufonina*. The mucosal layers of *L. ocellatus* and *L. podicipinus* are noticeably thinner (6-12 cells thick) than that of *L. savagei* and seem less densely packed with secretory cells. Interestingly their cell nuclei are smoothly elliptical or circular in contrast to the compressed, angular cells in *L. savagei*. The lobes of *L. ocellatus* and *L. podicipinus* are U-shaped, and as the arms of each lobe approach the lumen, the secretory cells are replaced by smaller cuboidal cells followed by columnar ciliated cells at the lumen. These ‘tufts’ of ciliated cells are pressed against each other to produce a continuous ciliated surface to the lumen.

The mucosal morphology of *Engystomops* is similar to those of *Leptodactylus ocellatus* and *L. podicipinus*, but only a few of the lobes are U-shaped; instead most are cut transversely and appear as circular ducts with tiny lumens surrounded by five to seven

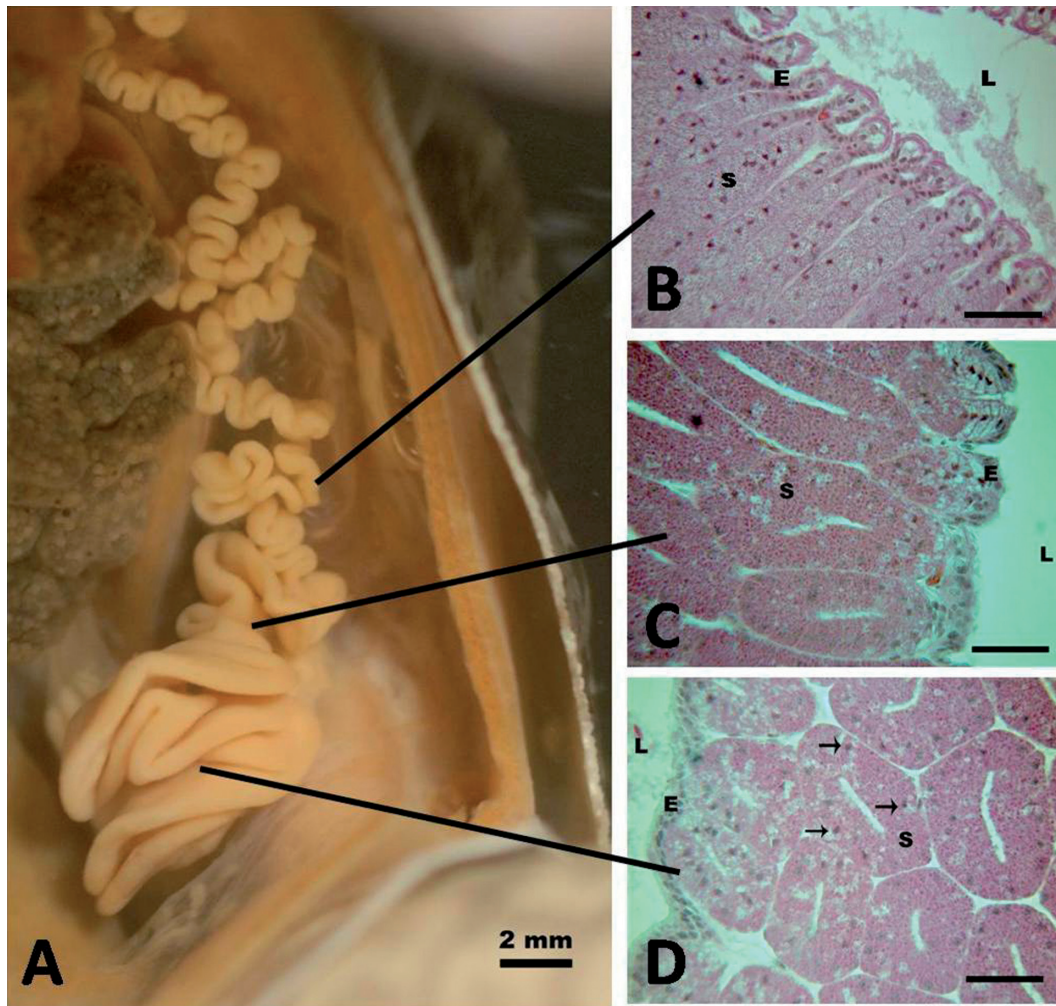


FIGURE 6. Photographs of the oviduct of *Leptodactylus ocellatus*, USNM 209231. A: *In situ* view of the left oviduct showing sites where histological sections were taken. Histological sections through B: *pars convoluta* (PC), C: anterior region of *pars convoluta dilata* (aPCD), and D: middle region of *pars convoluta dilata* (mPCD); arrows indicate nuclei. Abbreviations: E = epithelial cells, L = lumen, S = secretory cells. Scale bars in B, C, D = 50 microns.

large secretory cells. The mucosa of *Lithodytes* is the simplest of the seven taxa. The wall is relatively thin (8-10 cells thick) and comprised mostly of elliptical or circular lobes; few lobes open into the lumen. The ciliated cells also form a continuous, ciliated sheet around the lumen.

The mucosal layers in the two species of *Pleurodema* appear similar; however, the oviduct of *P. bufonina* is poorly preserved, and the cell structure has deteriorated. In *P. brachyops* (Fig. 8), the lumen is narrow and irregular and the mucosa is thick, nearly matching that of *L. savagei*; however, the secretory cell organization is strikingly different. The secretory cells in the basal layer of the mucosa have a typical lobular arrangement, but the lobes extend only half-way through the mucosa; above them, the secretory cells form a thick amorphous layer, occasionally with

a circular tubular structure. The ciliated epithelium appears continuous across the amorphous layer, but the irregular shape of the lumen makes confirmation of this observation difficult.

Pars convoluta dilata (aPCD, mPCD, pPCD). Grossly, the *pars convoluta* in *L. savagei* changes abruptly from a narrow to a very wide duct. An equally abrupt change occurs histologically. The secretory cells of the mucosa are approximately the same size throughout, but posteriorly, their numbers increase at least by a factor of 10, while the mucosa is thrown into a series of large folds, closely packed around the lumen of the oviduct. Each fold contains 30+ lobes. The lobes consist of columns of secretory cells densely packed with vacuoles, just as they are anteriorly in the *pars convoluta* (e.g., compare B and C in Figure 6 for *L. ocellatus*). Although each lobe has a central

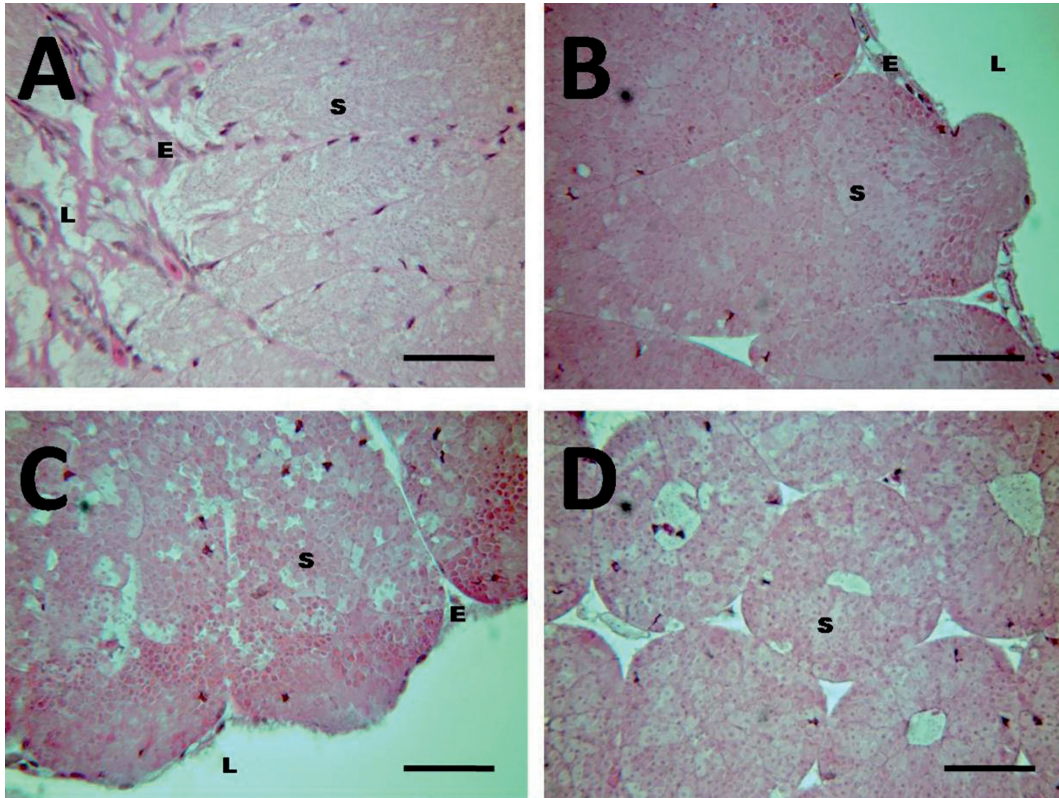


FIGURE 7. Histological sections showing changes at four points along the oviduct of *Leptodactylus savagei*, USNM 338121. A: *pars convoluta* (PC). B: anterior *pars convoluta dilata* (aPCD). C: middle *pars convoluta dilata* (mPCD). D: posterior *pars convoluta dilata* (pPCD). Abbreviations: E = epithelial cells, L = lumen, S = secretory cells. Scale bar = 50 microns.

duct that empties into the oviductal lumen, these openings are not commonly seen in the histological preparations.

Posteriorly in the PCD, the mucosal folds flatten, and the relative lengths of the lobes appear slightly shorter. Immediately anterior to the ovisac, the folds are broader and flatter. This flattening appears to result from both a narrowing of the lobes and a change in lobe orientation from perpendicular to the *lamina propria* to parallel to it. The central ducts of the lobes seem larger, and many ducts open into the oviductal lumen (Fig. 7B, C). Overall, the secretory cells enlarge from approximately 8-10 μm in diameter in the anterior *pars convoluta* and anterior most PCD to 12-14 μm in diameter in the posterior PCD. The secretory vacuoles also appear larger in the posterior region, and vacuole size may be the result of enlargement of the secretory cells. The number of vacuoles per cell seems to be the same throughout the length of the *pars convoluta*.

Comparisons of mucosal histology among all taxa emphasize the extreme development of the PCD in *L. savagei* and the likelihood that reproductive (endocrine) state greatly influences the development of the

mucosa and its secretory cycle. Three other *Leptodactylus* species either show post-peak secretory activity or alternatively, demonstrate interspecific variation. In *L. ocellatus* and *L. podicipinus*, the anterior PCD

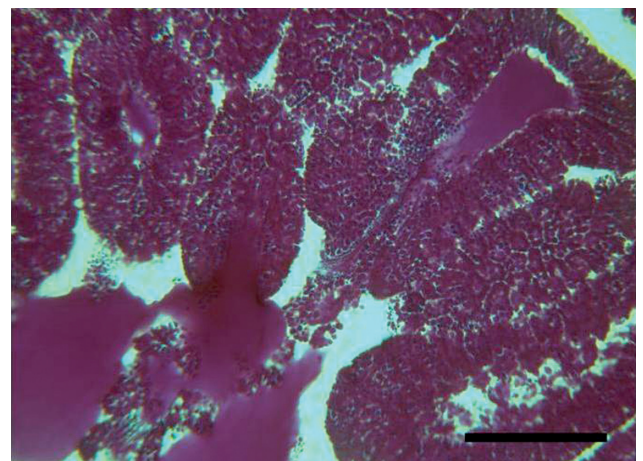


FIGURE 8. Histological section from the anterior *pars convoluta dilata* (aPCD) of *Pleurodema brachyops* stained with PASYM. This specimen (USNM 302128) had ova in its oviducts and it appears as though the collecting ducts were releasing secretions into the central lumen. Scale bar = 0.1 mm.

is of moderate size, and the mucosa forms a smooth wall throughout its entire length. The other three species have thinner walls (*i.e.*, fewer cell clusters comprising the lobes) than in *L. savagei*. In *L. ocellatus*, all secretory cells contain vacuoles; some cells in the lobular clusters of *L. podicipinus* have vacuoles, other cells are empty; and in *L. mystaceus*, vacuoles with ‘fluid’ are absent.

The mucosa in the anterior PCD of *Adenomera marmorata* is organized into a few broad folds, usually with the lobes lying parallel to the oviductal lumen. In *Pleurodema brachyops*, the mucosa of the anterior PCD is of modest height and equal to that anteriorly in the *pars convoluta*. The secretory lobes lie both perpendicular and parallel to the oviductal lumen, and most lobules show large ducts within them, although few ducts open into the lumen (similar to that seen in Fig. 8). Only in *P. brachyops* does the epithelial lining of the PCD lumen clearly show cilia; we presume cilia are found in all the species but the cilia were destroyed in the process of preparing the slides.

The *pars convoluta dilata* of *Engystomops pustulosus* and *Pleurodema bufonina* are represented only by histological sections from the posterior fifth of the pPCD. *Pleurodema bufonina* is not a foam-nester and lacks a PCD; therefore, the posterior section of the *pars convoluta* is what we describe. The mucosal wall of *P. bufonina* is moderately thin, consisting of small secretory lobes; the secretory cells are filled with vacuoles. In *E. pustulosus*, the mucosa is thick and folded. Lobes lie predominantly perpendicular basally in the mucosa and parallel adjacent to the lumen. The clusters of secretory cells are large, and the cells are packed with vacuoles.

DISCUSSION

Histological Considerations

Our observations agree with those of Alcaide de Pucci (1991a, b) and Alcaide *et al.* (2009) in that the histology of the oviducts in *Leptodactylus* spp. is essentially the same as that in other frogs. Despite the greatly differing external appearances of the oviducts, the histology appeared uniform in our study, especially among species of *Leptodactylus*. The PCD lacks any unique histo-morphological features – only the number and size of secretory cells appear to differ between species with or without morphologically obvious PCDs. As pointed out by Alcaide *et al.* (2009), noticeable differences in the histochemistry

of the PCD secretions exist among *Physalaemus biligonigerus*, *Pleurodema borellii*, and *Leptodactylus chaquensis*, and we suspect that future studies will demonstrate histochemical differences among the species we studied as well.

The interspecific variation we observed, including the arrangement of secretory cells in the anterior portion of the PCD in *L. podicipinus* contrasted with that in *L. ocellatus* and *L. savagei*, suggests that structural differences may exist at the tissue (but not cell) level. Also, some of the observed differences in appearance of cellular structures likely are due to variation in the quality of the preserved tissues. When well-preserved, the secretory cell structures are well defined, cell boundaries are distinct, and the ciliated epithelium lining is attached to the underlying mucosa.

Fernández and Ramos (2003) provided a detailed description of the histology of the oviduct in the toad *Rhinella arenarum* (as *Bufo arenarum*) in their review of the endocrinology of anuran reproduction. They noted that the *pars recta* is a relatively thin-walled tube with a mucosa consisting of one to two layers of secretory cells. These secretory cells apparently release an enzyme that modifies the ovum’s vitelline membrane to permit sperm penetration. The *pars convoluta* has a thicker mucosa, dominated by several layers of secretory cells, whose secretion forms the jelly layers of the egg. Within our sample of foam-nesting frogs, the general structure of the *pars convoluta* anterior to its expansion into the PCD appears similar to that of the *pars convoluta* in *R. arenarum*, although the mucosa of the latter taxon appears to consist of fewer layers of secretory cells. Fernández and Ramos (2003) used both “secretory” and “glandular” to refer to cells in the *pars convoluta*, but they did not differentiate between them. Distinct cells of these types are not evident in our samples, and Fernández and Ramos’s occasional use of the term “secretory glandular cells” suggests that they found only one type of cell in the toad as well. The secretory cells, as noted in our descriptive-histology section, are packed with secretory vacuoles. Fernández and Ramos described these structures as granules, as we did initially; we now recommend the use of vacuole, however, because these organelles contain fluid, not crystals (granules).

Horton (1984:67) looked at histological sections of the oviducts of a number of foam-nesting species (*Adelotus brevis*, *Lechriodus melanopyga*, *Limnodynastes ornatus*, *L. peroni*, *L. tasmaniensis*, and *Chironomantis petersii*) and found that “In each species the structure of the enlarged region is similar to that of

normal regions of *pars convoluta*. However, the tubular jelly-secreting glands are greatly enlarged and more numerous, often several layers deep, so that the oviduct walls are very much thicker than in more anterior regions.” Although the species that Horton examined are not closely related to those we examined, our findings are consistent with her results and those of Alcaide *et al.* (2009) – the number of secretory cells is much greater in the enlarged region of the oviduct.

Morphological and evolutionary considerations

The enlarged region of the posterior oviduct that we have termed the *pars convoluta dilata* (PCD), occurs in various sizes and forms in all mature females of foam-nesting species that we examined. The *pars convoluta* of *Pleurodema bufonina*, the only species examined that does not make foam nests, is not enlarged. In *Pleurodema bufonina* the *pars convoluta* gradually enlarges posteriorly; however, it never reaches the degree of enlargement and cohesiveness of the folded PCD evident in the foam-nesting species we examined. When this information is combined with data gathered by other authors, it seems likely that an enlarged *pars convoluta* (PCD) is characteristic of all foam-nesting species. The convergent evolution of similar morphological features in foam-nesting species is interesting when considered in light of the diversity of behavioral methods by which the secretion is beaten into foam.

Bhaduri (1953) reported a non-foam-nesting species, *Pachymedusa dacnicolor* that has an enlarged region in the posterior oviducts. However, this and related species of phyllomedusine frogs have an unusual reproductive mode. According to Pyburn (1970), females approach males that are calling from vegetation above a forest pond, and following amplexus, the pair descends to the pond where the female takes up water in her bladder. After a few minutes in the water the pair climbs back onto vegetation and deposits an egg mass on a leaf above the water. The female apparently releases the bladder water onto the egg mass as the eggs are being deposited (Pyburn, 1970). A single female of *Phyllomedusa callidryas* may split her clutch and deposit 3 to 5 batches at different but nearby sites on the same night. As with *Pachymedusa dacnicolor*, pairs of *Phyllomedusa callidryas* also visit the water before each egg laying event. Pyburn (1970) effectively showed that bladder water provided by the females of these two species is essential for

proper hydration of the egg jelly and normal development of the embryos. Even though our data indicate that all foam-nesting species have an enlarged region of the posterior *pars convoluta* (PCD), and that this modification directly corresponds to their reproductive mode, not every species with a PCD is necessarily a foam-nesting species. Although data are lacking, it seems likely that other species that produce considerable jelly or some other secretion during egg-laying may also have an enlarged posterior *pars convoluta*.

As far as is known, males of foam-nesting species in the genera *Rhacophorus*, *Polypedates*, and *Chiromantis* do not secrete any material necessary for the construction of foam nests. Literature reports indicate that some males within the family *Rhacophoridae* have highly coiled and secretory Wolffian ducts (Bhaduri 1932; Hoffman 1942; Iwasawa and Michibata 1972), but these secretory Wolffian ducts may be modified for sperm storage, as these arboreal species exhibit multi-male matings in vegetation above ponds. On the other hand, it may be that modified, secretory Wolffian ducts are found in species that exhibit a more terrestrial mode of reproduction. Whatever the case, behavioral and observational evidence suggest that males of these species do not contribute any type of secretion that is essential or beneficial to the construction of a foam nest. Jennions and Passmore (1993:217) reported that female *Chiromantis xerampelina* often add additional foam to their nests the night following spawning, and that this activity does not require the assistance of males. In *Rhacophorus arboreus* neither the number nor size of males involved in nest construction results in larger foam nests (Kasuya *et al.*, 1987). On the other hand, a general positive trend was found between the size of the female and that of the foam nest (Kasuya *et al.*, 1987). The two males that were examined in this study – *Leptodactylus podicipinus* and *Leptodactylus mystaceus* – showed no secretion-storing glands or other modifications of the reproductive system that would indicate production of additional secretion. Likewise, Bhaduri (1953) stated that males of *Eupemphix*, *Pleurodema*, and *Leptodactylus podicipinus* that he studied showed no modification of the Wolffian ducts that suggested a correlation with breeding habits.

A number of authors have described female foam-nesting frogs releasing a clear gelatinous substance prior to egg release. This material is undoubtedly the secretion produced in the PCD. Two plausible explanations about the nature of this secretion exist: it is egg jelly that for some reason does not become

incorporated into the jelly layers around individual ova, or it is some other secretion that is produced specifically to facilitate construction of a foam nest. Since foam-nesting has evolved independently a number of times, Heyer (1969) and Hödl (1990) hypothesized that foam-nest construction is mainly the result of behavioral modifications of existing reproductive behavior. Hödl (1990:553) stated: “Few behavioral (foam-beating) and physiological (jelly release before oviposition) changes appear to be necessary for the capability to shift from oviposition in water to foam-nest construction at the water’s surface.” To test the hypothesis that behavioral adaptations were primarily responsible for foam-nest construction, Haddad *et al.* (1990:226) manually “beat the mucus present in the spawn” of a non-foam-nesting species – *Scinax hie-malis*, and obtained ‘foam’. It is not clear to us what this ‘spawn mucus’ was (*e.g.*, egg jelly or something else), nor whether the resulting ‘foam’ consisted primarily of bubbles or actually had the consistency of ‘foam’ (also see discussion in Altig and McDiarmid, 2007:20).

Horton (1984) referred to the secretion as ‘oviducal mucopolysaccharides’ and later called it a ‘mucus’ or ‘foam-mucus.’ She (1984:101) suggested that the foam-mucus “must be secreted around the outside of the jelly capsule surrounding each egg.” We suspect that in making this suggestion that Horton was influenced by previous reports of egg jelly being beaten into foam (Heyer, 1969; Heyer and Rand, 1977). This statement however runs contrary to others’ observations (*e.g.*, Hödl, 1990; Liu, 1950) in which a transparent fluid passed out of the cloaca before the eggs. Horton further stated that mucus used in the construction of a foam nest is more fluid and less viscous than the jelly surrounding the eggs. This seems to be corroborated in *Rhacophorus bambusicola*, for which the secretion was described as a fluid (Liu, 1950). Coe (1974:19) commented that the secretion was a “pale fawn translucent viscous fluid.” Having a color other than clear suggests that the substance is not excess egg jelly. Kabisch *et al.* (1998) examined the chemical composition of foam from *Polypedates leucomystax* and found that the dried foam consisted of 93% protein; they also noted that the mucous secretion of the “foam gland” (as they called it) “consists of acid and neutral mucopolysaccharides, and the proteins develop a complex with carbohydrates” (Kabisch, *et al.*, 1998:11). Similar findings were reported for *Leptodactylus chaquensis*, *Physalaemus biligonigerus*, and *Pleurodema borellii* (Alcaide *et al.*, 2009). Is the secretory substance produced in

the PCD a unique material that is beaten by amplexant males to form the foam? A conclusive answer to this question will surely be found when someone looks at the chemical composition of the egg jelly, the secretion produced by the PCD, and the nest foam in a comparative context.

Most of the females examined in this study were mature and had ovaries with large oocytes, indicating that they were collected during the breeding season. We did not have females of one species collected at different times of the year, and thus were unable to determine if the size and shape of the PCD might vary through an annual cycle. Horton (1984) indicated that measurements of oviductal widths should be considered with caution because oviductal thickness may vary depending on the reproductive state of the female and whether or not she had bred previously. However, Horton (1984) found no evidence that the number of convolutions varied through a year or with the age of the frog. She raised frogs from eggs to juveniles and some to adults and concluded that (p. 96) “the number of convolutions is almost certainly determined at the onset of convolution and that there is no increase with increasing age of the individual.” In contrast, Bhaduri and Basu (1957) found the oviduct to be relatively straight in young or immature individuals but convoluted in adults. Although we did not count convolutions, observations (AF pers. obs.) support the idea that the number of convolutions is generally fewer in smaller females that are immature or have not bred. In the *Leptodactylus podicipinus* specimen that lacked visible oocytes (SVL = 39.6), the oviduct was less convoluted than was observed in the sexually mature female specimens (Table 2).

Although all the foam-nesting species examined in this study had PCDs, interspecific variation in the general size of the posterior region of the *pars convoluta*, the tightness of packing of the oviductal loops, and the abruptness of the enlargement was evident. The large *Leptodactylus savagei* (SVL 124.2 mm) had massive left and right PCDs formed by tightly compacted loops arranged in an oval-shaped structure. The moderate-sized species (SVLs 35–94 mm), *Leptodactylus insularum*, *L. leptodactyloides*, *L. ocellatus*, and *L. podicipinus*, had large to medium-sized PCDs that appeared similar in shape and size. The enlarged PCD regions of *Leptodactylus fuscus* and *Leptodactylus mystaceus* (SVLs 37–47 mm) were evident but comparatively small. Likewise, the PCDs of *L. fragilis* and *Adenomera hylaedactyla* (SVLs 25–38 mm) were comparatively small. It is likely that two factors, body size of the species and size and location of the

foam nest, are correlated with the relative size of the PCD. Larger frogs are likely to produce more eggs that occupy more body space and at the same time require a greater capacity for foam production. These two considerations will impact the packaging of the PCD in the body cavity. However, size of females cannot alone account for size of the PCD, because frogs of similar sizes that construct foam nests of different sizes and in different locations were found to have PCDs of different sizes.

For example, females of *L. podicipinus*, which are about the same size as those of *L. fuscus* and *L. mystaceus*, have much larger and more noticeable PCDs. This species reportedly produces foam nests on the surface of the water (Heyer, 1969), or in basins excavated by a male that are adjacent to a pond; clutch sizes of 1750–2953 eggs have been reported (Prado *et al.*, 2002). Alternatively, *Leptodactylus fuscus* builds chambered nests that are connected by tunnels, and each chamber is approximately 5 cm in diameter (Arzabe and Prado, 2006). *Leptodactylus mystaceus* reportedly has similar nests (Arzabe and Prado, 2006). These chamber-nesting species – *L. mystaceus* and *L. fuscus* – have comparatively small PCDs and have smaller clutches as well (up to about 300 eggs; Heyer and Bellin, 1973). Thus, some evidence indicates that the size and structure of the PCD reflects the relative clutch size of the species and the relative size and nature of the foam nest and its location. Confirmation of these observations is difficult, because accurate measurements of PCDs are difficult to obtain because the oviduct consists of soft flexible tissue and presumably varies in size and width depending on the time of year and breeding condition of the individual (AF pers. obs.; Horton, 1984). Unfortunately, no data are available on the oviductal morphology or size of the PCD in *Leptodactylus fallax*, a large species (maximum adult female SVL 167 mm, maximum adult male 159 mm, WRH, unpublished data) with trophic eggs and maternal care (Gibson and Buley, 2004). Interestingly, amplexant female *L. fallax* initially release few eggs (less than 100) for their size, and only about half of the eggs hatch. Following amplexus, the females remain close to their nests for up to 57 days as the tadpoles develop. During this period they provision their tadpoles with trophic eggs on up to 13 different occasions. With each provisioning event, the females increase the number of eggs provided as the tadpoles grow, with a total accumulated clutch size estimated at 10,000 to 25,000 eggs. During the first two or three provisioning events the tadpoles contain only creamy white fluid in their guts that is identical

in color to the trophic eggs. In subsequent feeding events the tadpoles ingest entire eggs with some eating up to 70 trophic eggs in a single bout. During this 33 day period the tadpoles grow from about 27 mm to about 127 mm total length. With each provisioning event the females release fluid mixed with trophic eggs and the tadpoles immediately crowd around the females' cloaca with rapid tail movements. Their wriggling motion together with swiping movements of the females' hind legs renew the foam in the nest. While many details of the reproductive biology of this amazing frog were well documented in the paper by Gibson and Buley (2004), many questions remain concerning the mechanistic responses of the mother frogs to the stimulation and growth needs of their tadpoles and the coordinated changes that must be reflected in the morphology of their reproductive systems.

We chose taxa that represent the known phylogenetic diversity within the *Leptodactylus* clade, and for comparative purposes included three species of leiuperids (one *Engystomops* and two *Pleurodema*, with one species of *Pleurodema* lacking a foam nest). The morphologies at the tissue and cellular levels that reflect an increase of secretory cells in the posterior parts of the *pars convoluta* are similar among the genera studied. The relatively straightforward increase of secretory cells that results in the PCD provides no information on whether the leiuperid and leptodactylid PCDs have been derived independently or arose from a common ancestor. The molecular data on relationships of the taxa we studied (Frost *et al.*, 2006; Grant *et al.*, 2006) support independent origins of the PCD in the leiuperids and leptodactylids. The limited histochemical data on posterior oviduct secretions suggest that considerable variation exists among species and it would be most profitable to evaluate this variability among a diverse sampling of foam nest producing frogs.

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