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Authors: Adriana M. Jeckel, Valentina Z. Caorsi, Taran Grant, and Márcio Borges-Martins

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The Nuptial Pads of *Melanophryniscus* (Anura: Bufonidae), with the Unexpected Occurrence of Nuptial-Pad–Like Structures in Females of Two Species

ADRIANA M. JECKEL,[1,2] VALENTINA Z. CAORSI,[3] TARAN GRANT,[1] AND MÁRCIO BORGES-MARTINS[3]

[1]*Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil*
[3]*Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil*

ABSTRACT.—**Nuptial pads are patches of thickened skin, associated with sexually dimorphic skin glands, present only in the thumbs and fingers of male anurans, and hence considered dimorphic sexual characters. Despite the morphological description for many species, the evolution and function of nuptial pads are still unclear. In this study, we describe the presence of nuptial-pad–like structures in females of two species of *Melanophryniscus* and compare their morphology and histology to males' nuptial pads. The epidermal modifications found in females are conical papillary epidermal projections, similar to the structures found in males; however, the density of these projections in males is twice the average found in females. Also, the nuptial-pad–like structure found in females cannot be considered an actual nuptial pad, because there are no specialized skin glands associated with the structure. This study brings the first records of epidermal modification in females of *Melanophryniscus* and provides the description of male nuptial pads for the genus.**

RESUMEN.—**Las callosidades nupciales son engrosamientos de la piel asociados a glándulas cutáneas sexualmente dimórficas, generalmente presentes sobre el dedo pulgar de anuros machos y por lo tanto considerados caracteres sexuales dimórficos. A pesar de su descripción morfológica para varias especies, la evolución y función de las callosidades nupciales aún no es bien comprendida. En este trabajo describimos la presencia de estructuras parecidas a callosidades nupciales en hembras de dos especies de *Melanophryniscus* y comparamos su morfología e histología con las estructuras encontradas en los machos. Las estructuras epidérmicas encontradas en las hembras son proyecciones epidérmicas papilares cónicas, similares a las encontradas en los machos; sin embargo, la densidad de estas proyecciones en los machos es el doble del promedio encontrado en las hembras. Además, la estructura que se encuentra en las hembras no puede considerarse una callosidad nupcial verdadera, ya que no presenta glándulas especializadas asociadas a ella. Este estudio presenta los primeros registros de modificaciones epidérmica en hembras de *Melanophryniscus* y la primera descripción detallada de las callosidades nupciales en machos del género.**

Secondary sexual characters are morphological traits that develop at maturity. They are usually sexually dimorphic in most anuran species and are present mostly in males; examples include vocal sacs, spines, and nuptial pads (Noble, 1931; Liu, 1936). Some of these characters remain present during the entire male adult life; others, like nuptial pads, can be associated with reproductive activity (Noble, 1931; Kurabuchi, 1993; Emerson et al., 1999; Kyriakopoulou-Sklavounou et al., 2012) and might depend on blood levels of testicular hormones (Greenberg, 1942; Izzo et al., 1982; Emerson et al. 1997; Epstein and Blackburn, 1997).

Nuptial pads are patches of thickened epidermis, usually with cornified projections of distinctive, often species-specific architectures (Kurabuchi, 1993; Kyriakopoulou-Sklavounou et al., 2012). They usually occur on the thumb (Finger II; Fabrezi and Alberch, 1996), occasionally extend to Fingers III and IV (e.g., Noble, 1931; Luna et al., 2018), and are always associated with large, sexually dimorphic skin glands (SDSGs; Fujikura et al., 1988; Thomas et al., 1993; Luna et al., 2018). These SDSGs are usually present in specific areas of the body (Thomas et al., 1993) and authors have described them in the nuptial pads of many anuran species as specialized mucous glands (SMGs) because of their morphological similarities with ordinary mucous glands (OMGs; Fujikura et al., 1988; Kurabuchi, 1993, 1994; Epstein and Blackburn, 1997; Brizzi et al., 2002; Luna et al., 2012). The actual content and function of these glands are yet to be understood; however, their ducts reach the surface of the pad epidermis and are usually distributed around the dermal spines (Kurabuchi, 1993, 1994; Brizzi et al., 2002; Luna et al., 2012, 2018), indicating an association of the glandular content with pad function.

Nuptial pads are considered secondary sexual characters that can be used to discriminate sexes because they are usually restricted to males (e.g., Kyriakopoulou-Sklavounou et al., 2012; Peloso et al., 2012). Many authors have hypothesized that nuptial pads have a role in grasping the female during amplexus and that the position of the nuptial pad might be related to the position of amplexus (Lataste, 1876; Noble, 1931; Duellman and Trueb, 1986; Epstein and Blackburn, 1997). Some other putative functions of this structure are male–male combat (Savage, 1961) and boosting the spread of secretions that attract and/or stimulate females (Thomas et al., 1993). Intriguingly, spines are present on the thumbs and hands of females of *Insuetophrynus acarpicus* (Diaz et al., 1983), *Xenopus laevis* (Kurabuchi and Inoue, 1981), and some species of *Crossodactylus* (Lutz, 1930; Pimenta et al., 2014).

We studied the nuptial pads of several species of *Melanophryniscus*. The genus currently comprises 29 recognized species (Frost, 2018) distributed in the Southern Cone of South America east of the Andes, from southern Brazil to Argentina, Paraguay, Bolivia, and Uruguay (Zank et al., 2014). *Melanophryniscus* is the sister clade of all other bufonids (Frost et al., 2006; Pyron and Wiens, 2011; Pyron, 2014), making it especially relevant for understanding character evolution within the family. In most species of *Melanophryniscus*, the nuptial pad is a sexually dimorphic character occurring in adult males on Finger II and frequently also on Fingers III and IV (Table 1) and is absent in juveniles and adult females (e.g., Braun and Braun, 1979; Baldo and Basso, 2004; Baldo et al., 2012; Peloso et al., 2012).

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[2]Corresponding Author. E-mail: amjeckel@gmail.com
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TABLE 1. Review of nuptial pad occurrence and structure in males of *Melanophryniscus*.

Species	Description
Melanophryniscus alipioi	Smooth, brown nuptial pads on Fingers II and III (Langone et al., 2008)
Melanophryniscus biancae	Several small brown-colored keratinized spines on Fingers III and IV (Bornschein et al., 2015)
Melanophryniscus cambaraensis	Conspicuous brown nuptial pad on Fingers II and III (Braun and Braun, 1979; Santos et al., 2010)
Melanophryniscus devincenzii	Pad composed by many minute, keratinized spines (Peloso et al., 2012)
Melanophryniscus krauczuki	Smooth, brown nuptial pads on Fingers II and III (Baldo and Basso, 2004)
Melanophryniscus macrogranulosus	Presence of nuptial pad (Braun, 1973; Caorsi et al., 2014)
Melanophryniscus milanoi	Several small brown-colored keratinized spines on Fingers III and IV (Bornschein et al., 2015)
Melanophryniscus moreirae	Pad composed by many minute, keratinized spines (Peloso et al., 2012)
Melanophryniscus pachyrhynus	Brown nuptial pads on Finger II and usually also on III and IV (Baldo et al., 2012)
Melanophryniscus rubriventris	Presence of nuptial pads (Peloso et al., 2012)
Melanophryniscus setiba	Enlarged, brown, keratinized spines at medial margin of Finger III (Peloso et al., 2012)
Melanophryniscus simplex	Pad composed by many minute, keratinized spines (Peloso et al., 2012)
Melanophryniscus stelzneri	Smooth, brown nuptial pads (Cei, 1980)
Melanophryniscus tumifrons	Pad composed by many minute, keratinized spines (Peloso et al., 2012)
Melanophryniscus xanthostomus	Several small brown-colored keratinized spines on Fingers III and IV (Bornschein et al., 2015)
Melanophryniscus setiba	Enlarged, brown, keratinized spines at medial margin of Finger III (Peloso et al., 2012)

Nevertheless, there are no published accounts of the detailed morphology of the nuptial pads of this genus. Hence, we provide the first histological description of nuptial pads for the genus. During our studies, we also observed a superficially identical nuptial-pad–like structure on the thumbs of females of two species that we describe and compare to the nuptial pads of males.

MATERIALS AND METHODS

Sample Data.—Given the lack of a comprehensive phylogenetic hypothesis for the genus, we sampled seven males and seven females of five *Melanophryniscus* species chosen on the basis of the phenetic groups of Cruz and Caramaschi (2003): *M. cambaraensis*, *M. macrogranulosus*, and *M. simplex* of the *M. tumifrons* group, *M. montevidensis* of the *M. stelzneri* group, and *M. sanmartini*, formerly of the *M. moreirae* group, but currently not placed in any group (Baldo et al., 2014). We analyzed the fingers of male and female *M. cambaraensis* and *M. macrogranulosus* with the use of optical stereo microscopy, scanning electron microscopy (SEM), and light microscopy; we submitted the females of remaining species to optical and scanning electron microscopy, and the males of the remaining species to optical microscopy (see below). We considered individuals with well-developed vocal slits to be adult males and those with convoluted oviducts and enlarged ova to be adult females.

All specimens in this study were deposited previously in the Amphibian Collection of the Departamento de Zoologia da Universidade Federal do Rio Grande do Sul (UFRGS) and the Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul (MCN), except two specimens of *M. macrogranulosus* (UFRGS 6587 and UFRGS 6588) that we collected specifically for this study. For the new specimens, we fixed whole hands and whole feet in modified Karnovsky's fixative (Ito and Karnovsky, 1968) at 4°C for 48 h, and processed the material for SEM analysis. We detailed the methods used to analyze each specimen and their corresponding voucher numbers in Table 2.

Optical Stereo Microscopy.—We examined all individuals of all species with the use of an optical stereomicroscope (OPTON TIM-2B, Anatomic, China) and obtained images with the use of a stereomicroscope coupled to a Nikon AZ100M (Nikon Corporation, Tokyo, Japan). We could not observe female structures with these instruments.

Scanning Electron Microscopy (SEM).—For SEM, we washed the whole hand and foot three times with Sorenson's phosphate buffer (0.2M; pH 7.2), dehydrated them with an increasing sequence of acetone concentrations, dried them with the critical point method with the use of liquid CO_2, and coated them with gold (adapted from Scotto, 1980 and Felgenhauer, 1987). We analyzed samples with the use of a JEOL JSM-6060 scanning electron microscope (JEOL Ltd., Tokyo, Japan). We estimated the density of protuberances for each pad of male *M. cambaraensis* and female *M. macrogranulosus* by counting three to six 200×200–μm^2 squares on each SEM photograph, with Adobe® Photoshop® software (San Jose, California, USA). The results are expressed as the mean ± standard deviation and range of protuberances per unit surface area (mm^2).

Light Microscopy and Histochemistry.—We embedded all thumbs used for light microscopy in historesin (Leica Biosystems Nussloch GmbH, Nussloch/Heidelberg, Germany), sectioned them transversely at 4–5 µm, and stained them with toluidine blue-fuchsine (TF) and hematoxylin-eosin (HE) for general morphological analysis. For histochemical study, we submitted the sections to the following reactions: bromophenol blue (BFB) for protein identification, alcian blue pH 2.5 (AB) for identification of acidic carbohydrates, and periodic acid-Schiff + alcian blue pH 2.5 (PAS + AB) for general identification of carbohydrates (Bancroft and Stevens, 1996; Kiernan, 2008). We photographed the sections with an Olympus BX51 (Olympus Corporation, Tokyo, Japan) light microscope equipped with a digital camera and Image-Pro Express software (Media Cybernetics, Rockville, Maryland, USA).

RESULTS

Adult males of all five species presented nuptial pads on the dorsum of Finger II and, less frequently, on Fingers III and IV (Fig. 1A–E). Some species presented dense brownish pads (Fig. 1C–E), and others presented more discrete sparse spines, difficult to see (Fig. 1A,B). Fingers of *Melanophryniscus montevidensis*, *M. sanmartini*, and *M. simplex* females lacked epidermal projections, but females of *M. cambaraensis* and *M. macrogranulosus* exhibited epidermal projections concentrated on the dorsolateral region of Finger II (Fig. 2, Table 3). However, these projections are indistinguishable on the optical stereomicroscope (Fig. 1F,G) because they are sparse and do not form a brownish cornified layer covering most of Finger II as on males.

TABLE 2. List of specimens, collection localities, sex, and histological methods employed.[a]

Specimen by species	Locality	Sex	OM	H&H	SEM
Melanophryniscus montevidensis					
UFRGS3256	Laguna de Rocha, Rocha, Uruguay	Female	×		×
UFRGS3258	Laguna de Rocha, Rocha, Uruguay	Male	×		
Melanophryniscus macrogranulosus					
MCN1693[b]	Morro da Gruta, Dom Pedro de Alcântara, RS, Brazil	Female	×	×	×
UFRGS6425	Garapiá, Maquiné, RS, Brazil	Female	×	×	
UFRGS6449	Morro da Gruta, Dom Pedro de Alcântara, RS, Brazil	Male	×	×	×
UFRGS6451	Morro da Gruta, Dom Pedro de Alcântara, RS, Brazil	Male	×		
UFRGS6587	Garapiá, Maquiné, RS, Brazil	Male	×	×	×
UFRGS6588	Garapiá, Maquiné, RS, Brazil	Female	×		×
Melanophryniscus cambaraensis					
MCN13477	FLONA São Francisco de Paula, RS, Brazil	Female	×	×	×
MCN9791[b]	PARNA Serra Geral, Cambará do Sul, RS, Brazil	Female	×	×	×
UFRGS4817	FLONA São Francisco de Paula, RS, Brazil	Female	×		
MCN9802[b]	PARNA Serra Geral, Cambará do Sul, RS, Brazil	Male	×	×	×
UFRGS6328	FLONA São Francisco de Paula, RS, Brazil	Male	×		
UFRGS6327	FLONA São Francisco de Paula, RS, Brazil	Male	×	×	×
Melanophryniscus sanmartini					
UFRGS6748	Pedras Altas, RS, Brazil	Male	×		
UFRGS6751	Pedras Altas, RS, Brazil	Female	×		×
Melanophryniscus simplex					
UFRGS4165	Bom Jesus, RS, Brazil	Female	×		×
UFRGS4296	Campo Belo do Sul, RS, Brazil	Male	×		

[a] OM: optical microscope; H&H: histology and histochemistry; SEM: scanning electron microscope.
[b] Species type series.

In fact, Finger II of female *M. cambaraensis* and *M. macrogranulosus* follows the blackish coloration and the spiny skin that are common in *Melanophryniscus* (Braun, 1973). The nuptial-pad–like structure that we refer to in this study are the minute cornified epidermal projections that we show in Figures 2A,B and 3. Epidermal projections were absent in all feet.

Externally, the modified epidermis of males and females of *M. cambaraensis* and *M. macrogranulosus* are mostly similar, both presenting conical epidermal projections; however, the density of the projections in males is twice the average found in females of both species (Table 3). Male epidermal projection density varied between fingers, being consistently higher on Finger II and variable on Fingers III and IV. Structural observations with SEM revealed a cornified ornamentation arranged in concentric circles around the apex of each epidermal projection in males that did not occur in females (Fig. 3).

Males present specialized mucous glands (SMGs) along the entire dorsolateral extension of the finger, with a few ordinary serous glands (OSGs) and OMGs (Fig. 4A), whereas females present only OSGs and OMGs along the entire finger (Fig. 4B). A dermal core occurs in the epidermal projections (Fig. 4C,D) in both sexes, characterizing them as conical papillary epidermal projections *sensu* Luna et al. (2018). The epidermis is structurally similar in males and females. As typically seen in anuran epidermis, four layers of epidermal cells surround the dermal core. The outermost layer (*stratum corneum*) consists of a monolayer of cornified cells that encloses the entire nuptial pad and forms the ornamentations. In males, these structures are characterized by a cornified projection that thickens from the base to the top (Figs. 5A, 4C), whereas in females the thickness is uniform around the entire epidermal projection (Fig. 4D). The subsequent layers are the *stratum granulosum*, formed by polygonal cells with flattened nuclei, which present some projections that seems to fit the cornified ornamentations of the *stratum corneum*; 2–3 cell layers of *stratum spinosum*, formed by cuboid cells with central nuclei; and the bottom layer, the *stratum basale*, connecting the epidermis to the dermis, formed by a layer of columnar cells.

Similar to OMGs, the SMGs of males are alveolar glands formed by a duct, an intermediate region (neck), and a secretory portion. The SMGs are almost double the size of the OMGs, however, and the secretory portion consists of a packed monolayer of columnar secretory cells containing eosinophilic granules, with their nuclei situated at the base, whereas the OMG secretory cells are usually cuboid. The secretory granules of the SMGs and OMGs were positive to AB, PAS + AB (Fig. 5), but negative to BFB; the only difference between both types of mucous glands is that SMG secretory granules are positive to eosin, whereas OMGs are not (Fig. 5). The duct is formed by a thin layer of cornified cells that evaginates through epidermis, connecting the neck (formed by two layers of flat cells) and the secretory portion of both kinds of glands with the exterior (Fig. 5C,D). Even though it was not possible to see the SMG duct pores through SEM, histological sections show that these ducts connect to the surface of the epidermis between the EPs.

DISCUSSION

Nuptial-Pad–Like Structures in Females.—Unlike females of other species of *Melanophryniscus*, females of *M. cambaraensis* and *M. macrogranulosus* presented conical papillary epidermal projections on the dorsolateral regions of the thumb that are externally similar to the nuptial pads observed in males of all studied species. Nevertheless, the female epidermal projections differed from those of males in being sparser, lacking the cornified ornamentation arranged in concentric circles around the apex of each epidermal projection in males, and, most significantly, SDSGs, which technically excludes them as nuptial pads (Luna et al., 2018).

The occurrence of typically sexually dimorphic structures in both sexes is rare, but has been reported in a few species of anurans. For example, in some species of *Crossodactylus*, cornified spines occur on the thumb of both sexes (Lutz, 1930;

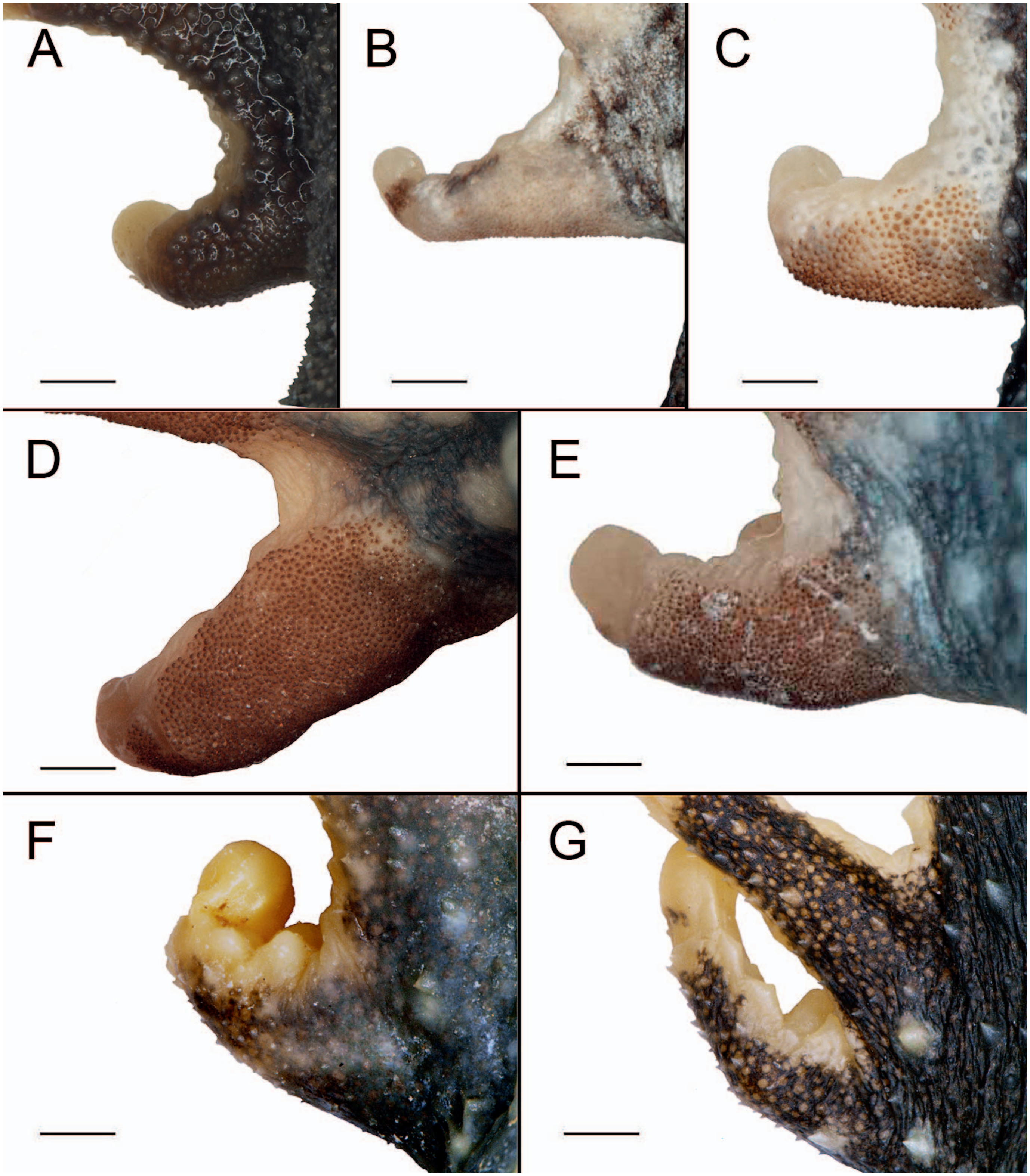

FIG. 1. Finger II of *Melanophryniscus* males. (A) *M. montevidensis*, (B) *M. sanmartini*, (C) *M. simplex*, (D) *M. cambaraensis*, and (E) *M. macrogranulosus*. Finger II of *Melanophryniscus* females (F) *M. cambaraensis*, and (G) *M. macrogranulosus*. Scale bar: 500 μm.

Pimenta et al., 2014). In *Crossodactylus grandis*, although there are no differences between sexes in the number of spines, the spines are more strongly developed in males than in females (Pimenta et al., 2014). Nevertheless, Luna et al. (2018) recently discovered that spines are not associated with SDSGs in either sex, bringing into question both the homology and functional significance of the keratinous epidermal projections of this group. Kurabuchi and Inoue (1981) reported that male and female *Xenopus laevis* develop spines from the finger to the forearm, being larger and fewer in females; however, SDSGs

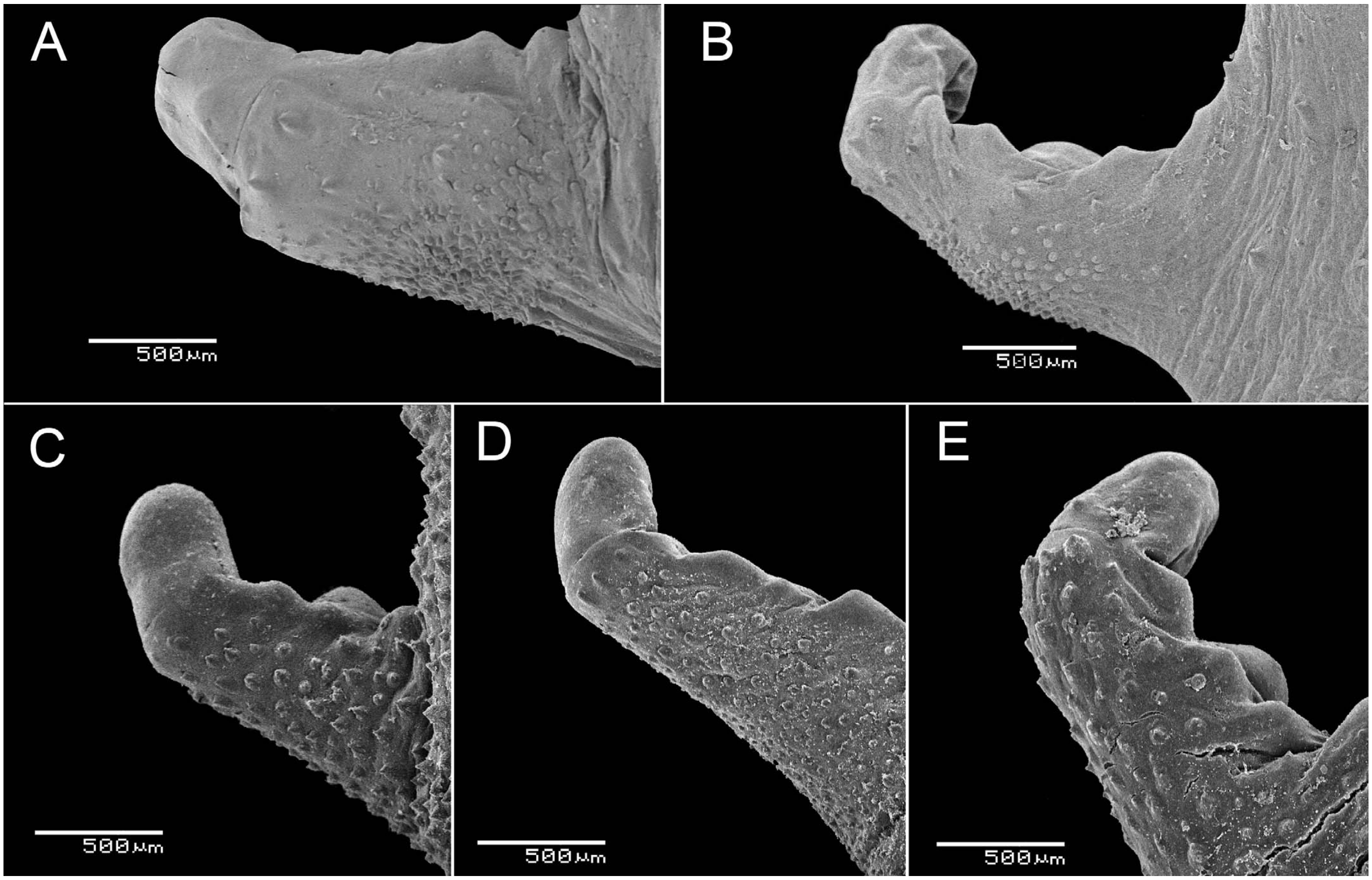

FIG. 2. Scanning electron micrograph of Finger I in the right hand of females from the studied species (A) *Melanophryniscus cambaraensis* and (B) *M. macrogranulosus*, and three other species from the genus—(C) *M. montevidensis*, (D) *M. sanmartini*, and (E) *M. simplex*. Scale bar: 500 µm.

occur only in males (Fujikura et al., 1988; Van Wyk et al., 2003), and the occurrence of only OMGs and OSGs in females led Luna et al. (2018) to exclude them as nuptial pads. Diaz et al. (1983) also described epidermal projections on female Finger II extending to Finger III in *Insuetophrynus acarpicus* (Rhinodermatidae). The structures were not as dense as in males, but the authors did not analyze them histologically and, therefore, could not report presence/absence of SDSGs.

Epidermal modifications are also present in females on the hind limbs of some other species. Some examples are the females of *Chiasmocleis avilapiresae* (Peloso and Sturaro, 2008), which have few dermal spines on toes, and of *Nelsonophryne aterrima* (Lehr and Trueb, 2007), which present few small spines on dorsum and on dorsolateral surfaces of their feet; however, none of these structures have been related to reproductive functions or to SDSGs. On the other hand, females of *Limnodynastes* and *Platyplectrum* have fleshy phalanges on some fingers of their forelimbs that provide a much greater surface area to the hands, used in paddling movements for stirring water and spawn into a foam nest (Martin, 1970). In these cases, the epidermal modifications have a clear reproduction function. Unfortunately, the function or origin of spines in thumbs of females, such as the ones found in *M. cambaraensis* and *M. macrogranulosus*, without association with SDSGs, is still unclear.

Nuptial Pads in Males.—In addition to reporting the unexpected occurrence of epidermal projections on the thumb in females of two species of *Melanophryniscus*, this study also provides the first detailed description of nuptial pads for male *Melanophryniscus*. The epidermal projections of these nuptial pads are characterized by cornified ornamentations arranged in concentric circles

TABLE 3. Nuptial pad data for male and female of *Melanophryniscus cambaraensis* and *M. macrogranulosus*. *n*: number of individuals analyzed; estimated density: mean ± SD of protuberances per square millimeter.

	M. cambaraensis		*M. macrogranulosus*	
	Male	Female	Male	Female
Description	Conspicuous brown nuptial pad on Fingers II and III and also on Finger IV ($n = 1$)	Few inconspicuous and minute structures only in Finger II ($n = 1$)	Conspicuous brown nuptial pad on Fingers II and III and also on Finger IV ($n = 2$)	Few inconspicuous and minute structures only in Finger II ($n = 1$)
Estimated density				
Finger II	583 ± 52	295 ± 85	754 ± 66	350 ± 79
Finger III	483.3 ± 52	No structures	729.1 ± 81.3	No structures
Finger IV	325	No structures	504.2 ± 329	No structures

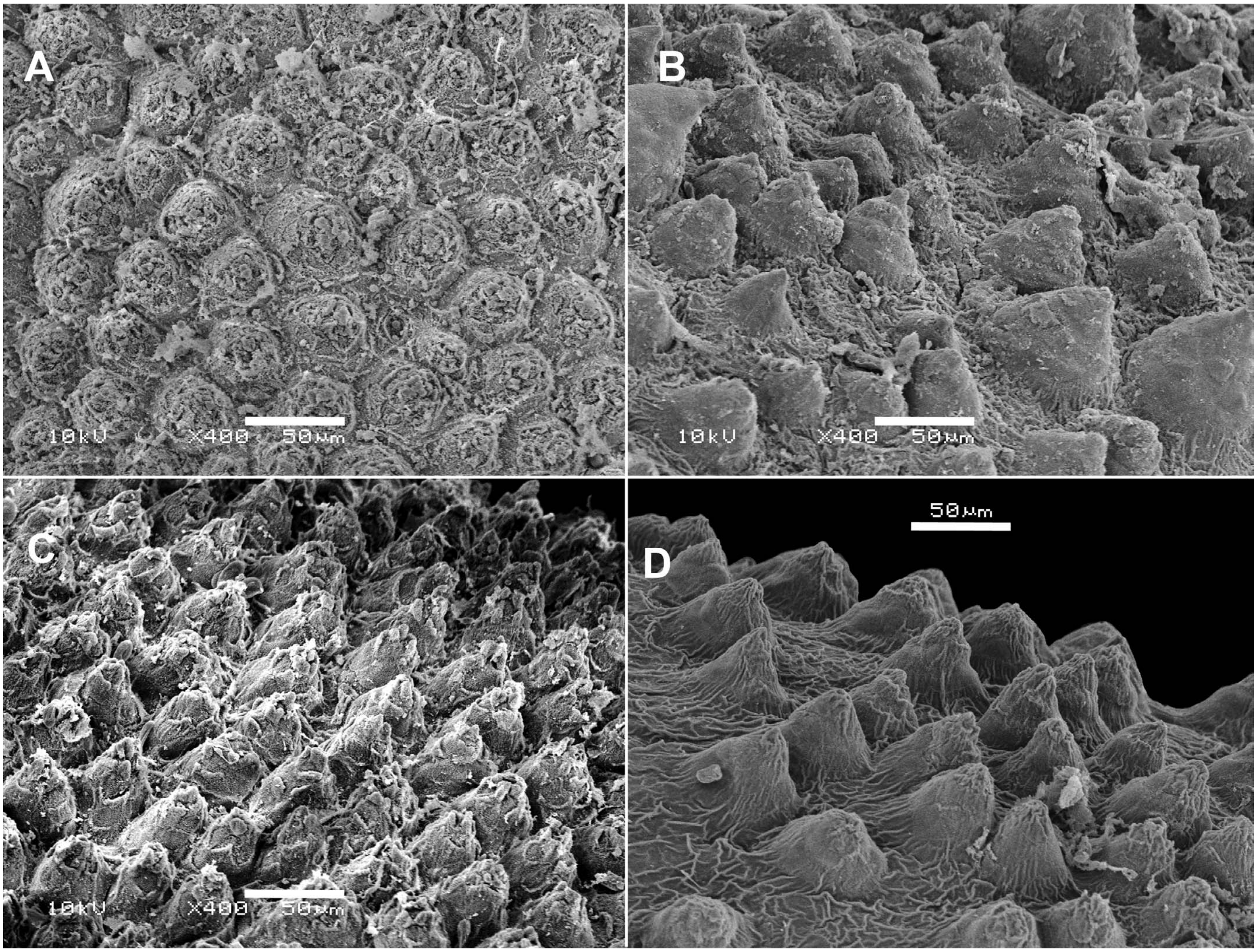

FIG. 3. Scanning electron microscopy of the epidermal projections on the first finger of (A) male and (B) female of *Melanophryniscus cambaraensis*, and of (C) male and (D) female of *M. macrogranulosus*. Scale bar: 50 µm.

around the apex of each projection. The functional significance, if any, of these ornamentations in the layer is unknown, but one hypothesis is that it might increase surface contact and better disperse glandular secretions around the pad (Thomas et al., 1993). According to previous studies, the precise arrangement of this keratinized ultrastructure might be species-specific (Kurabuchi, 1993; Kyriakopoulou-Sklavounou et al., 2012; Luna et al., 2012).

The morphology of SDSG described in our study is consistent with previous studies: alveolar glands consisting of a packed monolayer of columnar secretory cells containing eosinophilic granules, with a cell nucleus situated at the base (Epstein and Blackburn, 1997; Thomas et al., 1993; van Wyk et al., 2003; Luna et al., 2012). The SDSG ducts in *M. cambaraensis* and *M. macrogranulosus* are formed by a thin layer of keratinized cells that evaginate through the epidermis and open between the papillary epidermal projections of the nuptial pad. Luna et al. (2012) reported that, in four species of *Agalychnis*, the outermost duct cell protrudes beyond the limits of epidermis. In all 26 species of Phyllomedusidae analyzed by Luna et al. (2012), the ducts opened between the papillary epidermal projections and, in some cases, could not be seen by SEM. Luna et al. (2012) suggested that the exposition of the pores might have functional consequences to the spreading of the glands' secretions.

Some authors have suggested that the glands present in the nuptial pad are modified mucous glands because they are multicellular alveolar glands and they stain similarly to general histochemistry (Epstein and Blackburn, 1997; Brizzi et al., 2002). Our histochemical results also suggest that nuptial glands might be differentiated from OMGs. OMGs and SMGs stained positive to AB and PAS but did not react to BFB. Nevertheless, OMG granules did not stain as strongly to eosin as SMGs, suggesting different glandular contents. On the basis of previous studies, we were expecting the positive reaction for PAS stain for SMGs (Epstein and Blackburn, 1997; Kaptan and Bolkent, 2014; Luna et al., 2018). Until recently, only a few hylid and *Pelophylax* species shared the positive reaction to AB (Luna et al., 2012; Kaptan and Bolkent, 2014), but Luna et al. (2018) reported that many anuran species, from at least 23 families (including several bufonids), present secretions that stain with AB, whereas other species such as *Xenopus laevis* (Thomas et al., 1993), *Lithobates pipiens* (Epstein and Blackburn, 1997), *Dendropsophus labialis*, *Scinax perereca* (Luna et al., 2012), and all other species that Luna et al. (2018) reported did not react to AB. Further, *Xenopus laevis* had a positive reaction to the protein indicator ninhydrin-Schiff and performic acid–alcian blue, implying the presence of proteins in the glands (Thomas et al., 1993).

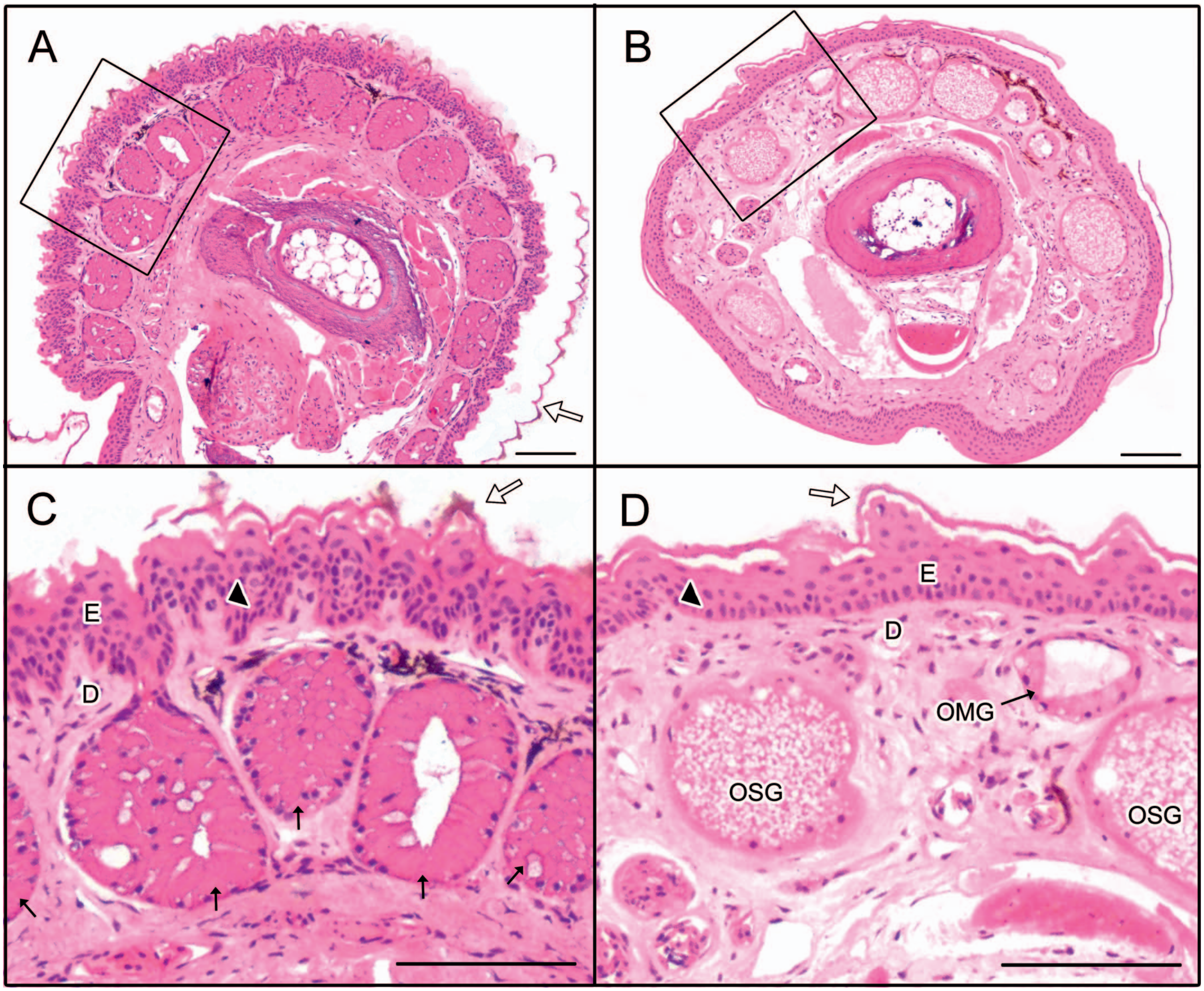

FIG. 4. Cross sections of *Melanophryniscus cambaraensis* male (MCN13477; [A,C]) and female (UFRGS6327; [B,D]) thumbs. Magnifications of selected areas show the presence of sexually dimorphic skin glands (black arrows) in males (C), and the presence of ordinary mucous (OMG) and ordinary serous glands (OSG) in females. D, dermis; E, epidermis; black arrow head, papillary epidermal projections; white arrow, *stratum corneum*. Scale bars: 100 μm.

The function and the origin of the skin modifications that shape the nuptial pads in anurans are still not well understood (Luna et al., 2018). The most cited and accepted hypothesis is that these excrescences aid males in holding females during amplexus (e.g., Lataste et al., 1876; Duellman and Trueb, 1986), and help them avoid being dislodged by other competing males (Savage, 1961). In fact, *Melanophryniscus* species are known to be explosive breeders; many individuals reproduce in temporary pools or streams after intense rains (Santos et al., 2010), and intense struggles among males occur during disputes for females, which sometimes are also carried into the mess (Caorsi et al., 2014). Unfortunately, the actual function (if there is one) of nuptial pads and epidermal projections in male and females, respectively, has not yet been tested empirically. There is also a lack of specific studies regarding the chemical content of the SDSGs associated with the nuptial pads, so their role in the reproductive behavior of males is not well understood. This study examined a novelty for the genus and new information on male and female structures of epidermal modification for anurans.

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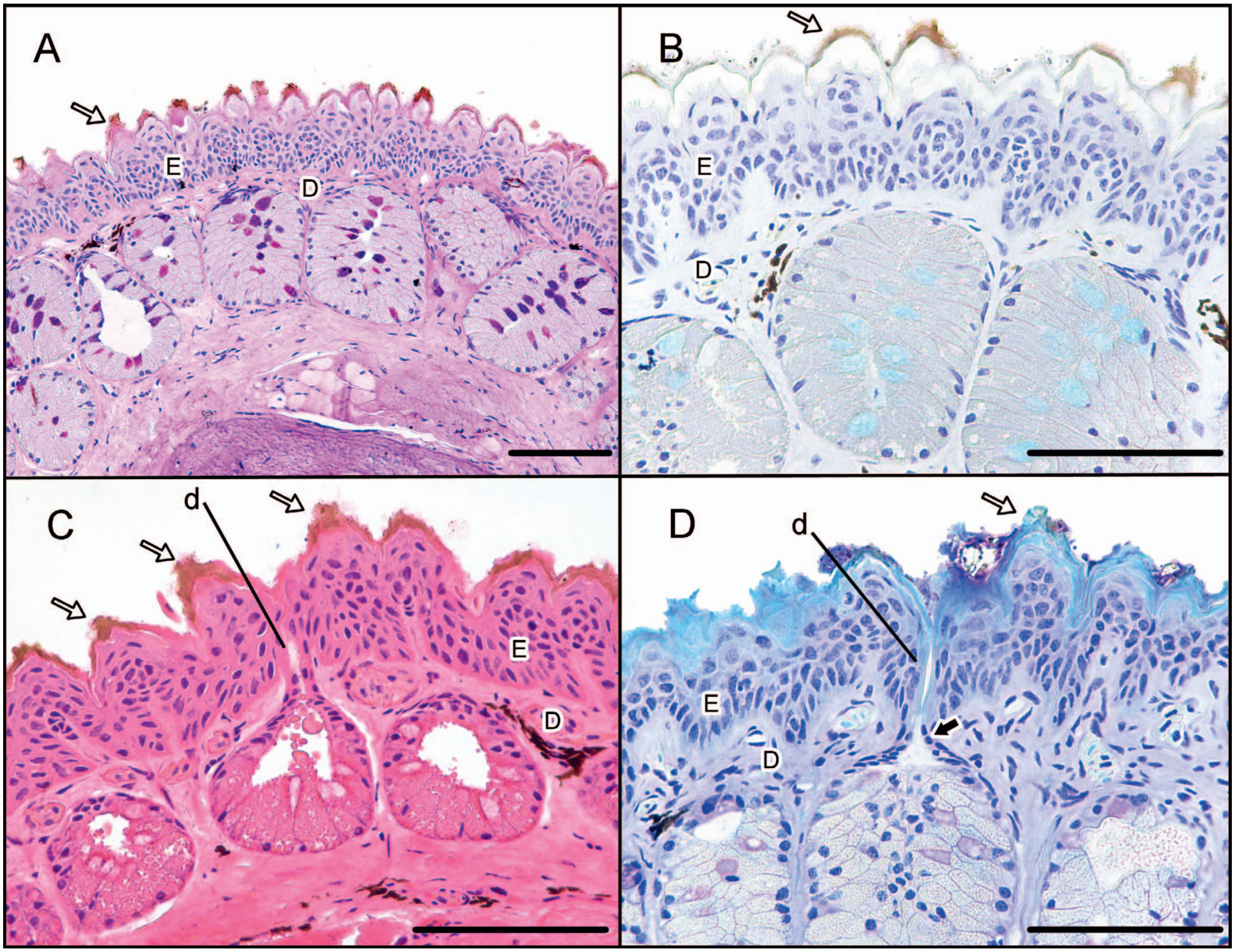

FIG. 5. Histochemistry of nuptial pads of *Melanophryniscus macrogranulosus*. (A) Male (UFRGS 6327), specialized mucous gland (SMG) positively stained with PAS + AB on the distal portion of each secretory cell. (B) Male (UFRGS 6327), SMG positively stained with AB. (C) Males (UFRGS 6586) present keratin ornamentation on top of papillae (white arrows). Gland ducts (d) open between papillae. Section stained with hematoxylin and eosin; SMG granules positively stained with eosin. (D) Detail of a SMG in male (UFRGS 6448), showing duct (d), formed by a thin layer of keratinized cells that cross the epidermis and opening between papillae; intermediate region (black arrow) and the secretory cells, filled with granules that positively stained with fuchsine but not with toluidine blue. Scale bars: (A,B) 100 μm; (C,D) 50 μm.

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