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SHORT NOTE

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New insights about ovarian pigmentation in Anura

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Amphibians have pigmented cells in organs beyond just skin. Their functions involve free radical neutralisation, early innate response, and a relationship with environmental temperature and UV light. In gonads, pigment containing-cells seem to be restricted mainly to the testes and related to sperm production. However, we report for the first time ovarian melanisation in Pseudis minuta and its ontogenetic changes in larval and postmetamorphic stages. Melanin containing-cells on the ovarian surface initially appear at early premetamorphic stages whereas in the cortex they occur later. In consecutive stages, melanin containing-cells were more evident among oocytes but without a clear pattern, being located randomly within the germinal epithelium or in the stroma. Although their function is unclear, a relationship with the fast acquisition of sexual maturity must be further explored.

Keywords: Anuran, germ cells, melanin, ovary, Pseudis minuta

Melanic pigment cells in internal organs have intrigued researchers for decades. In amphibians and fishes, pigment cells containing melanin occur in various tissues and organs such as skin, but also in liver, spleen, kidney, peritoneum, lung, heart, blood vessels, thymus, gonads, and meninges (Agius & Agbede, 1984; Zuasti et al., 1998; Gallone et al., 2002; Zieri et al., 2007; Franco-Belussi et al., 2011, 2012). All, but those in the skin, constitute the so-called extracutaneous pigmentary system (Breathnach, 1988).

The functions of the extracutaneous pigmentary system involve cytoprotection against free radicals and oxidative stress (Zuasti et al., 1998; McGraw, 2005), detoxification from pollutants (Fenoglio et al., 2005), and protection against bacteria (Franco-Belussi et al., 2013). Visceral melanin pigmentation in anurans seems to be related to other environmental factors too, since the amount of melanocytes responds to changes in temperature and UV light exposure (Franco-Belussi et al., 2016). However, coloration in all organs seems to be also correlated with the phylogeny (Franco-Belussi et al., 2009; Provete et al., 2012), but also responds differently to climatic variables depending on the lineage and locality in which species occur (Franco-Belussi et al., 2017).

Delmore et al. (2018), in a study of bat testicular pigmentation, called the melanin containing-cells in the tissues surrounding the gonads 'reproductive melanin', and the state of possessing this trait 'reproductive melanisation'. They suggested an association with sperm production or protection. In anurans, as in most tetrapods, reproductive melanisation seems to be restricted mainly to the testes (e.g., Guillette et al., 1983; Faivovich, 2002; De Oliveira et al., 2002; De Oliveira et al., 2003; De Oliveira & Zieri, 2005; Zieri et al., 2007; Franco-Belussi et al., 2009; Provete et al., 2012; Goldberg et al., 2020). When present in adults, testicular pigmentation begin to accumulate during the differentiation of the testes and it seems to be correlated with germinal cell differentiation (Goldberg et al., 2020). In those species with a fast rate of testicular differentiation, melanisation began at very early premetamorphic stages and increased at a continuous rate up to the juvenile period, when it acquired its maximum density (Goldberg et al., 2020). However, there is a mention of ovarian pigmentation in the ovarian surface of some specimens of Dendropsophus labialis (Pinto-Erazo et al., 2016), and a "black pigment" has been described inside degenerative germinal cells in adult anuran ovaries (Ogielska et al., 2010).

The comparative ontogeny of gonadal pigmentation is largely unstudied (but see Guillette et al., 1983; Goldberg et al., 2020). This is even more notable in ovaries. This lack of knowledge might be due to the paucity of studies that have focused on describing the ontogeny of melanin pigmentation. As a part of an ongoing research project describing and comparing gonadogenesis in different anuran species, we observed that ovaries of the lesser swimming frog *Pseudis minuta* presented a degree of melanisation during their development.

Pseudis minuta (Hylidae) is an aquatic species that inhabits temporary ponds in north-eastern Argentina, Uruguay and extreme southern Brazil (Frost, 2020). Highly pigmented testes that begin to accumulate melanocytes at premetamorphic stages have been described (Goldberg et al., 2020). Together with other species of the genus (*P. paradoxa* and *P. platensis*), *P. minuta* exhibits a highly accelerated rate of germ cell development and it has been hypothesised that the juvenile stage is included within their prolonged delayed

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Figure 1. Ovarian differentiation in *P. minuta.* a, d. Stage 29 (-Stage I). (a) external morphology with pigmented cells on the ovarian surface (white arrowheads); (d) differentiated ovary with a distinct central lumen (asterisk), and primordial germ cells in mitotic division placed in the cortex. b, e. Stage 32 (-Stage II). (b) external lobulation evident; (d) the ovarian cavity enlarged with the cortex formed by several layers of oocytes. A few melanocytes are evident among oocytes (black arrows). c, f. Stage 36 (-Stage III). Ovaries were externally pigmented (white arrowheads) whereas, histologically (f), few scattered melanocytes (black arrows) were located between the oocytes. g, j. Stage 42 (-Stage V). Ovarian size increase was attributable to an increase in the number and size of diplotene oocytes. More melanocytes can be distinguished among the oocytes. h, k. Stage 45 (-Stage X). By the end of metamorphosis external pigmentation increased. Melanocytes appeared located without a clear pattern. i, l. One-year-old postmetamorphic specimen. Externally, both ovaries appear highly pigmented whereas a few melanocytes can be found between maturing oocytes. do = diplotene oocytes. fb = fat bodies. k = kidney. ovd = oviduct. Scale bar equal to 1 mm in a, b, c, g, h, i; 50 μm in d; and 100 μm in e, f, j, k, l.

metamorphosis (Downie et al., 2009; Fabrezi et al., 2010; Goldberg et al., 2016). Sexual maturity is achieved in males as soon as the metamorphic period ends (Fabrezi et al., 2010; Goldberg et al., 2016).

Ontogenetic changes in ovarian pigmentation is a feature that has never been described before in any other anuran species. In this context, here we report for the first time the ontogenetic changes related to this feature in *P. minuta* and in pinpointing it, represent an important step in the comprehension of the ontogeny of gonadal pigmentation.

We studied larval (N = 29) and postmetamorphic (N = 8) specimens of *P. minuta*. Larval development was staged according to Gosner (1960). However, the approximate Fabrezi et al. (2009) Stage is also given for comparative purposes (~followed by the stage numbers

in Roman numerals). We used this table because it recognises, in P. platensis, several more metamorphic stages than those described in the commonly used table of Gosner (1960). Specimens were also classified as premetamorphic (up to Gosner stage 36; N = 9), prometamorphic (between stages 37 and 41; N = 10), metamorphic (between stages 42 and 45; N = 10), juvenile (without mature secondary sexual characters; N = 2), or adult (with mature gonads and secondary sexual characters; N = 5) following Etkin (1936) and Quinzio et al. (2015). Specimens were euthanised in an aqueous solution of chloretone, and fixed in 10 % formalin. Adults were then preserved in 70 % alcohol. All specimens were collected in temporary and semitemporary ponds near the interception of El Pescado River and Street 31 (35°1'19.81"'S, 57°51'10.11"W), La Plata department, and in Punta Indio (35°35'39.3"S, 57°29'07.41"W), Punta Indio department, both in province of Buenos Aires, Argentina. Larval specimens, accessioned as lots, and adult specimens, accessioned with individual numbers, are deposited in the Herpetological Collection of the Instituto de Bio y Geociencias (IBIGEO)-CCT-CONICET Salta with the following catalogue numbers and collecting dates: IBIGEO-A 1581 (08/12/2011), 1582 (15/12/2011), 1578 (17/06/2006), 1593 (16/10/2014), 1594 (16/10/2014), 1997 (16/10/2014), 1998 (16/10/2014), and 1599 (22/10/2014). This research adheres to The British Herpetological Society's Ethical Policy and Guidelines (British Herpetological Society, 2017). Specimen collection permits were issued by the Secretaría deFauna y Flora, Gobierno de la Provincia de Buenos Aires, Argentina (Res. 319/10 and 42/11).

Data were obtained from the following sources: (i) Manual dissection of larval and postmetamorphic specimens to describe changes in gonads. (ii) Histological sections of ovaries. To do this, ovaries were separated from preserved specimens, dehydrated, embedded in paraffin, and sectioned at 6 μ m. Sections were stained with hematoxylin and eosin. The age of postmetamorphic specimens were estimated by two independent observers by counting the number of lines of arrested growth (LAGs) in transverse section of phalangeal bones of toe IV, following Hemelaar (1986).

Early during premetamorphosis, at Gosner stage 29 (-Stage I), the first sign of ovarian differentiation is discernible with the incipient lobulation of the cords (Fig. 1a). Scattered pigmented cells appeared distributed on the midline surface of one or both ovaries (Fig. 1a). Histologically, a cortico-medullary structure is clearly, with a cortex composed of primordial germ cells and darkly stained somatic cells (Fig. 1d).

By stages 32-33 (-Stage II), the ovaries begin to show their distinctive morphology as they become divided into lobules (Fig. 1b). Melanocytes still occupy the midline of the ovarian surface (Fig. 1b). Histologically, the cortex is mainly composed of previtellogenetic primary oocytes (Fig. 1e). Each oocyte appear individualised and surrounded by proliferating prefollicular cells (Fig. 1e). A few melanocytes appear between the oocytes without a clear pattern (Fig. 1e).

By stage 36-37 (-Stage III), gonad size increases with an increasing number of germ cells (Fig. 1c, f). The

degree of lobulation, is more defined than in previous stages. Pigmentation, when present, appears as scattered brownish cells giving the organ a faint surface coloration (Fig. 1c). In some cases, the pigmentation is asymmetrical, with one ovary more pigmented than the other (Fig. 1c). In light microscopy, each oocyte appears individualised and surrounded by proliferating prefollicular cells (Fig. 1f). By these stages, a few spots of melanocytes accumulation are also discernible in the spaces between oocytes (Fig. 1f).

At the beginning of metamorphosis (Gosner stage 42; -Stage V), the ovaries continue to grow in size (Fig. 1g). Melanocytes are located all around the ovaries surface (Fig. 1g). Oocytes increase in size and present a highly basophilic cytoplasm and a larger nucleus with numerous nucleoli (Fig. 1j). Melanin containing-cells are more evident than in previous stages among oocytes but without a clear pattern, being located randomly within the germinal epithelium or in the stroma (Fig. 1j).

By the end of metamorphosis (Gosner stages 44-45; -Stages IX-X), lobules appear much wider than in previous stages (Fig. 1h). The presence of several pigmented cells gives a darker, like dotted brown, colour to the structure (Fig. 1h). Histologically, pigmentation remains as dark accumulations of melanocytes unevenly distributed around among oocytes (Fig. 1k). No pigmentation is observed in the oocytes in this phase. The external appearance of scattered melanocytes remains in juvenile and adult ovaries (Fig. 1i). Individual lobules contained developing oocytes of variable size in stages I to IV (sensu Dumont, 1972), encircled by follicular cells and a theca (internal and external) (Fig. 1l).

Ogielska & Kotusz (2004) distinguished three types of developmental rates of anuran ovaries and germ cells relative to somatic development: basic, retarded, and accelerated. In P. minuta, the ovarian cavity and diplotene oocytes are evident at premetamorphic stages; therefore the rate of ovarian differentiation of this species is accelerated. The same condition was described in Euphlyctis cyanophlyctis (Phuge & Gramapurohit, 2013), Pseudis paradoxa (Downie et al., 2009), Scinax fuscovarius (Goldberg, 2015), Microhyla ornata (Mali & Gramapurohit, 2015), Dendropsophus labialis (Pinto-Erazo et al., 2016) and in several ranid species (Ogielska & Kotusz, 2004; Gramapurohit et al., 2000). However, none of these species, with the exception of D. labialis, showed evidence of melanocytes on the surface or between oocytes.

Pigmented cells in anuran gonads have only been reported in testis of several species (De Oliveira et al., 2002, 2003; De Oliveira & Zieri, 2005; Zieri et al., 2007; Franco-Belussi et al., 2009), whereas in two bufonid species, *Rhinella diptycha* and *R. icterica*, pigmented cells were observed in the medullar region of the Bidder's organ (Farias, Carvalho-e-Silva & Brito-Gitirana, 2002; Silberschmidt Freitas et al., 2015). The only previous report in anuran ovaries only refer to those cases in *Dendropsophus labialis*, with no melanocytes among oocytes (Pinto-Erazo et al., 2016). In other vertebrates, ovarian pigmentation is also a rare trait and a review of the literature resulted in a single report in the Chinese silky fowl (*Gallus gallus domesticus* Brisson), which has hyperpigmentation in several organs (Muroya et al., 2000).

In P. minuta, melanocytes begin to accumulate in testes and ovaries during premetamorphosis (Goldberg et al., 2020; this study). All prometamorphic specimens had pigmented testes whereas ovaries showed a variable pattern with both or one ovary pigmented. At metamorphic climax, the testes had acquired their final ovoid shape and all specimens had highly pigmented testes whereas ovaries, although less pigmented than testes, present a large number of melanocytes (Goldberg et al., 2020). However, not all species with highly pigmented testis presents pigmented ovaries as we have seen in different species such as Physalaemus biligonigerus and Lysapsus limellum (Goldberg pers. obs.). In fact, Pseudis minuta, and those cases in D. labialis, are the only anuran species with this characteristic described so far. Interestingly, both species present an accelerated rate of ovarian differentiation (Pinto-Erazo et al., 2016).

Many anuran species produce pigmented eggs during vitellogenesis (Altig & McDiarmid, 2007), including P. minuta. In these mature oocytes, the animal hemisphere becomes dark brown with the deposit of melanin and the vegetal hemisphere remains an opaque, light yellow (Uribe Aranzábal, 2011). In some anurans species, the presence of dark pigment occurs in oocytes during degeneration (Ogielska et al., 2010). Melanin present in ovarian tissue could have functions related to protection of tissue against free radicals or potentially toxic agents produced during the process of cell degeneration (Zuasti et al., 1998; McGraw, 2005). However, ovarian and oocyte pigmentation seem to be two independent events, being the former possibly stimulated by steroids and the latter by gonadotropins (Jørgensen, 1992; Uribe Aranzábal, 2011). It is well known that melanocytes are capable of transferring melanin granules to epidermal cells (Nordlund et al., 1989), and therefore the source of oocyte could in theory follow that process. However, oocyte pigmentation is widespread among anuran species, but ovarian melanocytes are rare, indicating a different process.

The description of the ovarian differentiation in *P. minuta* presented here allowed the recognition for the first time in this species of reproductive melanisation in anuran ovaries. It is interesting to note that, regardless of sex, melanocytes can accumulate in gonads in a process regulated by an endocrine pathway (Zieri et al., 2015). Even when its function is unclear, and a relationship with the fast acquisition of sexual maturity must be further explored, it is evident that the present report represents a useful starting point to study a trait that in the past might have been unnoticed.

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REFERENCES

- Altig, R. & McDiarmid, R.W. (2007). Morphological diversity and evolution of egg and clutch structure in amphibians. *Herpetological Monographs* 21, 1–32.
- Agius, C. & Agbede, S.A. (1984). An electron microscopical study on the genesis of lipofuscin, melanin and haemosiderin in the haemopoietic tissues of fish. *Journal of Fish Biology* 24, 471–488.
- Breathnach, A.S. (1988). Extra-cutaneous melanin. *Pigment Cell Research* 1, 234–237.
- British Herpetological Society. (2017). Ethical Policy and Guidelines. *The Herpetological Bulletin* 141, 46–48.
- De Oliveira, C., Santana, A.C., Omena, P.M., Santos, L.R.S. & Zieri, R. (2003). Morphological considerations on the seminiferous structures and testes of anuran amphibians: *Bufo crucifer*, *Physalaemus cuvieri* and *Scinax fuscovarius*. *Biociencias* 11, 39–46.
- De Oliveira, C., Zanetoni, C. & Zieri, R. (2002). Morphological observations on the testes of *Physalaemus cuvieri* (Amphibia, Anura). *Revista Chilena de Anatomía* 20, 263–268.
- De Oliveira, C. & Zieri, R. (2005). Pigmentação testicular em *Physalaemus nattereri* (Steindachner) (Amphibia, Anura) com observações anatomicas sobre o sistema pigmentar extracutâneo. *Revista Brasileira de Zoologia* 22, 454–460.
- Delmore, J.L., Brennan, P.L.R. & Orr, T.J. (2018). Reproductive melanization may protect sperm from harmful solar radiation. *Evolutionary Ecology* 32, 127–139.
- Downie, J.R., Sams, K. & Walsh, P.T. (2009). The paradoxical frog *Pseudis paradoxa*: larval anatomical characteristics, including gonadal maturation. *Herpetological Journal* 19, 1–10.
- Dumont, J.N. (1972). Oogenesis in *Xenopus laevis* (Daudin).
 I. Stages of oocyte development in laboratory maintained animals. *Journal of Morphology* 136, 153–179.
- Etkin, W. (1936). The phenomena of the anuran metamorphosis.III. The development of the thyroid gland. *Journal of Morphology* 59, 68–89.
- Fabrezi, M., Quinzio, S.I. & Goldberg, J. (2009). Giant tadpole and delayed metamorphosis of *Pseudis platensis* Gallardo, 1961 (Anura, Hylidae). *Journal of Herpetology* 43, 228–243.
- Fabrezi, M., Quinzio, S.I. & Goldberg, J. (2010). The ontogeny of *Pseudis platensis* (Anura, Hylidae): Heterochrony and the effects of larval development on postmetamorphic life. *Journal of Morphology* 271, 496–510.
- Faivovich, J. (2002). A cladistic analysis of Scinax (Anura: Hylidae). *Cladistics* 18, 367–393.
- Farias, C.F., Carvalho-e-Silva, S.P. & Brito-Gitirana, L. (2002). Bidder's organ of *Bufo ictericus*: A light and electron microscopy analysis. *Micron* 33, 673–679.
- Fenoglio, C., Boncompagni, E., Fasola, M., Gandini, C., Comizzoli, S., Milanesi, G. & Barni, S. (2005). Effects of environmental pollution on the liver parenchymal cells and Kupffer-melanomacrophagic cells of the frog *Rana esculenta*. *Ecotoxicology and Environmental Safety* 60, 259–268.
- Franco-Belussi, L., Castrucci, A.M. de L. & De Oliveira, C. (2013). Responses of melanocytes and melanomacrophages of *Eupemphix nattereri* (Anura: Leiuperidae) to NIe4, D-Phe7-αmelanocyte stimulating hormone and lipopolysaccharides. *Zoology* 116, 316–324.

- Franco-Belussi, L., De Souza Santos, L.R., Zieri, R. & De Oliveira,C. (2012). Visceral pigmentation in three species of the genus *Scinax* (Anura: Hylidae): Distinct Morphological Pattern. *Anatomical Record* 295, 298–306.
- Franco-Belussi, L., Santos, L.R. de S., Zieri, R. & De Oliveira, C. (2011). Visceral pigmentation in four *Dendropsophus* species (Anura: Hylidae): Occurrence and comparison. *Zoologischer Anzeiger* 250, 102–110.
- Franco-Belussi, L., Nilsson Sköld, H. & De Oliveira, C. (2016). Internal pigment cells respond to external UV radiation in frogs. *Journal of Experimental Biology* 219, 1378–1383.
- Franco-Belussi, L., Provete D. & De Oliveira C. (2017). Environmental correlates of internal coloration in anurans vary throughout space and lineages. *Ecology and Evolution* 7, 9222–9233.
- Franco-Belussi, L., Zieri, R., Santos, L.R. de S., Moresco, R.M. & De Oliveira, C. (2009). Pigmentation in anuran testes: Anatomical pattern and variation. *Anatomical Record* 292, 178–182.
- Frost, D.R. (2020). Amphibian Species of the World: an Online Reference. Version 6.0 (Date of access: 23rd, January, 2020).
 Electronic Database accessible at http://research.amnh. org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA. doi.org/10.5531/ db.vz.0001.
- Gallone, A., Guida, G., Maida, I. & Cicero, R. (2002). Spleen and liver pigmented macrophages of *Rana esculenta L*. A new melanogenic system? *Pigment Cell Research* 15, 32–40.
- Goldberg, J. (2015). Gonadal differentiation and development in the snouted treefrog, *Scinax fuscovarius* (Amphibia, Anura, Hylidae). *Journal of Herpetology* 49, 468–478.
- Goldberg, J., Barrasso, D.A., Agostini, M.G. & Quinzio, S. (2016).
 Vocal sac development and accelerated sexual maturity in the lesser swimming frog, *Pseudis minuta* (Anura, Hylidae).
 Zoology 119, 489–499.
- Goldberg, J., Valverde, B.S.L. & Franco-Belussi, L. (2020). Testicular melanization in anuran species: ontogeny and sexual maturity. *Amphibia-Reptilia* 41, 75–86.
- Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183–190.
- Gramapurohit, N.P., Shanbhag, B.A. & Saidapur, S.K. (2000). Pattern of gonadal sex differentiation, development, and onset of steroidogenesis in the frog, *Rana curtipes. General and Comparative Endocrinology* 119, 256–264.
- Guillette, L.J. Jr., Weigel, J. & Flater, G. (1983). Unilateral testicular pigmentation in the Mexican lizard *Sceloporus variabilis*. *Copeia* 1, 155–161.
- Hemelaar, A. (1986). Demographic study of Bufo bufo L. (Anura, Amphibia) from different climates, by means of skeletochronology (Ph.D. Thesis). University of Nijmegen, Nijmegen, Netherlands.
- Jørgensen, C.B. (1992). Growth and reproduction. Pp. 439– 466, in: Feder, M.E. & Burggren, W.W. (eds). Environmental Physiology of the Amphibians. USA: The University of Chicago Press.

- Mali, P.V., Gramapurohit, N.P. (2015): Pattern of gonadal differentiation and development up to sexual maturity in the frogs, *Microhyla ornata* and *Hylarana malabarica*: a comparative study. *Journal of Experimental Zoology Part A* 323, 666– 678.
- McGraw, K.J. (2005). The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? *Animal Behaviour* 69, 757–764.
- Muroya, S., Tanabe, R., Nakajima, I. & Chikuni, K. (2000). Molecular characteristics and site specific distribution of the pigment of the Silky Fowl. *Journal of Veterinary Medical Science* 62, 391–395.
- Nordlund, J.J., Zalfa, M.D., Abdel-Malek, A., Boissy, R.E. & Rheins, L.A. (1989). Pigment Cell Biology: An Historical Review. *The Journal of Investigative Biology* 92, 535–635
- Ogielska, M. & Kotusz, A. (2004). Pattern of ovary differentiation with reference to somatic development in anuran amphibians. *Journal of Morphology* 259, 41–54.
- Ogielska, M., Rozenblut, B., Augustyńska, R., & Kotusz, A. (2010). Degeneration of germ line cells in amphibian ovary. *Acta Zoologica* 91, 319–327.
- Phuge, S.K. & Gramapurohit, N.P. (2013). Gonadal sex differentiation, development up to sexual maturity and steroidogenesis in the skipper frog, *Euphlyctis cyanophlyctis*. *General and Comparative Endocrinology* 181, 65–71.
- Pinto-Erazo, M.A., Goldberg, J. & Jerez, A. (2016). Gonadal development in the Neotropical high Andean frog *Dendropsophus labialis* (Amphibia: Hylidae). *Cuadernos de Herpetología* 30, 57–68.
- Provete, D.B., Franco-Belussi, L., De Souza Santos, L.R., Zieri, R., Moresco, R.M., Martins, I.A., De Almeida, S.C. & De Oliveira, C. (2012). Phylogenetic signal and variation of visceral pigmentation in eight anuran families. *Zoologica Scripta* 41, 547–556.
- Quinzio, S., Goldberg, J., Quinzio, S.I., Goldberg, J., Cruz, J.C., Pereyra, M.C. & Fabrezi, M. (2015). La morfología de los Anuros: pasado, presente y futuro de nuestras investigaciones. *Cuadernos de Herpetología* 29, 51–67.
- Silberschmidt Freitas; J., Franco-Belussi, L. & de Oliveira, C. (2015). Morphological and histochemical studies of Bidder's organ in *Rhinella schneideri* (Amphibia: Anura) males. *Italian Journal of Zoology* 82, 479–488.
- Uribe Aranzabal, M. C. (2011). Hormones and the female reproductive system of amphibians. Pp. 55–81, in: Norris, D.O. & Lopez, K.H. (eds.). Hormones and Reproduction of Vertebrates. Amphibians. Vol. 2. USA: Academic Press.
- Zieri, R., Taboga, S.R. & De Oliveira, C. (2007). Melanocytes in the testes of *Eupemphix nattereri* (Anura, Leiuperidae): Histological, stereological, and ultrastructural aspects. *Anatomical Record* 290, 795–800.
- Zuasti, A., Jiménez-Cervantes, C., García-Borrón, J.C. & Ferrer, C. (1998). The melanogenic system of Xenopus laevis. *Archives of Histology and Cytology* 61, 305–316.

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