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Visceral pigmentation in four *Dendropsophus* species (Anura: Hylidae): Occurrence and comparison

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Abstract

Amphibians share with other ectothermic vertebrates an extracutaneous pigmentary system consisting of melanin-containing cells in various organs and tissues. This paper describes the interspecific variation in the visceral pigmentation and extracutaneous pigment system in four species of the genus *Dendropsophus* [i.e., *D. elianeae*, *D. minutus*, *D. nanus*, and *D. sanborni* (Anura: Hylidae)]. Fifteen adult males from each species were collected in the region of São José do Rio Preto (State of São Paulo, Brazil), and their visceral pigmentation was analyzed during the reproductive period. The individuals were weighed and measured, and the pigmented visceral cells were classified and documented in photographs. The shape and quantity of the pigment cells differed among the various structures of the same individual, as well as among the same structures of different species. Similarities and differences among these species were observed, and a visceral pigmentation pattern for anatomical structures was detected. In the digestive system of all species analyzed, there was an absence of pigment cells on the stomach and middle intestine. However, the pigmentation of the final portion of the intestine (i.e., the rectum) showed interspecific variation, with *D. minutus* presenting intense pigmentation, whereas the other species presented no pigmentation. Significant differences were detected also in the cardio-respiratory system, mesentery, and lumbosacral peritoneum.

Keywords: Anurans; Extracutaneous pigmentary system; Melanocytes

1. Introduction

Amphibians and other ectothermic vertebrates possess an extracutaneous pigmentary system in various tissues and organs, which comprises cells, whose cytoplasm contains melanin (Gallone et al., 2002). These melanin-containing cells are frequently found in the liver, spleen, kidneys, peritoneum, lung, heart, blood vessels, thymus, gonads, and meninges of fishes, amphibians, and reptiles (Agius, 1980; Agius and Agbede, 1984; Zuasti et al., 1990, 1998; Christiansen et al., 1996; Bagnara and Matsumoto, 2006; Gallone et al., 2002; Zieri et al., 2007; Pederzoli and Trevisan, 1990; Trevisan et al., 1991). These cells are similar to melanocytes (Zuasti et al., 1998; Agius and Agbede, 1984), which are derived from the neural crest (Bagnara and Matsumoto, 2006) and are able to produce and store melanin inside their cytoplasm (Agius and Roberts, 2003). In organs

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with hematopoietic function, there are also cell types with phagocytic activity similar to that of macrophages (Agius, 1980). These cells are derived from hematopoietic stem cells (Sichel et al., 1997), which often conglomerate to form pigmented nodules called melanomacrophage centers (Agius, 1981).

Current knowledge about visceral pigmentation, be its structural features or its diversity, is superficial, with the exception of a few morphological studies in anurans, such as *Physalaemus marmoratus* (Aoki et al., 1969; Oliveira and Zieri, 2005), *Physalaemus cuvieri* (Oliveira et al., 2002, 2003), *Eupemphix nattereri* (Oliveira and Zieri, 2005; Zieri et al., 2007), *Rana esculenta* (Cicero et al., 1989; Sichel et al., 1997; Barni et al., 1999; Gallone et al., 2002; Barni et al., 2002), *Rana ridibunda* (Akulenko, 1998) and, *Xenopus laevis* (Sichel et al., 1997; Zuasti et al., 1998).

Our aim in this study was to characterize the visceral pigmentation in the cardiorespiratory, digestive and urogenital systems and their associated serosal membranes in four tree-frog species of the hylid genus *Dendropsophus* (i.e., *D. elianeae*, *D. minutus*, *D. nanus*, and *D. sanborni*) during their reproductive periods. We also evaluated the intra- and interspecific differences and searched for patterns of occurrence of pigmentation that could help to distinguish related species. Such an approach will increase our knowledge about the extracutaneous pigmentary system in neotropical anurans.

The specimens were treated according to the ethical guidelines of São Paulo State University – UNESP/Botucatu, following the *Guide for Care and Use of Laboratory Animals* (Protocol No. 001/06-CEEA). In the laboratory, the specimens were anesthetized and euthanized by submersion in 20% ethanol. All specimens had the organs of the abdominal cavity exposed by medial incision for macroscopic documentation under a stereomicroscope (Leica-MZ16), using the program Image Manager 5.0 (Version 4.0.5, Media Cybernetics Inc., Bethesda, MD, USA) to capture the images.

2. Materials and methods

Fifteen male specimens of each species (*D. elianeae*, *D. minutus*, *D. nanus*, and *D. sanborni*) were collected in the region of São José do Rio Preto (State of São Paulo, Brazil) during their reproductive period from October 2006 to March 2007. Some of the specimens used were from the Amphibian Collection of the Department of Botany and Zoology (DZSJRP-Amphibia) at the São Paulo State University-IBILCE/UNESP (*D. elianeae:* DZSJRP 6368, 7646, 7890, 7965, 7968–9, 8016, 8037, 8089, 8124, 8474–6, 8232, 8532).

Visceral pigment cells were classified according to the protocol of Franco-Belussi et al. (2009), based on the differences in the intensity of pigmentation present on the gonads of anurans, and applied to other organs and tissues in this study. The pigmentary classification is based on the intensity of pigmentation, ranging from absence (category 0) to entirely pigmented, when an intense black coloration is observed (category 3 = maximal intensity), with the categories 1 and 2 representing a gradual increase in the intensity of pigmentation.

For this study, the protocol of classification for the visceral pigmentation was applied to the following anatomical structures and regions of the specimens as follows: (1) pericardium and blood vessels at the basis of the heart; (2) heart; (3) lungs; (4) stomach; (5) middle intestine; (6) rectum; (7) intestinal mesentery, (8) kidney and renal blood vessels; (9) testes; (10) nerves of the lumbar plexus; (11) lumbosacral parietal peritoneum.

To compare the differences between categories of pigmentation in each organ or region we used the G test for goodness of fit, with the corrections by Yates and Williams (Sokal and Rohlf, 1995). This test was implemented using the code provided by Prof. Peter Hurd available at http://www.psych.ualberta.ca/~phurd/cruft/g.test.r. The test was run using the R software v. 2.11.1 (R Development Core Team, Informer Technology Inc. 2010).

3. Results

Visceral pigment cells in the various organs and membranes of the abdominal cavity varied significantly in their occurrence and distribution among the species analyzed, allowing a classification of this pigmentation according to the intensity of pigmentation on an organ's surface. This pigmentary classification is shown for *D. elianeae*, *D. minutus*, *D. nanus*, and *D. sanborni* in Fig. 1. There is no pigmentation (category 0) on the pericardium, heart, vertebral column, and kidneys of *D. minutus*, and on the lungs of *D. sanborni*. There is an intense black pigmentation (category 3) on the rectum of *D. minutus.* There are a few pigment cells creating only a little pigmentation (category 1) on the pericardium, heart, and vertebral column of *D. nanus* and on the lungs, kidneys, and rectum of *D. elianeae*. There is a large number of pigmented cells, which mask the color of the organ (category 2), on the kidneys of *D. elianeae.* The pigmentation on organs and structures of the abdominal cavity in the four species (*D. elianeae*, *D. minutus*, *D. nanus* and *D. sanborni*) are summarized in Figs. 2–5 and Table 1.

The pigmentation of the pericardium revealed significant interspecific differences (*G*-test: $G = 23.56$; df = 9; $p = 0.005$), heart (*G*-test: $G = 61.93$; df = 9; $p < 0.01$), and lungs (*G*-test: $G = 49.54$; df = 9; $p < 0.01$) (Fig. 2). All 15 individuals of *D. elianeae* presented no visceral melanocytes (category 0) on the pericardium and cardiac blood vessels. In addition, 14 out of 15 individuals of *D. minutus* showed no visceral melanocytes, although one specimen presented only a little pigmentation (category 1). On the other hand, only 8 out of 15 specimens of *D. nanus* and 6 out of 15 specimens of *D. sanborni* presented no pigmentation. Only a little pigmentation (category 1) was found in 6 specimens of *D. nanus* and in 8 specimens of *D. sanborni*, and one specimen of each of those species presented a large number of pigmented cells