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OVARIAN MORPHOLOGY IN TWO SPECIES OF *Pseudonannolene* (DIPLOPODA, ARTHROPODA)

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ABSTRACT

The anatomy of the gametes and reproductive system of millipedes is important in establishing the phylogenetic relationships of this group with other arthropods. The genus *Pseudonannolene* (Spirostreptida) has the most derived male reproductive system among millipedes. In contrast, little is known about the female reproductive system. In this study, we describe the ovarian morphology of two species of *Pseudonannolene*. In both species, the ovaries consisted of a single tube in which oocytes in different developmental stages were observed. These oocytes were randomly distributed throughout the gonad. A region similar to a germarium was present at the anterior extremity of the tube, which suggested the presence of a single apical germination center. Since the pairing of ovarian structures is considered a primitive character, the two species examined here showed the most derived model of ovarian structure described so far among millipedes. This conclusion agrees with similar observations for the male reproductive system of this genus.

Key words: Diplopoda, morphology, ovary, *Pseudonannolene*, reproductive system

INTRODUCTION

The class Diplopoda which was formerly included in the Myriapoda, contains the millipedes known in Brazil as *piolhos-de-cobra, embuás*, *gôngolos* and *caramujis*. There have been few studies of the internal morphology of millipedes, with most of them dealing with Asian species. Analysis of the anatomy of the gametes and reproductive systems of millipedes has generated discussion concerning the phylogenetic relationships of this group with other arthropods [1,4,18].

In most myriapods (except the Symphyla), the ovary is a simple tubular organ that is considered secondary and appears independently as an evolutionary specialization in some groups of the Myriapoda [7,14]. Several derivations of this structure occur in millipedes, such as the paired condition displayed by *Craspedosoma* sp., the morphologically simple but highly organized internal structure of the ovaries in *Orthomorpha*, and the simple, unpaired, sac seen in *Iulus*, in which the germination regions are the only areas of the primary paired condition that remain [7].

In this report, we describe the ovarian morphology of two species of *Pseudonannolene*, a genus restricted to South America and which has the most derived male reproductive system among millipedes [4].

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MATERIAL AND METHODS

Young adult females of *Pseudonannolene tricolor* Brolemann, 1901 (n=7) and *P. tocaiensis* Fontanetti, 1996 (n=12) were collected near the city of Rio Claro and in the cave "Caverna da Toca" in Itirapina, respectively (São Paulo State, Brazil). The specimens were dissected in physiological solution and the ovaries were removed and freed of excess fat bodies. The material was fixed in 4% paraformaldehyde and processed by a routine procedure for embedding in historesin. Sections 7 μm thick were cut on a Sorvall JB-4 Bio Rad microtome and then hydrated and mounted on microscope slides. Once dry, the sections were stained with hematoxylin and eosin. Oocyte development was assessed using the classification of Camargo-Mathias *et al.* [2]: stage I – early oocyte, stage II – intermediary oocyte, stage III – vitellogenic oocyte.

RESULTS

The ovaries of *P. tocaiensis* and *P. tricolor* consisted of a single tube (Fig. 1A-C) located ventrally to the gut and surrounded by a simple epithelium or external membrane (Fig. 1D). This membrane separated the ovary from the surrounding fat bodies, which were well-developed in this region (Fig. 1A,E). Oocytes in different developmental stages were observed along the entire ovary.

These stages varied from early oocytes (stage I in Fig. 1A,C) to well-developed oocytes, in which vitellogenesis was practically complete and the

chorion had been deposited (stage III in Fig. 1E). The distribution of the oocytes bore no relationships to the developmental stages.

The anterior region of the tube which was very likely an extension of the external membrane, contained globular cells with large nuclei that were similar to oogonia (Fig. 2A-C). This arrangement suggested the existence of a single apical germination center. Similar cells undergoing meiotic division were also observed in the external membrane, although fewer in number (* in Fig. 2E), and gradually acquired the appearence typical of type I oocytes (*in Fig. 2D).

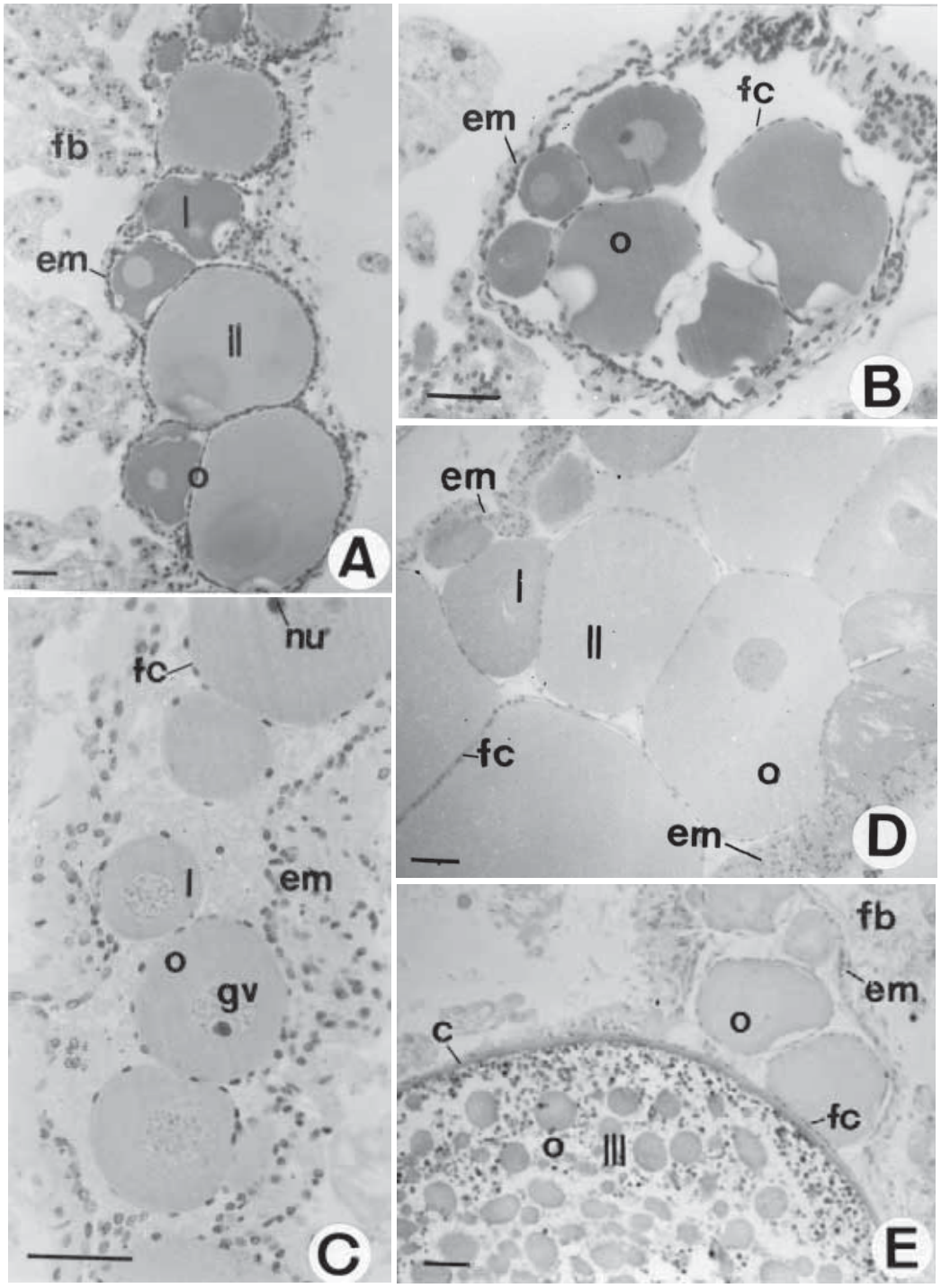

Figure 1. Longitudinal (A, C, D, E) and transversal (B) sections of the ovaries of *Pseudonannolene tocaiensis* (**A, B, C, E**) and *Pseudonannolene tricolor* (**D**) showing the random distribution of oocytes in different stages of development. Note the absence of paired structures. **I** – early oocyte, **II** – intermediary oocyte, **III** – vitellogenic oocyte, **c** – chorium, **fc** – follicular cells, **fb** – fat body, **em** – external membrane, **nu** – nucleolus, **o** – oocyte, **gv** – germination vesicle. HE staining. Bar = 50 µm.

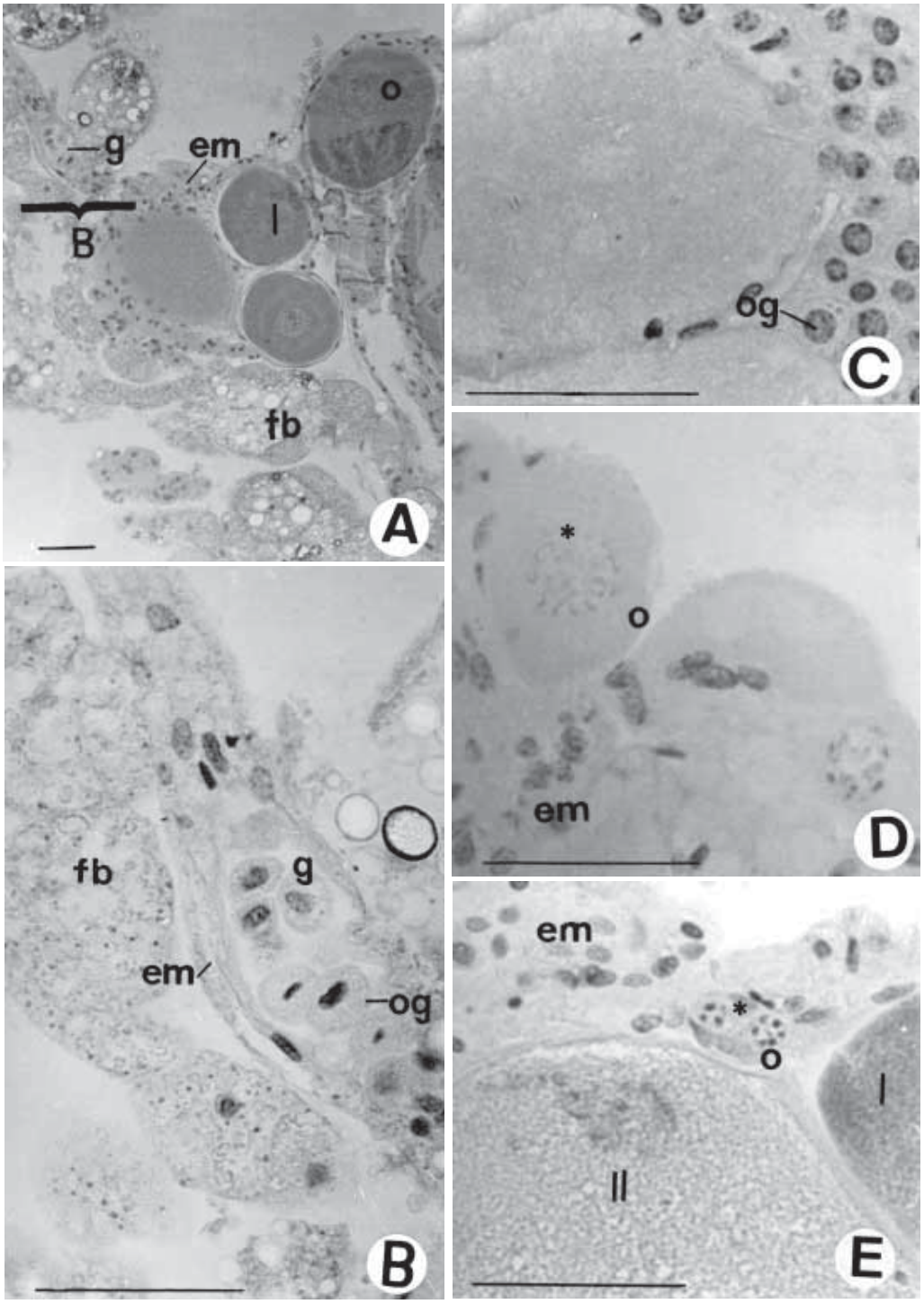

Figure 2. Longitudinal sections of the ovaries of *Pseudonannolene tricolor* (A, B, E) and *Pseudonannolene tocaiensis* (C, D). (**A**) shows the germarium of *P. tricolor* (detailed in **B**) surrounded by the fat body. (**C**) the germarium of *P. tocaiensis.* (**D**) and (**E**) - note the initial oocytes in the external membrane. **I**, **II** – stages of oocytes development, **fb** – fat body, **g** – germarium, **em** – external membrane, **og** – oogonia, **o** – oocyte, (*****) – oocytes undergoing meiosis. HE staining. Bar = 50 µm.

DISCUSSION

The ovaries of millipedes have been described as single [11,13] or paired [3,10,15] structures. In *Jonespeltis splendidus* [12] and *Rhinocricus padbergi* [5], the immature ovaries are paired but gradually lose this symmetry to become a sac containing a single mass of oocytes. This characteristic has been observed in several species of iulids, polydesmids, and penicelates [8,9,13,14,16].

In most millipedes species, the oocytes are arranged in ovisacs that normally retain the paired condition [7,11,17]. As shown here for *P. tocaiensis* and *P. tricolor*, the oocytes are not grouped but are distributed at random. This arrangement also occurs in *Cathamicrophyllum* sp. [6,16].

No sign of pairing was seen among the ovarian structures of *P. tocaiensis* and *P. tricolor*, neither at the beginning of ovary development nor in the region of the germarium. Since pairing of the ovarian structures is considered a primitive characteristic in this group [7,14], we suggest that these two species show the most derived ovarian model described for millipedes. In addition to not showing "Iulus type" pairing [7] during development of the oocytes, these species also lacked pairing of the germination regions. Thus, the ovarian condition found in *P. tocaiensis* and *P. tricolor* can be considered a step towards the derivation of this system. A similar condition was observed in the morphology of the reproductive system of male *Pseudonannolene* [4], with this genus having the most derived testicular arrangement among millipedes.

Kubrakiewcz [9] described oogonia at the germination sites as an oocyte buried within a cluster of cells in the germarium of the ovarian wall. *P. tocaiensis* and *P. tricolor* also contained cells under development in the ovarian wall, but most of these were in the stage of oocyte differentiation, whereas the oogonia were observed in higher numbers at the extremities of the ovary.

Nadarajalingam and Thanumalaya [11] distinguished three regions in the ovaries of some iulids and spirostreptids: an apical germination zone, a middle pre-vitellogenic zone and a terminal vitellogenic zone. However, Yahata and Makioka [18] reported that in Penicelatta the germarium contained oogonia, pre-vitellogenic oocytes, and a vitellarium which had the most developed oocytes. No pre-vitellogenic or vitellogenic zones were seen in *P. tocaiensis* and *P. tricolor*. Rather, oocytes were found in all stages of development, but without any specific or well-defined distribution. The random distribution of oocytes has also been observed in *Iulus*, in contrast to *Orthomorpha*, in which there is a regular pattern of distribution [7]. These results indicate an unusual ovarian organization in *Pseudonannolen*e.

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REFERENCES

1. Baccetti B, Dallai R (1978) The evolution of myriapod spermatozoa. *Abh. Verh. Naturw. Ver. Hamburg* **21**, 203-217.
2. Camargo-Mathias MI, Fontanetti CS, Micó-Balaguer E (1998) Histochemical studies of *Rhinocricus padbergi* Verhoeff ovaries (Diplopoda, Spirobolida, Rhinocricidae). *Cytobios* **94**, 169-184.
3. Fabre L (1855) Recherches sur l'anatomie des organes reproducteurs et sur le développement des myriapodes. *Ann. Sci. Nat. (Zool.)* **3**, 256-320.
4. Fontanetti CS (1990) Morphology of testicles of some Brazilian species of Diplopoda and their phylogenetic relations. *Rev. Bras. Zool*. **7**, 541-544.
5. Fontanetti CS, Staurengo da Cunha MA (1993) Morfologia ovariana e desenvolvimento dos ovócitos de *Rhinocricus padbergi* Verhoeff (Diplopoda, Spirobolida, Rhinocricidae). *Rev. Bras. Zool.* **53**, 7-12.
6. Gealekman O, Tichomirova E, Warburg M (1996) Phenology and reproduction of the iulid millipedes *Catamicrophyllum* spp inhabiting the Mediterranean region in northern Israel. *Isr. J. Zool.* **42**, 66.
7. Kubrakiewicz J (1987) The ovary structure in two species of millipedes, *Iulus scandinavius* and *Orthomorpha gracilis* (Myriapoda, Diplopoda). *Zool. Polon.* **34**, 251-260.
8. Kubrakiewicz J (1991a) Ultrastructural investigation of the ovary structure of *Ophyiulus pilosus* (Myriapoda, Diplopoda). *Zoomorphology* **110**, 133-138.
9. Kubrakiewicz J (1991b) Ovary structure and oogenesis of *Polyxenus lagurus* (L.) (Diplopoda: Pseulaphognatha). An ultrastructural study. *Zool. Jb. Anat.* **121**, 81-93.
10. Miley HH (1930) Internal anatomy of *Euryurus erytropygus* (Brandt, Diplopoda*). Ohio J. Sci*. **40**, 229-254.
11. Nadarajalingam K, Thanumalaya P (1984) Oogenesis in a millipede *Spirostreptus asthenes* (Myriapoda, Diplopoda). *Zool. Anz.* **3/4**, 229-239.
12. Nair VSK (1981) Oocyte development and vitellogenesis in *Jonespeltis splendidus* (Myriapoda: Diplopoda). *J. Anim. Morphol. Physiol*. **28**, 186-194.
13. Newport B (1841) On the organs of reproduction and the development of the Myriapoda. *Phil. Trans. R. Soc. of London,* 99-130.
14. Sareen ML, Adiyodi KG (1983) Arthropoda-Myriapoda. 1. Oogenesis, oviposition and oosorption. In: *Reproductive Biology of Invertebrates* (Adiyodi KG, Adiyodi RG, eds). pp. 497-520. J. Wiley & Sons: Chichester.
15. Seifert B (1932) Anatomie und Biologie des Diplopoden *Strongylosoma pallipes Oliv. Zeits. f. Morph. Okol. Tiere* **25**, 362-507.
16. Warburg MR, Gealekman O (2000) Ovarian structure and oogenetic cycle in the millipede *Catamicrophyllum caifanum* (Diplopoda: Iulidae). *J. Zool. (London)* **250**, 79-85.
17. Warburg MR, Rosenberg M (1994) Ovarian structure in a millipede*, Eumastigonus sp*. (Diplopoda; Cambalidae). *N. Z. J. Zool.* **25**, 301-306.
18. Yahata K, Makioka T (1994) Phylogenetic implications of structure of adult ovary and oogenesis in the penicillate diplopod *Eudigraphis nigricans* (Miyosi) (Diplopoda, Myriapoda). *J. Morphol*. **222**, 223-230.

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