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Comparative Study of Ovarian Structures in Polydesmid Diplopods (Diplopoda: Polydesmida) with Special Reference to the Arrangement of Germ Cells

Kensuke YAHATA

Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan
E-mail: yahata@biol.tsukuba.ac.jp

Abstract

The ovarian structures of four polydesmid diplopods (*Nedyopus tambanus*, *Oxidus gracilis*, *Epanerchodus orientalis*, and *Eucondylodesmus elegans*) are described with particular attention paid to the arrangement of germ cells within the ovary. A pair of longitudinally oriented germ zones, containing oogonia and connecting growing oocytes via follicle epithelia, were present within the dorso-lateral region of these polydesmid ovaries. Within each germ zone, oogonia and growing oocytes were arranged according to their volume, with oogonia and younger oocytes located at the medial ends, and larger oocytes located more laterally. In some species, ovarian epithelia of paired germ zones formed paired folds that extended toward the ovarian lumen and made paired longitudinal grooves on the dorsal surface of the ovary along both sides of its median axis. Orderly arrangement of germ cells is regarded as a distinct common ovarian feature among polydesmid diplopods. The germ zone folds likely enable many oocytes to develop at once.

Introduction

The class Diplopoda is divided into two subclasses, the Penicillata and the Chilognatha. The former is regarded as the more primitive subclass with a single order, while the latter is more advanced and is composed of 13 or 14 orders (Enghoff, 1984).

Previous studies have suggested that the ovarian structure of chilognathan diplopods can be characterized by the following features: 1) a single long, sac-like ovary; 2) a lateral pair of longitudinal germ zones for oogonial proliferation and oocyte growth within the ovarian epithelium; and 3) follicular connections between the growing oocytes in the ovarian lumen and the germ zones (on the Glomerida: Yahata and Makioka, 1997; on the Spirostreptida: Sareen, 1967; on the Julida: Kubrakiewicz, 1987, 1991a, 1991b; on the Polydesmida: Seifert, 1932; Kubrakiewicz, 1987). Despite the presence of these distinguishing characteristics, controversy remains regarding the basic ovarian structure in polydesmid diplopods. Previous studies have been consentaneous in their assertion that polydesmid diplopods display "paired (tubular) ovaries" accompanied by growing oocytes on their outer surfaces and surrounded by a "single common ovitube" (Versluys and Demoll, 1923; Radhaklishnan and Adiyodi, 1977; Nair, 1981). Such composition is peculiar among chilognathan diplopods.

In addition to the characteristics of the polydesmid ovary mentioned above, Kubrakiewicz (1987) observed the presence of an orderly arrangement of oocytes oriented according to oogenetic growth in the ventro-laterally paired germ zones of a polydesmid, *Oxidus gracilis* (formerly *Orthomorpha gracilis*). A similar orderly arrangement of germ cells according to volume was observed in the ovary of a glomerid diplopod, *Hyleoglomeris japonica*, (Yahata and Makioka, 1997); however, according to cladistic analysis by Enghoff (1984), the Glomerida are one of the most primitive chilognathan orders and are only distantly related to the more advanced Polydesmida. Despite previous research by Kubrakiewicz (1987), detailed information regarding the location and arrangement of germ cells in polydesmid ovaries is still lacking.

The present study describes the ovarian structure in four polydesmid species. The order Polydesmida is composed of four suborders: the Chelodesmidea, the Paradoxosomatidea, the Polydesmidea and the Dalodesmidea (Hoffman, 1979). In previous studies, three paradoxosomatidean species (Seifert, 1932; Radhaklishnan and Adiyodi, 1977; Nair, 1981; Kubrakiewicz, 1987) and a polydesmidean species (Versluys and Demoll, 1923) have been examined. The present study examines two paradoxosomatidean species, including those examined by Kubrakiewicz (1987), *Nedyopus tambanus* and *Oxidus gracilis*, and two polydesmidean species from different

families, *Epanerchodus orientalis* (Polydesmidae) and *Eucondylodesmus elegans* (Doratodesmidae). Special attention is paid to the arrangement of germ cells within ovaries in order to reveal any features common among polydesmids.

Materials and Methods

Adult of *Nedyopus tambanus* (Attems) were collected from a vegetable garden in Nanto City, Toyama Prefecture. Adults and subadults of *Oxidus gracilis* (Koch) were collected from leaf litter at a site located at Mt. Tsukuba, Ibaraki Prefecture. Adults of *Epanerchodus orientalis* (Attems) were collected from leaf litter gathered in Nanto City, Toyama Prefecture, and Ueda City, Nagano Prefecture. Adults of *Eucondylodesmus elegans* Miyosi were collected from leaf litter gathered in Iwaki City, Fukushima Prefecture.

Adult females of the four species were fixed in Bouin's solution for a few days after excision of the head parts with a razor blade in a physiological saline solution. Fixed specimens were decalcified in a 5% formic acid, 70% ethanol solution for a few days, dehydrated in a graded ethanol-*n*-butanol series, and cleared with 100% methyl benzoate. After being embedded in paraffin, serial sections of 5 μm were stained using either Mayer's hematoxylin and eosin, Heidenhain's azan, or McManus's alcian blue–periodic acid Schiff (PAS)–hematoxylin technique.

Results

Nedyopus tambanus *and* Oxidus gracilis

Nedyopus tambanus and *Oxidus gracilis*, both belonging to the family Paradoxosomatidae, displayed very similar structures in the reproductive systems of adult females.

The adult ovary in both species was comprised of a single median, long, sac-like organ running between the alimentary canal and the ventral nerve cord. The ovary of *N. tambanus* was present within the fifth through the 19th body segments, while that of *O. gracilis* was present within the fourth through the 18th segments. The ovarian wall of both species, which surrounded a wide ovarian lumen, consisted of an ovarian epithelium ranging from 1–4 μm in thickness (Figs. 1–4). A pair of germ zones was found in the dorso-lateral region of the ovary in both species (Figs. 1, 3). The ovarian epithelium of each germ zone formed a shallow and narrow fold that extended toward the ovarian lumen (Figs. 1–4), creating a longitudinal groove on the surface of the ovary (arrows in Figs. 1–4).

Oogonia and several growing oocytes were arranged in order of increasing volume starting at a medial position and continuing into the lateral region of each folded germ zone (Figs. 1–4). Within each folded germ zone, oogonia and younger oocytes were located along the medial end, nearest to the dorsomedian line of the ovary; larger oocytes were located along the fold of the germ zone, first towards the innermost region of the ovary, and then moving more laterally; and the largest oocytes were located nearest to the lateral end of each germ zone (Figs. 2, 4).

Oogonia approximately 8 μm in diameter, together with several very early previtellogenic oocytes less than 10 μm in diameter, were observed among germ zone epithelial cells (Figs. 2, 4). Growing oocytes larger than 10 μm in diameter were surrounded by a layer of extremely flattened follicle cells. The follicular epithelium of these oocytes was connected with the ovarian epithelium of the germ zones. Early previtellogenic oocytes larger than 20 μm in diameter had consistently strong basophilic ooplasm and one or two distinct nucleoli in their large germinal vesicles (Figs. 2, 4). Larger oocytes in late previtellogenic and vitellogenic stages were located more laterally, far from the oogonial area, in each germ zone.

Epanerchodus orientalis

The adult ovary in *Epanerchodus orientalis* was comprised of a single median, long, wide and sac-like organ extending from about the fourth to the 19th body segment. The ovarian wall, by which a wide ovarian lumen was surrounded, consisted of an ovarian epithelium ranging from 1–7 μm in thickness (Figs. 5, 6). Ovarian epithelial cells were somewhat flattened, each with a spheroidal nucleus approximately 6 μm in diameter (Fig. 7).

A pair of germ zones was found in the dorso-lateral region of the ovary (Fig. 5). The ovarian epithelium of each germ zone formed a deep and wide fold that extended toward the ovarian lumen, creating a longitudinal groove on the surface of the ovary (arrows in Figs. 5, 6).

Within each germ zone, oogonia and several growing oocytes were arranged in order of increasing volume. Oogonia as well as the smallest oocytes were located along the medial end of the germ zone, nearest to the dorsomedian line of the ovary; larger oocytes were oriented along the germ zone, first towards the innermost region of the ovary, then moving laterally, towards the lateral end of the germ zone; and the largest oocytes were located at the most dorso-lateral position, opposite side to the oogonial area (Figs. 5, 6). Oogonia approximately 10 μm in diameter contained a nucleus with basophilic chromatin granules but lacked a nucleolus (Fig. 7). These oogonia were only found in the region of the germ zone nearest to the dorsomedian line of the ovary (Fig. 6). Several early previtellogenic oocytes, less than 20 μm in diameter with a distinct nucleolus in the germinal vesicle, were also found within this same part of the germ zone (Fig. 7).

Growing oocytes larger than 30 μm in diameter were surrounded by a thin layer of follicle epithelium. These folliculated oocytes were connected to each germ zone via their follicles (Fig. 8). Early previtellogenic oocytes larger than 30 μm in diameter had a distinct nucleolus in their

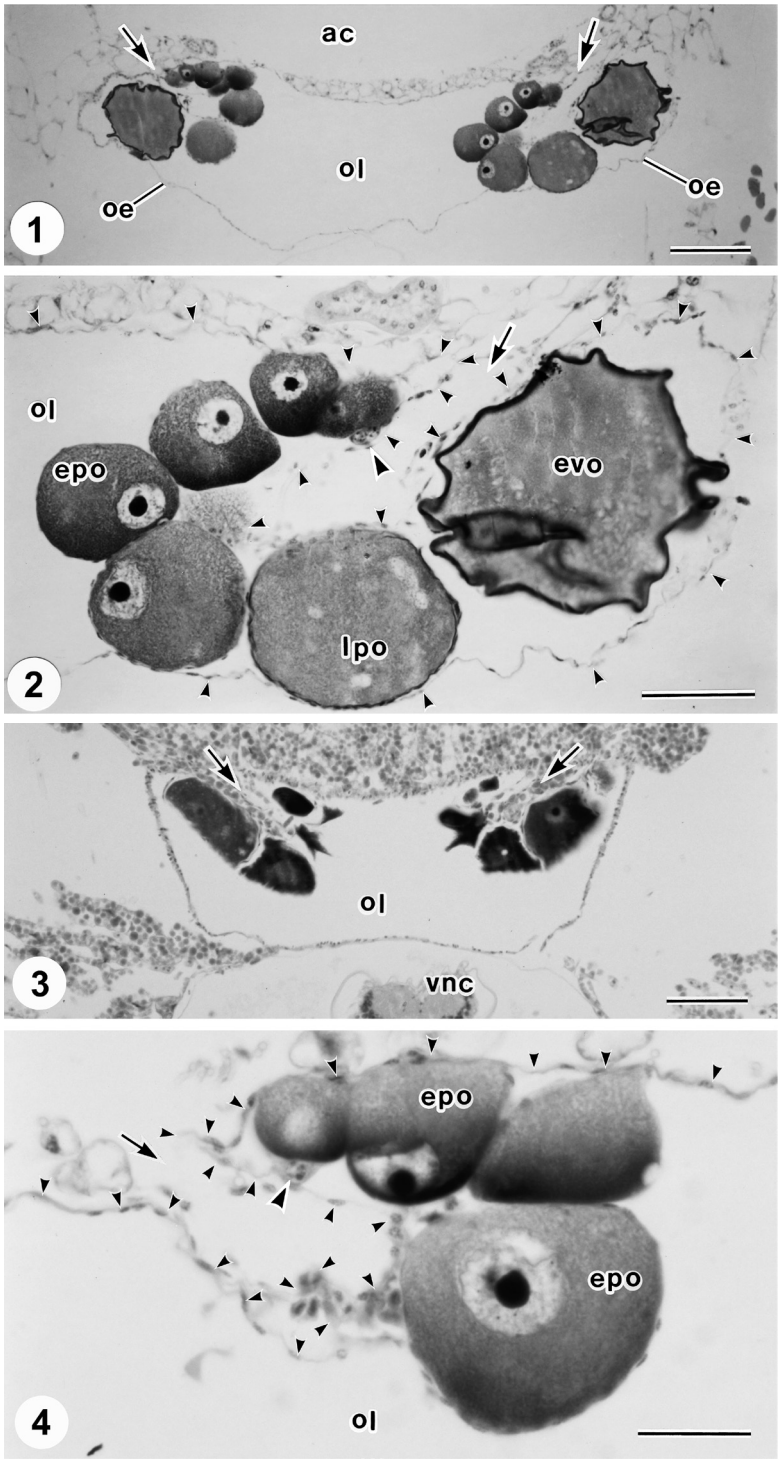


Fig. 1 Cross-section of adult ovary in *Nedyopus tambanus*. Dorsal side is oriented towards top of the image. Specimen prepared using hematoxylin and eosin staining. Section shows paired germ zones. Ovarian epithelium of each germ zone forms a shallow and narrow fold that extends towards the ovarian lumen. Arrows indicate the narrow openings of these folds, which create a pair of longitudinal grooves on the dorsal surface of the ovary. ac: alimentary canal, oe: ovarian epithelium, ol: ovarian lumen. Scale = 100 μm.

Fig. 2 Cross-section of adult ovary in *Nedyopus tambanus*. Dorsal side is oriented towards top of the image. Magnification of the right germ zone shown in Fig. 1. Small arrowheads indicate ovarian epithelium. Arrow indicates a narrow opening of the germ zone fold, which creates a longitudinal groove on the dorsal surface of the ovary. Large arrowhead indicates position of oogonia. Growing oocytes are arranged in order of increasing volume. epo: early previtellogenic oocyte, evo: early vitellogenic oocyte, lpo: late previtellogenic oocyte, ol: ovarian lumen. Scale = 50 μm.

Fig. 3 Cross-section of adult ovary in *Oxidus gracilis*. Dorsal side is oriented towards top of the image. Specimen prepared using hematoxylin and eosin staining. Section shows paired germ zones. Ovarian epithelium of each germ zone forms a shallow and narrow fold that extends towards the ovarian lumen. Arrows indicate narrow openings of these folds, which create a pair of longitudinal grooves on the dorsal surface of the ovary. ol: ovarian lumen, vnc: ventral nerve cord. Scale = 50 μm.

Fig. 4 Cross-section of young adult ovary in *Oxidus gracilis*. Dorsal side is oriented towards top of the image. Specimen prepared using hematoxylin and eosin staining. Extended part of left germ zone shows growing oocytes arranged in order of increasing volume. Small arrowheads indicate ovarian epithelium. Arrow indicates a narrow opening of the germ zone fold, which creates a longitudinal groove on the dorsal surface of the ovary. Large arrowhead indicates position of oogonia. epo: early previtellogenic oocyte, ol: ovarian lumen. Scale = 10 μm.

large germinal vesicle as well as weakly basophilic ooplasm. In late previtellogenic oocytes larger than 80 μm in diameter, a number of very small lipid droplets appeared within the ooplasm. These larger oocytes were located more laterally, far from the oogonial area, in each germ zone.

Eucondylodesmus elegans

The adult ovary in *Eucondylodesmus elegans* was composed of a single median long and sac-like organ, extending from about the fourth through to the 17th body segment. Ovarian epithelium ranging from 1–2 μm in thickness surrounded a wide ovarian lumen (Fig. 9). A pair of germ zones was found in the dorso-lateral region of the ovary (Fig. 9). Oogonia with spherical nuclei approximately 4 μm in diameter were found along the medial end of each germ zone (Fig. 10). Several growing oocytes larger than 10 μm in diameter were surrounded by a layer of follicle epithelium. These folliculated oocytes were connected to the germ zones via their follicles (Fig. 11). Within each germ zone, oogonia and several growing oocytes were arranged in order of increasing volume starting at a medial position and continuing into the lateral region (Figs. 9, 10). No folded structures were observed around either of the germ zones, resulting in the surface of the ovary having a smooth appearance.

All specimens of *E. elegans* examined in the present study were young and no information regarding vitellogenic growth of oocytes could be obtained. Only approximately 40–50 oocytes in late previtellogenic stages of oogenetic development were present within each ovary.

Discussion

Comparison of ovarian structure within polydesmid diplopods

Previous studies (Versluys and Demoll, 1923; Radhaklishnan and Adiyodi, 1977; Nair, 1981) have described some polydesmid ovaries as "paired (tubular) ovaries" that connect various sizes of developing oocytes via their outer surfaces while being surrounded by "single common ovitube" epithelium that is continuous with the oviductal epithelium. The present study, on the other hand, noted ovarian epithelia of paired germ zones forming a pair of deep folds that extended toward the ovarian lumen and created paired longitudinal grooves running along both sides of the median axis on the outer dorsal surface of the ovary. It is possible that previous studies have misidentified these paired germ zone folds as "paired tubular ovaries"

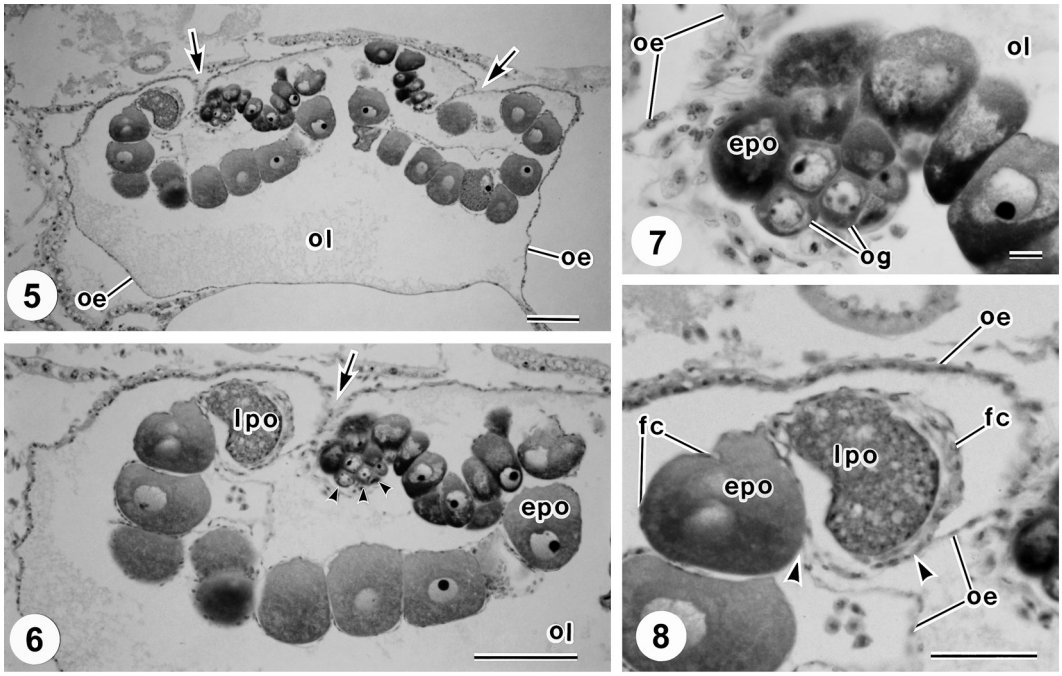


Fig. 5 Cross-section of adult ovary in *Epanerchodus orientalis*. Dorsal side is oriented towards top of the image. Specimen prepared using hematoxylin and eosin staining. Section shows a pair of germ zones. Ovarian epithelium of each germ zone forms a deep and wide fold that extends towards the ovarian lumen. Arrows indicate the narrow openings of these folds, which create a pair of longitudinal grooves on the dorsal surface of the ovary. oe: ovarian epithelium, ol: ovarian lumen. Scale = 100 μm.

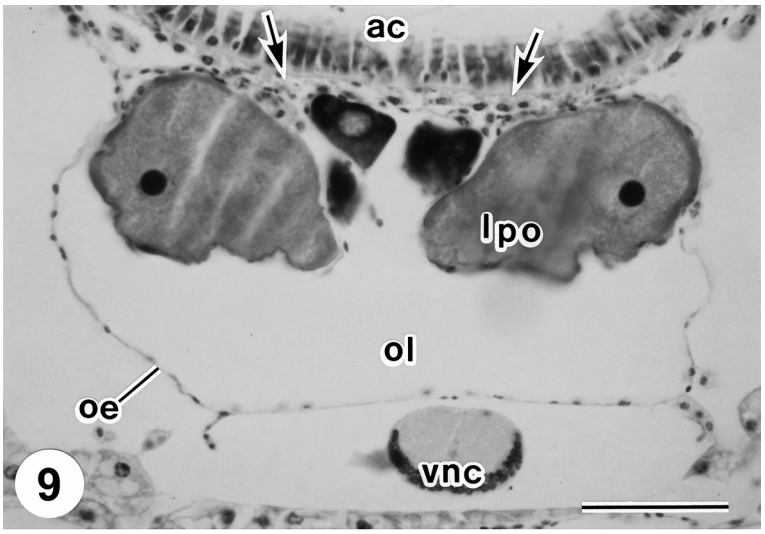
Fig. 6 Cross-section of adult ovary in *Epanerchodus orientalis*. Dorsal side is oriented towards top of the image. Magnification of the left germ zone shown in Fig. 5 showing growing oocytes arranged in order of increasing volume. Arrow indicates the narrow opening of the germ zone fold. Arrowheads indicate position of oogonia. epo: early previtellogenic oocytes, lpo: late previtellogenic oocyte, ol: ovarian lumen. Scale = 100 μm.

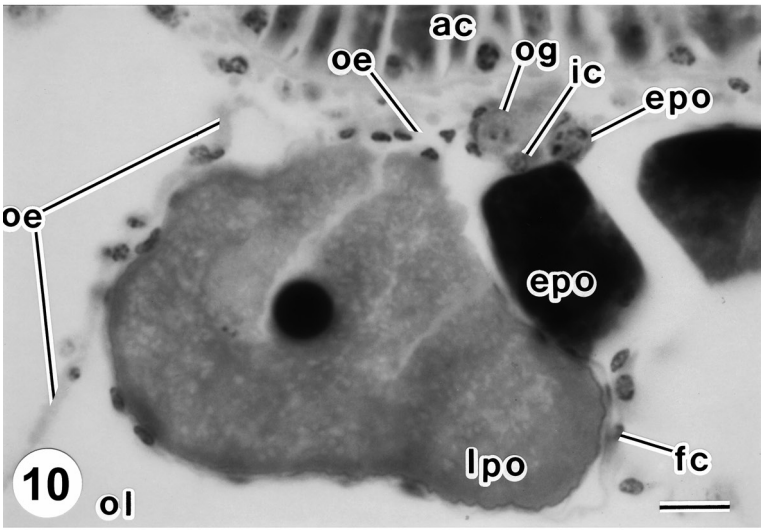
Fig. 7 Cross-section of adult ovary in *Epanerchodus orientalis*. Dorsal side is oriented towards top of the image. Extended part of germ zone, including oogonia and early previtellogenic oocytes, connecting with ovarian epithelium and forming young germ cell-cluster. epo: early previtellogenic oocyte, oe: ovarian epithelium, og: oogonium, ol: ovarian lumen. Scale = 10 μm.

Fig. 8 Cross-section of adult ovary in *Epanerchodus orientalis*. Dorsal side is oriented towards top of the image. Extended part of germ zone, connecting early previtellogenic oocyte (epo) and late previtellogenic oocytes (lpo) to the ovarian epithelium (oe) of the germ zone via their own follicle layers. Arrowheads indicate follicular connections. fc: follicle cell. Scale = 50 μm.

with a single median sac-like ovary serving as a common "ovitube".

Kubrakiewicz (1987) observed ovarian structure in the polydesmid *Oxidus gracilis*, one of the species examined in the present study, and illustrated the paired germ zones as being located in the ventro-lateral parts of the ovary, while failing to mention any folded structure around them. The present study, however, revealed that *O. gracilis* possessed paired germ zones in the dorso-lateral parts of the ovary and that paired germ zone folds extended toward the ovarian lumen, as seen in other polydesmids. Results of the present study strongly suggest that





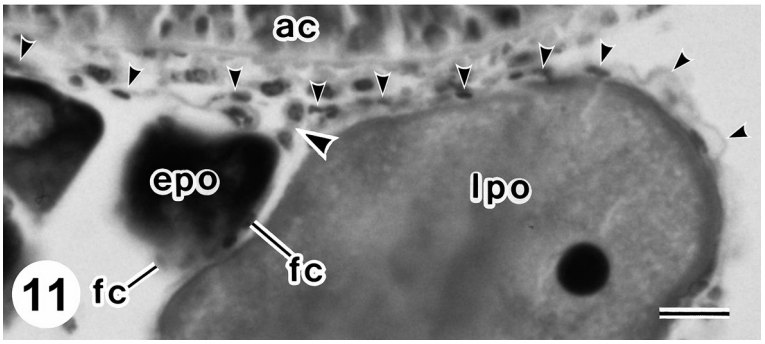


Fig. 9 Cross-section of adult ovary in *Eucondylodesmus elegans*. Dorsal side is oriented towards top of the image. Specimen prepared using hematoxylin and eosin staining. Section shows a pair of germ zones (arrows). ac: alimentary canal, lpo: late previtellogenic oocyte, oe: ovarian epithelium, ol: ovarian lumen, vnc: ventral nerve cord. Scale = 50 μm.

Fig. 10 Cross-section of adult ovary in *Eucondylodesmus elegans*. Dorsal side is oriented towards top of the image. Extended part of left germ zone showing growing oocytes arranged in order of increasing volume. ac: alimentary canal, epo: early previtellogenic oocyte, fc: follicle cell, ic: interstitial cell, lpo: late previtellogenic oocyte, oe: ovarian epithelium, og: oogonium, ol: ovarian lumen. Scale = 10 μm.

Fig. 11 Cross-section of adult ovary in *Eucondylodesmus elegans*. Dorsal side is oriented towards top of the image. Extended part of right germ zone showing folliculated, early previtellogenic oocyte (epo) connected to the ovarian epithelium (small arrowheads) via its own follicle layer. Large arrowhead indicates the follicular connection. ac: alimentary canal, fc: follicle cell, lpo: late previtellogenic oocyte. Scale = 10 μm.

Kubrakiewicz (1987) misread the dorso-ventral axis of the ovary and failed to notice the folded structures of the germ zone epithelium. Kubrakiewicz (1987) observed ovaries after they had been removed from the bodies of specimens, possibly allowing the dorso-ventral axis to be misread and the folded structures to be either partially or entirely unfastened.

The present study described that the depth and width of germ zone folds varied among the four polydesmid species examined. *Epanerchodus orientalis* exhibited the most developed, deep and wide germ zone folds, while *Eucondylodesmus elegans* exhibited no folds around the germ zones whatsoever. It is possible that the *E. elegans* specimens used in the present study were too young to form germ zone folds, and that mature specimens would have developed these folds; however, young specimens of the other polydesmid species examined always showed adequately developed folds (*e. g.*, the specimen of *Oxidus gracilis* shown in Fig. 4). Alternatively, structural differences in germ zone folds could be related to differences in fecundity or in the number of eggs per clutch. *E. elegans*, whose ovaries lacked germ zone folds, had only several tens of late previtellogenic oocytes in the adult ovary; conversely, *E. orientalis*, *Nedyopus tambanus*, and *O. gracilis* had well developed germ zone folds and are known to lay several hundreds of eggs at once (*e. g.*, Causey, 1943; Shinohara, 1957; Murakami, 1962; Komoto and Matsumoto, 1967). Wider germ zones forming folded structures likely enable more oocytes to develop simultaneously.

Comparison with other chilognathan diplopods

The present study identifies several features that can be used to distinguish polydesmid ovaries from those of other chilognathans. In polydesmid germ zones, the orderly arrangement of oogonia and growing oocytes according to their volume was observed. Within each germ zone, oogonia and younger oocytes were found within the dorso-medial regions, while larger oocytes were located more laterally. Kubrakiewicz (1987) described a similar orderly arrangement of germ cells in the ovary of *Oxidus gracilis*. Based on the results of Kubrakiewicz (1987) and those of the present study, the orderly arrangement of germ cells from the dorso-medial region towards the lateral region according to volume should now be recognized as an ovarian feature common among polydesmid diplopods. To ascertain this, future research should be undertaken in order to examine chelodesmidean and dalodesmidean ovaries.

Similar orderly germ cell arrangement has also been observed in the ovary of *Hyleoglomeris japonica*, a chilognathan species belonging to the order Glomedida (Yahata and Makioka, 1997). However, germ cell arrangement in the ovary of *H. japonica* clearly differs from arrangements observed in polydesmid ovaries. In *H. japonica*, oogonia and smaller oocytes are located at the dorsal and ventral edges of each germ zone, while larger oocytes are located more laterally (Yahata and Makioka, 1997). Phylogenetically, the Glomerida and the Polydesmida are distantly related to one another (Enghoff, 1984), and similar ordered arrangements of germ cells have not been observed in other diplopod orders. Therefore, the orderly germ cell arrangements observed in glomerid and polydesmid ovaries likely do not share a common evolutionary origin.

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