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Morphology of the internal reproductive organs of *Archegozetes longisetosus* Aoki (Acari, Oribatida)

Paavo Bergmann[1]*, Michael Laumann[1], Peter Cloetens[2] & Michael Heethoff[1]

[1]*Eberhard–Karls-Universität Tübingen, Abteilung für Evolutionsbiologie der Invertebraten, Auf der Morgenstelle 28 E, 72076 Tübingen, Germany; e-mail: bergmann_paavo@yahoo.de*

[2]*European Synchrotron Radiation Facility (ESRF), 38043 Grenoble, France*

**Corresponding author*

Abstract

The morphology and three-dimensional organisation of the internal reproductive system of *Archegozetes longisetosus* is described, using a combination of conventional microscopic methods and the non-invasive technique of synchrotron X-ray microtomography. The reproductive system is described at various points of its development and the differentiation of its components observed. The genital duct forms during tritonymphal stage close to the ventral body wall from different precursors. Previtellogenesis and the differentiation of eugenital lobes start prior to the last moult. Flap-like structures separate the oviducts from the uterus and the uterus from the vagina in the adult stage. We propose a nomenclature for the subdivisions of the ovary.

Keywords: development, ovary, synchrotron X-ray tomography, holotomography

Zusammenfassung

Wir stellen die Morphologie und die dreidimensionale Organisation des internen Reproduktionssystems von *Archegozetes longisetosus* vor. Hierbei kommen konventionelle mikroskopische Methoden und die nicht invasive Technik der Synchrotron-Röntgen-Mikrotomographie zur Anwendung. Das Reproduktionssystem wird zu verschiedenen Zeitpunkten seiner Entwicklung beschrieben und die Differenzierung seiner Komponenten verfolgt. Die Genitaltrakte bilden sich während des Tritonymphen-Stadiums nahe der ventralen Leibeswand aus unterschiedlichen Vorläufern heraus. Die Prävitellogenese und die Differenzierung der eugenitalen Loben beginnen noch vor der letzten Häutung. Klappenartige Strukturen trennen im Adultus die Ovidukte vom Uterus und den Uterus von der Vagina. Wir schlagen eine Nomenklatur für die Untereinheiten des Ovars vor.

1. Introduction

Oribatid mites (Acari, Oribatida) comprise about 9000 described species (Schatz 2002).

They are unique among animals for their exceptionally high rate of parthenogenesis: 10 % of the species reproduce by obligate thelytoky (Norton et al. 1993). These species form several clusters, which presumably radiated while being parthenogenetic (Palmer & Norton 1992, Norton et al. 1993, Maraun et al. 2004, Laumann et al. 2007). According to molecular analyses, this parthenogenetic radiation took place at least 100 million years ago (Heethoff et al. 2007b). Hence, they are excellent examples for so-called 'ancient asexuals', i.e. long-term stable parthenogenetic systems, and it was suggested that the second big group of Sarcoptiformes, the Astigmata, may have emanated from one of the thelytokous clusters (O´Connor 1984, Norton & Kethley 1994, Norton 1998, Maraun et al. 2004), although molecular data on this aspect are contradictory (Murrell et al. 2005, Domes et al. 2007). The suggested stem group of Astigmata within the oribatid mites is the Trhypochthoniidae (Norton et al. 1993), a taxon consisting of more than 50 obligatory parthenogenetic species (Heethoff et al. 2007b). One member of this family is the species *Archegozetes longisetosus* Aoki, 1965 which was referred to as the most-studied oribatid mite under laboratory conditions (Smrž & Norton 2004, Heethoff et al. 2007a). Studies investigated various aspects of its biology such as life history, embryonic development, anatomy of the digestive system, toxicology and functional morphology (Heethoff & Koerner 2007, Heethoff et al. 2007a and cited references). However, except for the gross morphology of the reproductive organs and some information on reproductive rates (Heethoff et al. 2007a), the whole complex of reproductive biology remains largely unknown from its form to its function. The majority of studies on the anatomy of the genital system of mites or their reproductive biology refer to taxa other than Oribatida, with an emphasis on pests, parasites and their possible control agents (Alberti & Coons 1999, and e.g. Di Palma & Alberti 2001, Shatrov 2002, de Oliveira et al. 2007). Comparative studies of Sarcoptiformes are scarce (Alberti & Coons 1999, Walzl et al. 2004).

The genital system of oribatid mites usually consists of a massive, unpaired ovary located posterior to the eugenital orifice and close to the ventral body wall, surrounded by tissue of nutritive nature (Alberti & Coons 1999, Alberti et al. 2003). Adjacent are paired oviducts that fuse to an unpaired uterus, leading to the vagina. The vagina continues into a long, cuticular ovipositor that is double-folded at its insertion and at the circular fold at about half-length inside the idiosoma when not in use. At its tip, it splits into three eugenital lobes that surround the genital orifice. Eugenital lobes and the circular fold that separates the proximal and distal portions of the ovipositor bear setae (Alberti & Coons 1999). As to the adopted nomenclature for the portions of the genital system, please refer to the discussion. In the present study, we used synchrotron X-ray microtomography (Cloetens et al. 1999, Cloetens et al. 2006, Betz et al. 2007) to analyse the spatial organisation of the reproductive system in *A. longisetosus*. To gain insight in developmental processes, whole specimens of various ages were examined, revealing remarkable changes of the organ system during development.

2. Materials and methods

2.1. Rearing

Specimens were taken from our laboratory culture of *Archegozetes longisetosus* ran (Heethoff et al. 2007a). Clutches of eggs were removed from the culture and placed in the wells of tissue culture plates (Tissue Culture Cluster^{24}, Costar) for further development. Wells were filled 1 cm high with plaster-of-paris:charcoal (6:1) mixture. The plates were kept in constant dark; air humidity was kept at 90 % and temperature at 20 °C. Bark of different trees, covered with green algae (mainly *Protococcus*) was supplied as food.

The wells were checked daily for sufficient food supply and moisture, and specimens were removed with a fine brush at appropriate times (tritonymphs: two and five days after moulting to the instar; tritonymph in quiescent period prior to last moult; adults: one and five days after last moult).

2.2. Synchrotron-X-ray-Micro-Computer-Tomography (µCT)

Specimens collected from the culture were fixed in a 6:3:1 mixture of 80 % ethanol, 35 – 38 % formaldehyde (standard solution, Merck) and 100 % acetic acid for at least 24 hours. After dehydration in a graded ethanol series of 80 %, 85 %, 90 %, 95 % and 100 % with three times 10 minutes each, the specimens where subjected to critical point drying in liquid carbon dioxide (E 3000 Series II Critical Point Drying Apparatus, Polaron Equipment Limited). Dried specimens were glued with cyanoacrylate to the tip of plastic stubs (3 mm diameter, 12 mm length) and stored in an exsiccator.

Measurements at the European Synchrotron Radiation Facility (ESRF) were conducted at beamline ID 19 (experiment number SC2127) with an energy level of 20.5 keV. Samples were scanned for holotomography (Cloetens et al. 1999, 2006) at 10, 20, and 45 mm distances to the detector with 1300 projections over 180°. X-Rays were converted to visible light by a scintillator and projections were recorded with 0.35 s exposure time on a cooled CCD (ESRF FreLoN) with 2048 x 2048 pixels and an effective spatial resolution of 0.7 µm. Holotomography includes a phase retrieval step and the grey level in the tomographic slices is proportional to the local electron density (dark corresponding to a higher density in the representation used).

Phase-enhanced tomography was performed at a sample-detector distance of 20 mm. It enhances all abrupt density changes and boundaries in the sample.

A detailed description of the technique is given by Betz et al. (2007).

Voxeldata were visualised with the software VGStudio Max 2.1 (Volume Graphics, Heidelberg, Germany) and segmented with amira 4 (Mercury Computer Systems, Inc., Berlin, Germany) on a 64bit-Dual-Opteron computer system.

2.3. Light and electron microscopy

Whole specimens and dissected genital organs for serial sectioning were fixed in 2.5 % glutaraldehyde in Na-cacodylate with 1.66 % sucrose at pH 7.2 overnight at 4 °C, and postfixed in 1 % of osmium tetroxide in Na-cacodylate for 2 hours at 0 °C. Samples were dehydrated in a graded ethanol series (AGAR LV) or isopropanol (SPURR) at 70 %, 75 %, 80 %, 85 %, 90 %, 95 % and 100 % for three times ten minutes each and placed in fresh

100 % alcohol overnight. Then, the alcohol was replaced by propylene dioxide (2 x 1 h) and samples were gradually infiltrated (propylene dioxide / resin mixture 1:1, 1:3, 1:7 and pure resin for 1.5 h each, pure resin overnight) and embedded in epoxy resin (SPURR´s medium / AGAR LV). Polymerisation was conducted at 60 °C for 48 h. Semithin (0.5 µm) and ultrathin (70 nm) sections were cut using a Reichert Ultracut microtome and diamond knives (Diatome 45°). Semithin sections were stained in toluidine blue 0.13 % / alkaline fuchsine 1 %, or methylene blue / Azur II according to Richardson et al. (1960) and light microscopy was conducted with a Zeiss Axioplan light microscope and a Zeiss MrC5 digital camera. Ultrathin sections for transmission electron microscopy (TEM) were contrasted with uranyl acetate for 20 min and lead citrate for 10 min. TEM was conducted on a Philips Technai 10 electron microscope. Images were digitally captured by a MegaVision II digital camera.

2.4. Preparations of genital tracts

Fresh specimens were dissected using sharpened tungsten needles (Norton & Sanders 1985) under stereomicroscopic control on a glass slide, either in a drop of glycerol, for immediate observation with phase contrast / DIC light microscopy, or they were dissected submersed in pre-cooled glutaraldehyd-fixative and then processed for serial semithin and ultrathin sectioning as described in the previous paragraph.

3. Results

The results of the analysis are presented in chronological order, although denomination of components and their respective precursors in younger specimens is based upon the interpretation of the adult state, as the features are most clearly distinguishable in reproducing females, and terms are generally defined for adult specimens.

3.1. Tritonymphal stages

Specimens as young as 48 h after moulting to the tritonymph already show a development of the genital bud (Fig. 1a) that allows for observation of the duplicated wall of the ovipositor, precursors of eugenital lobes (Fig. 1b) and precursors of oviducts apparently originating close to the ventral body wall (Fig. 1c). The ovary at this age lacks any structure discernible by µCT, except for numerous small and dense particles that are likely oogonial nuclei (Figs 1a, b)

Specimens collected on day five after moulting of the tritonymph already show a developed genital system with invaginated ovipositor bearing three eugenital lobes as differentiations of its distalmost portion, an unpaired portion (vagina / uterus) (Fig. 2c) and paired oviducts showing a free lumen and making contact to a roughly spherical ovary (Figs 2b, c), although the features are restricted to a more medio-ventral part of the notogaster and are less well defined compared to specimens taken later in development (Fig. 2). The tissue forming the oviducts appears lighter, indicating lesser density, than the tissue of the developing ovipositor. No oocytes had started previtellogenesis at this age.

Specimens collected from the middle of the quiescent phase associated with moulting to the adult already show distinct features of the internal reproductive organs (Fig. 3): the ovipositor is clearly developed, a distinct cuticular intima is not yet recognisable. It already features

distinct eugenital lobes. The unpaired portion of the distal genital tract now appears as two distinct portions, distally the vagina and proximally the uterus. The vagina appears as a hollow structure protruding antero-ventrad from the dorsal rim of the invaginated ovipositor. Its walls are of the same density as the walls of the ovipositor. The lumen of the vagina is not yet continuous with that of the oviducts and uterus (Figs 3b, e). The latter two have wall epithelia of lesser density than the vagina. The paired oviducts are clearly visible, as hollow structures, taking a course from the base of the proximal wall of the ovipositor laterad along the latero-ventral body wall and again mediad to merge with the tissue surrounding the ovary (Fig. 4). The ovary does not show thighs, yet a single layer of previtellogenetic oocytes encompasses the central zone of the medulla and radially organised small cells.

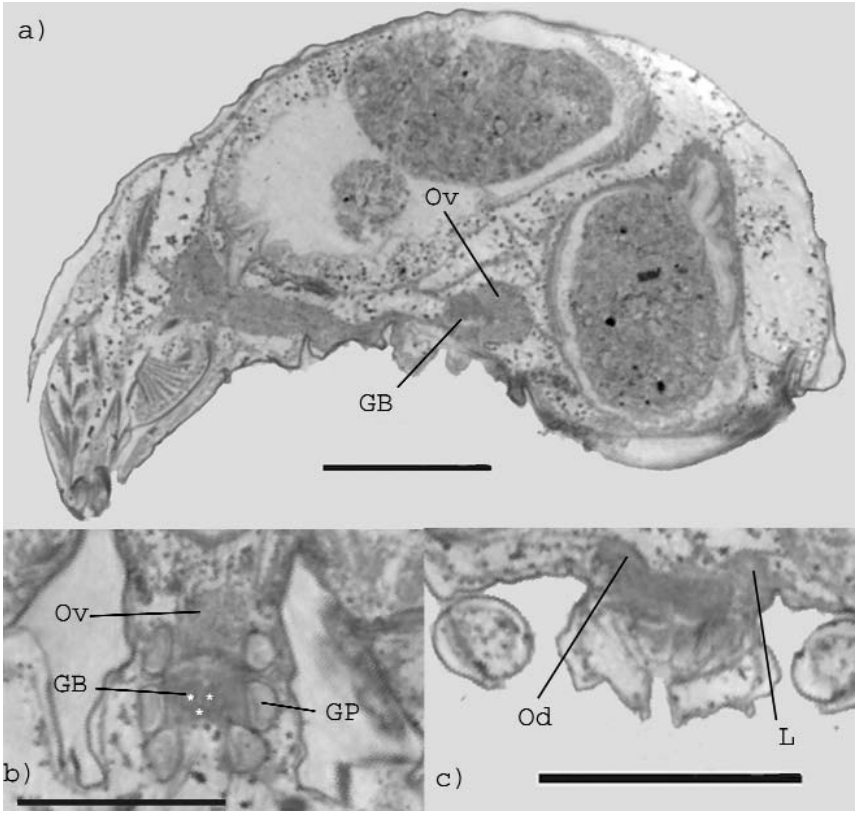

Fig. 1 Tritonymph, two days after moulting. Virtual holotomographic slices, a: sagittal plane; b: horizontal plane; c: cross-sectional plane. The genital bud invaginates into the opisthosoma, the central part shows already the development of eugenital lobes (indicated by white asterisks in b). Oviducts are already being formed as hollow tubes from the rim of the genital bud. The ovary is still undifferentiated. Abbrevations: GB: genital bud; GP: genital papilla; L: lumen of oviduct; Od: oviduct; Ov: ovary. Scale bar = 100 µm.

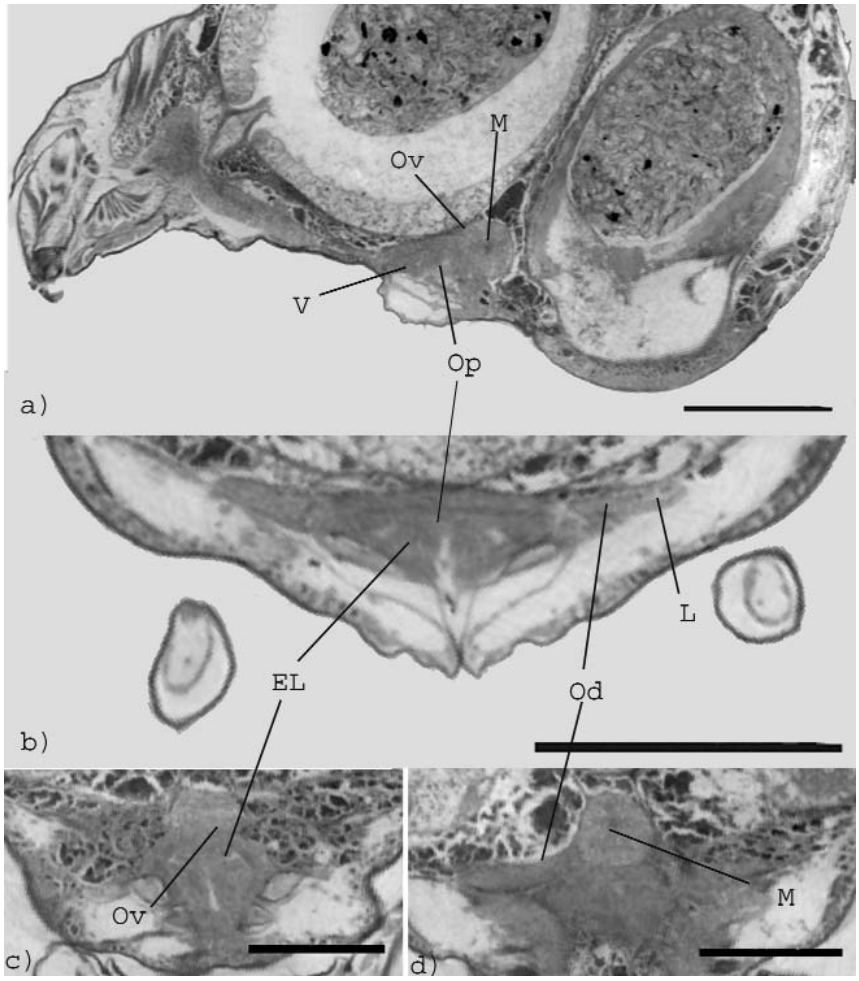

Fig. 2 Tritonymph, five days after moulting. Virtual holotomographic slices: a: sagittal plane; b: cross-sectional plane; c: horizontal plane, close to ventral body wall; d: horizontal plane, more dorsally than c). Ovipositor already with differentiated eugenital lobes, oviducts with free lumen making contact to ball-shaped ovary. No oocytes have started previtellogenesis. EL: eugenital lobes; L: lumen; M: medulla; Od: oviduct; Op: ovipositor; Ov: ovary; V: vagina. Scale bar = 100 µm.

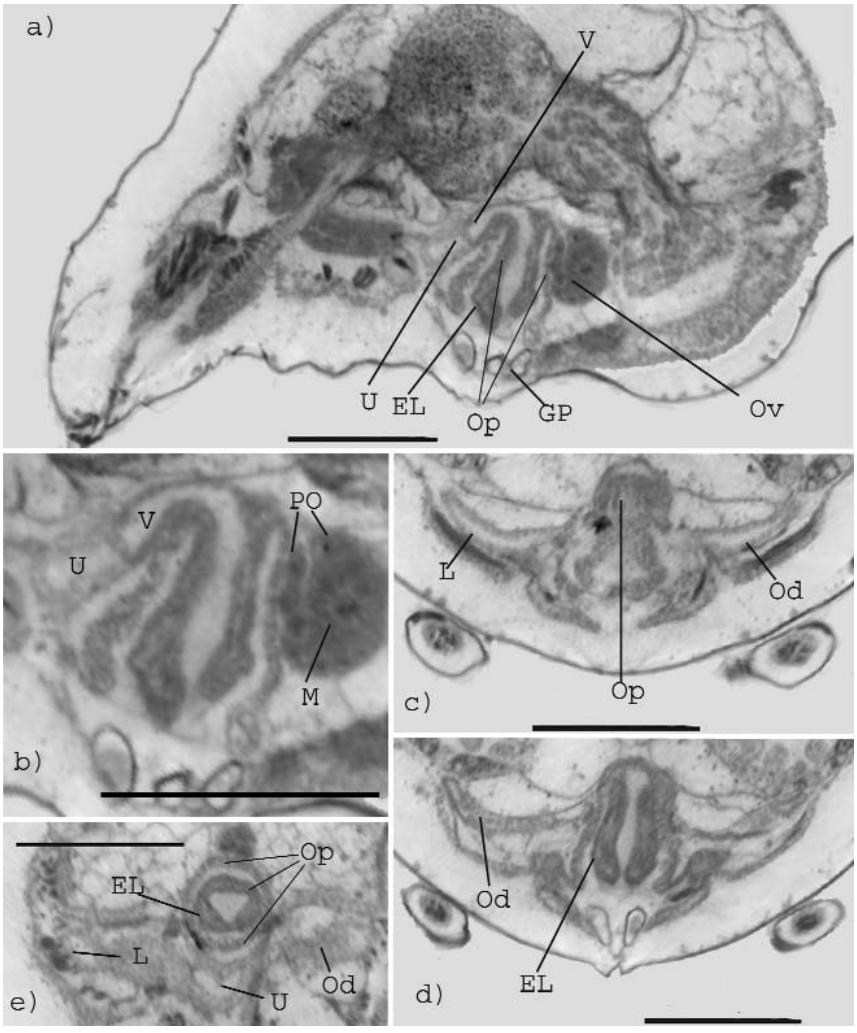

Fig. 3 Tritonymph from the quiescent phase prior to moulting to the adult. Virtual holotomographic slices. a: sagittal plane; b: detail from Fig. a; c: cross-sectional plane, just anterior of genital papilla I; d: cross-sectional plane at genital papilla II; e: horizontal plane. Ovipositor fully developed with three eugenital lobes, yet still lacking a discernible cuticle. Ovary ball shaped, at its periphery are oocytes starting previtellogenesis. Vagina, uterus and oviducts with free lumen. EL: eugenital lobes; GP: genital papilla; L: lumen; M: medulla; Od: oviduct; Op: ovipositor; Ov: Ovary; PO: previtellogenetic oocyte; U: uterus; V: vagina. Scale bar = 100 µm.

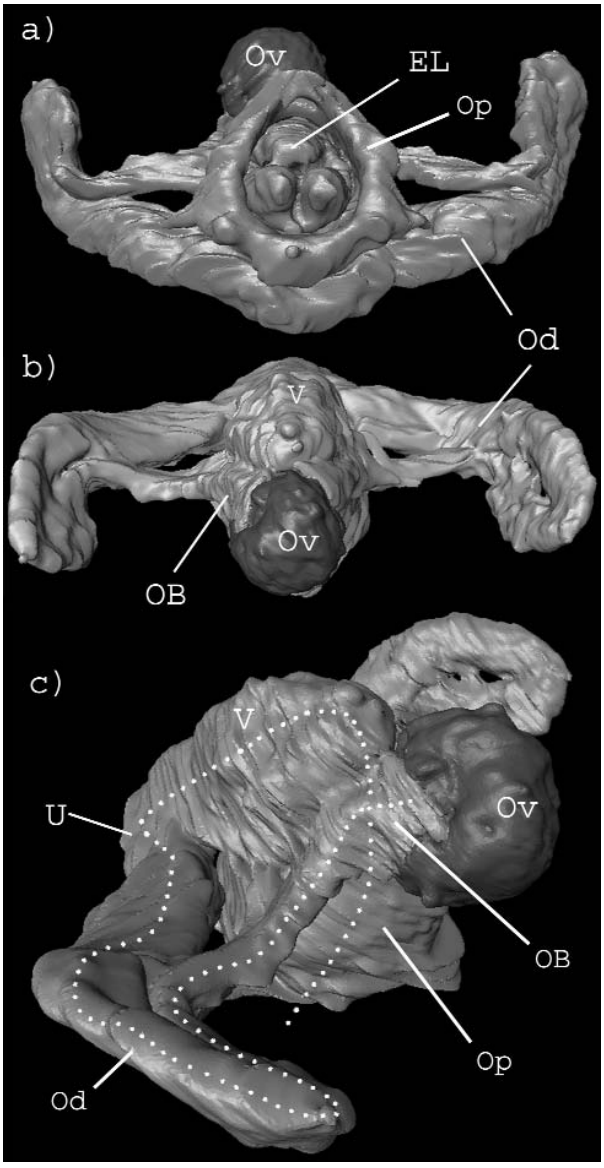

Fig. 4 Tritonymph from the quiescent phase prior to moulting to the adult. Surface model of the genital system, modelled from holotomographic data. a: ventral view; b: dorsal view; c: postero-dorso-lateral view. Ovipositor with three eugenital lobes continues to unpaired uterus and vagina, which leads into paired oviducts. These show lateral S-curvature and make contact to the ball-shaped ovary via ovarial bulbs. Dotted line depicts course of left half of the genital tract. EL: eugenital lobes; OB: ovarial bulb; Od: oviduct; Ov: ovary; Op: ovipositor; U: uterus; V: vagina.

3.2. Adult, on first day after moulting

Phase-enhanced tomography of an adult 24 h after moulting clearly shows the duplicated, corrugated wall of the ovipositor, cuticular structures of the eugenital lobes as well as their setae (Figs 5a, b, c). The epithelium lining the inner surface of the ovipositor continues into the vagina, which proceeds anteriorly ventrad towards the base of the ovipositor, in the region of genital papilla I. The epithelium lining the inner surface of the ovipositor shows no sign of a cuticular intima proximal to the base of the eugenital lobes in μCT scans. It continues into the vagina, which actually forms the inner surface of the proximal portion of the ovipositor in its extended state. The walls of the vagina are of a wrinkled appearance, relatively thin (approx. 5 μm) and envelope a clearly distinguishable lumen (Figs 5a, b, c).

The vagina continues proximally into the uterus from which the paired oviducts take their course close to the ventro-lateral body wall of the idiosoma (Fig. 6). The distal parts of the oviducts show no discernible lumen, but resemble the vagina lining in terms of tissue structure. They project latero-anad until they reach the vicinity of the point where the thighs of the ovary take a turn posteriorly (Fig. 6b). At this point, the proximal oviducts slightly bend anad, accompanying the row of the most distal oocytes until they make contact with the ovary, i.e. the distalmost oocyte and its sheath, forming the ovarial bulb of the oviduct (Woodring & Cook 1962, see discussion). The proximal oviducts, especially the ovarial bulb, show thick walls of a wrinkled, compressed appearance and a clearly discernible, smoothly outlined lumen (Figs 5c, d).

The unpaired ovary is located posterior to the invaginated, cuticular ovipositor. The central zone of the ovary is a distinctive ball-shaped structure. This structure is radially symmetrical in any plane of sectioning. It is surrounded by a layer of previtellogenetic oocytes enveloped in a thin layer of a follicular epithelium. Previtellogenetic oocytes and follicular epithelium form a pair of rostro-laterad protrusions or ‘thighs’ of the ovary, with the oocytes in triple to double row proximally and in single row distally (Fig. 5c). The follicular epithelium wrapping the previtellogenetic oocytes appears smooth and is not continuously discernible in μCT. At this developmental stage, no oocytes have so far reached the oviduct or even accumulated visible yolk vesicles or a vitelline envelope (Fig. 5). The oocytes are spherical, 20 – 30 μm in diameter and possess large, irregularly shaped germinal vesicles (~8 μm) containing a single dense mass of chromatin. The central zone surrounding the medulla shows numerous small, dense nuclei, but no cell borders were discernible in μCT.

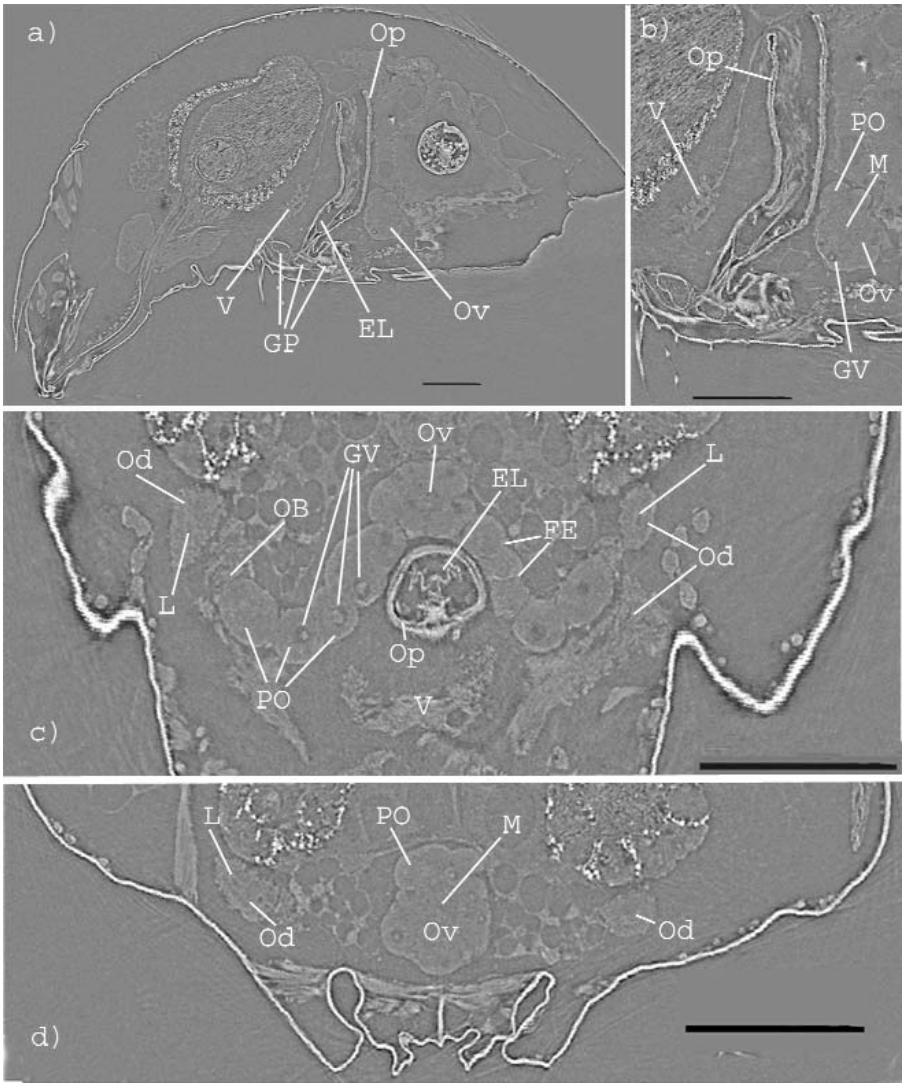

Fig. 5 Adult one day after moulting. Virtual slices from phase-enhanced tomography a: sagittal plane; b: detail from Fig. a; c: horizontal plane; d: cross-sectional plane. Numerous oocytes are in previtellogenesis and show large germinal vesicles. These cells are wrapped in smooth, thin follicular epithelium and form lateral thighs of the ovary. None has started vitellogenesis or passed to the lumen of the oviduct at the ovarial bulb. The oviducts are restricted to an area close to the ventral body wall and are still relatively short. Their walls are thick and have a wrinkled appearance. A fine lumen is smoothly outlined. EL: eugenital lobes; FE: follicular epithelium; GP: genital papilla; GV: germinal vesicle; L: lumen; M: medulla; OB: ovarial bulb; Od: oviduct; Op: ovipositor; Ov: ovary; PO: previtellogenetic oocyte; V: vagina. Scale bar = 100 μm.

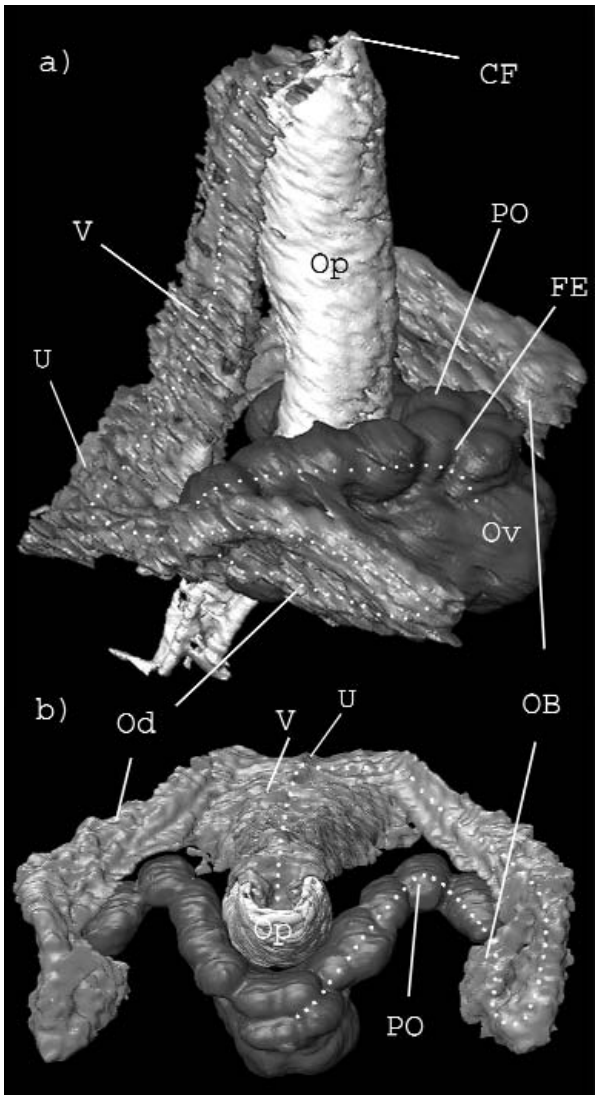

Fig. 6 Adult, one day after moulting. Surface model of the genital system, obtained from phase-enhanced tomographic data: a: dorsolateral view; b: dorsal view. Previtellogenetic oocytes tightly wrapped in follicular epithelium form the lateral thighs of the ovary. These protrude latero-rostrad and then turn to latero-anad. They are contacted by the ovarial bulb of the oviducts, which lead to the vagina. The vagina can be seen to continue into the lining of the ovipositor at the circular fold. The distal portion of the ovipositor bearing the eugenital lobes is situated inside the proximal portion visible in this surface model (see Figs 5a, b, c; 7) Dotted line depicts course of genital tract (left side in Fig. a, right side in Fig. b). CF: circular fold; FE: follicular epithelium; OB: ovarial bulb; Od: oviduct; Op: ovipositor; Ov: ovary; PO: previtellogenetic oocyte; U: Uterus; V: vagina.

3.3. Adult, on fifth day after moulting

The oviducts of a female collected five days after moulting to the adult contained six developed eggs, packed with large, dense yolk vesicles and wrapped in a smooth eggshell (Figs 7b, 8). The analysis of the exact course of eggshell formation, i.e., the origin and formation of vitelline membrane and chorion was not subject of this study, yet resembles closely the situation described by Witalińsky (Witalińsky 1986, 1993. See discussion for further detail).

The eggs considerably stretch the oviduct wall, up to the limit of spatial resolution of the scans (0.7 μm), bulging into the surroundings. Hence, there is some amount of free lumen between and even around the eggs, indicating the presence of an acellular substance that appears in semithin sectionings (Figs 8, 11c). In comparison to freshly moulted adults, the oviduct is shifted to a more dorsal position in the animal, up to the level of the caeca and opisthosomal glands. The most proximal parts of the genital tracts show a peculiar S-curvature (rostrad – anad – rostrad) before reaching dorso-rostrad between the epithelium of the caeca and the body wall (Figs 7b, 8). The eggs are positioned towards the distal part of the paired oviducts, not entering the uterus or vagina and leaving the proximal part of the oviduct with the ovarial bulb yet unstretched and free of eggs (Fig 8). Duplicatures of the wall epithelium form one median and two lateral flap or valve-like structures, providing a marked distinction between the lumina of the paired oviducts, that of the vagina and a short, unpaired section that may represent the uterus (Figs 7a, c, 14). The epithelium forming the distal wall of this section, including the flap at the base of the vagina appears thicker than that of the proximal wall, which in holotomographic scans resembles the thin wall of the oviduct (Fig. 7). A dense substance covers the internal surface of the thicker part of the epithelium that connects to the vagina at the flap mentioned before (Fig. 7a, c). All eggs within the oviduct have completed vitellogenesis and show a rigid eggshell. The ovarial bulb marks the point where the anad curving part of the ovarial thighs contacts the most proximal part of the oviduct. Holotomographic virtual slices show the tissue of the ovarial bulb to be of different structure and considerably less dense than the adjacent follicular epithelium surrounding the vitellogenetic oocytes (Fig. 9). Vitellogenetic oocytes occupy the distal part of the ovarial thighs. No free lumen is visible between or around the densely packed cells in this region. The oocytes show a progression in the accumulation of yolk and at the same time, the formation of the eggshell. Their shape is irregular, occupying virtually all available volume. To a lesser extent, this also applies to the previtellogenetic oocytes in the ventrally adjacent proximal thigh of the ovary (Fig 7b).

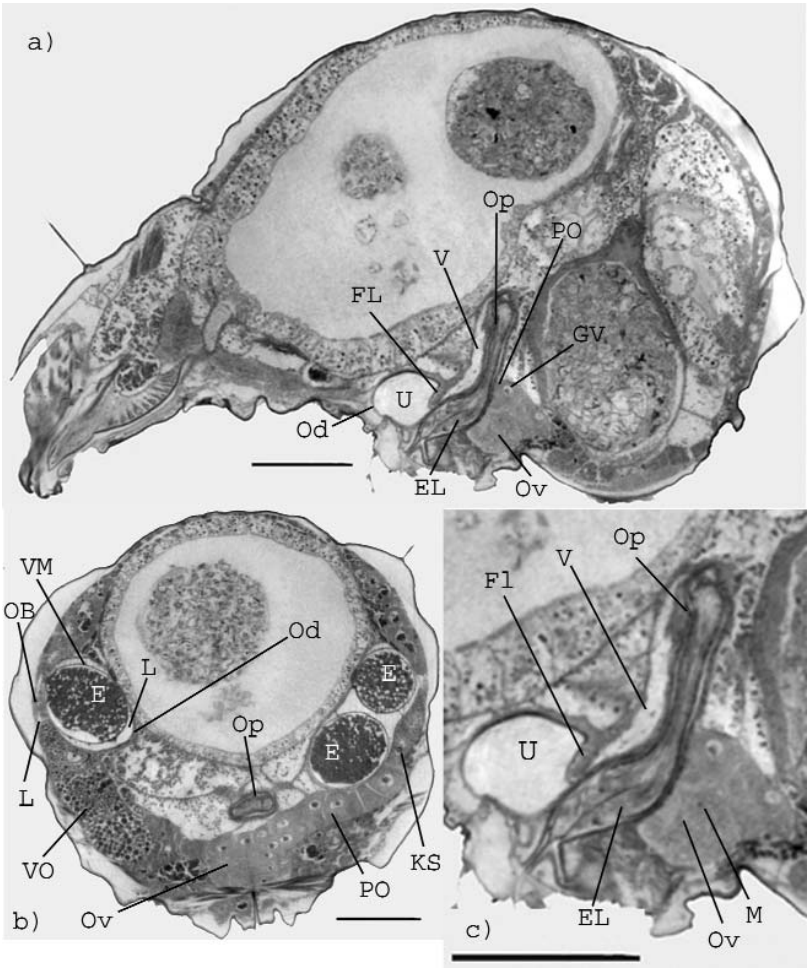

Fig. 7 Adult, five days after last moulting. Virtual holotomographic slices: a: sagittal plane; b: cross-sectional plane; c: detail from Fig. a. Ovary with thighs of previtellogenetic and vitellogenetic oocytes wrapped in thin follicular epithelium (not visible). Karyospheres visible in germinal vesicles as dense, hollow structures. Oviducts stretched and filled with developing eggs. The lumen of the uterus is separated from that of the vagina by a flap-like fold of the wall. Free lumen around chorionated eggs. Folded into the proximal portion of the ovipositor, cuticular structures of the eugenital lobes and their setae are visible, as indicated in a and c. E: egg; EL: eugenital lobes; Fl: flap between uterus and vagina; GV: germinal vesicle; KS: karyosphere; L: lumen; M: medulla; OB: ovarial bulb; Od: oviduct; Op: ovipositor; Ov: ovary (central part); PO: previtellogenetic oocyte in proximal thigh of ovary; U: uterus; V: vagina; VM: vitelline membrane; VO: vitellogenetic oocyte in distal part of ovary. Scale bar = 100 μm.

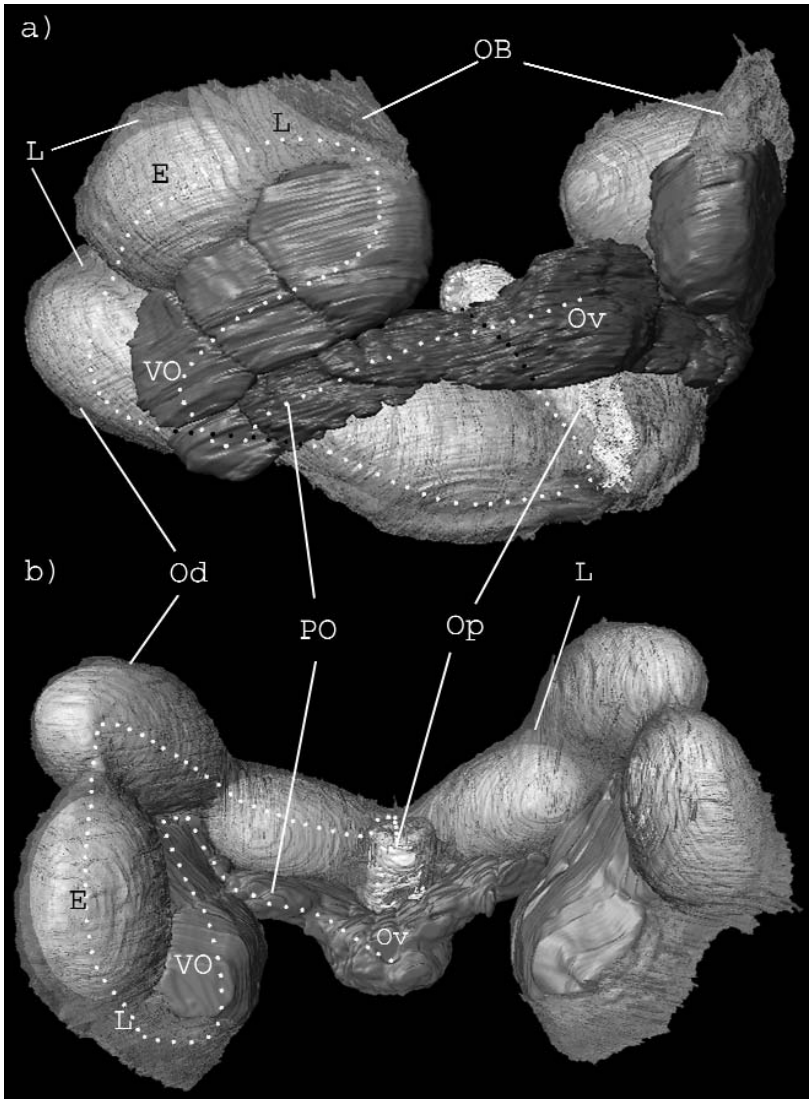

Fig. 8 Adult, five days after last moulting. Surface model of the genital system, obtained from holotomographic data: a: ventrolateral view; b: dorsal view. Four vitellogenetic oocytes and three eggs on either side of the genital tract. The eggs stretch the oviduct walls, yet a free lumen is visible around eggs, and especially in the ovarial bulb, as eggs are collected in the distal part of the oviduct. No eggs have entered the vagina. Dotted line depicts course of the left side of the genital tract. E: egg; L: lumen; Od: oviduct; Op: ovipositor; Ov: ovary (central part); PO: previtellogenetic oocyte; VO: vitellogenetic oocyte.

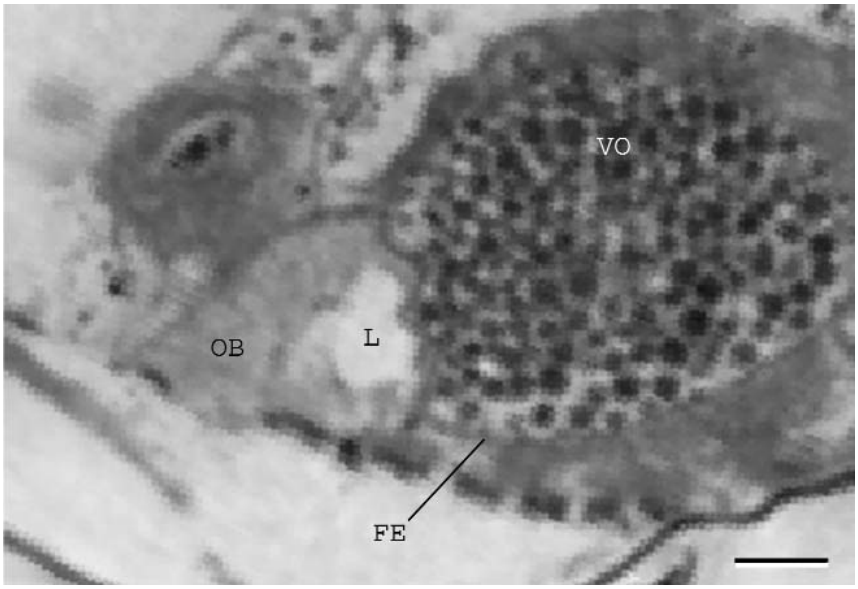

Fig. 9 Adult, five days after last moult. Magnified virtual holotomographic slice. Resolution of image is resolution of scan (0.7 μm). Detail of ovarial bulbs contacting distalmost vitellogenetic oocytes. Note difference in tissue density and structure between OB and FE. L: lumen; OB: ovarial bulb; FE: follicular epithelium; VO: vitellogenetic oocyte. Scale bar = 10 μm.

3.4. Ovarial thighs and oviduct wall

Preparations of the genital tract indicated a continuous resilient lining, and only loose contact to surrounding organs. No oocytes or eggs were found in the open haemolymph space, and developing eggs show a gradual tanning of the eggshell, indicating ongoing development and / or modification of the eggshell (Fig. 10).

A 3-D-model of the thigh of the ovary obtained from semithin sectionings of a prepared genital tract showed the previtellogenetic oocytes tightly wrapped in flattened follicular cells that extend between the individual oocytes (Fig. 11a). Previtellogenetic oocytes start as a single layer surrounding the central zone of the ovary. While they grow in volume and their nuclei develop into a large germinal vesicle with a prominent nucleolus, two ‘streams’ of these cells reach out laterally to form the ovarial thighs. Within the thighs, the ‘stream’ of germ cells narrows down to a single file of previtellogenetic oocytes, one after another (compare Fig. 7b). In this region, dense material likely to be chromatin condenses to a hollow structure within the germinal vesicle, indicating the presence of a karyosphere (Figs 5, 7b, 10, 12, 15). Germinal vesicles of previtellogenetic and vitellogenetic oocytes largely occupy a central position. The follicular cells form a delicate layer of less than 1 μm in thickness, separating the germ cells from the surrounding nutritive tissue as well as from each other

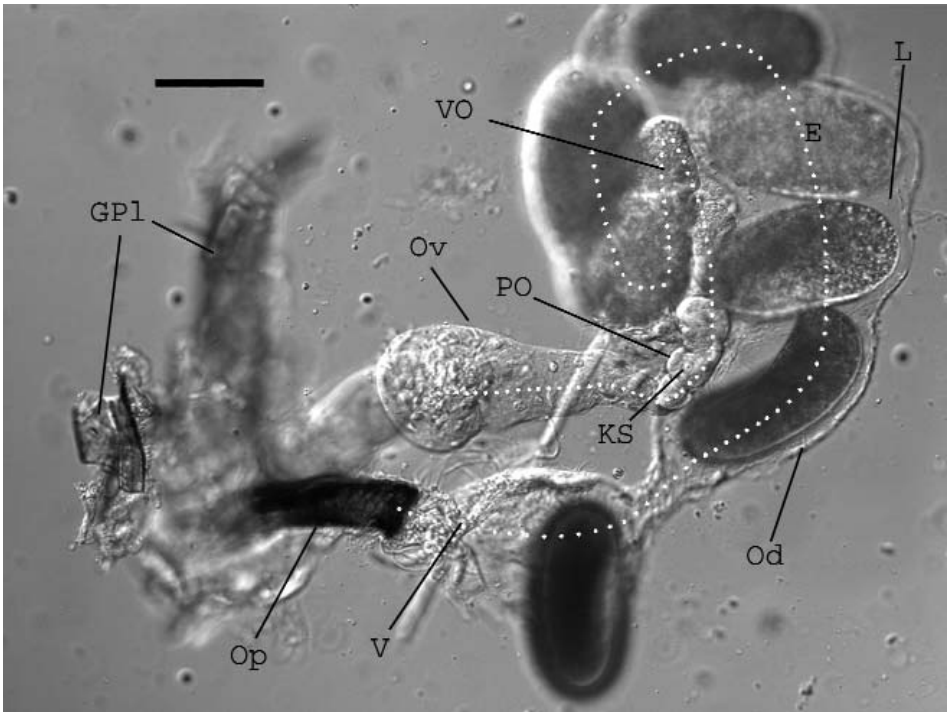

Fig. 10 Adult female. Differential interference contrast. Genital tract, obtained by dissection of fresh specimen. Left branch of oviduct removed. Lumen of oviduct visible around chorionated eggs. Eggshells gradually tanned towards vagina. Previtellogenetic oocytes with karyosphere and vitellogenetic oocytes in S-curvature of genital tract. Dotted line depicts course of genital tract. E: egg; GPl: genital plate; KS: karyosphere; L: Lumen; Od: oviduct; Op: ovipositor; Ov: ovary (central part); PO: previtellogenetic oocyte; V: vagina; VO: vitellogenetic oocyte. Scale bar = 100 µm.

(Figs 11a, b). The follicular cells fill up the spaces between previtellogenetic oocytes and extend proximally to the central zone, that consists of the medulla and radially organised strains of small, polygon cells delineated by lighter staining, nucleus-free cords that originate from the medulla. No cell borders could be traced within the medulla (Figs 5, 12). The cords seem to connect individual cells to the medulla. No tubular arranged tissue or contingent lumen could be observed in this part of the genital tract (Fig. 12). A free lumen appears for the first time, when a change in tissue structure also marks the beginning of the oviduct at the ovarial bulb (Fig. 13).

Fig. 15 gives a schematic overall impression of the features in the adult genital system of *A. longisetosus*.

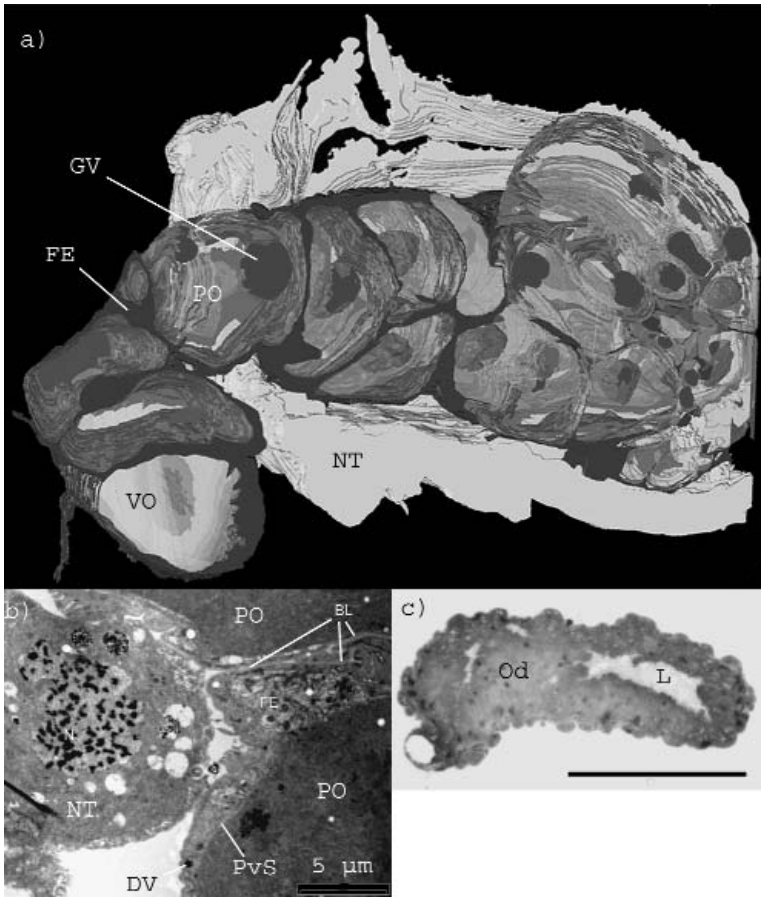

Fig. 11 Adult female. Sections of embedded genital tract obtained from dissection of fresh specimen: a: surface model of the left lateral thigh of the ovary, obtained from semithin sections. The follicular epithelium wraps the individual oocytes and leaves no lumen. Its thickness increases with the onset of vitellogenesis. Germinal vesicles are large and in central position. The ovary is surrounded by nutritive tissue. Previtellogenetic oocytes form a 'stream' that narrows to a single file of oocytes distally. Oocyte surfaces set to transparency; b: TEM micrograph of the same specimen, from the region of the proximal border of the cell labelled 'PO' in Fig. a. Follicular epithelium comprising of flattened cells with folded basal lamina. Distal oocyte developing perivitelline space. Its follicular cells with dense vesicles. c: Semithin sectioning of the same object from an empty part of the oviduct. The oviduct wall appears folded, granulose and vacuolated with rough surfaces. The lumen is filled with an amorphic substance that stains lightly. Stain: Richardson.

BL: basal lamina; DV: dense vesicles; FE: follicular epithelium; GV: germinal vesicle; L: lumen; N: nucleus; NT: nutritive tissue; Od: oviduct; PO: previtellogenetic oocyte; PvS: perivitellin space; VO: vitellogenetic oocyte. Scale bar = 50 µm.

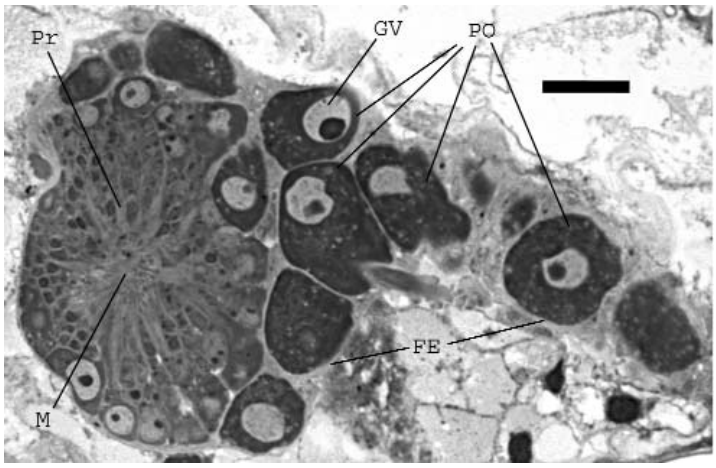

Fig. 12 Adult female. Semithin section of the ovary. Central zone with medulla and radially arranged protrusions and small cells, periphery with previtellogenetic oocytes wrapped in follicular epithelium. These oocytes develop germinal vesicles and reach out to form the lateral thigh of the ovary. FE: follicular epithelium; GV: germinal vesicle; M: medulla; PO: previtellogenetic oocytes; Pr: protrusions of medulla. Slice thickness: 0.5 µm, Stain: toluidine blue / basic fuchsine. Scale bar = 20 µm.

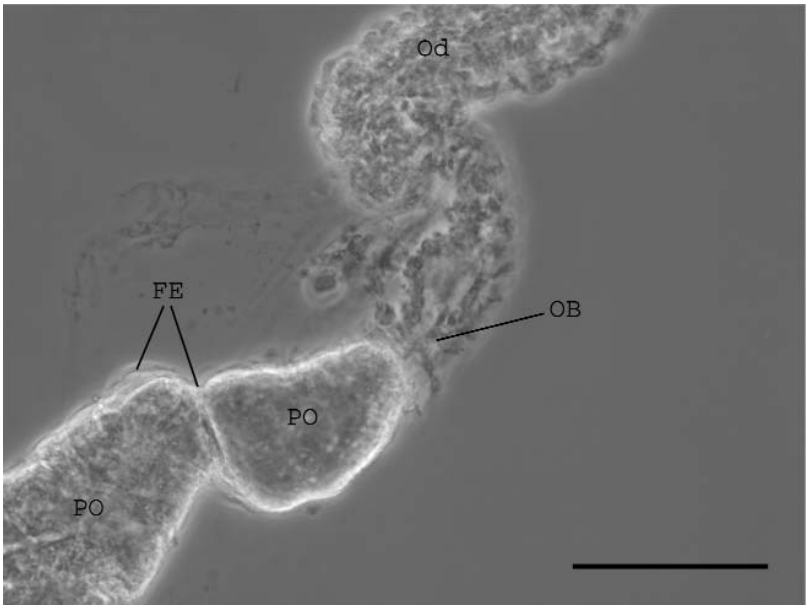

Fig. 13 Adult female. Genital tract, obtained by dissection of fresh specimen. Previtellogenetic oocytes wrapped in smooth follicular epithelium and the ovarial bulb as the beginning of the oviduct. Note differences in tissue structure. PO: previtellogenetic oocyte; FE: follicular epithelium; Od: oviduct; OB: ovarial bulb. Light microscopy; phase contrast. Scale bar = 50 µm.

4. Discussion

In general, the results fit in with the data already available on the gross anatomy of the female reproductive organs of oribatid mites (Michael 1884, Woodring & Cook 1962, Baker 1985, Witaliński 1986, Taberly 1987a, b, c, Witaliński et al. 1990, Witaliński 1993, Heethoff et al. 2007a). All studies cited above describe a massive, unpaired ovary medioventrally in the opisthosoma, connected to paired, tube-like oviducts that converge into an unpaired uterus that leads to an unpaired vagina just anterior to the base of the ovipositor. Differences exist as to what processes take place within which part of the system, as will be discussed below. Ultrastructural analysis as well as additional developmental data are still needed for a sound discrimination of organs and tissues forming the genital duct, especially for the nature of the transition from follicular epithelium to the oviduct wall and from the oviduct wall to the vagina. At the current state, developmental data from younger adult and tritonymphal stages suggest that the paired oviduct forms simultaneously with the unpaired vagina and the invaginating ovipositor at the ventral body wall. As µCT did not reveal a cuticular intima of the ovipositor or the vagina with certainty, ultrastructural analysis of developmental stages is needed to trace the boundary between the tissues forming the unpaired portions of the genital tract, i.e. uterus, vagina and ovipositor. Density differences suggest different physiology that, on the basis of developmental data available so far, may coincide with a different origin of the tissue forming the uterus and vagina, as has already been noted by Taberly 1987b (Fig. 3). The flap-like structure at the basis of the vagina described for the adult would be part of the section of the genital tract that also forms the vagina as well as the roof of the uterus lumen, whereas the rest of the uterus together with the flaps that separate its lumen from those of the oviducts would share its properties and / or origin with the oviduct walls. A structure separating the vagina from the uterus and the uterus from the oviducts is not yet described for the genital systems of any oribatid mite. Its functional significance could lie in proving a guidance system that coordinates the transmission of eggs from the left and right oviduct into the vagina, together with the constrictions between oviducts and uterus (Fig. 14). This is further indicated by the fact that eggs seem to be passed down to the most distal part of the oviducts first, leaving the proximal part free, yet do not enter uterus nor vagina instantaneously (Fig. 8). Histological confirmation of contractile elements would make it possible to develop a functional model of the instant of oviposition. Previtellogenetic and vitellogenetic oocytes show large germinal vesicles in a more or less central position and distinct karyospheres, except for a small portion of cells around the curvature from the proximal rostrad to the distal anad section of the ovarial thigh (see Laumann et al. in this issue). The localisation of vitellogenesis in a lateral s-curvature of the genital duct resembles the situation shown by Walzl et al. (2004) for Astigmata, which possess paired ovaries.

While in certain species structures are reported that suggest a paired predecessor for the generally unpaired oribatid ovary, like the bi-lobated ovary of *Xenillus tegeocranus* (Warren 1947, cited in (Taberly 1987b), or germaria arranged in a two-by-two fashion as in *Hafenrefferia gilvipes* (Witaliński 1986), no traces of a paired origin were found in studies on *Ceratozetes cisalpinus* (Woodring & Cook 1962), or mites of the group Desmonomata, like *Plathynothrus peltifer, Trhypochthonius tectorum* (Taberly 1987b) and *A. longisetosus* (this study).

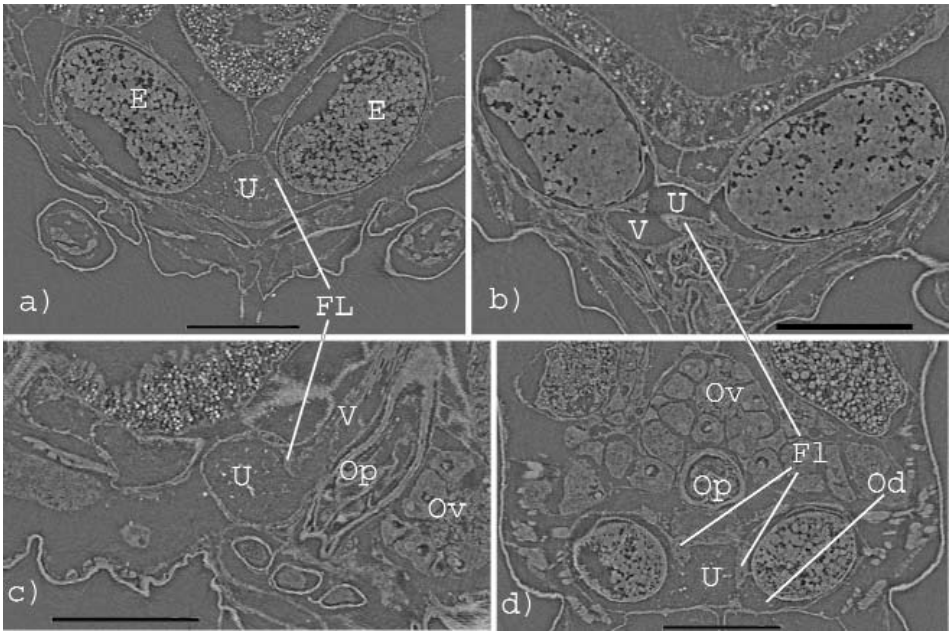

Fig. 14 Adult females, various specimens. Virtual slices of phase-enhanced tomography: a: transversal plane, adult of unknown age, flaps between oviducts and uterus; b: transversal, adult five days after last moult, flap between uterus and vagina; c: Sagittal plane, adult of unknown age, flap between uterus and vagina; d: horizontal plane, flaps between oviducts and uterus. Flap-like structures marking the boundaries between oviducts, uterus and vagina. Fl: flaps; Od: oviduct; Ov: ovary; Op: ovipositor; U: uterus; V: vagina. Scale bar = 100 µm.

A double sheathing of the ovary by a tunica propria and peritoneum, as reported for *C. cisalpinus* (Woodring & Cook 1962) or *Hydrozetes* sp. (Baker 1985) was not revealed in *A. longisetosus*. The authors, however, give no notice as to the nature of these layers. In accordance with Taberly (1987b) and Witaliński (1986), a thin follicle epithelium could be traced by the help of LM and TEM reaching from the periphery of the central zone to the tip of the ovarial thighs closest to the ovarial bulb. This ovarial bulb is described by Woodring & Cook 1962 as the slightly swollen part of the oviduct that makes contact to the ovary, i.e. the sheating of the oocytes. The follicular epithelium is delimited against the haemolymph space by a fine basal lamina, folded in intricate patterns (Fig. 11b). So, potentially the terms 'peritoneum' and 'tunica propria' are mere synonyms for the follicular epithelium and its basal lamina (Shatrov 2002), or may include the surrounding, putatively nutritive (Alberti et al. 2003) tissue. In the developing genital tract, the growing oviduct makes contact as an ovarial bulb at first with the developing central zone of the ovary (Fig. 4b). Later the developing previtellogenetic and vitellogenetic oocytes grow in paired extensions of the ovary, apparently following the path of the dorso-caudad retreating oviducts. Taberly (1987b, c) stated that previtellogenetic oocytes are tightly wrapped in follicular epithelium, and are therefore still being enclosed in the ovary, where vitellogenesis begins. The oocytes are then

passed into the lumen of the oviduct, and the completion of vitellogenesis and choriogenesis take place in the oviduct in *T. tectorum* and *P. peltifer*. In *A. longisetosus*, the follicular epithelium is involved in vitellogenesis and eggshell formation. Although no detailed analysis of eggshell formation was undertaken in this study, the situation found closely resembles the results published by Witaliński (1986) for *H. gilvipes*. Upon formation of a perivitelline space, dense vesicles appear in the follicular epithelium, while a granular vitellar membrane starts to build up and yolk vesicles accumulate and grow in a centripetal manner, as described for Astigmata by Walzl et al. 2004 (Fig. 11b). As described by Witaliński (1986) for *H. gilvipes*, these processes take place in the distalmost parts of the ovary. In discriminating ovary and oviduct, we adopt the nomenclature used by Taberly (1987b), based on the presence of a lumen and differences in tissue structure between follicular epithelium and oviduct wall. In all adult specimens of *A. longisetosus* analysed so far, this demarcation coincides with the presence of smooth, developed eggs in final size that seem to have completed vitellogenesis and the eggshells of which seem to have been already deposited and are merely subject to tanning. As the reported impermeability of the vitelline envelope (Aeshlimann & Hess 1984, Yastrebtsov 1992) would hinder communication between the egg and the mother, this demarcation would probably also mark the beginning of the following generation as separate biological units (Witaliński 1993). Yet this leaves the structure referred to as 'ovary' with two easily distinguished subdivisions: First, the central zone, consisting of the medulla and radially arranged nutritive cords and small cells, which have a flower-like appearance in cross sections.

Second, the zone of previtellogenetic and, in the case of *A. longisetosus*, vitellogenetic oocytes tightly wrapped in follicular epithelium and forming two paired lateral extensions (thighs) that reach out along the lateral body wall in rostrad orientation in its proximal and anad orientation in its distal part.

To avoid further confusion, a solution could be to propose useful, not mistakable terms for these subdivisions. With reference to their overall appearance in light microscopy and µCT, the latter portion could be denominated the 'Meros' of the ovary (as Greek 'ό μερός' = 'thigh, hip; part, side'), the first one 'Rhodoid' (as Greek 'τό ροδον' = 'rose') (Fig. 15). The term 'Rhodoid' does not interfere with 'Germarium', as it refers to the persisting structure solely and does not imply the existence of oogonia, which has been denied by Taberly (1987c) for adult specimens of *T. tectorum* and *P. peltifer*.

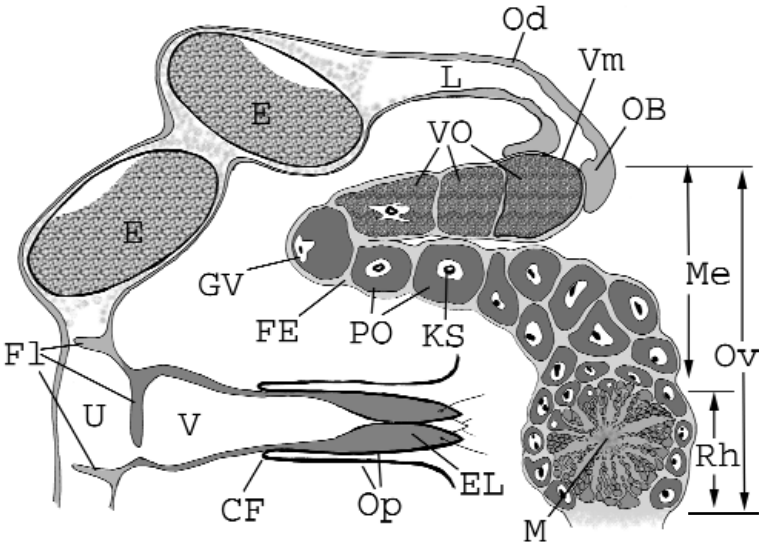

Fig. 15 Schematic drawing of the genital system of an adult female of *A. longisetosus*. The general arrangement of the major subdivisions is shown along with anatomical features mentioned in this work. Right portion only. CF: circular fold; E: egg; EL: eugenital lobes; FE: follicular epithelium; Fl: flaps, subdividing the genital tract; GV: germinal vesicle; KS: karyosphere; L: lumen; M: medulla; Me: meros (thigh) of the ovary; OB: ovarial bulb; Od: oviduct; Op: ovipositor; Ov: ovary; PO: previtellogenetic oocyte; Rh: rhodoid (central part) of the ovary; U: uterus; V: vagina; VM: vitellar membrane; VO: vitellogenetic oocyte.

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