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Titel der Diplomarbeit

Ultrastructural Investigation on the Female (resp. Hermaphroditic)
Reproductive System and some Aspects of Oogenesis
in 5 Marine Arthrotardigrada (Heterotardigrada)

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1 Introduction

Abstract

Few investigations exist on the reproduction of **marine** arthrotardigrades up today. Reproduction and in particular **oogenesis** is a neglected field of tardigrade biology. The aim of this study is to contribute to the ultrastructure of the genital apparatus (gonopore, gonoduct, gonads) and the stages of gametogenesis.

In this study, single stages of oogenesis and female (respectively hermaphroditic) reproductive organs of the marine arthrotardigrades (heterotardigrades) are investigated.

Five species of marine arthrotardigrades of two families are compared with each other (as far as possible, because some of the available specimens are in different stages of oogenesis).

Of special interest in this study are: different stages of germinal cells in the ovary, oviduct, seminal receptacle and gonopore of the four female arthrotardigrade species *Actinarctus doryphorus*, *Halechiniscus greveni*, *Styraconyx* n.sp. and *Batillipes noerrevangi*.

Additionally the first description of the ultrastructure of the hermaphroditic gonad and stages of oogenesis in the (only hermaphrodite known so far) marine tardigrade *Orzeliscus belopus* is given.

Because of the little sampling size it was difficult to compare different stages of oogenesis.

This work is only a first attempt to ultrastructurally elucidate the structures mentioned, further studies should be made to understand the entire process of oogenesis in marine arthrotardigrades.

1.1 Phylum Tardigrada

(RAMAZZOTTI & MAUCCI 1983; KINCHIN 1984; HANSEN 2004)

1.1.1 General remarks

Tardigrades are aquatic micro-metazoans.

First discovered in 1773 by J.A.E. GOEZE, they were called „*kleiner Wasser Bär*“, and they since then have been controversial as to their systematical position.

In 1962, RAMAZZOTTI erected the phylum „*Tardigrada*“ which is consistent till today.

Taxonomically, the phylum is related to the **arthropod – annelid** complex (BACCETTI & ROSATI 1969, 1971; BUSSERS & JEUNIAUX (1973), GREVEN (1980), KRISTENSEN (1976, 1978b, 1979) or, by other authors, to the **nematodes** (CROWE et al 1970, DEWEL & CLARK (1973). KINCHIN (1984) gives a good overview on the tardigrade phylogenic position.

Active tardigrades are always surrounded by a liquid layer of water. Under unfavourable conditions, many tardigrades (especially the semi-terrestrial species) have the special ability to undergo cryptobiosis to form a so-called „*tun*“. At this stage, the metabolism of the animal is standing still. This special physiological state is not an exclusive feature of tardigrades: other animals living in habitats characteristic of inconstant conditions, like Rotatoria and Nematoda have developed the ability of undergoing such resting stages as well.

The exceeding 900 species of tardigrades known to date have conquered a diversity of marine, freshwater and (semi-)terrestrial habitats. They live in the interstitial mesopsammon of marine and freshwater sites, in cryonit holes at the poles or hot hydrothermal springs. They are found on algae, mosses, liverworts, lichens or rather seldom on flowering plants; they also live in leaf litter, sediments or soil.

Some species show a cosmopolitan distribution, while others seem to be rare or endemic.

The geographic distribution of tardigrades still leaves many white spots to be worked on (especially the mesopsammon of freshwater and marine habitats).

Most tardigrades are herbivorous, few terrestrial species are true carnivores.

2 species of marine tardigrades have been described as truly ectoparasitic (*Tetrakentron synaptae* on *Leptosynapta gallienni* (Holothuroidea), *Echiniscoides hoepneri* on *Balanus balanus* (Cirripectida); KRISTENSEN 1980).

The range of body size of tardigrades reaches from 50 µm up to 1200µm total length. The average size is from 250 to 300µm in Echiniscidae (Heterotardigrada), and 350 to 500µm in Macrobiotidae (Eutardigrada). Marine species are usually smaller than terrestrial or freshwater species.



1.1.2 Anatomical and morphological remarks

Tardigrades are easily recognized, since their body shows general characters:

The body is bilateral symmetrical: flattened on the ventral, more or less convex on the dorsal side. It normally possesses 4 pairs of legs, which terminate from two up to multiple claws of varying shapes, or digits with claws or sucking discs. The legs usually are segmented (which is clearly recognizable in the telescopic legs of arthrotardigrades).

Tardigrades display a metameric organization since their body is separated in 5 indistinct segments: one cephalic segment, three body segments (each bearing one pair of legs, which direct lateroventrally) and one terminal segment (bearing the last pair of legs).

The nervous system that reminds of the arthropods consists of a tripartite large brain (divided into proto-, deuto- and tritocerebrum) connected via commissures to a subpharyngeal ganglion, followed by four trunk ganglia.

The covering chitinous cuticle is secreted by the epidermis and shows different sculptures and projecting spines or filaments. In some species, the thickened cuticle forms distinct plates (Echiniscoidea and Arthrotardigrada). The animals undergo periodical moults, in which the ectodermal parts of the gut and even the claws (and toes if existent) of the legs are renewed. Tardigrades in moult without the buccopharyngeal apparatus have been described as “*simplex stage*” (KRISTENSEN 1976). The synthesis of new claws (and toes) occurs in special claw glands of the legs, while the pharyngeal apparatus is rebuilt by stylet glands (in previous descriptions termed “*salivary glands*”).

Tardigrades are often colourless or greyish and thus are more or less transparent. Sometimes, the pigmentation of the epidermis or an accumulation of reserve matter in the coelomocytes cause lively colours, such as brownish, yellow, orange, pink, violet, red or rarely green.

True circulatory and respiratory systems are lacking in tardigrades, due to their small size. Between the muscle traits and the internal organs coelomocytes are floating in a fluid-filled body cavity (haemocoel); their main function is storage and mobilisation of polysaccharides and lipids (KINCHIN 1994). As concluded from its development, the body cavity is a pseudocoelom or haemocoel, it is not lined by any epithelium (BERTOLANI & REBECCHI 1999).

The digestive tract is divided into three parts: the foregut (buccopharyngeal apparatus), the midgut and the hindgut.

Separate muscle bands, each composed of a single cell comprise the muscular system of the tardigrades. Somatic muscles are truly cross-striated, they may be obliquely striated as well.

1.1.3 Systematical remarks

The phylum consists of two large classes:

Heterotardigrada (with about 379 species in 55 genera)

and **Eutardigrada** (with 551 species in 46 genera) (HANSEN 2004).

A third class (Mesotardigrada) is considered dubious today, for it has been created to include a monotypic species, whose type locality has been destroyed by an earthquake before more specified descriptions could be made of the species (RAMAZZOTTI & MAUCCI 1983).

Eutardigrades, organized in two orders (Parachela & Apochela) generally have a uniform body shape; the segmentation of the legs is reduced which gives them a stumpy appearance. Most eutardigrades' legs terminate in double claws without digits. They have a true cloaca, three ectodermal Malpighian tubules between the mid- and hindgut. Their cephalic appendages are mostly reduced. The most frequent characters used in determination are the morphology of the bucco-pharyngeal apparatus and the form of the (double-)claws. Eutardigrade species are almost entirely (semi-)terrestrial or limnic, only few marine species have been described in some genera (e.g. *Halobiotus*).

Heterotardigrades, organized in two orders (Echiniscoidea & Arthrotardigrada) exhibit a wide range of body shapes and different leg terminating structures (claws, digits bearing claws or sucking discs). Their intestinal and genital ducts have separate orifices, the gonopore lies posterior to the three-lobed anus, which is situated between the fourth pair of legs. They have distinct cephalic, trunk and leg appendages that are important for species determination. Moreover the type of bucco-pharyngeal apparatus, morphology of the foot with its terminating structures and presence or absence of cuticular plates are brought in for heterotardigrade systematics. Heterotardigrades live in terrestrial, limnic and marine environments.

The majority of the authors agree on the fact, that marine heterotardigrades are the most apomorphic of the tardigrades.

**Arthrotardigrada** (HANSEN 2004)

Arthrotardigrade heterotardigrades show a wide array of diversity in body shape - reaching from simplified vermiform body shape with an unornamented cuticle (*Coronarctus*, *Angursa*, *Styraconyx*) to cuticular expansions in form of wings (*Florarctinae*, *Paradoxipus*, *Chrysoarctus*), elongated pillars (*Actinarctus*, *Rhomboarctus*, *Raiarctus*), balloons or leaf-like structures (*Tanarctus*), projections (Batillipedidae, *Halechiniscus*) and dorsal plates (Renaudarctidae, Stygarctidae, Neostygarctidae).

Some arthrotardigrades were found to have cuticular (cephalic) vesicles containing symbiotic bacteria (e.g. *Wingstrandarctus* KRISTENSEN 1984)

Most arthrotardigrades are characterized by a complete set of cephalic appendages, which in most cases are of the segmented type (consist of cirrophorus, seta and flagellum).

This set of cephalic appendages, surrounding the mouth opening, is composed of

- an unpaired median cirrus,
- paired internal cirri,
- paired external cirri,
- paired primary clavae (normally with terminating pores),
- paired lateral cirri (= cirri A) and
- two sensory plates (=cephalic papillae, sometimes referred to as tertiary clavae)

A variability of cirrus like sensory organs located on the body is of taxonomic value (e.g. leg spines, paired terminal cirrus E).

KRISTENSEN & HIGGINS (1989) give a good ecological overview on marine tardigrades (in relation to sediments).

Only few scientists have focused their interest on the biology and electron microscopy of marine arthrotardigrades (R.M KRISTENSEN, J. RENAUD-MORNANT, S. DEGRIMALDI-ZIO and others of her group, L.R. POLLOCK)

1.2 Reproduction of Tardigrades

(BERTOLANI 1983, 1992, BERTOLANI & REBECCHI 1999, Kinchin 1984)

Reproduction in tardigrades only appears through gametes (fertilized or unfertilized eggs). Tardigrades are generally **gonochoristic** (bi- and unisexual). Predominantly in eutardigrades (e.g. genera *Macrobiotus*, *Hypsibius*, *Milnesium*), meiotic and ameiotic **parthenogenesis** (unisexual populations of non-marine tardigrades, with diploid, triploid and tetraploid biotypes) are important strategies, often occurring in instable environments. Parthenogenesis allows a rapid colonization of environments after passive dispersal in connection with the special feature of cryptobiosis (BERTOLANI 1994). Some populations totally lack male specimens.

Rarely, **Hermaphroditism** is occurring in some – mostly eutardigrade - species (e.g. *Macrobiotus*, *Amphibolus*), and up to date in two marine heterotardigrade populations of *Orzeliscus* found by KRISTENSEN, (unpublished) and *Batillipes pennaki* (GRIMALDI DE ZIO & D'ADDABBO GALLO, 1975) (BERTOLANI 1987, 1992). Sparse studies indicate that hermaphroditism is simultaneous (female gametes appear in the ovotestis besides male ones without barriers) and that self-fertilization is possible (BERTOLANI & REBECCHI 1999).

1.2.1 Gonadal morphology and sexual dimorphism

The always unpaired, sac-shaped **gonad** is situated dorsally of the gut in the pseudocoel. The gonad fuses during embryonic development from paired mesodermal anlagen (eucoelomate rudiments), which originally were thought to be formed by enterocoely (MARCUS 1928 & 1929), modern authors revised the description of gastrulation and discovered schizocoely (BERTOLANI & REBECCHI 1999).

The gonad has a very thin cellular lining (sometimes only consisting of the basal lamina in most of the heterotardigrades) and is held up and fixed to the body wall dorsally by a single middle anterior/cranial **ligament** (in heterotardigrades) or by 2 anterior/cranial ligaments (in eutardigrades). The dimension/size of the gonad depends on the age of the animal and the gonadal stage in the reproductive cycle – e.g. the gonad of mature females covers mid-gut and rectum completely, especially when it is filled with mature eggs before deposition. (WEGLARSKA 1979) Male gonads (e.g. of eutardigrade *Milnesium tardigradum*) are small, spheroidal and flattened. Some degree of species-specific variation in the shape of the gonad occurs for example in marine species (REBECCHI et al. 2000b).

Sexual differentiation occurs during post-embryonic development and the germ cell maturation begins after the first or second moult.



The gonads resemble each other in both sexes. Only the caudal **gonoducts** allow sexual determination, if no ripe gonial cells are visible inside the gonad: paired deferent ducts distinguish the male testis from the female ovary, which only has one single oviduct. The gonoduct of hermaphrodite species resembles the unpaired oviduct of the females. The gonoducts only display a very narrow lumen and have a thin lining of flattened cells resembling those of the ovarial border.

Some ripe adult males of hetero- and eutardigrade species show a dilated distal portion of each deferent duct or paired caudal bulges formed by the testis which both contain high numbers of active spermatozoa (seminal vesicles) (BERTOLANI 1992, JØRGENSEN et al 1999).

Ultrastructural studies on spermatozoa and spermiogenesis are still limited among the heterotardigrades (KRISTENSEN 1979 & 1984; KRISTENSEN & HALLAS 1980; JØRGENSEN et al 1999; REBECCHI et al 2000b).

Heterotardigrade spermatozoa lack a well defined midpiece which is consistent in eutardigrades; most heterotardigrade spermatozoa bear two unmodified “free” mitochondria.

The genital ducts of heterotardigrades end in a ventral **gonopore**, situated anterior to the anus (which is situated ventrally between the 4th pair of legs). Those of heterotardigrades, may as well serve to distinguish the sexes in most of the gonochoric species: the male gonopore simply displays a small circular or oval opening, while the female genital opening consists of 6 cuticular valves, forming a rosette which is situated more anterior than the male opening (KRISTENSEN 1980, POLLOCK 1970).

The genital duct(s) of eutardigrades open(s) into the rectum, thus forming a **cloaca** with a ventral orifice between the 4th pair of legs in both sexes.

Many tardigrade females have **seminal receptacles** (primarily described as “*annex glands*” with excretory and osmoregulatory functions by RENAUD-MORNANT 1967 and POLLOCK 1970; later, it has been recognized as cuticular structure for storing spermatozoa by KRISTENSEN (1984)): most marine arthrotardigrade females (except for Batillipedidae) bear paired cuticle-lined vesicles situated ventro-laterally, with sinuous ducts which open via separate outlets on either side of the female rosette gonopore. (GRIMALDI DE ZIO et al. 1990; RENAUD-MORNANT & DEROUX 1976).

JØRGENSEN et al. (1999) describe in their work the spermiogenesis of *Actinarctus doryphorus* and investigate the postcopulatory modifications of spermatozoa inside the female seminal receptacles. They are shed during moult with all their content - mostly few degenerated spermatozoa (KRISTENSEN 1984).

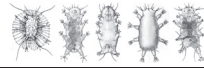
In a few eutardigrade females (e.g. *Macrobotus hufelandi*) (MARCUS 1928 & 1929, BERTOLANI 1983), and in one heterotardigrade (*Batillipes pennaki* - GRIMALDI DE ZIO & D'ADDABBO GALLO 1975), a single internal organ devoid of cuticle has been found, leading into the rectum via a short duct besides the orifice of the genital duct(s). The origin of this epidermal organ obviously different from that of the seminal receptacle of heterotardigrades, and has been referred to as **spermatheca**.

The seminal receptacle and spermatheca are best observed when they are filled with spermatozoa. Terrestrial tardigrades (exception *Oreella*) and some heterotardigrades (like *Batillipes*) lack cuticular seminal receptacles.

In some instances dimorphism was reported in secondary sexual characters (BERTOLANI 1992). In heterotardigrades, the **sexual dimorphism** has been recognized earlier than in eutardigrades (not only - as mentioned - in the sexes different gonopores) and as here it also may be used to distinguish males from females. The heterotardigrade males are thus often smaller than the females, their cephalic clavae (chemoreceptive sensory appendages) may be longer and larger (e.g. *Halechiniscus greveni*, *Actinarctus doryphorus*), and the epicuticle may be more massive in some marine species.

In eutardigrades, sexual dimorphism occurs in a minority of species and it is always associated with the legs (claw shapes), but it is not valid to allow a sexual diagnosis, since the feature can change while moulting in relation to egg deposition or mating (REBECCHI & NELSON 1998).

In few marine species, dwarf males have been seen, which exist besides normal sized males in some populations (e.g. *Tetrakentron synaptae* see KRISTENSEN 1980; *Tholoarctus natans* see KRISTENSEN & RENAUD-MORNANT 1983).



1.2.2 Mating, fertilization and embryogenesis

(BERTOLANI 1990, BERTOLANI & REBECCHI 1999)

The knowledge of mating, fertilization processes and embryonic development is mostly based on chance observations, because up to date rearing tardigrades in the laboratory has been little successful (and has mostly been done on parthenogenetic species of terrestrial or freshwater eutardigrades).

Internal fertilization occurs in most gonochoristic non-marine species (with copulation preceded by a complex courtship behaviour). Species that possess seminal receptacles fertilize their eggs externally since the lumen of the receptacle is lined with cuticle and the ducts open just beside the gonopore. In some cases, egg deposition occurs shortly after fertilization and in most cases, moulting is related to it as well.

Some populations of terrestrial, mainly, heterotardigrada have no males and there is no evidence of how fertilization occurs in cases of hermaphroditism.

Observations of mating have been described in some eutardigrade species, the females were in the process of moulting while copulating. In eutardigrades no copulatory organ has been observed, the gonopores of some marine and terrestrial heterotardigrades seem to form a small externally protruding tube, which may help copulation.

The egg is homolecithal, the first cleavages are total and equal with an early loss of symmetry, which indicates a modified spiral cleavage. The resulting blastula has a small blastocoel and is composed of equal sized cells. The endoderm delaminates at a very early developmental stage and fills the blastocoel.

MARCUS (1929) first described embryogenesis in detail, he mentioned five enterocoelous somatic pouches which form from the intestine walls, the frontal pairs disaggregate to form isolated cells, only the last pair fuses into a single anlage to form the gonad - thus the only eucoelomic structure. Embryogenesis has not been entirely covered by ultrastructural surveys, but actual findings indicate an schizocoelic origin from pre-existing mesoderm without enterocoely.

The post-embryonic development of many heterotardigrade species is an indirect one, with "larval" stages, differing from adults mostly related to cuticular structures (e.g. claw number or anatomy, spikes, plates, presence of gonopore and or anus etc.); the adult stage is normally reached after the 2nd moult. The eutardigrades display a direct development, only the secondary sexual characters differ in adults.

1.3 Oogenesis

Animal eggs are highly differentiated cells, since they contain the full developmental program to produce a new individual.

Oogenesis is the first and maybe important phase of embryogenesis. In this stage the sexual recombination takes place (in meiosis), organelles are replicated and stored, metabolites (mainly yolk) for embryogenesis are produced and accumulated and important information for further development is stored in nucleus, cytoplasm, cortex and plasma membrane (WOURMS 1987).

1.3.1 Oogenesis in tardigrades

The studies on oogenesis in tardigrades are very scarce till today. MARCUS (1928 and 1929) described the tardigrade reproduction in very detailed light microscopic studies.

Several light microscopical studies deal with eggs of eutardigrades. B. WEGLARSKA published four electron microscopic studies on the female and hermaphrodite reproduction of eutardigrades (WEGLARSKA 1975, 1979, 1982 and 1987).

While there are quite a few light and ultrastructural studies on spermiogenesis of both eu- and heterotardigrades (for review, see REBECCHI et al. 2000b; for example also see JØRGENSEN et al. 1999), the reproduction of the few known hermaphrodite species has not been studied by means of electron microscopical techniques, only ecological and light microscopical studies have been published till now (REBECCHI et al. 2000a, REBECCHI & BERTOLANI 1994).

Up to date, no publication has dealt with the ultrastructure of oogenesis and female gonadal structure in heterotardigrades, which maybe due to the fact that heterotardigrade specimens generally are difficult to prepare due to their thick cuticle and arthrotardigrades generally are difficult to collect. JØRGENSEN et al. 1999 describe seminal receptacles of *Actinarctus doryphorus* in chapter 3.4. of their paper in connection with postcopulatory reductions of spermatozoa.

Fertilization takes place internally of the female body, some authors have described the occurrence of a vitelline membrane, in marine heterotardigrades a micropyle seems to be absent in the egg shell.

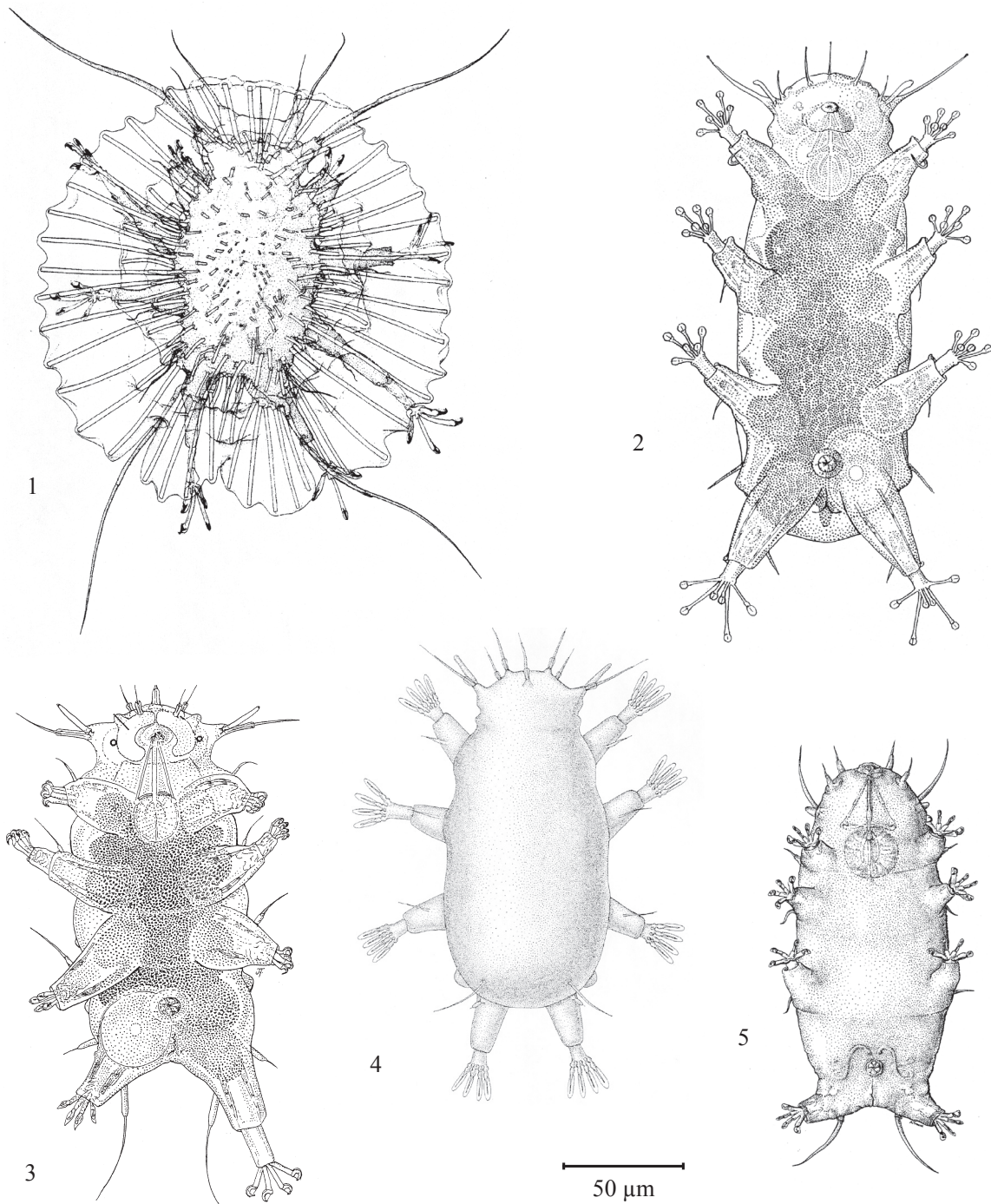
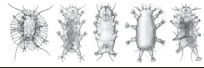
Oogenesis usually begins after the second moult and the moult cycle is synchronized with the successive oocyte ripening (BERTOLANI & REBECCHI 1999).

The tardigrade females mainly are iteroparous (=breeding several times per lifetime) (REBECCHI & BERTOLANI 1994)



Sometimes only one (*Wingstrandarctus* - KRISTENSEN 1984) to few eggs are deposited, some species may lay more eggs (*Echiniscoides* - KRISTENSEN & HALLAS 1980).

Eggs are of spherical or ovoid shape and have a smooth or ornamented shell (referred to as **chorion**). Marine heterotardigrades normally deposit their smooth sticky eggs freely on the substratum (plesiomorphic character), as do the limnic or terrestrial (mostly eu)tardigrades with their ornamented eggs, or if the eggs are smooth-shelled, they are left in the exuvium during the moult (apomorphic character - ornamentation probably was lost). In some eutardigrade genera, the egg-shell ornamentation is an important systematic feature. (RAMAZZOTTI & MAUCCI 1983 ; BERTOLANI et al. 1996, D'ADDABBO GALLO et al. 1999).



Figs. 1 - 5: Habitus of female marine Arthrotardigrades (Species, resp. genus mentioned in this study):

Fig. 1: *Actinarctus doryphorus* (from: D'ADDABBO-GALLO et al. 1999, p. 22), dorsal view.

Fig. 2: *Batillipes noerrevangi* (from: KRISTENSEN 1978a, p. 5), ventral view.

Fig. 3: *Halechiniscus greveni* (from: KRISTENSEN 1981, p. 28), ventral view.

Fig. 4: *Orzeliscus belopus* (from: MCKIRDY et al. 1976, p. 14), dorsal view.

Fig. 5: *Styraconyx sardiniae*, a species comparable to *S. n. sp.* (from: D'ADDABBO-GALLO et al. 1989, p. 19).



2 Material and Methods

2.1 Specimen

The following species (sampling sites in brackets) were examined (habitus see Figs. 1 - 5):

- *Halechiniscus greveni* (Frederikshavn, Denmark)
- *Actinarctus doryphorus*, SCHULZ 1935 - (Roscoff, France)
- *Styraconyx* n. sp. (Roscoff, France)
- *Batillipes noerrevangi*, KRISTENSEN 1978 - (Niva, Denmark)
- *Orzeliscus belopus* (Bermuda)

For a tabular description and comparison of the five mentioned species see Table 2a - 2e (pp. 24 - 29).

Systematic position:

Phylum Tardigrada	
Classis Heterotardigrada	
Ordo Arthrotardigrada	
Familia Halechiniscidae	
Subfamilia Halechiniscinae	<i>Halechiniscus greveni</i>
Subfamilia Tanarctinae	<i>Actinarctus doryphorus</i>
Subfamilia Styraconyxinae	<i>Styraconyx</i> n.sp.
Familia Orzeliscidae	<i>Orzeliscus belopus</i>
Familia Batillipedidae	<i>Batillipes noerrevangi</i>

Tab.1: Systematic position of the observed species

2.2 Specimen treatment

The tardigrades were collected and prepared for examination with the transmission electron microscope by Reinhardt Møbjerg KRISTENSEN from the Zoological Museum in Copenhagen as described in JØRGENSEN et al. (1999) (p. 236 f)

The ultrathin sections on filmed grids were examined in a Zeiss EM 9 S-2 (at ZMUC, Copenhagen) and in a Zeiss EM 902 (at Vienna University, Department for Ultrastructure Research).

The material is in property of the Zoological Museum, University of Copenhagen (ZMUC).

	<i>Halechiniscus greveni</i>	<i>Styraconyx n. sp.</i>	<i>Actinarctus doryphorus</i>	<i>Orzeliscus belopus</i>	<i>Batillipes noerrevangi</i>
family		Halechiniscidae		Orzeliscidae	Batillipedidae
subfamily	Halechiniscinae	Styraconyxinae	Tanarctinae		
collected from	Roscoff, France	Roscoff, France	Frederikshavn, Denmark	Bermuda	Nivå, Denmark
1st description by	RENAUD-MORNANT & DEROUX 1976	not described yet	SCHULZ 1935	DUBOIS-REYMOND MARCUS 1952	KRISTENSEN 1976
found	Northern Sea, Mediterranean	Northern Sea	Northern sea (Helgoland) ^{1,2} , Mediterranean Sea ³	in the interstitium of sandy sediments	Northern Sea (Denmark) ^{1,2}
biotope	mesopsammon, sink in biological station Roscoff ^{1,2} in subtidal sediment ³		mesopsammon on Amphioxus sand ¹ , in Polygordius Bruchschill ² or in first 5 cm of coarse biogenous (coralligenous) detritus ³ ; accidentally found on an echiniscid (thus postulated as facultatively parasitic) ¹		in shallow waters, at surface of the sandy sediment, large aggregations in connection with vegetation ¹ , interstitially in sandy beaches ³
colour			colourless, transparent ²		
length	125 μm^1 , 90 - 133 μm^3		125 μm^2 , 164 μm^3 (without expansion); 170 μm^1 , 185 μm^2 , 210 μm^3 (with cuticular expansions)	about 200 μm	163 - 217 μm^1
width	60 μm^1 , 42 - 61 μm^3		70 μm^2 , 98 μm^3 (without expansion), 170 μm^2 , 230 μm^3 (with expansion)		

Tab. 2a: Comparison of five species of arthrotardigrada.

	<i>Halechiniscus greveni</i>	<i>Styraconyx n. sp.</i>	<i>Actinarctus doryphorus</i>	<i>Orzeliscus belopus</i>	<i>Batillipes noerrevangi</i>
cephalic appendages full set:	yes ^{1,2} large cirrophori except for the ventral cirri ³	yes	yes ² , with long scapus, short flagellum and strong cirrophorus (except for ventral ones) + ² , short ³	yes cirri of tripartite type	yes cephalic cirri are not segmented ⁴
median cirrus (up)	+ ^{1,2}	+	+ ² , short ³	+	+ with distinct socket ¹
internal cirri (p)	+ ^{1,2}	+	+ ^{1,2,3}	+	+ with distinct socket ¹
external cirri (p)	+ ^{1,2}		+ ^{1,2,3}	+	+ ¹
lateral cirri (p) (= cirrus A)	+ at end of lateral lobes ¹	+ on common cirrophore with primary clavae ²	+ ^{1,3} (described as bristle at lateral cirrus ²)	+	+ ¹
primary clavae (p)	+ at end of lateral lobes, may be longer than lateral cirri ¹ , banana-shaped, with pore at the end ^{2,3}	+ on common cirrophore with lateral cirri ²	+ ³ longer than lateral cirri (described as lateral cirri ^{1,2})	+	+ arising from same socket as the lateral cirri, club-shaped, slightly s-formed, with opening at distal end ¹
cephalic papillae (p) = tertiary clavae	+ flat, kidney-shaped, partially surrounding mouth opening (referred to as secondary clavae) ³	small ¹ , lenticular-shaped (secondary clavae) ² ?	+ with terminal pore ³ club shaped ³ (described as primary clavae ²)	+	+ ⁴ low, domelike structures around the mouth cone

Tab. 2b: Comparison of five species of arthrotardigrada.

	<i>Halechiniscus greveni</i>	<i>Styracomyx n. sp.</i>	<i>Actinarctus doryphorus</i>	<i>Orzeliscus belopus</i>	<i>Baillipes noerrevangi</i>
pharynx	with 3 placoids, ventral one longer with knob-shaped apophysis ³	with 3 distinct articulated placoids (ventral one longer) ²	bearing 3 pharyngeal bars ²		large circular pharynx bulb ¹
stylets			weakly curved ²		
stylet supports	+ ¹	+	+ ^{1,2}	+	+
mouth opening	ventral ¹	subterminal ²	ventral ¹ , on slight conical projection ² , surrounded by strong sucker-ring ³	subterminal flat mouth cone	ventral, pouting, triangular, small cuticular teeth in mouth opening ¹
head	flat, frontally rounded ¹ , lateral expansions (lobes - on the edges lateral cirrus and primary clava) ¹			head delimited from body by slight lateral body projection posterior to common bases of clavae and lateral cirri	clearly demarcated through constriction in front of 1st pair of legs ¹
eyespot	2 present		7 - 10 lipoid droplets around buccal area ^{1,3} in some populations (described as light receptive organs)		2 lipoid eyes present ¹
body cuticle	no lateral expansions ³ strongly segmented plates lacking	no dorsal segmental plates; punctuated by uniformly arranged pores ^{1,2}	no strongly sclerotic dorsal segmental plates ^{1,2,3} ; dorsal cuticular extensions suspended by elongated slender cuticular pillars (lateral longer than dorsal) („Röhrchen“ ⁴¹) forming wing-like appendage („Heiligenschein“ ⁴¹ = gloriole) around body ^{2,3}	transparent with a fairly regular punctation, small body projection between 3rd and 4th legs possible	typical caudal spike (0 or 2 - temporal variation), body shows 3 lateral projections (may vary) ¹

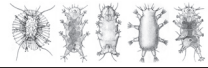
Tab. 2c: Comparison of five species of arthrotardigrada.

	<i>Halechiniscus greveni</i>	<i>Styraconyx n. sp.</i>	<i>Actinarctus doryphorus</i>	<i>Orzeliscus belopus</i>	<i>Batillipes noerrevangi</i>
legs: long, telescopically retractable	+		+ ^{1,3} tibia lance-shaped, tarsus conical ³	+	
legs terminating in		4 claws		4 genus characteristic elongate adhesive expansions	6 (4 juvenile) small adhesive sucking discs (totally circular) ¹
toes / digits	yes (4)	yes (4) ¹ internal digits with heart-shaped proximal pads ²	yes (4) ^{1,2,3}	yes (4)	6 (4 juvenile), of different length, some extremely long ¹
claws	4 hook-shaped ¹ : 2 external (simple) 2 internal (spurred) without calcar	4 with peduncles and 2 accessory points ^{1,2} large claw sheaths ² ?	4 hook-shaped ^{1,3} : 2 external (simple) 2 internal (spurred) all with claw sheath ¹ and strong calcar ³	no	no
sense organs	cirri on coxae of first to third legs ¹ , papillae on all 4 legs (4th leg with large bell-shaped cirrophorus) ³	appendages on all legs ¹	thin setae on each leg, longer on 4th leg ³	spines on all 4 legs, oval papilla with very fine distal thread on 4th leg, tripartite type	spines on all 4 legs ¹
caudal cirri	+ (cirri E with articulation) ^{1,3}		+ ^{1,2} , (cirri E with articulation pleat-shaped basal part) ³	+ cirrus E with thick base tapering continuously to a very fine point	+ strongly sculptured cirrus E ¹
dorsal cirri	+		+ between 3rd and 4th leg ^{1,2}		

Tab. 2d: Comparison of five species of arthrotardigrada.

	<i>Halechiniscus greveni</i>	<i>Styraconyx n. sp.</i>	<i>Actinartacus doryphorus</i>	<i>Orzeliscus belopus</i>	<i>Batillipes noerrevangi</i>
seminal receptacle (p)	simple vesicles with sinuous ducts opening on either side of female gonopore ³	paired with opening located upon female gonopore ²	dorso-lateral, filled with male sperm, openings lateral to female gonopore ³		
female gonopore	ventral rosetta with 6 plaques ¹	ventral rosetta with 6 petals ²	rosette-like, 22 µm from anus ³		system of 6 voluminous epithelial muscle cells, small eggs laid singly on sand grains, smooth and very sticky ¹
male gonopore			covered with crescent shaped cuticular fold 9 µm from anus ³		oval ¹
sexual dimorphism	gonopores, primary clavae larger in males than in females ^{2,3}		gonopores, primary clavae's basal portion stouter in males than in females ³		males smaller than females, gonopore
references	RENAUD-MORNANT & DEROUX 1976 ¹ ; KRISTENSEN 1981 ² ; GRIMALDI DE ZIO et al 1990 ³	KRISTENSEN 1977 ¹ ; D'ADDABBO GALLO et al 1989 (S. sardiniae) ² ; KRISTENSEN & HIGGINS 1984; KRISTENSEN (personal communication)	SCHULZ 1935 ¹ ; GRELL 1937 ² ; D'ADDABBO GALLO et al 1999 ³	McKIRDY et al 1976	KRISTENSEN 1978a ¹ , KRISTENSEN 1978b ² KRISTENSEN 1979 ³ KRISTENSEN 1981 ⁴

Tab. 2e: Comparison of five species of arthrotardigrada.



3 Results

3.1 Gonopore

In all five species considered, the female (resp. hermaphroditic) gonopore comprises a regular preanal rosette formed by six myoepithelial cells (seen in Figs. 7, 10, 13, 15 & 16) that are covered by cuticle. During the moult, the gonopore and its cuticle is rebuilt underneath the old gonopore by surrounding epidermis cells as it can be seen in *Actinarctus* specimens (Fig. 6).

The six leaved pattern of the epidermis rosette cells may be followed deeply under the epidermis (Fig. 16).

The gonopore is covered by a cuticular fold in *Actinarctus doryphorus* (Fig. 7) or in *Styraconyx* n.sp. females.

At a short distance to the gonopore there often may be found nerve cells (ganglia) with their typical nuclei (Fig. 7).

Also in-between the epidermis cells, nerve structures are to be detected (Fig. 9).

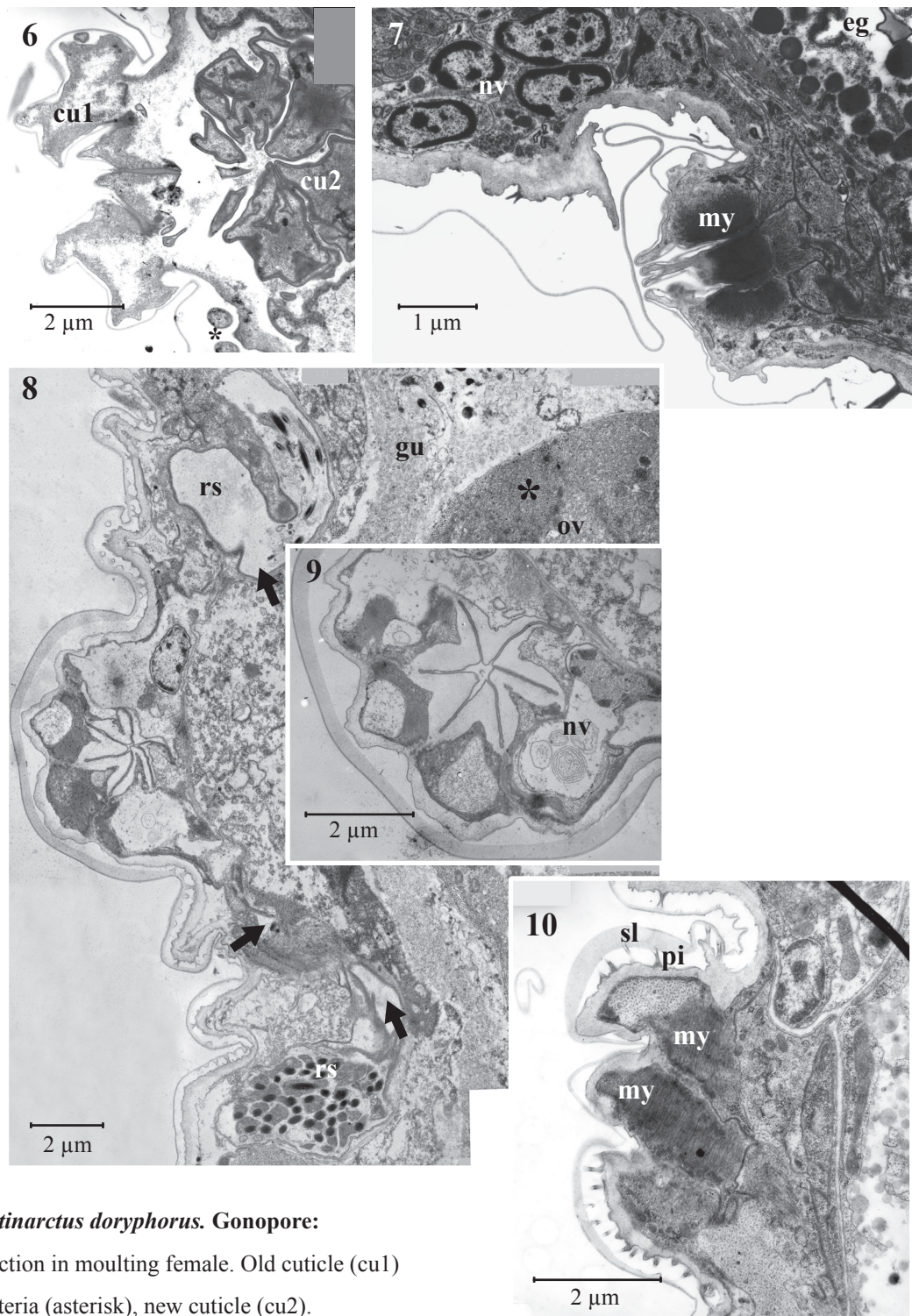
In some sections (of *Actinarctus doryphorus* and *Halechiniscus greveni*), the ovary is visible close to the gonopore complex (yolk granules), in some instances the gut is squeezed to only a very thin layer in-between the gonopore and the ovary, and may be hardly detected (Figs. 8 & 14).

The gonopore of *Styraconyx* n.sp. is a little elevated (as seen in Fig.11).

In *Batillipes noerrevangi* only the outer structure of the gonopore was investigated by REM (Figs. 12 & 13).

The seminal receptacles are in the vicinity of the gonopore, as could be seen in *Halechiniscus greveni* (Fig. 8); the gonopore and seminal receptacle were not in the same section plane in *Orzeliscus belopus*, but very close to each other (Figs. 21 & 22).

In *Orzeliscus*, some spermatozoa were found in a ventromedian space in the region of the gonopore (Figs. 15 and 17).



Figs. 6 & 7: *Actinarctus doryphorus*. Gonopore:

Fig. 6: Cross-section in moulting female. Old cuticle (cu1) with bacteria (asterisk), new cuticle (cu2).

Fig. 7: Sagittal section. Nerve cells (nv) with nuclei, myoepithelial cells (my) form the gonopore. Ovum (eg).

Figs. 8 - 10: *Halechiniscus greveni*. Gonopore:

Fig. 8: Cross-section. Ovary (ov) with microvilli-like structure (asterisk), gut (gu). Note the seminal receptacles (rs) and their ducts (arrows) on the ventral body side.

Fig. 9: Detail of gonopore rosetta. Note the nerval structures (nv) between the epidermis-cells.

Fig. 10: Sagittal section; myoepithelial cells (my), cuticle with striated layer (sl) and pillars (pi).

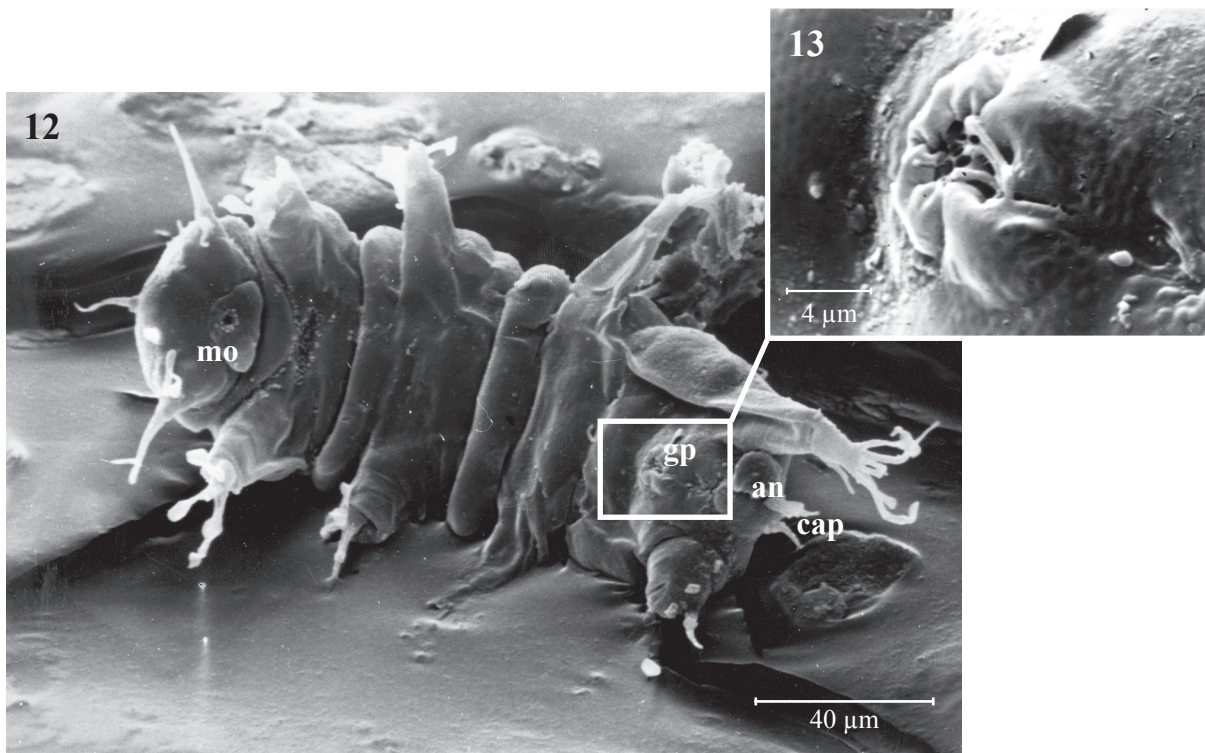
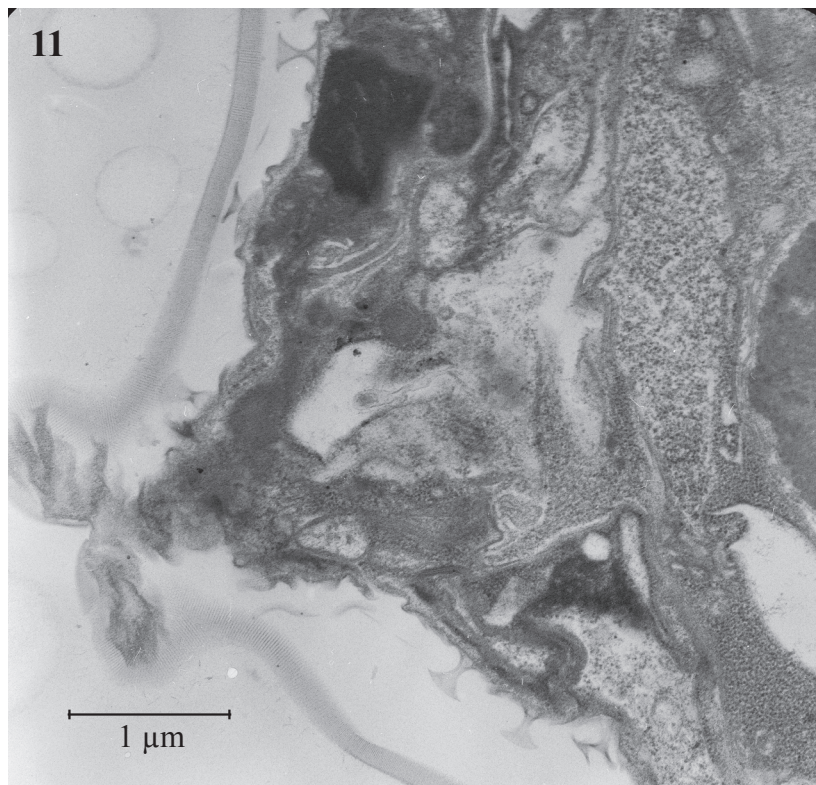


Fig. 11: *Styraconyx* n.sp. Elevated gonopore.

Fig. 12 & 13: SEM pictures of *Batillipes noerrevangi*. Gonopore (gp) from ventral side, survey (12) and detail (13); anus (an), mouth plate (mo); caudal appendages (cap).

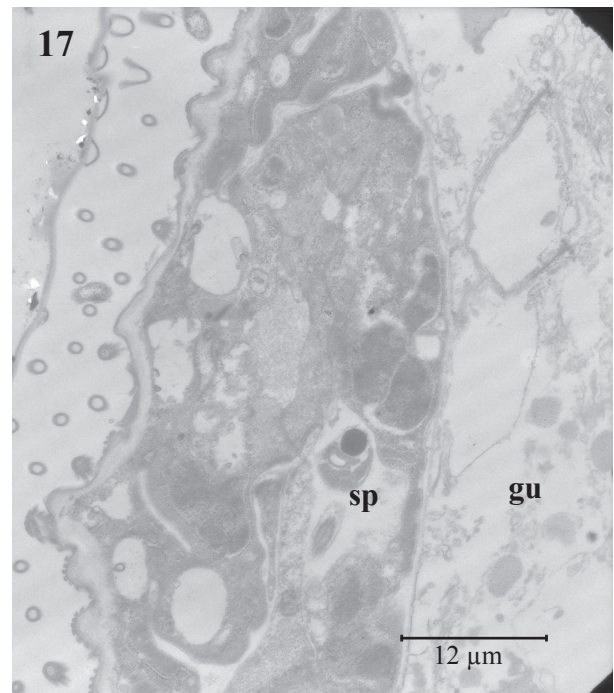
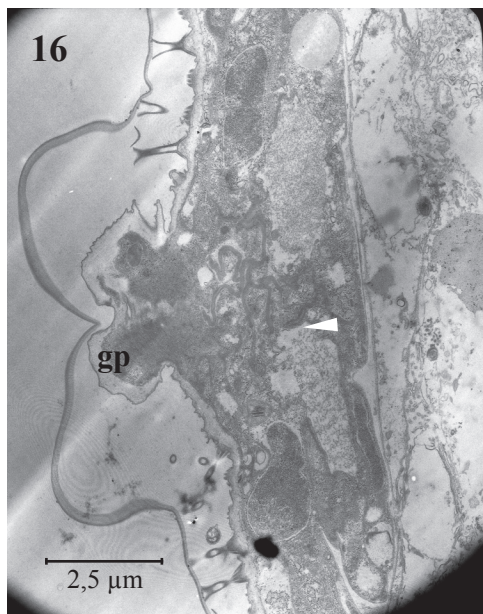
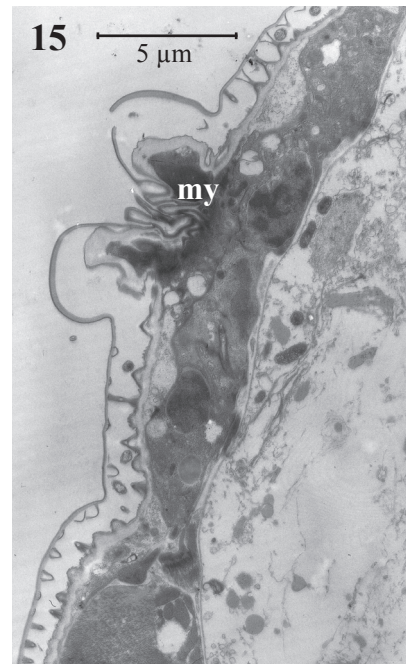
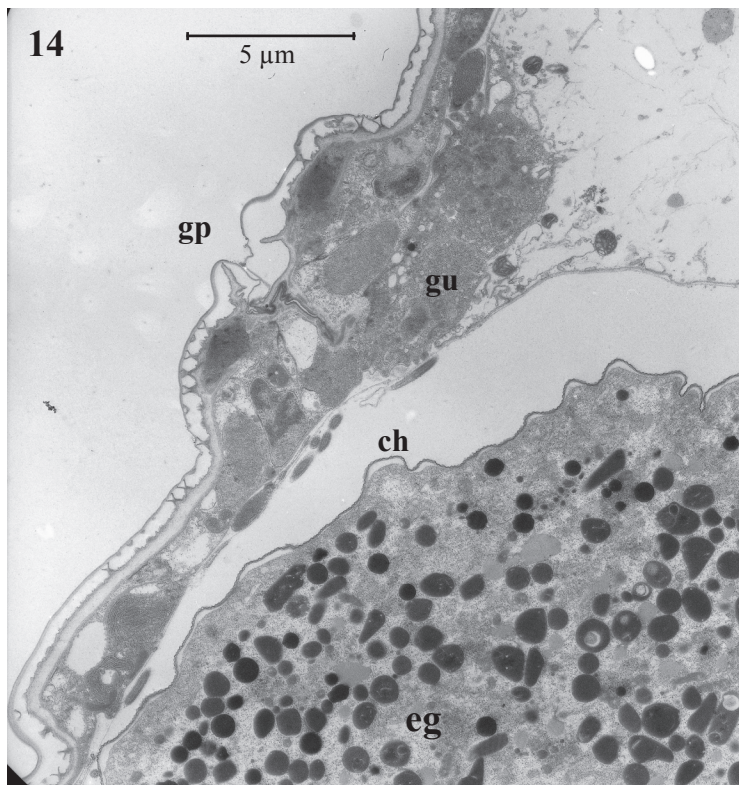


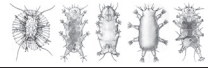
Fig. 14 - 17: *Orzeliscus belopus* female gonopore in cross-section:

Fig. 14: Ripe ovum (eg) beneath gonopore (gp), stuffed with yolk and covered by intact chorion (ch); gut (gu).

Fig. 15: Myoepithelial cells (my) of gonopore.

Fig. 16: Six-leaved structure visible (gp) beneath gonopore (white arrowhead).

Fig. 17: Section in plane close to gonopore; note the spermatozoa (sp), which lie beneath gonopore; gut (gu).



3.2 Seminal receptacle

Halechiniscus, *Styraconyx*, *Actinarctus* and *Orzeliscus* possess a paired cuticle-lined seminal receptacle. The separate orifices of the receptacles could be observed in *Halechiniscus greveni* and *Orzeliscus belopus* on either sides of the gonopore (Fig 8 & 22). No resembling structure has been encountered in *Batillipes noerrevangi*.

The opening of the duct on the ventral body surface was just missed in the section of *Halechiniscus greveni* (Fig. 8). In close vicinity of the receptacle, muscles are detected (Fig. 19).

The lumen of the receptacle is always lined by a thin layer of cuticle and quite frequently filled with few to numerous spermatozoa. The heads of the spermatozoa assemble in close contact to each other mainly in the bulbus (Fig. 19), while the cilia of the tails are extending into the duct of the receptacle. (Figs. 8, 18 & 20).

In one lateral bulbus of the seminal receptacle of *Halechiniscus greveni*, in cross section there are approximately 20 to 40 spermatozoa per seminal receptacle (Fig. 8).

In *Actinarctus doryphorus* the bulbus and the duct of the receptacle are found close to the cuticle and the surrounding epidermis cells contain many parallel membranous structures. Underneath the receptacle, an empty lumen lined with cuticle is embedded in epidermis cells with a big active nucleus.

The ducts of *Orzeliscus' belopus* receptacles lie quite superficially and their small lumina contain one single spermatozoon only per receptacle. The striated layer of the thick cuticular lining is evident (Figs. 21 & 22). The cilium of the spermatozoon is surrounded by a kind of heterogeneous granular matrix, as may be found in *Actinarctus doryphorus* (Fig. 20). In Fig. 22 the orifice of the duct is visible on the left side. It is little distant from the section plain of the gonopore. The head of the sperm has not been encountered in the observed section planes.

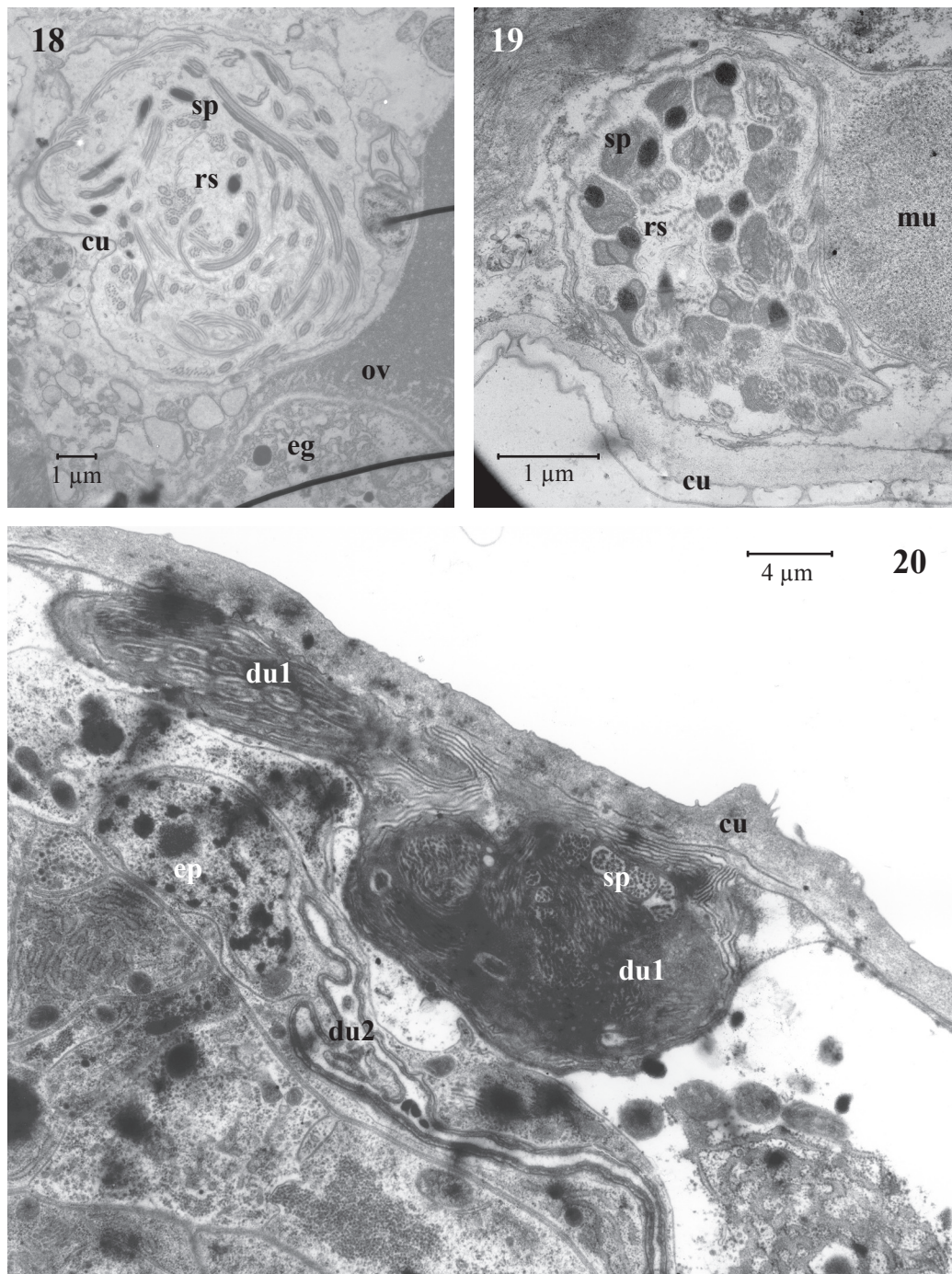
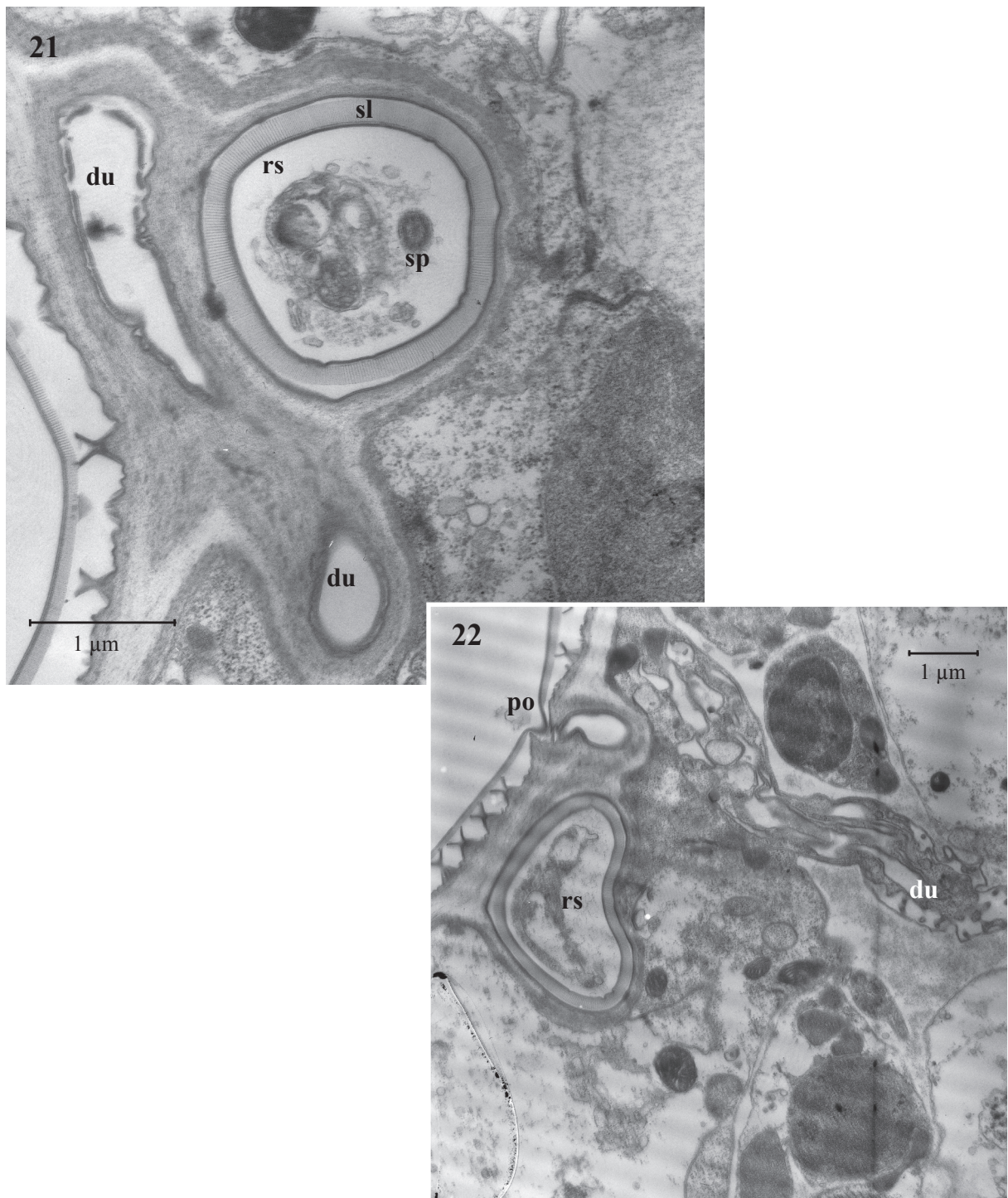


Fig. 18: *Styraconyx* n.sp. Seminal receptacle (rs) containing spermatozoa (sp), near ovary (ov) with single ovum (eg); cuticle of receptacle (cu).

Fig. 19: *Halechiniscus greveni*. Seminal receptacle (rs) of female containing spermatozoa (sp), nearby cuticle (cu). Prominent muscle (mu) in direct vicinity of receptacle.

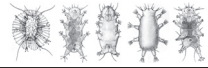
Fig. 20: *Actinarctus doryphorus* female seminal receptacle nearby moulted cuticle (cu); spermatozoa (sp) embedded in inside dense matrix inside the sections of the duct (du1). Beneath, new cuticle lined duct of empty seminal receptacle (du2) is formed by epidermis cells (ep).



Figs. 21 & 22: *Orzeliscus belopus* female seminal receptacle (rs) near ventral cuticle.

Fig. 21: Sections of the bulbus and duct (du) of seminal receptacle (rs); bulbus containing only one spermatozoon (sp). Note the striated cuticle layer (sl) inside the seminal receptacle.

Fig. 22: Other section nearby the long section of the duct (du). Note the opening of the seminal receptacle (rs) in the ventral cuticle (po).



3.3 Ovary and gonial cells

3.3.1 Structures of the ovary

The ovary is surrounded by a very thin wall, which is not continuously visible, because usually it is reduced to the basal lamina - especially in the region of the ripe ovum (see Figs. 24, 32, 37, 48, 50, 53, 58 & 61).

The ovary of *Styraconyx* reaches almost to the height of the pharynx bulb. The anterior end of the ovary is always characterized by a typical electron-dense osmiophilic matrix reaching into the cuticle, which probably serves as attachment for the frontal part of the ovary inside the body cavity (Figs. 30 & 31).

The ovotestis of *Orzeliscus belopus* also reaches the pharynx bulb; in the most anterior portion there are only encountered test cells with early spermatogonia and single spermatozoa.

3.3.2 Stages of oogenesis

The cells in the anterior ovary have small round nuclei with surrounding systems of sometimes little dilated cisternae of RER. The mitochondria are often arranged in groups.

In-between the oocytes of *Styraconyx* n.sp., mainly at the ovary margins, there are sometimes cells with cytoplasm of fairly granular appearance (Fig. 34).

The sister cells of the oocytes are connected via intercellular bridges among each other as well as with the oocytes. An evident electron dense substance always accompanies the connecting membranes at the outside (Figs. 33 & 45). Inside the cytoplasmic bridges, organelles like ribosomes, mitochondria and endoplasmic reticulum may be observed (Figs. 28, 37). During the early stages of oogenesis, it is not clear, which cell will be the prospective oocyte.

In *Orzeliscus belopus*, even the nucleus (or part of it) has been found in the connecting cytoplasmic bridge between the two sister cells (Fig. 45). At this stage, the oocytes' nuclei were irregularly formed, very huge and studded by many regular nuclear pores (Figs. 38 & 45).

In the last third of the advanced ovary there is a characteristic caudal isthmus, where the nurse cell seems to spread out towards the mature ovum lying posteriorly. This ripe egg occupies most of the space in the caudal part of the ovary; only sometimes there are thin layers of a nurse cell visible (or the cells, which form the lining of the ovary) (Figs. 32, 43).

The mature ovum (reaching a diameter up to 50 μm) is stuffed with many yolk granules (or platelets) of heterogeneous appearance, surrounded by a single membrane (Figs. 32, 47, 60) that is not always easily visible. Many free ribosomes give the cytoplasm a very granulated

appearance, mitochondria are often arranged in groups and some cisternae of RER mostly lie in the vicinity of the nucleus. The outer nuclear membrane forms blebs going out into the cytoplasm (Fig. 36), becoming part of the endoplasmic apparatus, which is less developed than in the oocytal stages before.

In the species, where the eggs were in a very advanced stage, nurse cells could only rarely be proved; mostly they have been discovered in an anterior or a fairly posterior region of the ovary (Figs. 25 and 47).

The yolk of all species is quite heterogeneous in appearance; the big electron dense homogeneous yolk granules in ripe oocytes and ova may bear different heterogeneous inclusions (Figs. 32, 39, 42, 58). Yolk globules multiply during vitellogenesis and increase markedly in size (up to about 1 μm). Besides, there are less electron dense lipid yolk bodies around by which the membrane is clearly visible (sometimes with aggregates of ribosomes).

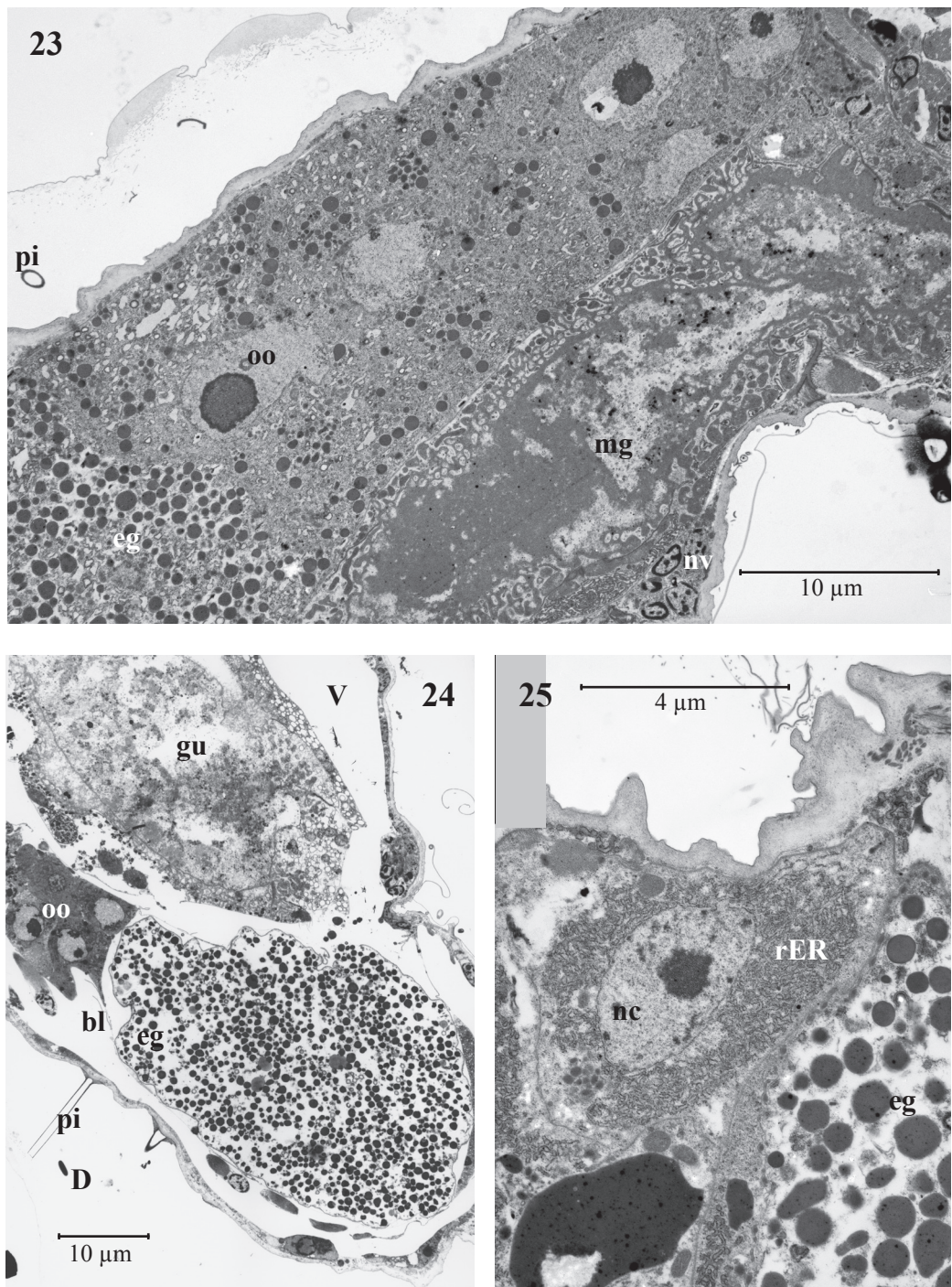
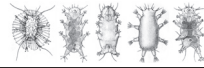
In *Actinarctus* (Figs. 23 & 24) and *Styraconyx* (Fig. 31) this succession of stages in oogenesis can be easily followed from the anterior to the most posterior part of the ovum. Cells increase in size towards the posterior end, and changes appear in organelle distribution, size and morphology. In the most posterior section one mature ovum with abundant yolk platelets dilates the ovary and thus pretends a preceding narrow section (isthmus).

Also in *Orzeliscus*, a big egg filled with many lipid and heterogeneous yolk granules, covered by chorion occupies the posterior part of the ovary (Fig. 42). This egg has been found constricted by a dorsoventral muscle in one instance (Fig. 41).

In *Halechiniscus greveni*, the clear sequence of oogenesis is not clearly visible in one specimen - most of the oocytes are at the same stage of oogenesis (Fig. 26). In the previtellogenetic oocytes, rough ER is found in parallel sheets or in whorls (with vesiculated margins) like dictyosomes, but all the membranes are studded with abundant ribosomes.

In another specimen, the ovary contained three advanced oocytes separated by elaborate microvillar structures (Figs. 49 & 50). The anterior part of the ovary with the earlier stages could not be detected in the latter individual.

In *Batillipes belopus* oocytes and nurse cells, an elaborate Golgi is frequently located in the vicinity of the electron-lucent nucleus; mitochondria group in the same region close to the dictyosomes and the nucleus (Figs. 38 and 39). Because of the sparse material, no comments on egg numbers ripening at once can be given.

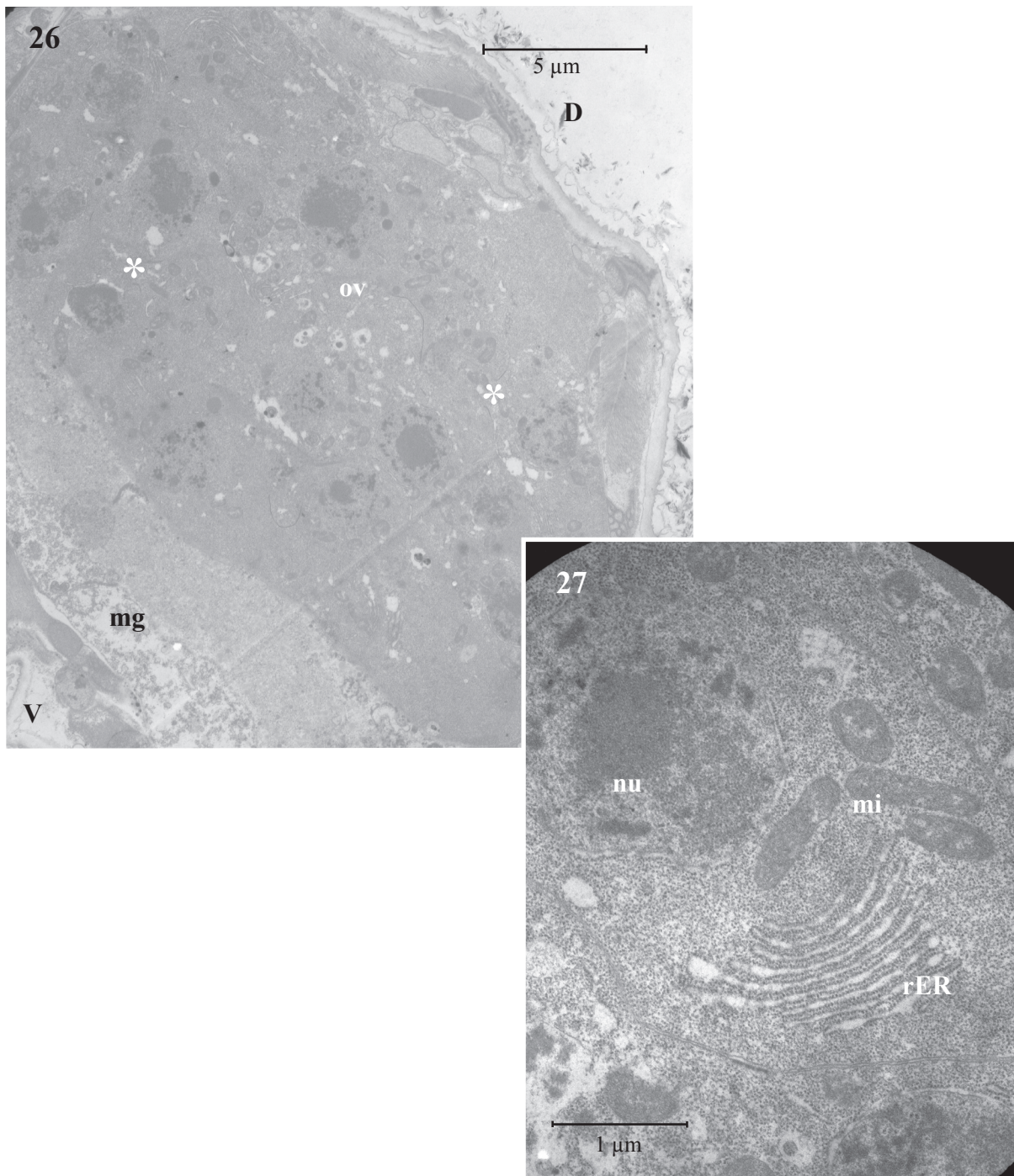


Figs. 23 - 25: *Actinarctus doryphorus*. Ovary, containing ripe ovum with yolk caudally:

Fig. 23: Survey; oocytes followed by ripe ovum (eg) caudally, ventral midgut (mg) and ganglion (nv). Cross-section of epicuticular pillar (pi).

Fig. 24: Ovum (eg) with many yolk granules, on the dorsally of the ventral (V) gut (gu) note the typical oocytes (oo) inside the ovary. Ovarial basal lamina (bl). Dorsally (D) long epicuticular pillar (pi).

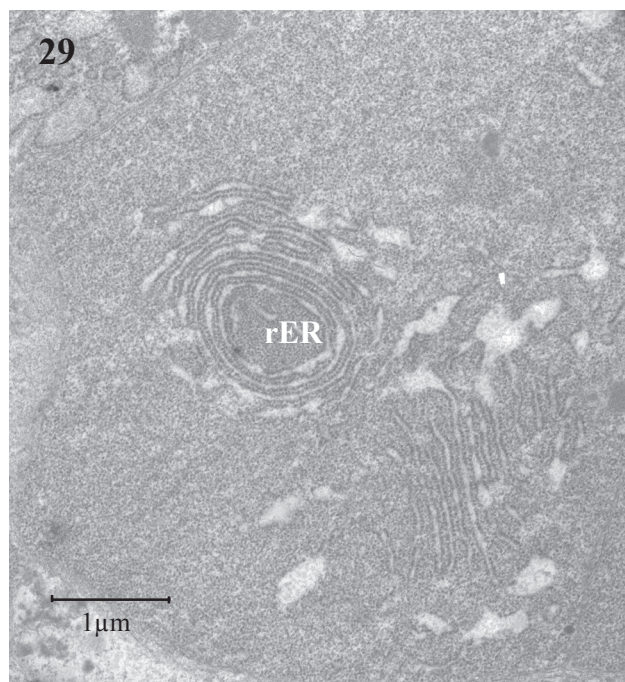
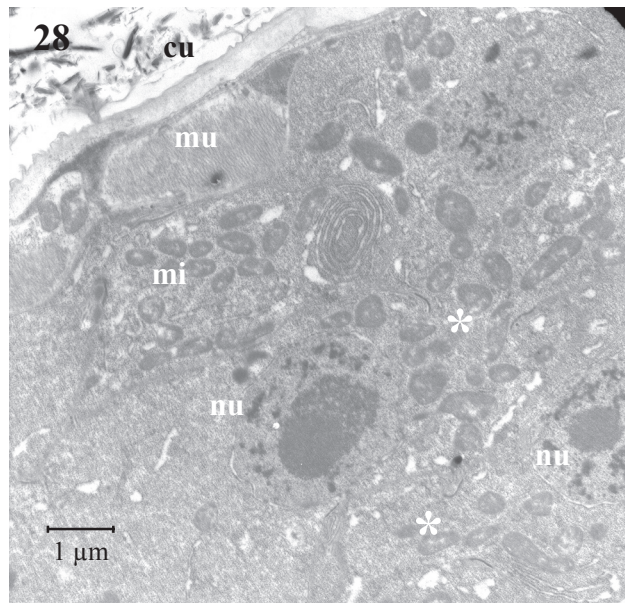
Fig. 25: Caudal nurse cell (nc) besides the ripe ovum (eg). Note the abundant rough endoplasmic reticulum (rER) inside the nurse cell (nc).



Figs. 26 & 27: Previtellogenetic ovary of *Halechiniscus greveni*:

Fig. 26: Ovary (ov) with intercellular bridges (asterisks) in-between the not yet differentiated cells; ventrally midgut (mg).

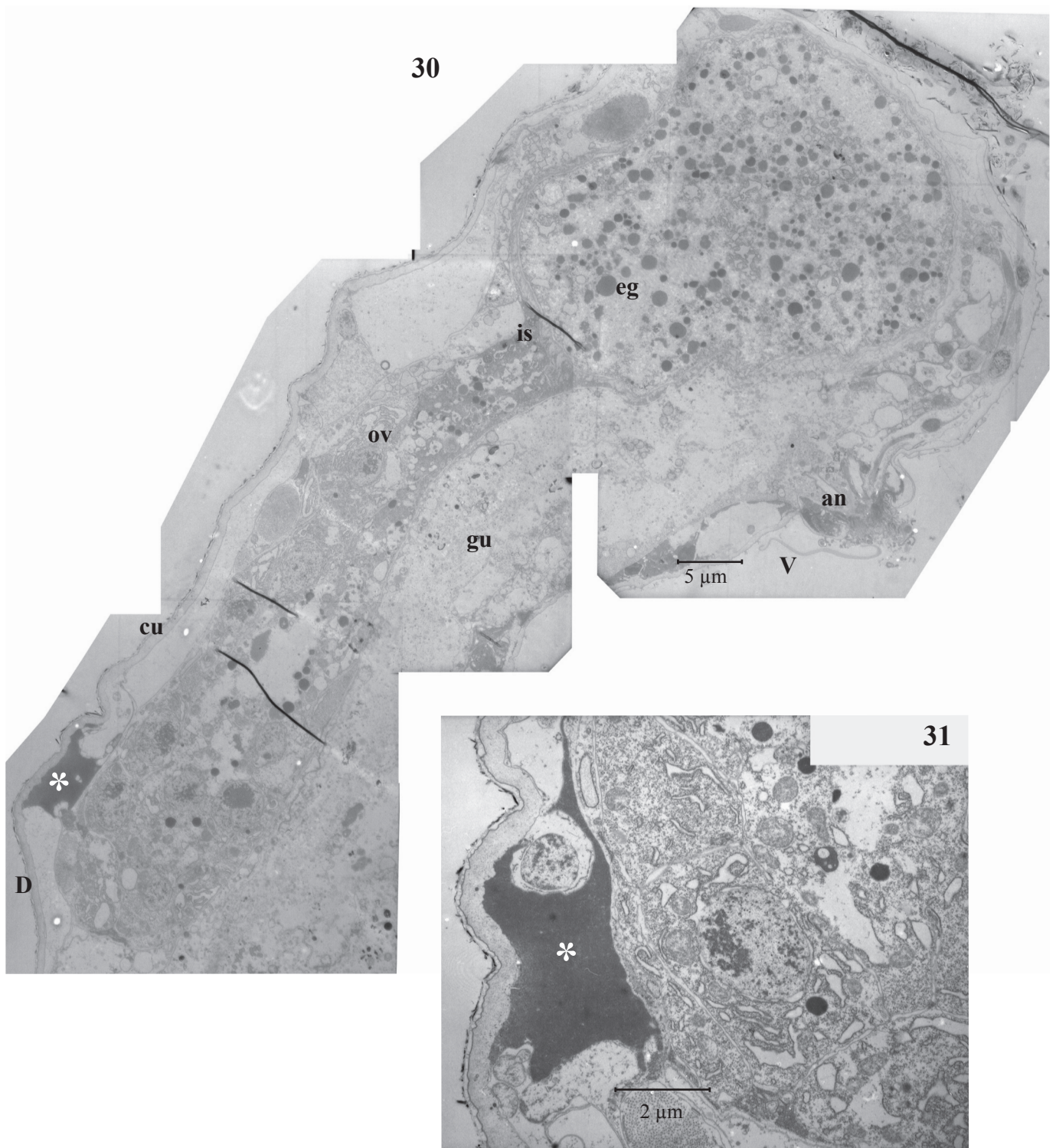
Fig. 27: Detail: Aggregation of mitochondria (mi) and well developed system of rough endoplasmic reticulum (rER) close to nucleus (nu).



Figs. 28 & 29: Previtellogenetic ovary of *Halechiniscus greveni*:

Fig. 28: Detail: Crowd of electron dense mitochondria (mi), nucleus (nu) with nucleolus, intercellular bridges (asterisks), cuticle (cu), muscle (mu).

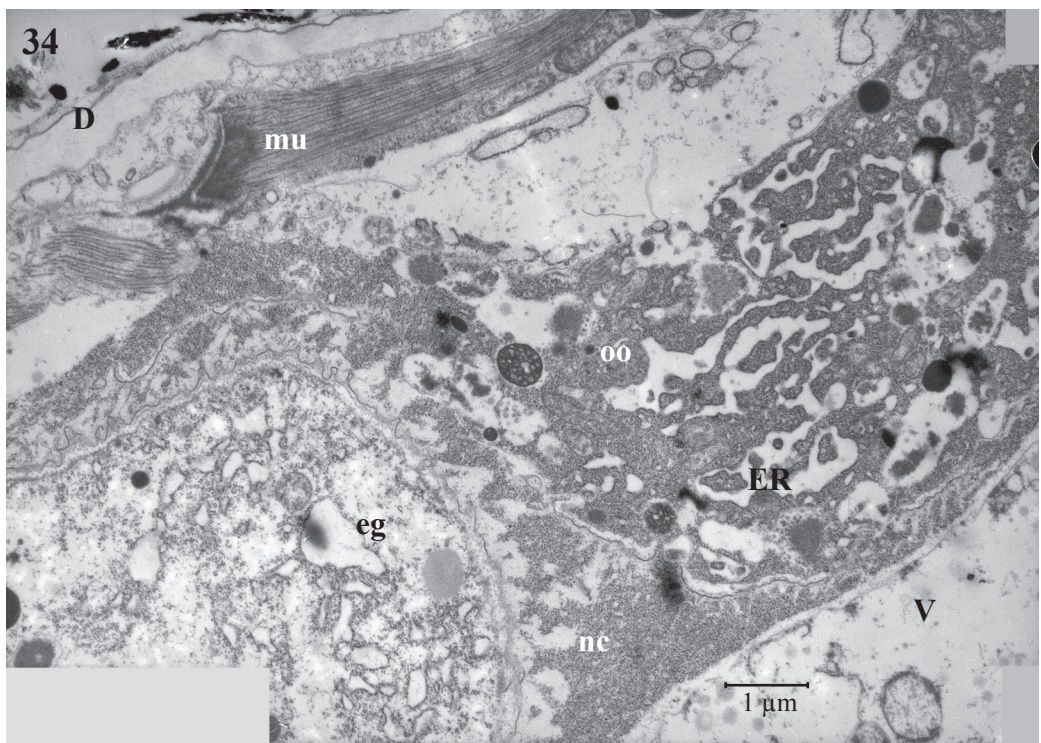
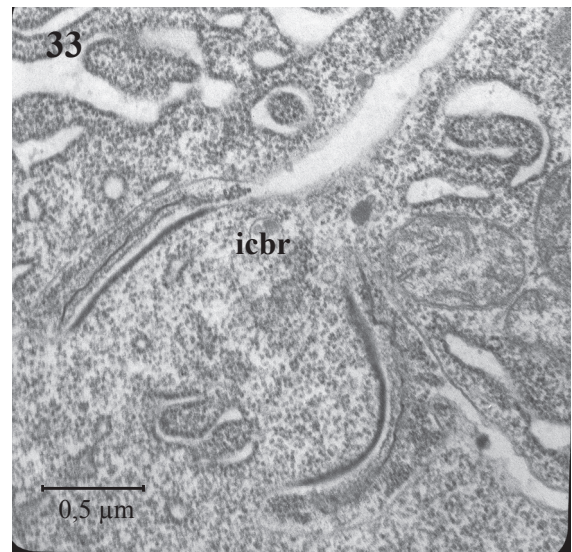
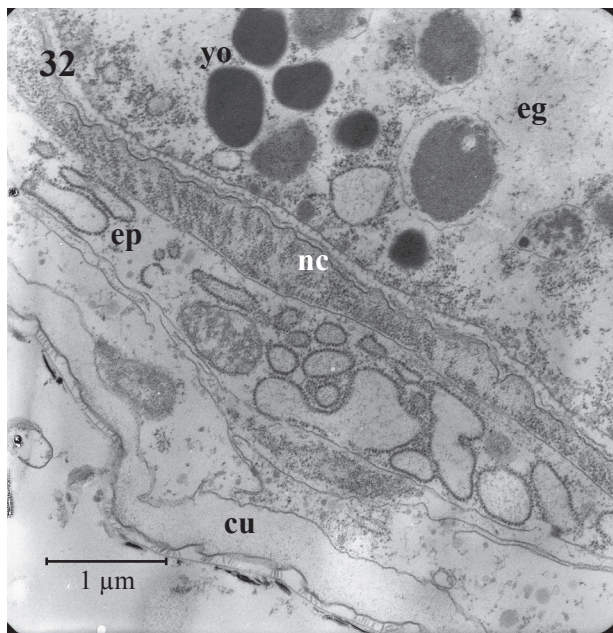
Fig. 29: Detail: Concentric whorl of endoplasmic reticulum (rER).



Figs. 30 & 31: Ovary of *Styraconyx n.sp*

Fig. 30: Survey of ovary (ov); caudally ripe ovum (eg) with yolk; followed by isthmus (is) cranial attachment (asterisk) in dorsal epidermis (D); dorsal cuticle (cu). Gut (gu) and anus (an) visible ventrally (V).

Fig. 31: Most cranial part of ovary with attachment in dorsal cuticle (white asterisk).



Figs 32 - 34: Ovary of *Styraconyx* n.sp:

Fig. 32: Detail: Remnants of nurse cell (nc) around the ripe ovum (eg) with yolk (yo). Cuticle (cu) and epidermis cells (ep).

Fig. 33: Detail of the more cranial part: Intercellular bridge (icbr). Note the cisternae of endoplasmic reticulum inside the oocytes.

Fig. 34: Isthmus before ripe ovum (eg). System of endoplasmic reticulum (ER) inside oocyte (oo) and ovum (eg). Remnants of nurse cell (nc). Dorsal (D) muscle attachment (mu). V ventral

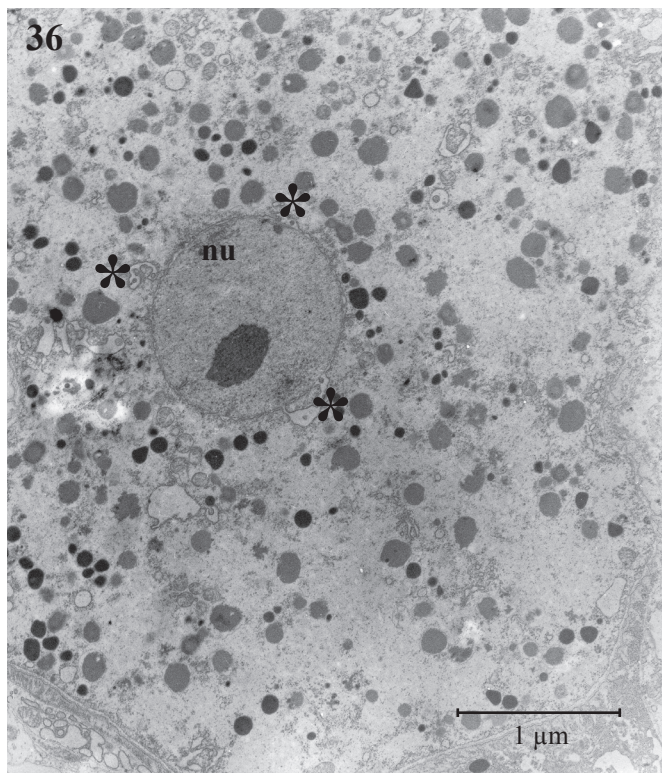
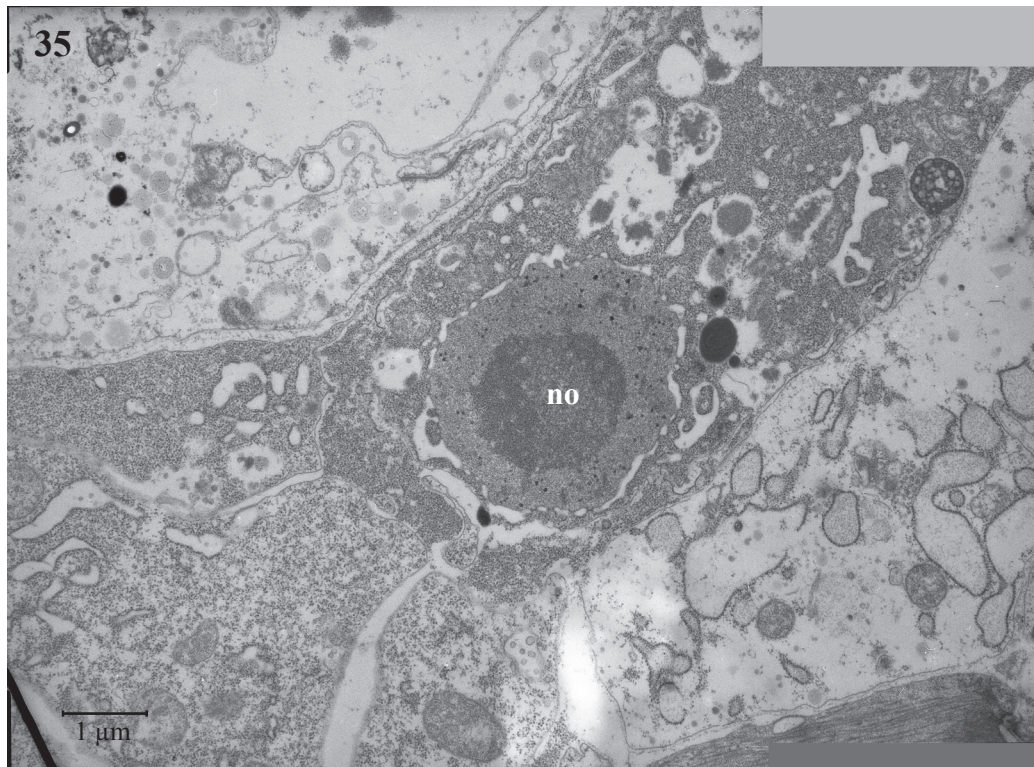


Fig. 35: Ovary of *Styraconyx* n.sp. Isthmus with big oocyte nucleus and nucleolus (no).

Fig. 36: Ripe caudal ovum of *Styraconyx* n.sp. with big nucleus (nu). Note the blebbing outer nuclear membrane (asterisks).

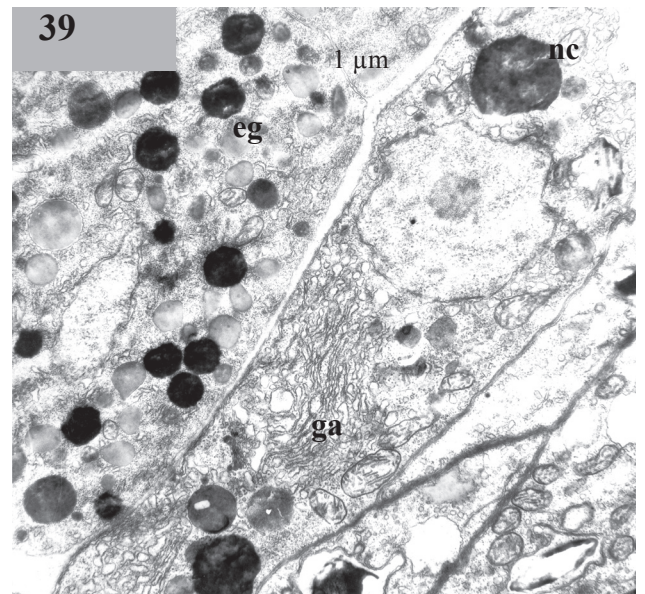
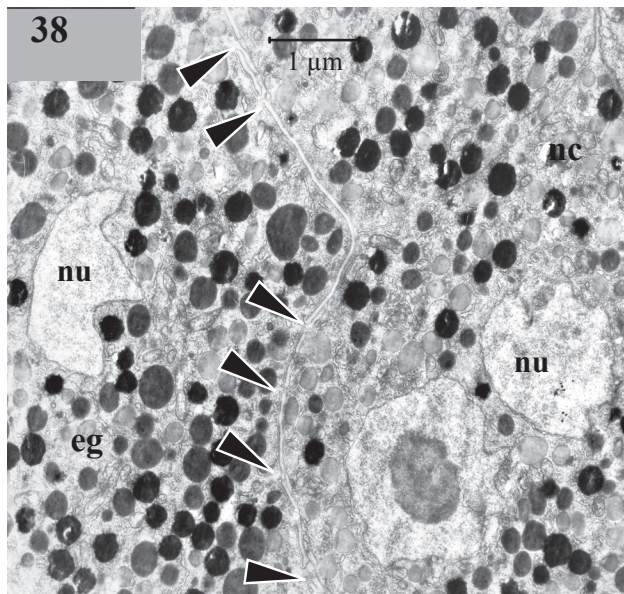
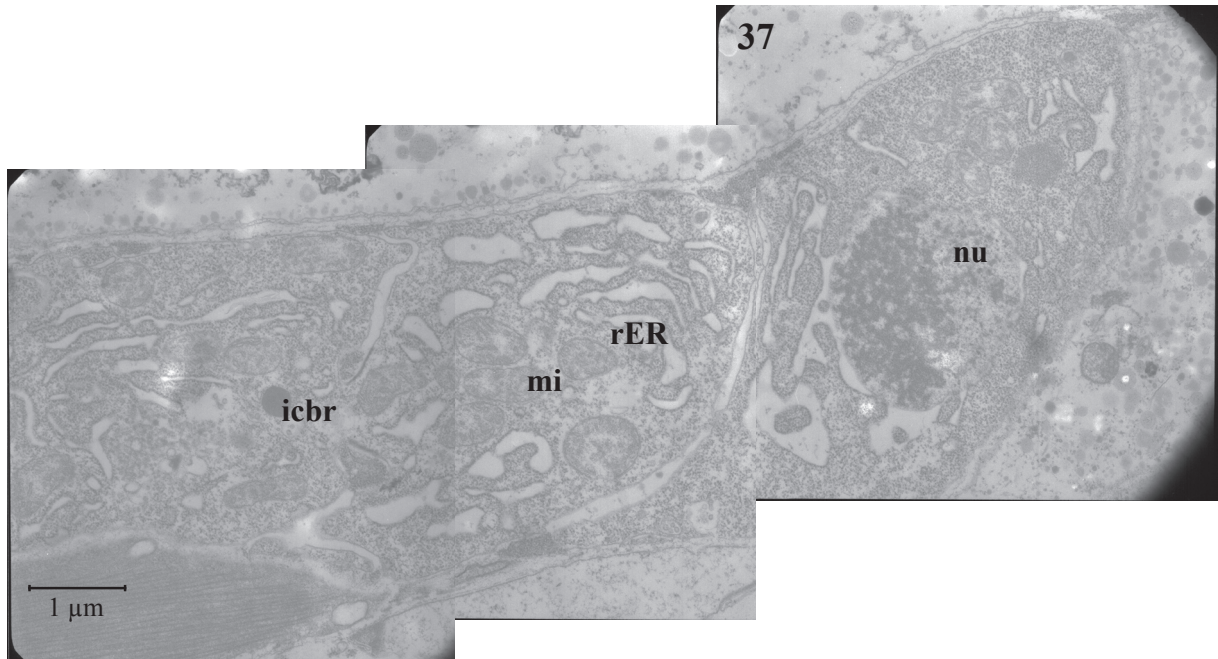


Fig. 37: Ovary of younger *Styraconyx* n.sp, most cranial part. Note the intercellular bridge (icbr), mitochondria (mi) and the elaborate rough endoplasmic reticulum (rER). Nucleus (nu).

Figs. 38 & 39: Ovary of *Batillipes noerrevangi* with ripe ova

Fig. 38: Ovary with ovum (eg) and nurse cell (nc), pseudopodia (arrowheads), both with big irregular nuclei (nu), with abundant nuclear pores.

Fig. 39: Ovum (eg) and nurse cell (nc); predominant Golgi apparatus (ga).

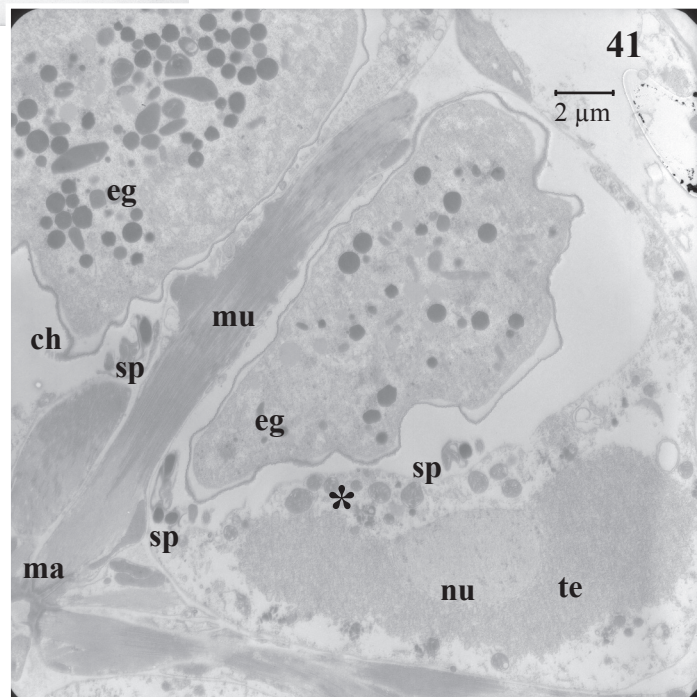
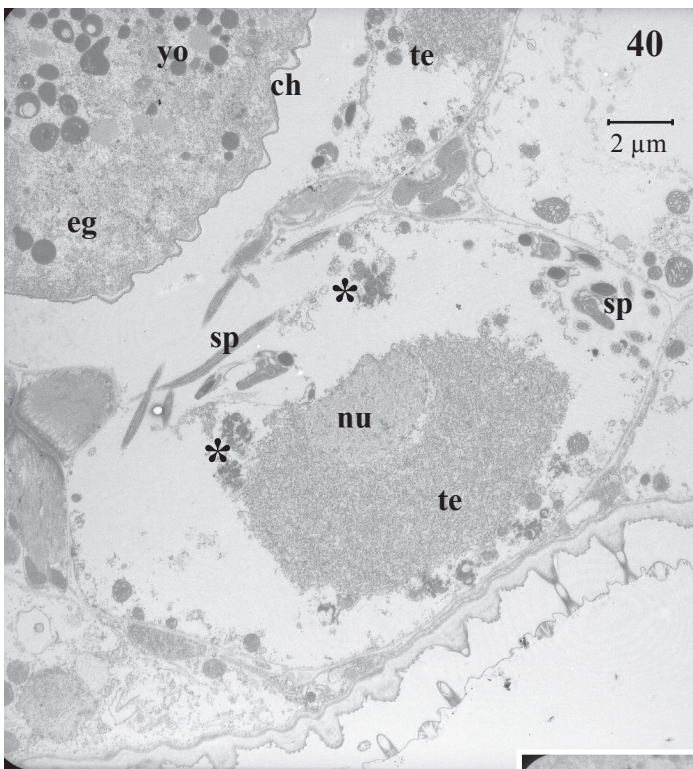
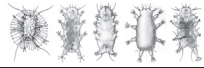
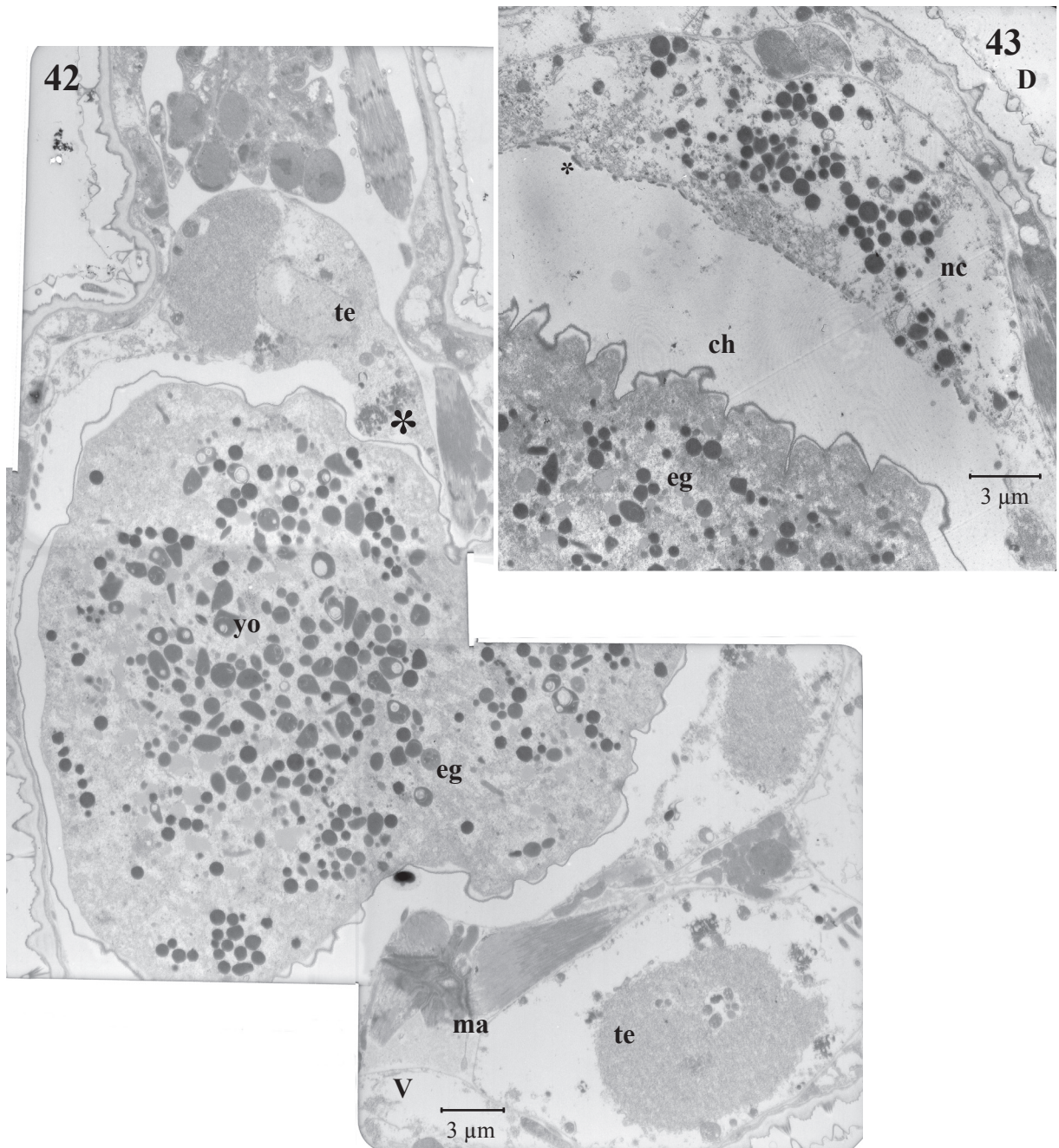


Fig. 40: *Orzeliscus belopus*. Cranial lateral pouch with test cells (te) and spermatozoa (sp); note the typical dark structure (asterisk). Central big ripe ovum (eg) with yolk granules (yo), surrounded by chorion (ch).

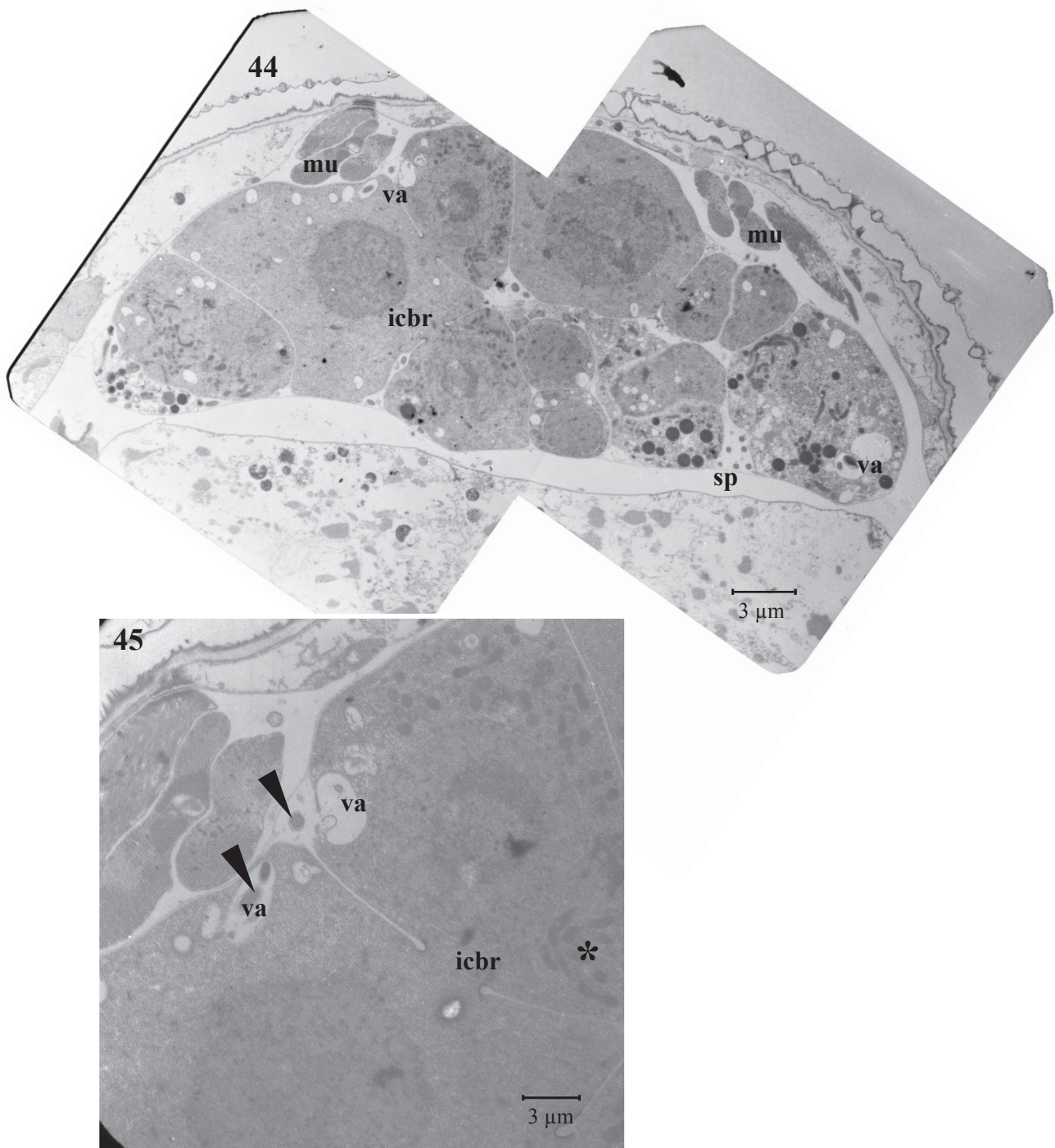
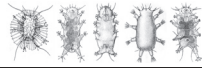
Fig. 41: *Orzeliscus belopus*. Cranial part of ovotestis. Ripe ovum (eg) filling the free space around a muscle (mu), ventral muscle attachment (ma). Lateral pouch with test cell (te) with mitochondria (asterisk), nucleus (nu) and elaborate cisternae of endoplasmic reticulum.



Figs. 42 & 43: *Orzeliscus belopus* ovotestis.

Fig. 42: Lateral and dorsal test cells (te) with typical dark structures (asterisk). Central big ovum (eg) with heterogeneous yolk granules (yo). Muscle attachment in-between egg and test cell (ma).

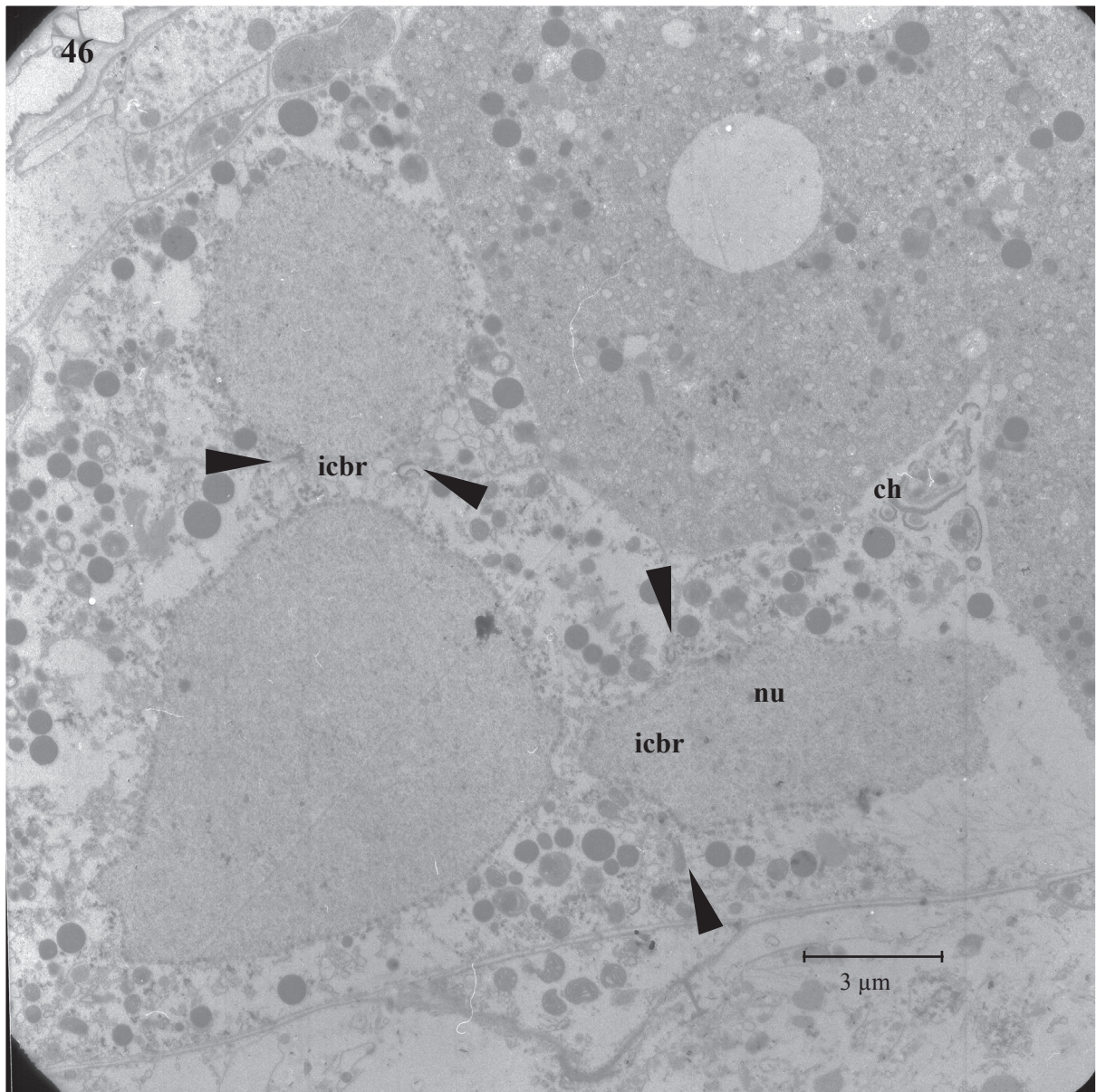
Fig. 43: Detail: female part of ovotestis. Ventral ripe ovum with yolk and intact chorion (ch). Dorsal (D) nurse cell (nc) with yolk and parts of chorion (asterisk).



Figs. 44 & 45: *Orzeliscus belopus*. Ovotestis:

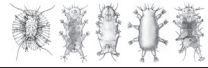
Fig. 44: Survey of frontal part with mostly male parts. Spermatozoa (sp) are visible inside and released from vacuoles (va). Note the intercellular bridges (icbr). Dorsal there are two muscle aggregations (mu).

Fig. 45: Detail: Male part of ovotestis. Spermatozoa (arrowhead) are released from vacuoles (va), group of mitochondria (asterisk). Typical intercellular bridge (icbr) in-between test cells.



Figs. 46: *Orzeliscus belopus*. Ovotestis:

Fig. 46: Detail: Female part of ovotestis. Note the intercellular bridges (icbr and arrowheads) and beginning chorion formation (ch) in peaces. The huge nucleus (nu) partly is situated inside the intercellular bridge, the nuclear pores are visible.



3.3.3 Chorion formation and microvillar structures

The big mature ovum takes up most of the available space and displays minute (20 to 40 nm long) stubby microvilli that regularly cover the membrane beneath the developing, but already closed chorion. This can be seen in *Batillipes noerrevangi* (Fig. 61), *Styraconyx* n.sp. (see superficial sections of the ovum with the microvilli and chorion in Fig. 54), *Halechiniscus greveni* (Figs. 47, 49 & 50) and *Orzeliscus belopus* (Figs. 57 & 58). Only in *Actinarctus doryphorus* the chorion and microvilli could not be encountered in the few available specimens.

The chorion is characterized in the four species where it could be seen as follows:

Batillipes noerrevangi has a regularly striped chorion of approximately 100 to 150 nm around the very far developed egg, but it is sometimes still disrupted by „pseudopodia“, which lead to the remnants of the covering nurse cells situated outside the chorion.

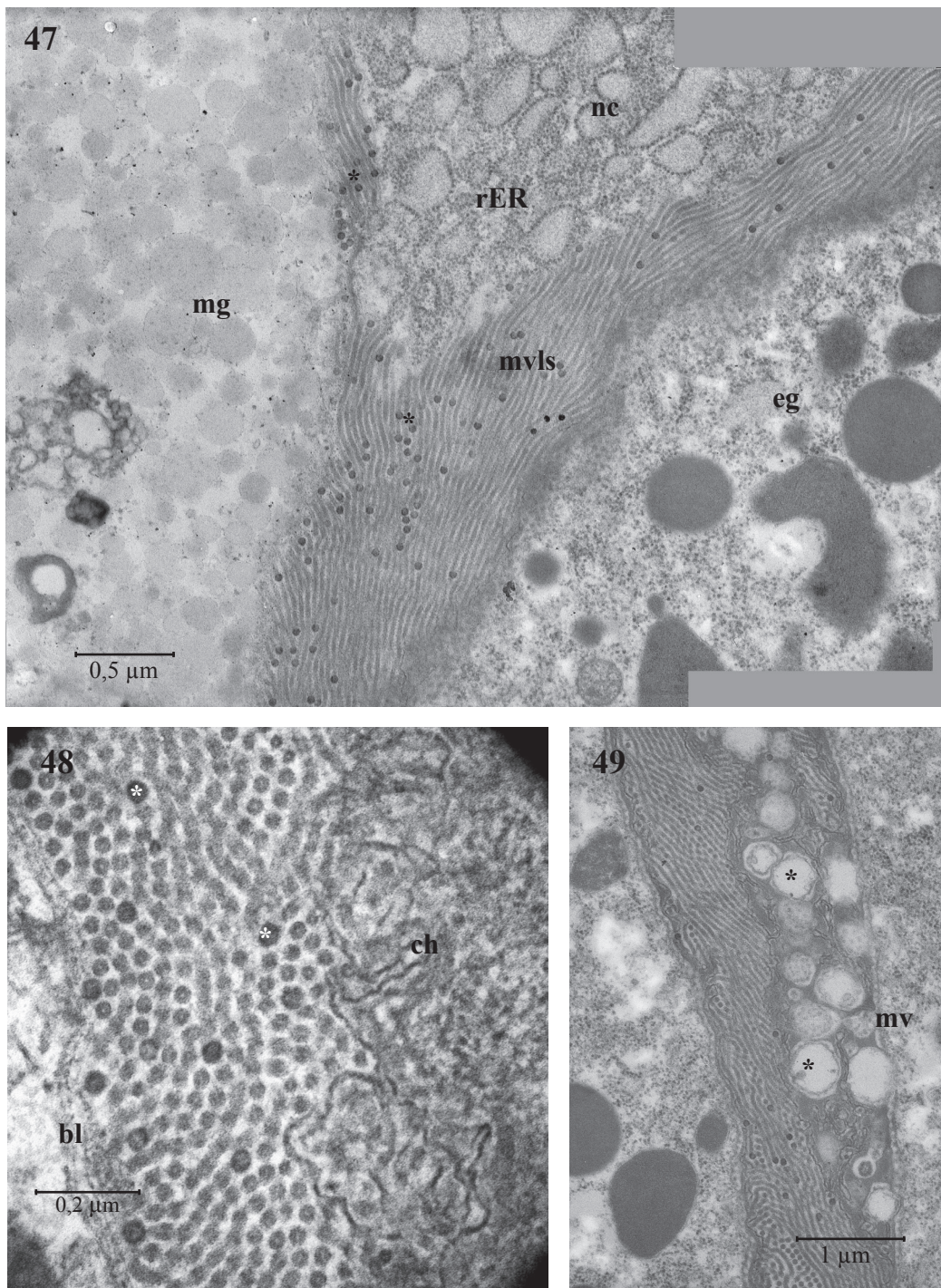
Styraconyx begins to build up its chorion in one thin regular, electron dense layer, which is first present in several isolated spots, but conjoins later to form a regular layer around the ovum which is about 40 nm thick (Figs. 53, 54 & 55). The eggs in the specimens available seemed to be not fully developed (this means ready to be laid), so there could be a further development (with may be striations or other structures).

In one specimen, tubular structures were visible around the mature ovum. They are arranged in a kind of well-defined “nests” and have a slightly chaotic arrangement (Figs. 53, 55 & 56). The origin of these structures is not very clear.

Halechiniscus’ chorion begins to show up in isolated lenticular spots, regularly closed together around the late stage oocyte, resembling pearls on a necklace. Particular microvillar structures of great length in a highly ordered state, often in parallel arrangement, cover the entire surface of mature ovum at the state of chorion formation. Their tips terminate in extremely osmiophilic vesicles (match-tip-structure). Sometimes the vesicles are caught in wandering along the microvilli and are fused with the membrane of the ovum to deposit their content on the surface of the ovum.

The provenance of the microvillar structures is not very easy to determine, in most of the cases coming from the nurse cells, they cover the ovum’s surface in parallel array; in some instances they may come out of the ovum in-between thicker chorion preformations. The thickness of the chorion is difficult to determine, since there is no regular layer in the specimens and it is not clear, if the microvilli-like structure is to become a part of the chorion structure; including the microvilli-like layer the chorion measures up to 600 nm, without it is beneath 200 nm thick (Fig. 48).

Orzeliscus has its very typical chorion that shows little knobs. These knobs first appear in groups of two to four knobs (between 0,2 and 0,5 μm long) in the more central part of the gonad in-between the cells and are to be detected in the direct vicinity of exocytotic or endocytotic vesicles. (see Fig. 58). Isolated pieces of chorion form circular droplets of chorion and fuse at the caudal end of the ovary to surround the ovum in a regular layer studded with knobs. Most caudally, one ovum or few ova is/are surrounded by an entire chorion. Thickness with knobs is from 150 to 200 nm.

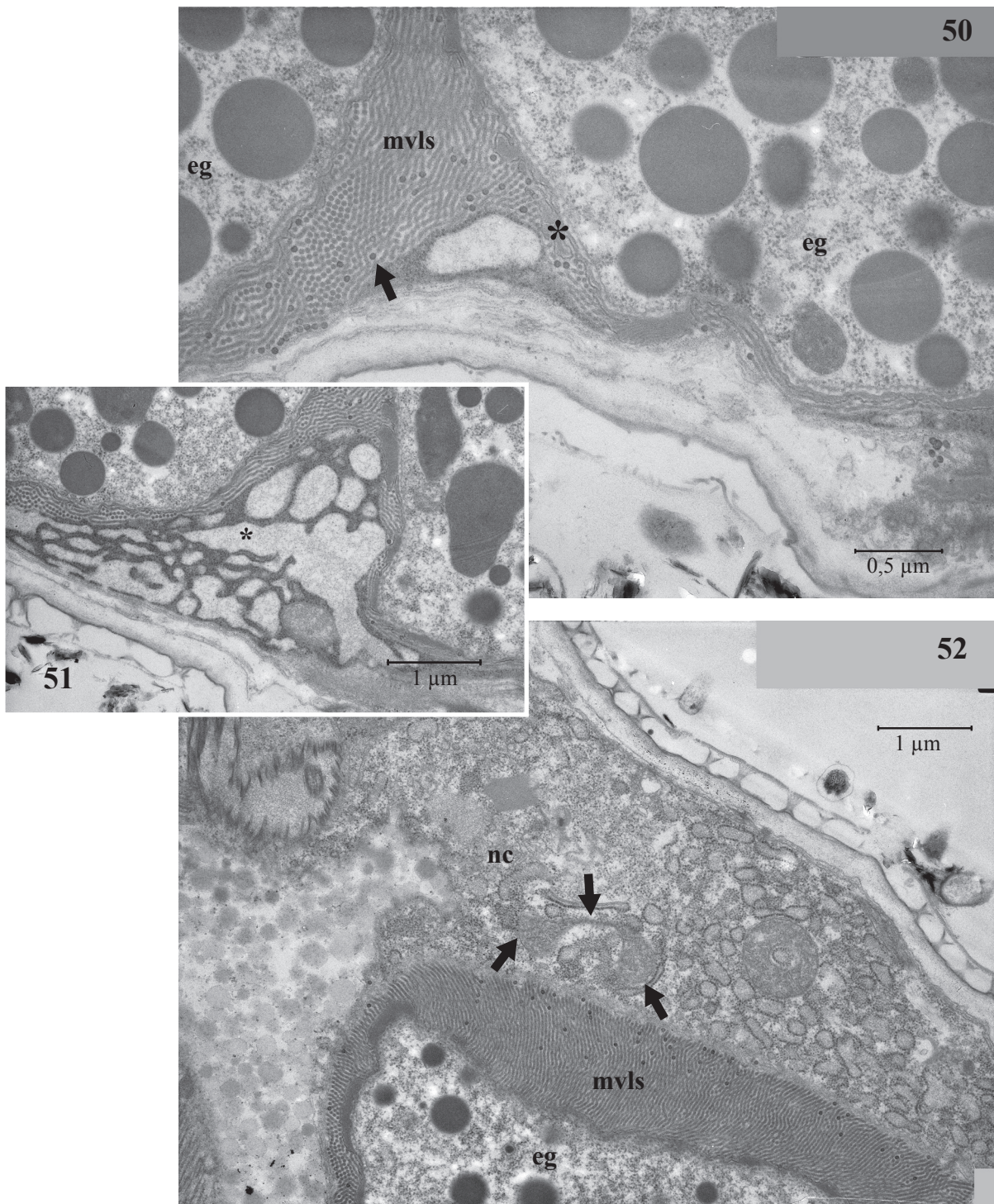


Figs. 47 - 49: Microvilli-like structure surrounding the oocytes of *Halechiniscus greveni*:

Fig. 47: Nurse cell (nc) with elaborate rough endoplasmic reticulum (rER), sending out bulk of microvilli-like structure (mvls) ending in dark vesicular tips (asterisks). Midgut (mg) on one side, ripe ovum (eg) on the other side of structure. Border of ovary not clearly visible.

Fig. 48: Detail of the microvilli-like structure with electron dark vesicles (asterisks); chorion (ch) and basal lamina (bl) of ovarian wall.

Fig. 49: Microvilli-like structure with big hollow vesicle like structures (asterisks) in-between two oocytes. Note the short microvilli (mv) at oocyte surface.

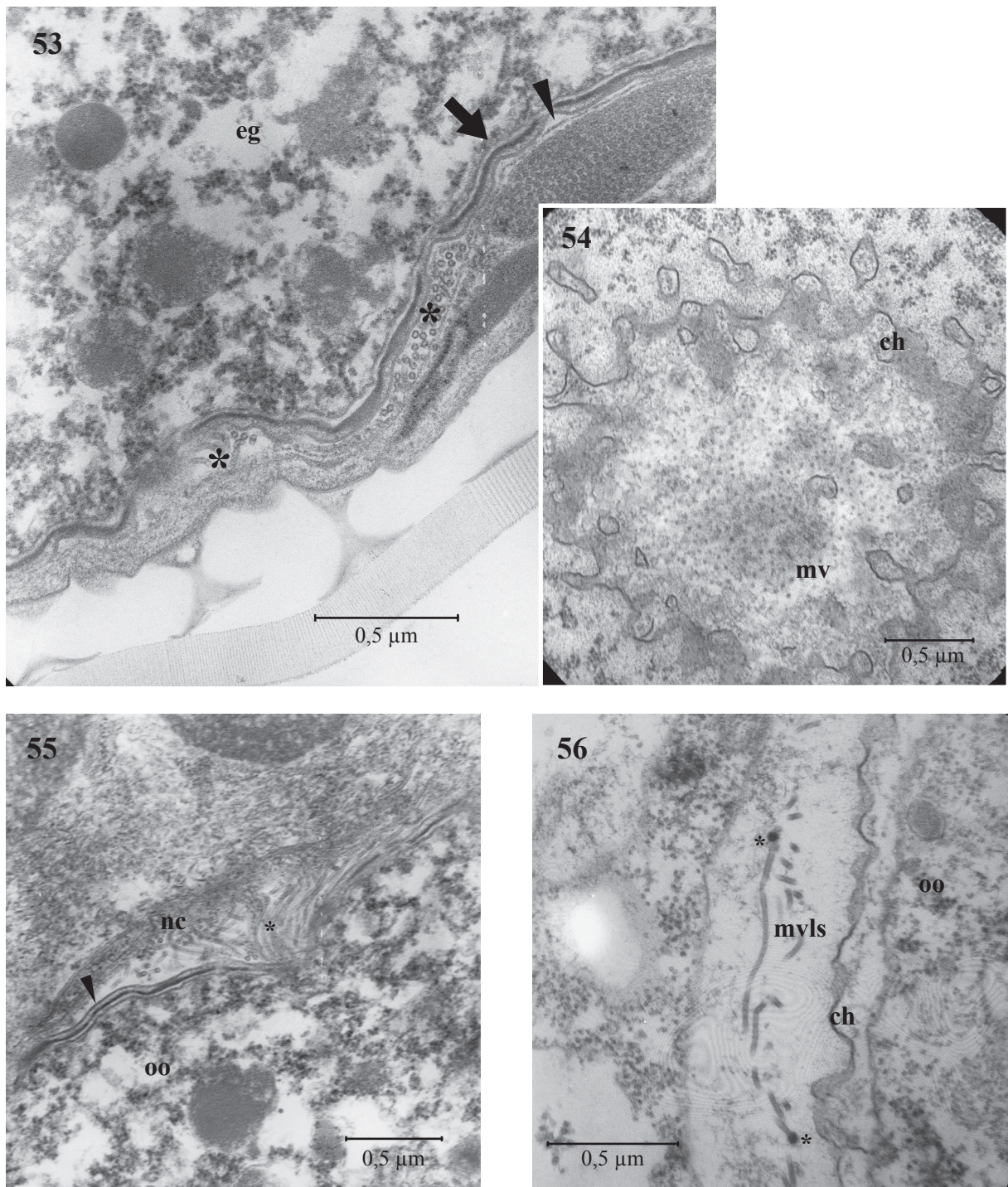


Figs. 50 - 52: *Halechiniscus greveni*:

Figs. 50 & 51: Microvilli-like structure (mvls) in-between two ripe ova (eg), forming a highly ordered pattern.

Reticulate structure (asterisk) may originate from nurse cell. The arrow points at one of the electron-dense vesicles

Fig. 52: Microvilli-like structure (mvls) around ripe ovum (eg), besides nurse cell (nc). Note the budding mitochondrion (arrows)



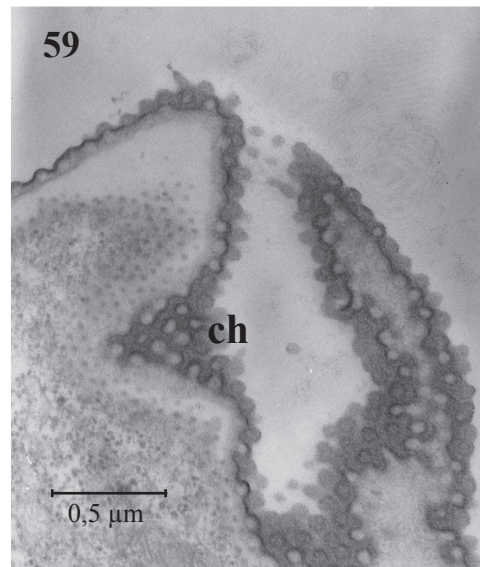
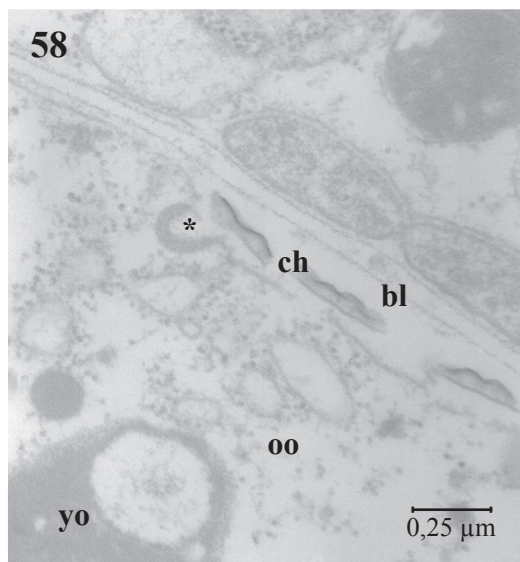
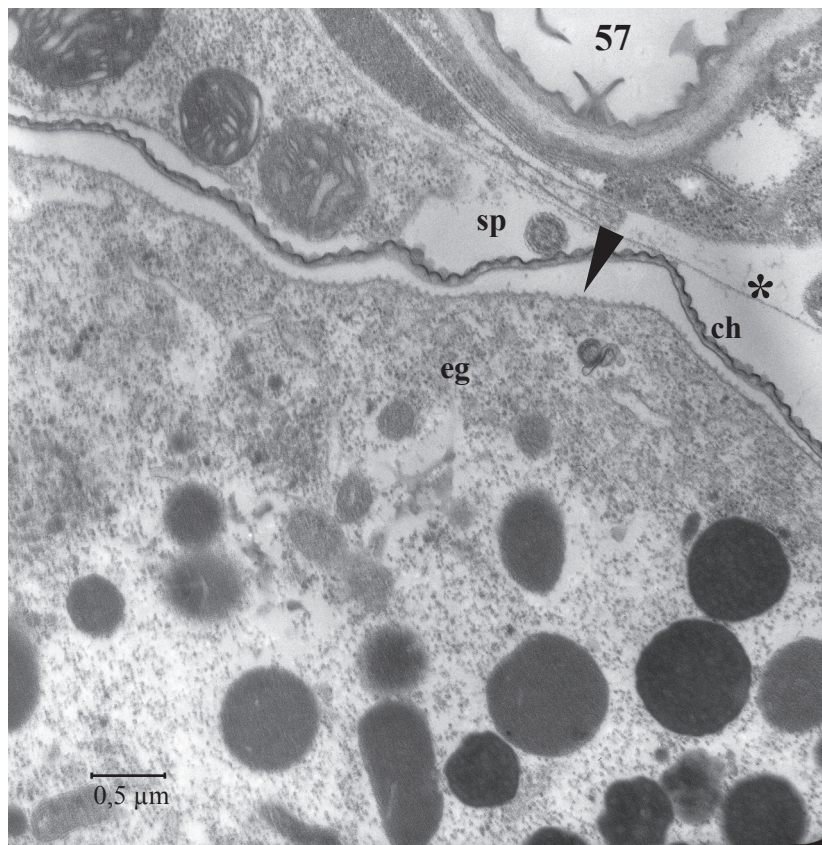
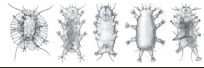
Figs. 53 - 56: *Styraconyx* n.sp. Microvilli- like structure and chorion formation:

Fig. 53: Detail: Chorion formation, chorion (arrow). Nests of microvilli-like structure (asterisks). Basal lamina of ovary (arrowhead)

Fig. 54: Ripe ovum, cut superficially. Sections of short stubby microvilli (mv) formed by oolemma, chorion (ch).

Fig. 55: Nest of microvilli-like structure (asterisk), probably leading to nurse cell (nc), chorion (arrowhead); oocyte (oo).

Fig. 56: Scattered microvilli-like structures (mvls) around oocyte (oo) with two osmiophilic tips (asterisks).



Figs. 57 - 59: *Orzeliscus belopus*:

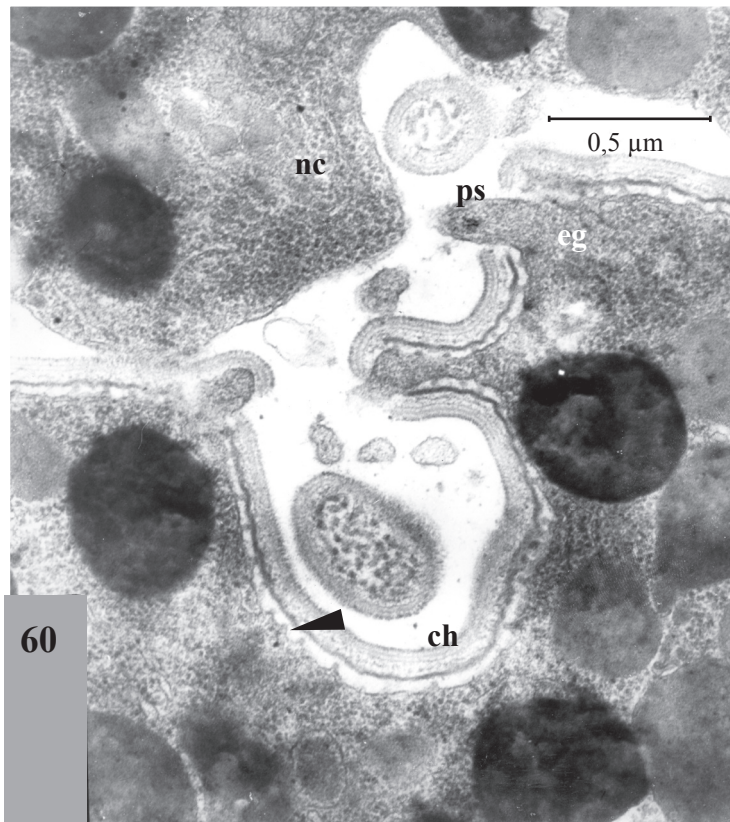
Fig. 57: Ovum (eg), oolemma studded with small microvilli (arrowhead), surrounded by intact chorion (ch).

Note the spermatozoon (sp) next to the chorion and the basal lamina of the ovarian wall (asterisk).

Fig. 58: Chorion formation via exocytosis (asterisk) from oocyte (oo). Heterogeneous yolk granule (yo). Chorion

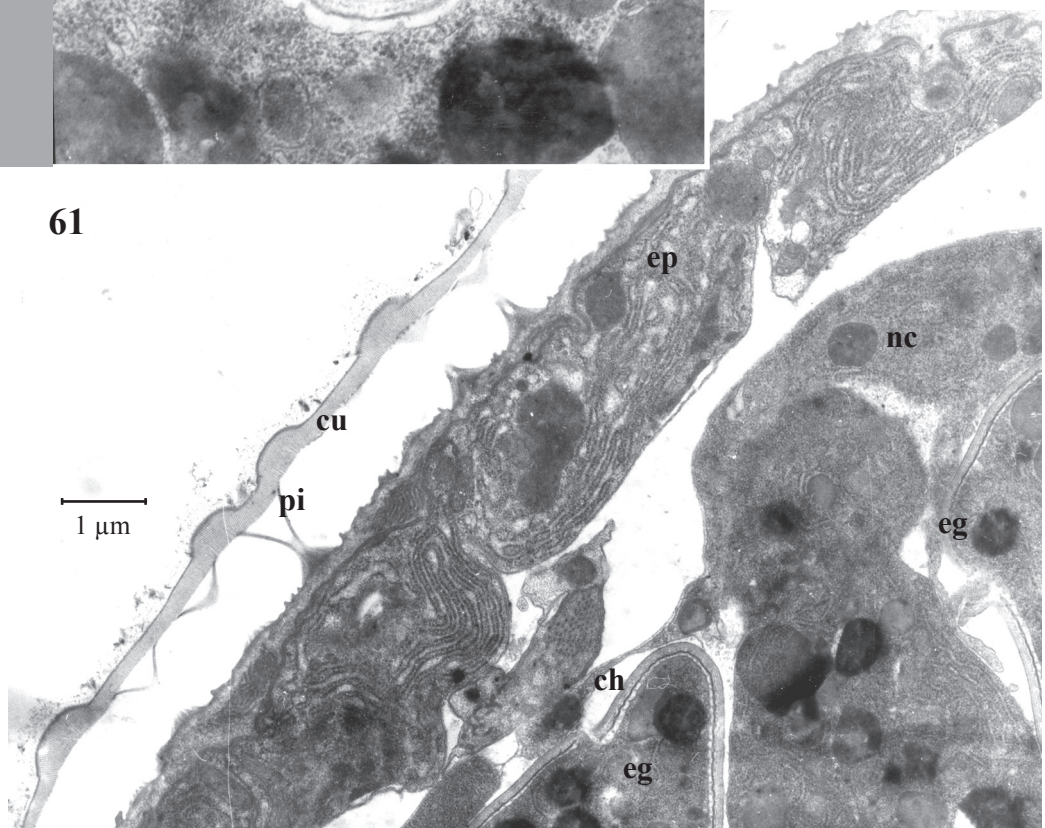
(ch) in pieces, very thin basal lamina (bl) of ovotestis.

Fig. 59: Detail of chorion (ch), which shows beautiful knobs.



60

61



Figs. 60 & 61: Ovary of *Batillipes noerrevangi* (Photos by R.M. Kristensen):

Fig. 60: Detail: Ripe ovum (eg) forming pseudopodia (ps) coming out of striated thick chorium (ch), underneath which microvilli are visible (arrowhead). Remnants of nurse cells (nc).

Fig. 61: Ovary with ripe ova (eg), surrounded by thick chorium (ch) and nurse cell (nc). Cuticle (cu) with pillar (pi), epidermis cells (ep).

List of Abbreviations:

an	anus	mg	midgut
bl	basal lamina	mo	mouth
bu	bulbus of rs	mu	muscle
cap	caudal appendages	mv	microvilli
ch	chorion	mvls	microvilli-like structure
ci	cilium	my	myo-epithelial cell
cu	cuticle	nc	nurse cell
D	dorsal	no	nucleolus
du	duct	nu	nucleus
ec	epicuticle	nv	nerve cell
eg	egg, ovum	oo	oocyte
ep	epidermis (cell)	ov	ovary
er	endoplasmic reticulum	pi	pillars of the epicuticle
ga	Golgi apparatus	po	pore
gp	gonopore	ps	pseudopodia
gu	gut	rER	rough endoplasmic reticulum
hg	hindgut	rs	seminal receptacle
ho	honey comb layer	sl	striated layer of cuticle (honeycomb)
icbr	intercellular bridge	sp	spermatozoon
li	lipid droplet	V	ventral
mg	midgut	va	vacuoles
mi	mitochondrion	yo	yolk



4 Discussion

All of the few existing studies on tardigrade reproduction deal with eutardigrade species. Especially the ultrastructure of oogenesis and female reproductive structures of marine Arthrotardigrades have been neglected so far. The reasons for this are, that the preparation of marine tardigrades is difficult and the aquirement of material is limited.

	<i>Halechiniscus greveni</i>	<i>Actinarctus doryphorus</i>	<i>Batillipes noerrevangi</i>	<i>Styraconyx</i> n.sp.	<i>Orzeliscus belopus</i>
female gonopore	6 - 7 rosetta cells covered by cuticle, comprising myofilaments				
paired cuticular sem rec	present (20 - 40 spermatozoa)	present (15 - 20 (?) spermatozoa)	absent	present (35 - 45 spermatozoa)	present only 1 spermatozoon
yolk	many heterogeneous inclusions of different e-density, enclosed in single membrane				
ic bridges	present	probably yes	not observed	present	present
nurse cell			probably consumed by ovum	probably consumed by ovum	role in chorion formation
mv-l str	highly ordered with osmophilic vesicular tip	not observed	broad (120 nm) pseudopodia brake up chorion	in well defined, but chaotic nests, slender	not observed
short mv of oolemma	present in all the species				
chorion	single accumulations of chorionic material fuse subsequently	lentiform fragments, later fusing to consistent e-dense layer	thick regular striped layer	thin regular layered with e-dense middle-layer	knob fragments join together to form undulated chorion
number of eggs laid at one period	1 to few (5)	probably 1	probably 1	1 single	1 single
deposition	freely on substratum				
egg morph	smooth shelled and sticky				

Tab. 3: Structures associated with reproduction in the investigated five arthrotardigrade species

(sem rec = seminal receptacle, ic = intercellular, mv-l str = microvilli-like structure, mv = microvilli, morph = morphology)



4.1 Gonopore

The rosetta shaped gonopore in marine female heterotardigrades was mentioned by many authors before (POLLOCK 1970, KRISTENSEN 1980 (*Tetrakentron synaptae*), KRISTENSEN 1984 (*Wingstrandarctus*), KRISTENSEN & HIGGINS 1989 (*Paradoxipus orzeliscoides* - p. 270 fig.9), in some cases it was mistaken as male opening. In fact, the male opening is a simple oval orifice, which in most species, lies more distant from the anus than the gonopore in females.

All five species possess a 6 leaved rosetta gonopore in the female (resp. hermaphrodite). The rosette cells of the gonopore are myoepithelial cells, most likely of ectodermal origin (KRISTENSEN 1978b), as is known for *Actinarctus doryphorus*, *Halechiniscus greveni* and *Orzeliscus belopus* and supported by the fact that these epidermal cells secrete their own cuticle.

This work confirms the widely agreed special constitution of the female gonopore in marine heterotardigrades and reveals the homologous ultrastructure in *Orzeliscus belopus*, the only hermaphrodite marine heterotardigrade known until now (in the original descriptions, the hermaphrodite constellation of this species was not distinguished, the individuals were mistaken for females due to their gonopore). For a SEM-picture of female gonopore of *Orzeliscus belopus* from Bermuda see KRISTENSEN & NEUHAUS 1999 (p. 272, Fig. 34.); only later, the first author recognized the hermaphroditic constitution of *O. belopus* specimens he had collected at Bermuda.

The concentration of nerve cells, which is frequently found proximately, probably constitutes the ganglion of the fourth pair of legs, or it could also be the innervation of the gonopore, but for this it seems quite too large.

In *Wingstrandarctus*, KRISTENSEN (1984) described two small pores anterior to the gonopore with unknown function, but none of these pores were encountered in the investigated species.



4.2 Seminal receptacle

Little abundance of marine tardigrades leads to the need of storing the spermatozoa acquired during copulation, which is not always probable in the marine ecosystem.

Fertilization must be external, since the entire lumen of the seminal receptacle is lined with cuticle and the ducts open in the ventral gonopore to the either side of the gonopore.

For some *Batillipes* species, internal receptacles probably formed by the gonoduct have been mentioned (BERTOLANI 2001), but these could not be seen in the *Batillipes noerrevangi* specimen surveyed.

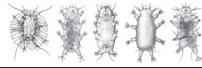
One of the females of *Actinarctus doryphorus* was in moult. It has one big egg in the most posterior part of its ovary ready to deposit. The seminal receptacles are filled with many spermatozoa that are embedded in an electron dense matrix and seem to be degenerated.

JØRGENSEN et al. (1999) describe in their work the spermiogenesis of *Actinarctus doryphorus* and investigate the postcopulatory modifications of spermatozoa inside the female seminal receptacle.

In-plane of the gonopore of *Orzeliscus belopus*, a paired lateral structure with ventral openings has been detected. The orifices are at a big distance lateral to the gonopore. Both lumina of the structure are lined with cuticle (clearly recognizable by the striated layer of the epicuticle) and in each one isolated cellular structure with a clear 9+2 ciliar structure is evident. This could indicate a ciliar sense organ nearby the gonopore or - more probably - it is a paired seminal receptacle containing just one single spermatozoon per side. There has been no work so far reporting this particularity, but KRISTENSEN (personal communication) also confirms the latter explanation. It is not clear yet why there is only a single spermatozoon per receptacle.

KRISTENSEN & HIGGINS (1989) postulate complex seminal receptacles as being plesiomorphic in Halechiniscidae.

The seminal receptacles are innervated by a small nerve (KRISTENSEN 1984), which could not be detected in the material available.



4.3 Gonad

4.3.1 Structure of the gonad

The delineation of the ovary has been described by SUZUKI (2006) as basal lamina lined by a single discontinuous layer of thin epithelial cells in Eutardigrada, which also was stated for *Macrobotus richtersi* by WEGLARSKA (1979).

There are no ultrastructural reports in heterotardigrades, but KRISTENSEN (1979) observed, that the male reproductive organ of *Batillipes noerrevangi* is surrounded by a basal lamina only. This is also true for the females of marine arthrotardigrades; no epithelial cells were perceived and thus seem to be largely reduced in the ovarian wall of arthrotardigrades.

The oviduct is composed of the same flattened cells as the ovarian wall and has a very narrow lumen (BERTOLANI 1983) and thus is difficult to encounter.

Two ligaments from the apical ovarian part towards the body wall, hold the ovary into place in Eutardigrades; at the insertion sites the cuticle is thickened and forms a support shaped like a transverse rod (BERTOLANI 1983). In Heterotardigrades, there is only a single ligament, which was only detected in *Styraconyx* n.sp. ovary as an extremely electron-dense structure with cellular elements on its edge, which could correspond to the cuticular thickening (since the reinforcement structures of the cuticle for muscle attachments in tardigrades display highly osmiophilic substances as well).

WEGLARSKA (1979) characterised the ovary of *Macrobotus richtersi* as meroistic-polytrophic. Cystoblasts in the ovary divide and form a cluster of cystocytes; in early vitellogenesis, the nurse cells resemble very much the appearance of the oocyte. Distinct egg chambers as in insects' ovarioles are not apparent.

If the terminology of WEGLARSKA (1975, 1979 & 1987) is followed, one can characterise the anterior part (reaching up to the isthmus) as germarium, and the most posterior part (containing the yolk laden ovum) as vitellarium. But this seems not adequate for the five examined Arthrotardigrades, since the boundary in-between this structures is not clear.

Later, DEWEL et al. (1993) also describe a central multinuclear mass (trophocyte) in the two Eutardigrades *Milnesium tardigradum* and *Halechiniscus perfectus* that is surrounded by peripheral mononuclear oocytes. Also SUZUKI (2006) reconstructs several large multinuclear cells, surrounded by many mononuclear oocytes in the centre of the ovary in the eutardigrade



Milnesium tardigradum. The multinuclear cells are interconnected by intercellular bridges, and they are connected to the oocytes as well.

In contrast to these findings in Eutardigrades (which normally mature more than one oocyte at a period), the relation to the nurse cells is inverse in the examined Arthrotardigrades: the prospective egg cell is surrounded by its nurse cells.

Cytoplasmic bridges (fusosomes) connect the developing oocytes to a defined number of nurse cells, as WEGLARSKA demonstrated in 1979 and 1987. This can be confirmed for *Orzeliscus*, *Styraconyx* and *Halechiniscus*. Around the fusosome, electron dense lamellae are formed (WEGLARSKA 1979) and often serve to clearly identify the intercellular bridges.

Through the connections remaining from an incomplete cytokinesis, presumably yolk precursors (pro-yolk-bodies), lipids, different granules and vesicles, rRNA and organelles (like ribosomes and mitochondria) are transferred into the developing oocytes. The ovum increases in size, while the nurse cells seem to diminish or are consumed by the egg cell. As the single ripe ovum occupies most of the space of the caudal portion of the ovary, the remnants of the nurse cells are congregated in an isthmus of the ovary, which directly precedes the dilated portion with the big ovum in the most posterior part. Sometimes, slight remains of the nurse cells partly surround the ovum.

Ovotestis of *Orzeliscus belopus*:

REBECCHI et al. 2000a report, that the hermaphroditic tardigrade species develop their male gametes in the anterior and posterior poles of their gonad. This can be corroborated for *Orzeliscus belopus*. No barriers separating the female and male portions of the gonad could be detected - on the contrary, both parts of the gonad were found to be in very close contact, sometimes difficult to distinguish from each other (see Fig. 44)

The lining of the ovary seems to be reduced to the basal lamina, in contrast to the hermaphroditic Eutardigrade *Isohypsibius granulifer* which sac-like ovotestis is surrounded by a continuous single cell-layer (WEGLARSKA 1987).

In the most anterior part of the gonad of *Orzeliscus belopus* mainly undifferentiated gonial cells predominate, they are connected via prominent intercellular bridges; some of them can be identified as test cells, others seem to bear ovarian attributes like pro-yolk bodies. Maybe this is the appropriate section, WEGLARSKA (1987) describes as the germarium in *Isohypsibius granulifer*. The central and posterior ovotestis is occupied by the developing oocyte and its nurse cells, titled vitellarium in *Isohypsibius granulifer* (WEGLARSKA 1987). Spermatozoa are most common in the very posterior part, they are located around the ripe ovum with chorion and are detectable caudally in a lateroventral position kind of seminal vesicles. Besides several



spermatozoa, they contain a cellular mass with a huge nucleus and abundant endoplasmic reticulum and mitochondria. This may be an analogue structure to the giant “nurse” cells found in the testis of *Actinarctus doryphorus* (JØRGENSEN et al. (1999).

Ripe spermatozoa are located around the ripe ovum with chorion. It is doubtful that this lateral structure is homologous to the caudal sac-like evagination found in *Macrobiotus joannae* (REBECCHI et al. 2000a) or even the seminal vesicles described in male testes, e.g. in *Batillipes noerrevangi* (KRISTENSEN 1979), or in *Actinarctus doryphorus* (JØRGENSEN et al. 1999).

The mode of fertilization (self- or cross-fertilization) is not known yet.

4.3.2 Oogenesis

Tardigrade females are generally described to be **iteroparous** (breeding several times per life). Most tardigrades have a synchronous maturation of few to many oocytes per maturation cycle, whereas many arthrotardigrades display an **asynchronous maturation** (REBECCHI & BERTOLANI 1994). This could be clearly proved for two of the five investigated species: The ovaries of *Actinarctus doryphorus* and *Styraconyx* n.sp. display a clear progressive sequence from oogonia in the most anterior part, to the single big mature ovum occupying the space of the most posterior ovarian section.

This seems to be true for *Orzeliscus*, since the most caudal egg is the most mature; but the ovotestis of this species certainly demands further investigations.

In several specimens of *Halechiniscus greveni*, more than one egg was encountered in the hind ovary - in one case as many as three very big and yolk-stuffed ova with dividing nuclei could be observed (no figure in this work). The species thus may be capable of laying more than one egg.

This confirms that there are some exceptions to the asynchronous egg ripening in arthrotardigrades, which were reported in some *Batillipes* species (*B. pennaki* and *B. mirus* - POLLOCK 1970; *B. lesteri* - KRISTENSEN & MACKNESS 2000). KRISTENSEN (1978a) mentioned in his first description of *Batillipes noerrevangi* that females he reared in culture only laid one sticky smooth shelled egg a time, which never contained embryos.

KRISTENSEN 1980 described females of *Tetrakentron synaptae* bearing from 2 to 7 oocytes inside their ovaries. But this species too, normally deposits one single egg at a time. So the common oviposition in arthrotardigrades consists of one egg a time. Only some species (e.g. in *Batillipedidae*) are capable of laying multiple eggs per clutch, which is an apomorphic character.



SUZUKI 2006 stated that the nutritional condition influences the clutch size in a parthenogenetic strain of the semiterrestrial eutardigrade *Milnesium tardigradum*.

Even oosorption may appear regularly in animals that are in a bad nutritional state (WEGLARSKA 1987), which leads to a reduced egg number or even no egg deposition at all. The process of oosorption was not properly described so far and could not be encountered in the small sample of this study. One could tend to interpret some structures (e.g. the chaotic nest of microvilli towards the oocyte of *Styraconyx* n.sp. in Fig. 55) towards oosorption, but this would need further examination.

MARCUS (1929) defined the oogenesis of tardigrades as alimentary and nutrimentary, which has been supported by WEGLARSKA (1979) - during their development, the oocytes use material which is supplied by the nurse cells (= trophocytes) first recognized by WEGLARSKA (1979) in a study about eutardigrade oogenesis.

Nurse cells are seen in all five species investigated in this study. At some stages, there are apparent intercellular bridges in-between female germinal cells and nurse cells, which thus are the sister cells of the ova. In the early stages it is not easy to decide which cells later become the egg cells and which will form the nourishing nurse cells.

Many authors agree in subdividing oogenesis into 4 stages (WEGLARSKA 1979 & 1987; REBECCHI & BERTOLANI 1994):

- previtellogenesis,
- early vitellogenesis,
- late vitellogenesis and
- mature oocytes.

In eutardigrades, yolk proteins are mainly formed by autosynthesis, but may be supported by micropinocytosis and nurse cell contribution (WEGLARSKA 1979, BERTOLANI & REBECCHI 1999). The first two modes can also be assumed for the five arthrotardigrades studied, but the indications for pinocytosis for acquiring yolk material are not conclusive in this work.

In *Macrobiotus richtersi*, WEGLARSKA (1979) stated 2 different kinds of yolk bodies with different origins, which cannot be observed in the arthrotardigrades of this study.

The oogonia of the five arthrotardigrade species show the typical appearance as described by other authors. They are crowded together in the ovary, contain a big nucleus with large nucleolus (WEGLARSKA 1975) and have abundant free ribosomes (typical for cells with protein

accumulation). The other organelles (ER, mitochondria and Golgi) are sparse; the first nutrients to appear are lipid droplets resembling in the ooplasm without any perceivable connection to organelles or membranes (WEGLARSKA 1979 & 1987, NØRREVANG 1972).

In the early oocytes the first pro-yolk bodies and lipid droplets appear, the nucleus and its nucleolus increase in volume and the outer nuclear membrane forms blebs. Most of the organelles multiply, e.g. the mitochondria. The cytoplasm of young oocytes is filled with numerous mitochondria, cisternae of smooth and rough reticulum, dictyosomes, ribosomes, annulate lamellae (WEGLARSKA 1975). All of these are found in the oocytes of the five arthrotardigrade species, but due to the preparation it is difficult to detect smooth endoplasmic reticulum and dictyosomes. WEGLARSKA (1987) observed massive changes in the structure of the mitochondria of *Macrobotus richtersi* at the onset of vitellogenesis, but not in *Isohypsibius granulifer*. Those mitochondrial modifications were never present in the five arthrotardigrade species of this work.

During vitellogenesis yolk platelets appear and grow significantly by fusion of yolk spheres and incorporation of lipid, but in the five arthrotardigrade species there is no pronounced correlation to dictyosomes of the Golgi apparatus like SUZUKI (2006) observed in the eutardigrade *Milnesium tardigradum* or WEGLARSKA (1979) stated for *Macrobotus richtersi*.

The cortical granules and annulate lamellae WEGLARSKA (1987) detected in *Isohypsibius granulifer*, could not be seen in the vitellogenesis of the five arthrotardigrade species.

The mature egg contains mainly yolk bodies, most of the organelles are reduced. Its surface is covered by the chorion, a vitelline membrane like WEGLARSKA (1979) depicted for *Macrobotus richtersi* could not be followed for the specimens of this study, maybe it is secreted in a later stage by the small microvilli of the oolemma.



4.3.3 Chorion formation and microvilli structure

MARCUS (1929) reported the ovarian wall cells to be responsible for the egg-shell (chorion) formation outside the vitelline envelope (yolk membrane) in tardigrades. His only light microscopical descriptions of the anatomy and the physiology of mainly eutardigrades were excellent at his time, but the electron microscope has enabled to encounter another level of the size scale. Since the wall of the ovaries is very reduced in all the five species, it may be excluded as source of the chorion in arthrotardigrades.

WEGLARSKA (1975 and 1987) observed the formation of the chorion in advanced vitellogenesis, in *Macrobotus richtersi* and *Isohypsibius granulifer* she connected sporadic microvilli at the surface of the oolemma to its genesis.

Some *Tetrakentron synaptae* (KRISTENSEN 1980) showed a thickened and sculptured chorion instead of the normal thin sticky shell and earlier two different patterns of egg maturation were observed in *Batillipes pennaki* (POLLOCK 1970) - the explanation to these findings was a seasonal or weather-dependent variation which is also supposed in some eutardigrade species. Such a specialized egg envelope was not seen in the material studied. To elucidate such a correlation periodic samplings and additional light microscopical surveys on living animals are needed.

Few differences were noticed in the chorion formation of the observed species: lentiform-shaped isolated islets in *Halechiniscus* and *Actinarctus* (chorion seems to be quite thin in the ripe ovum), pearl-necklace fragments, obviously discharged by exocytosis (Fig. 58) by the oocyte or its nurse in *Orzeliscus* which fuse into a regular layer of napped appearance (Fig 57), thin plate shape pieces in *Styraconyx* fuse to a regular layer, one continuous thick striated layer around ova of *Batillipes* (which probably represents a very progressed stage).

In a polychaete, ECKELBARGER (1980) described the egg envelope formation as patches along the oolemma.

WEGLARSKA (1975) noted microvilli on the oocyte surface of *Macrobotus richtersi* and SUZUKI (2006) observed microvilli on the cell surface of later oocytes and their adjacent multinuclear nurse-cells in *Milnesium tardigradum*. Coincidentally he found electron-dense amorphous bodies around the oocyte that appear to fuse to the later chorion.

In all five arthrotardigrade species the surface of the mature oocytes is studded with numerous small and low microvilli extending to the chorion; in *Halechiniscus greveni* they are difficult to detect.



This microvillar enlargement of the oolemma and outer membrane of the nurse cell could either have a function related to the ovum's nutrition (HUEBNER & ANDERSON 1976) or to the chorion-formation or both.

The microvilli-like structures, which have been found in *Styraconyx* probably originate in the nurse cells lying around the ovum in a small layer or in the lining of the ovary. They could either have the function of providing nutrition for the egg in stage of chorion formation (maybe also assist in chorion formation) or maybe they are indicating oosorption, because the ovum surrounded by these microvilli-like structures looked a little hollow and apoptotic.

So it is difficult to judge if this structure may be homologous with the regular microvilli-like border found in *Halechiniscus*. But the occurrence of osmiophilic tips indicates (at least functional) homology.

4.4 Conclusion and outlook

Comparison of the specimens in this work is difficult because the limited material was in different stages.

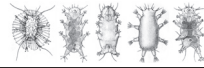
Marine tardigrades are considered to be the most plesiomorphic of all tardigrades. No case of parthenogenesis has been registered and only one species with hermaphrodite condition has been described so far. These apomorphic conditions only developed, when tardigrades changed to the more instable environments (BERTOLANI & REBECCHI 1999).

It is moreover difficult to compare tardigrades to other groups because of their minimization (several organic systems are reduced, like respiratory and circulatory systems).

In Onychophora little is known about oogenesis, therefore it is very difficult to draw any comparison. RUHBERG (1990) stated, that the oocytes have microvilli.

In the five tardigrade species no indication of the presence of follicle cells was found. The oogenesis of the examined species may be confirmed as alimentary and nutrimentary as suggested by previous studies (BERTOLANI & REBECCHI 1999, MARCUS 1928 & 1929, WEGLARSKA 1975 & 1987)

The cytoplasmic continuity in-between oocytes and nurse cells via intercellular bridges has been described for many different systematic groups: e.g. in polychaetes (OLIVE 1983) and other spiralian (HUEBNER & ANDERSON 1976), some crustaceans (ADIYODI & SUBRAMONIAM 1983,



ZERBIB 1980). Cytoplasmic bridges occur between nurse cell and oocyte, but also in-between nurse cells. Their predominant function in most of the species is the transfer of material and organelles from the nurse cell to the oocyte

But one has to be very careful in drawing phylogenetic implications, because these modes probably have evolved several times independently and thus are just functional analogies; this type of oogenesis is mostly developed in small animals that produce small numbers of relatively large eggs (OLIVE 1983). I agree with ECKELBARGER (1980): some important mechanisms have evolved independently several times (like pillar structures of cuticle (KRISTENSEN & NEUHAUS 1999) in order to answer similar requirements - and may therefore be only termed analogous.

Simultaneous hermaphroditism as postulated by REBECCHI et al. (2000a) is true for *Orzeliscus belopus* as well. Self-fertilization is considered possible by these authors, but the presence of a seminal receptacle in *Orzeliscus belopus* disproves that (although it is not too clear from the present results, if the single spermatozoon has been received from another individual).

Microvilli in oogenesis appear in several groups (NØRREVANG 1968) - echinoderms, crustaceans, insects, annelids, amphibians and mammals. They represent an apparent enlargement of the oocyte surface, which facilitates metabolic exchange, e.g. pinocytosis. They may as well participate in the chorion formation, as supposed in the case of polychaetes by ANDERSON & HUEBNER (1968), or they may provide for the nutrition of the oocyte during the construction of the thick egg envelope, as is assumed in a study on pentastomida by NØRREVANG (1972). OLIVE (1983) reported for crustaceans, that granules or vesicles derived from microvillar tips form the precursors of the later chorion; and NØRREVANG (1972) encountered microvilli with swollen tips, which in some preparations of pentastomidan oogenesis he considered artefactuous. This could be an analogy to the osmiophilic tips of *Halechiniscus greveni* and *Styraconyx* n.sp.

It is not clear, if the pseudopodia in *Batillipes* can be compared to the microvilli-like structure of the 3 halechiniscid species. There has to be done further research, because this work can only spotlight on different stages of development, which are difficult to compare with each other.

This work offers the first small insight into the processes of arthrotardigrade oogenesis. Future studies may deal with a successive row of stages of oogenesis. Additional histochemical surveys may clarify the cellular components and their interactions in different oogonial stages.



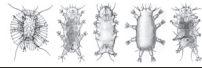
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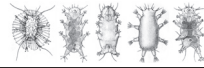
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Zusammenfassung

Diese Arbeit behandelt die Ultrastruktur des weiblichen (bzw. zwittrigen) Genitalapparates und Stadien der Eientwicklung von 5 Arten mariner Arthrotardigraden (Klasse Heterotardigrada):

Actinarctus doryphorus, *Batillipes noerrevangi*, *Halechiniscus greveni*, *Orzeliscus belopus* und *Styraconyx* n.sp.

Orzeliscus belopus ist die einzige marine Art, die bisher als Zwitter beschrieben wurde.

Strukturen, die untersucht und gegenübergestellt werden sind: Gonopore, Receptaculum seminis und die Gonaden.

Alle Gonoporen zeigen das typische rosettenförmige Muster aus 6 - 7 myoepidermalen Zellen, auch die des Zitters *Orzeliscus belopus*.

Nur in *Batillipes noerrevangi* konnte kein Receptaculum seminis gefunden werden, die übrigen Arten besitzen eine paarige, mit Cuticula ausgekleidete Einstülpung mit separaten Öffnungen beiderseits der Gonopore. Im Receptaculum von *Orzeliscus belopus* wurde pro Seite nur je ein Spermatozoon festgestellt, bei den anderen 3 Arten konnten zahlreiche, zum Teil modifizierte Spermatozoen beobachtet werden.

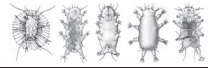
Das Ovar war in allen Fällen nur von einer Basallamina umgeben, es bestand eine klare Abfolge von Oogonien im vorderen, bis hin zu einem reifen Ei im hintersten Abschnitt.

Die Oocyten sind mit mehreren Nährzellen durch Zellbrücken verbunden, durch die verschiedene Organellen (z.B. Mitochondrien, Ribosomen) und RNA in die zukünftige Eizelle gelangen.

Die Eireifung ist asynchron, es wird anscheinend immer nur ein Ei herangereift, nur bei *Halechiniscus greveni* konnten mehrere reife Eizellen beobachtet werden.

Die Chorionbildung erfolgt in allen beschriebenen Arten ähnlich: zunächst sind nur Bruchstücke der jeweils arttypisch gestalteten Eihülle an der Oberfläche der heranreifenden Eizelle zu sehen, die später zu einer durchgängigen Schicht um das Ovum fusionieren.

Bei *Halechiniscus greveni* wurden bemerkenswerte parallele „microvilli-like structures“ um die Eizelle beobachtet, die wahrscheinlich mit der Ausbildung des Chorions zu tun haben.



Außerdem werden die Ergebnisse der Arbeit mit den wenigen bereits vorhandenen Studien über die Eientwicklung und Reproduktionsbiologie der zweiten Klasse der Tardigrada, den Eutardigrada, verglichen.

Die Beschaffung und Präparation mariner Bärtierchen ist immer noch schwierig. Aufgrund des wenigen Materials konnten nur Ovarien in unterschiedlichen Stadien beschrieben werden, einige vergleichbare Ergebnisse konnten auch zur Eihüllen-(Chorion) Bildung gefunden werden.

Deshalb ist diese Arbeit nur als erster kleiner Einblick in die Feinstruktur der Reproduktion von Arthrotardigraden zu sehen, weitere Studien mit histochemischen Methoden und eine genaue Abfolge der Stadien der Eientwicklung wären anzustreben.

Curriculum vitae

born 12th of August 1975 at Horn / Lower Austria

1981 - 1984 elementary school at Horn

1984 - 1993 grammar school at Horn; 1993: final exam

1993 - 2008 University of Vienna

(Zoologie, Biologie LA (Erdwissenschaften AHS; Warenlehre HAK)

2008 V - XII work placement at the educational service of the

Senckenberg Naturmuseum at Frankfurt am Main

additional skills:

- Qualified waste management officer (Abfallbeauftragte WU Wien 2002)
- Wiener Fiakerin (since 1999, 6 years of experience)
- Open Water Diver (SSI and PADI)
- Driving licence (B)

hobbies:

music, books, languages, sports, horses, scouts

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