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UNFERTILIZED OOCYTES IN STREPTOCEPHALIDS: RESORBED OR RELEASED?

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ABSTRACT

Short interval monitoring of isolated females of *Streptocephalus dichotomus* Baird, *S. proboscideus* (Frauenfeld), and *S. torvicornis* (Waga) revealed no resorption of unfertilized oocytes in the lateral pouches of the oviducts or in the ovisac. Oocytes expelled by the female disintegrate in the medium. This finding is in disagreement with earlier studies on *S. dichotomus* and *S. proboscideus,* but corroborates findings on *S. vitreus* (Brauer) and *S. rubricaudatus* (Klunzinger). Light and electron microscopic studies of unfertilized and fertilized oocytes show polar-body extrusion, vitelline-membrane formation, and egg-shell deposition in fertilized oocytes only. The streptocephalids studied here differ, in this respect, from two other anostracans, *Tanymastix* and *Artemia,* where unfertilized oocytes develop a vitelline membrane, and sometimes an egg shell.

Most species of fairy shrimps live in ephemeral ponds. Through the production of dormant offspring in the form of encapsulated gastrulas, they are able to exploit unstable environments.

Studies on reproduction in anostracans started with von Siebold (1873) on *Artemia* and Gissler (1881) on *Eubranchipus.* More detailed work was published by Linder (1959) on *Eubranchipus,* by Bowen (1962) and Criel (1980, 1992) on *Artemia,* by Munuswamy and Subramoniam (1985a, b, c) on *Streptocephalus dichotomus* Baird, and by Brendonck (1991) on *Streptocephalus proboscideus* (Frauenfeld). In 1991, Belk gave an overview of reproductive behavior in the Anostraca.

The presence of diverticula of the oviducts, i.e., lateral pouches, where the oocytes are temporarily stored before entering the ovisac, is typical for Anostraca (see Claus, 1886, Valousek, 1952, and Criel, 1980). In many Anostraca, copulation is the natural trigger for the transit of the oocytes from the lateral pouches to the ovisac (*Branchipus* (Claus, 1886), *Artemia* (Benesch, 1969), *Eubranchipus serratus* Forbes (see Belk, 1991)). There is, however, divergence of opinion on the need of copulation for this transit to occur, and on the fate of the unfertilized oocytes: in *E. serratus,* Belk (1991) found no transit, and neither did Claus (1886) in *Branchipus*; Prophet (1963) reported resorption of unfertilized oocytes in *Streptocephalus sealii* Ryder, and Munuswamy and Subramoniam (1985b) noticed complete oocyte resorption in the lateral pouches and in the ovisac of *S. dichotomus.* In *S. proboscideus,* Brendonck (1991) claimed to have seen resorption of unfertilized oocytes based on the presence of an opaque sludge formed by the degenerating oocytes. In contrast, females of *Streptocephalus vitreus* (Brauer) and *Streptocephalus rubricaudatus* (Klunzinger) released unshelled oocytes in the absence of males (Hildrew, 1985, and L. M. Adriaens, personal communication, respectively). Garreau de Loubresse (1980) studied the effect of isolation in another fairy shrimp, *Tanymastix.* She noted that 65–70% of females passed unfertilized oocytes spontaneously into the ovisac, where they became coated with shell-gland material. She did not mention the fate of the blocked oocytes in the remaining females.

Unfertilized oocytes of *Artemia* also pass to the ovisac (Bowen, 1962) where a fertilization membrane is formed (Criel, 1980). The oocyte is apparently activated, since it proceeds to the maturation divisions and extrudes at least one polar body (G. Criel, personal observation).

In the laboratory, casual observations of unmated females of *S. proboscideus* expelling unfertilized oocytes prompted us to follow more closely the reproductive cycles of three species of *Streptocephalus,* namely, *S. dichotomus, S. proboscideus,* and *S. torvicornis.* We not only checked whether or not

Table 1. Residence time (in minutes) of oocytes in the lateral pouches, oocytes/cysts in the ovisac, and the interval lapsing between oocyte/cyst release and molting in *Streptocephalus dichotomus, S. torvicornis* and *S. proboscideus*. [*N* = number of cycles observed when all four replicates were combined (mean ± SD)]. (Cycle = time lapsing from oocyte release into the lateral pouches to molting).

Species/cases	*N*	Oocytes in the lateral pouches	Oocytes/cysts in the ovisac	Between oocytes/cysts release and molting
S. dichotomus				
Isolated female	**16**	516 ± 211	806 ± 188	208 ± 99
Female with one male	**21**	468 ± 148	907 ± 229	192 ± 94
Female with two males	**18**	413 ± 202	918 ± 242	181 ± 109
S. torvicornis				
Isolated female	**12**	526 ± 154	928 ± 172	340 ± 240
Female with one male	**16**	434 ± 137	903 ± 145	320 ± 85
Female with two males	**19**	451 ± 129	982 ± 210	252 ± 116
S. proboscideus				
Isolated female	**12**	329 ± 120	1,010 ± 114	365 ± 122
Female with one male	**19**	435 ± 100	1,093 ± 161	363 ± 90
Fertilized cycles	2	360 ± 254	1,470 ± 127	210 ± 42

there was resorption of the unfertilized oocytes in the lateral pouches of the oviduct or in the ovisac, but also recorded the duration of three different stages of the reproductive cycle.

Materials and Methods

Cysts of *S. proboscideus, S. torvicornis*, and *S. dichotomus* were obtained from collections at the Laboratory of Animal Ecology, University of Gent. They were hatched in EPA medium (USEPA, 1985) at 25°C and nauplii of each species were reared in an aquarium using the alga *Scenedesmus* as food.

Reproductively active animals were subjected to 1 of the following treatments: females alone, females in the presence of 1 male, and females with 2 males. In *S. proboscideus*, the combination of 1 female with 2 males was not studied. Per treatment, we used 4 replicates in polystyrene containers with 150 ml of aerated, filtered (50 μm) tap water as culture medium. All the containers were kept in a thermal water bath at 29 ± 1°C under continuous fluorescent (1,500 lux) illumination. Before the start of the observations, animals were acclimated for 3 days. The animals were changed daily to clean containers with fresh medium. Food was supplied twice a day: 10^5 algal cells ml^{-1} immediately after transfer to fresh medium, and again 10^5 algal cells ml^{-1} 12 h later.

All females were monitored at high-frequency: observations were at hourly or shorter intervals, and lasted for 3–6 cycles (from ovulation to molting). All data were taken by visual observation in order to avoid stressing the animals. Oocytes from some isolated females not included in the observational study were dissected out of the ovisac after 1 h and 5 h of descent and were fixed overnight with glutaraldehyde-paraformaldehyde in cacodylate buffer 0.1 M (Karnovsky, 1965) and postfixed with 2% osmium tetroxide in the same buffer. After dehydration, they were embedded in LX resin. Semithin and ultrathin sections were examined by light or transmission electron microscopy. The same procedure was followed to study the oocytes of mated females, immediately after (<30 min) and 5 h of fertilization.

Duration of the reproductive cycle was studied from ovulation onward. Residence time of oocytes in the lateral pouches, in the ovisac, and time lapse between oocyte or cyst release, and molting in all treatments were recorded and analyzed statistically.

Results

Table 1 gives the quantitative data, and an analysis of variance is presented in Table 2. In the three species, we found no significant difference among treatments in storage time of oocytes in the lateral pouches (Tables 1, 2). After these oocytes passed to the ovisac, they remained there for more than 50% of the total duration of a cycle (Table 1). Isolation did not significantly influence the duration of this stage in *S. dichotomus* or *S. torvicornis*, but in females of *S. proboscideus* the unfertilized oocytes were expelled at 75% of the normal duration time ($P < 0.001$) (Tables 1, 2).

Among treatments with males, except for two clutches in *S. proboscideus*, the rest of the clutches in this species and all clutches in *S. dichotomus* and *S. torvicornis* were not fertilized.

From the ovisac, females released oocytes or cysts into the medium. After expulsion, oocytes disintegrated individually (Fig. 1), in small patches (Fig. 2), or as a mass (Fig. 3). All females molted after releasing oocytes or cysts. The three treat-

Table 2. ANOVA of the residence time of oocytes in the lateral pouches, oocytes/cysts in the ovisac, and the interval between oocyte/cyst release and molting among three treatments within species of *Streptocephalus*.

Species	Parameter	Source	*SS*	*d.f.*	*MS*	*F*	*P*
S. dichotomus	oocyte storage in the lateral pouches	between groups	90761	2	45380	1.035	0.280
		within groups	1807959	52	34768		
	oocyte retention in the ovisac	between groups	129346	2	64673	1.302	0.281
		within groups	2582905	52	49671		
	duration between oocyte release and molting	between groups	6268	2	3134	0.307	0.737
		within groups	530853	52	10208		
S. torvicornis	oocyte storage in the lateral pouches	between groups	64061	2	32030	1.750	0.186
		within groups	805513	44	18307		
	oocyte retention in the ovisac	between groups	56969	2	28484	0.870	0.426
		within groups	1439826	44	32723		
	duration between oocyte release and molting	between groups	68018	2	34009	1.512	0.232
		within groups	989718	44	22493		
S. proboscideus	oocyte storage in the lateral pouches	between groups	84357	2	42178	3.121	0.059
		within groups	405446	30	13514		
	oocyte retention in the ovisac	between groups	364586	2	182293	8.676	0.001
		within groups	630315	30	21010		
	duration between oocyte release and molting	between groups	44613	2	22306	2.121	0.138
		within groups	315483	30	10516		

ments did not show significant differences in this stage (Tables 1, 2).

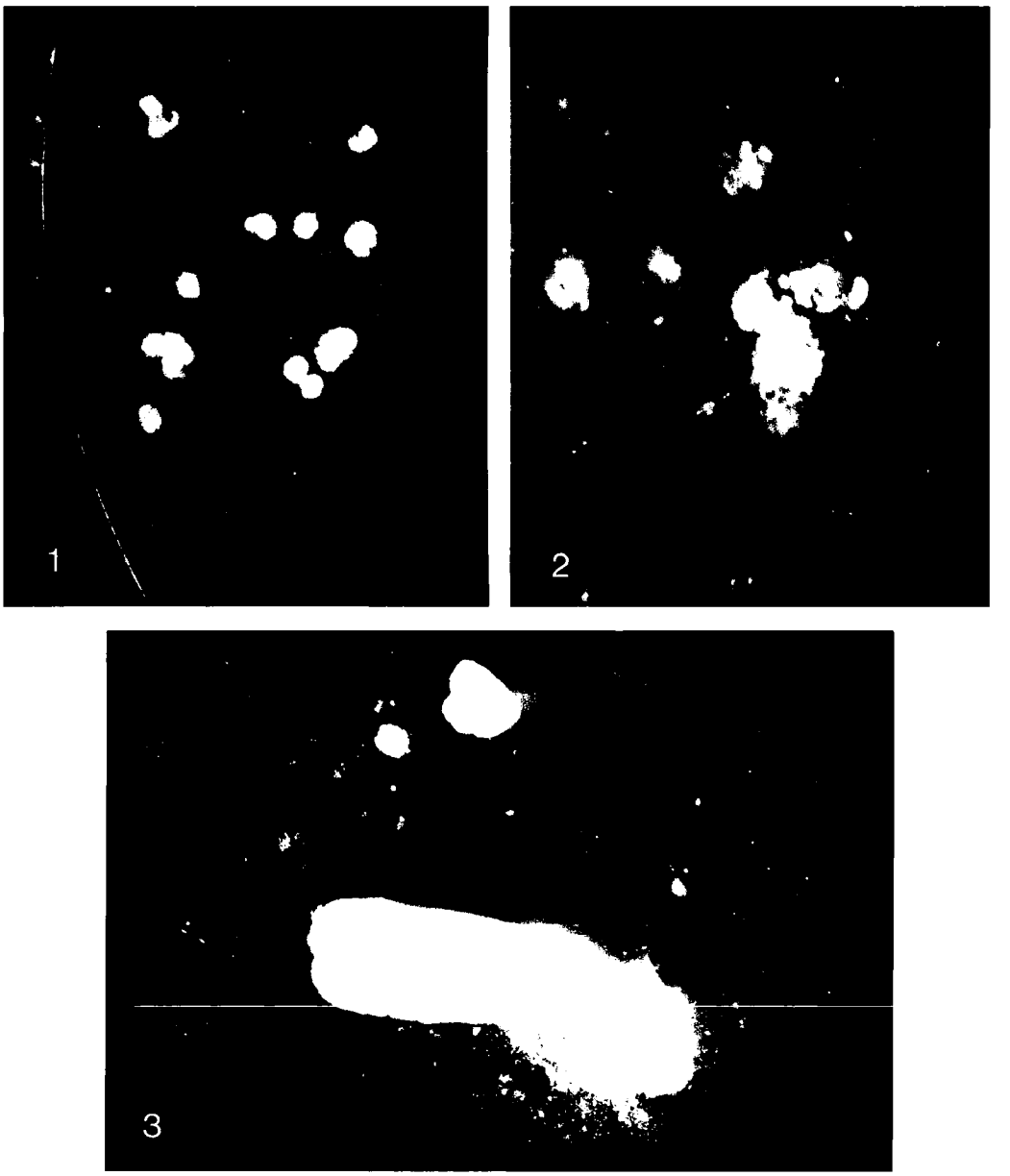
Microscopic studies showed chromosomes at metaphase and the lack of vitelline membrane in unfertilized oocytes of isolated females (Fig. 4a, b). The fertilized oocytes, to the contrary, had extruded a polar body half an hour after fertilization (Fig. 5a), and after five hours had formed a vitelline membrane and exhibited early embryonic development (Fig. 5b).

Discussion

All isolated females of the three species observed were able to pass oocytes from the lateral pouches to the ovisac. However, release of shell-gland material was not triggered by this transit, so that naked oocytes were expelled into the medium where they soon disintegrated. These observations seem to indicate that, although no stimulus, like clasping of a male, or mating, or introduction of the penes in the genital pore, is required to move the oocytes (Belk, 1991), some such stimulus is nonetheless needed for release of the shell-gland material. This contrasts with the findings of Munuswamy and Subramoniam (1985b) and of Brendonck (1991) who reported resorption of oocytes from the lateral pouches of the oviduct in *S. dichotomus* and *S. proboscideus*, respectively. Munuswamy and Subramoniam (1985b) also mentioned that only in a few cases did unmated females pass oocytes into the ovisac, where they were then resorbed. In the present study, not only in *S. dichotomus* and *S. proboscideus*, but also in *S. torvicornis*, such a resorption did not occur. Isolated females of the congeneric species *S. vitreus*, as reported by Hildrew (1985), and *S. rubricaudatus* (L. M. Adriaens, personal communication) showed no oocyte resorption.

Because of our close monitoring, we actually saw females releasing oocytes from the ovisac many times. In doing this, they turned the ventral side of their body down and by frequent body contractions expelled the oocytes. Such oocytes rapidly disappeared from the container, because females swimming at the bottom of the container facilitated the physical disintegration of the oocytes. This is probably the reason why Prophet (1963), Munuswamy and Subramoniam (1985b), and Brendonck (1991) failed to notice the expelled unfertilized oocytes in their work.

On the other hand, our work is in line with findings in *Tanymastix*, where Garreau de Loubresse (1980) found only 65–70% of the isolated females of *Tanymastix* moving oocytes into the ovisac. In contrast with our results, these oocytes were coated with shell-gland material. Garreau de Loubresse reported 2 or 3 cycles of this kind which she called abnormal because of differences in shell structure. She did not mention the fate of oocytes in the remaining 30–35% of



Figs. 1–3. Unfertilized oocytes released by *Streptocephalus proboscideus* found disintegrating individually (Fig. 1), or in groups (Fig. 2) at bottom of container. Females of *S. torvicornis* sometimes expelled all unfertilized oocytes in form of a mass (Fig. 3).

females. We found that all females of the three species of streptocephalids pass oocytes in all the reproductive cycles studied with no release of shell-gland material.

In isolated females of *Artemia,* Bowen (1962) observed descent of oocytes from the lateral pouches to the median ovisac and did not mention any signs of oosorption. This finding led Belk (1991) to hypothesize an "endogenous egg cycle" in *Artemia* which he related to the occurrence of parthenogenesis in this genus. The activation of unfertilized oocytes upon descent in the ovisac in *Artemia* observed by one of us (GC) corroborates his hypothesis. However, a comparison needs to be made with the

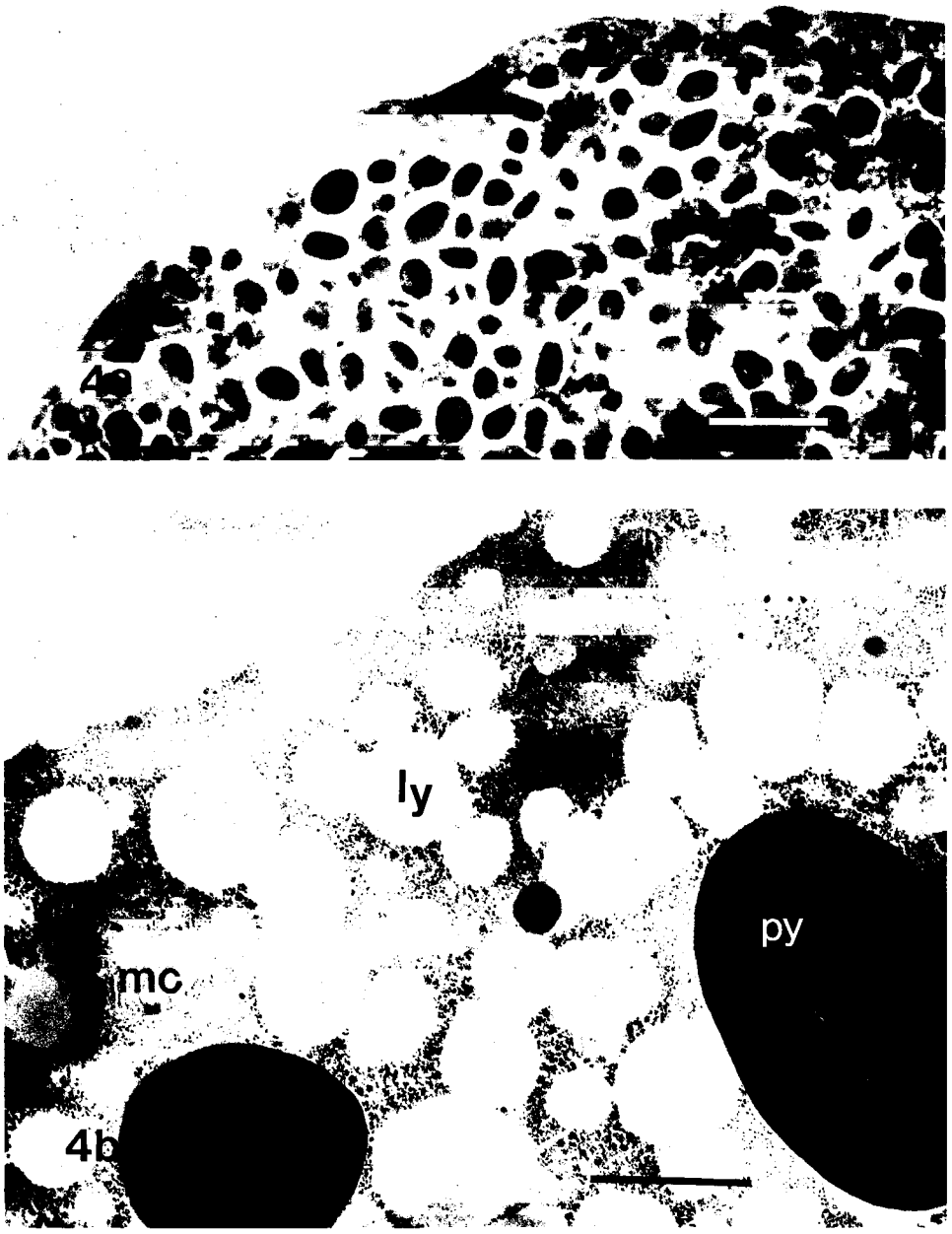


Fig. 4a, b. Unfertilized oocyte of *Streptocephalus proboscideus* 5 h after descent into ovisac: a, light micrograph showing persistence of metaphase chromosomes (arrows) (bar = 10 μm). b, electron micrograph showing absence of vitelline membrane (bar = 1 μm). ly = lipid yolk droplets, mc = mitochondria, py = proteinaceous yolk droplets.

unfertilized oocytes of *Tanymastix,* a genus where no parthenogenesis occurs.

Females of *S. proboscideus, S. dichotomus,* and *S. torvicornis* releasing unfertilized oocytes, even in the presence of males, may be ascribed to the artificial nature of laboratory conditions. A similar finding has been reported by Hildrew (1985) in *S. vitreus.*

We conclude that (1) short-interval monitoring of other anostracan groups will be required to take stock of variation existing

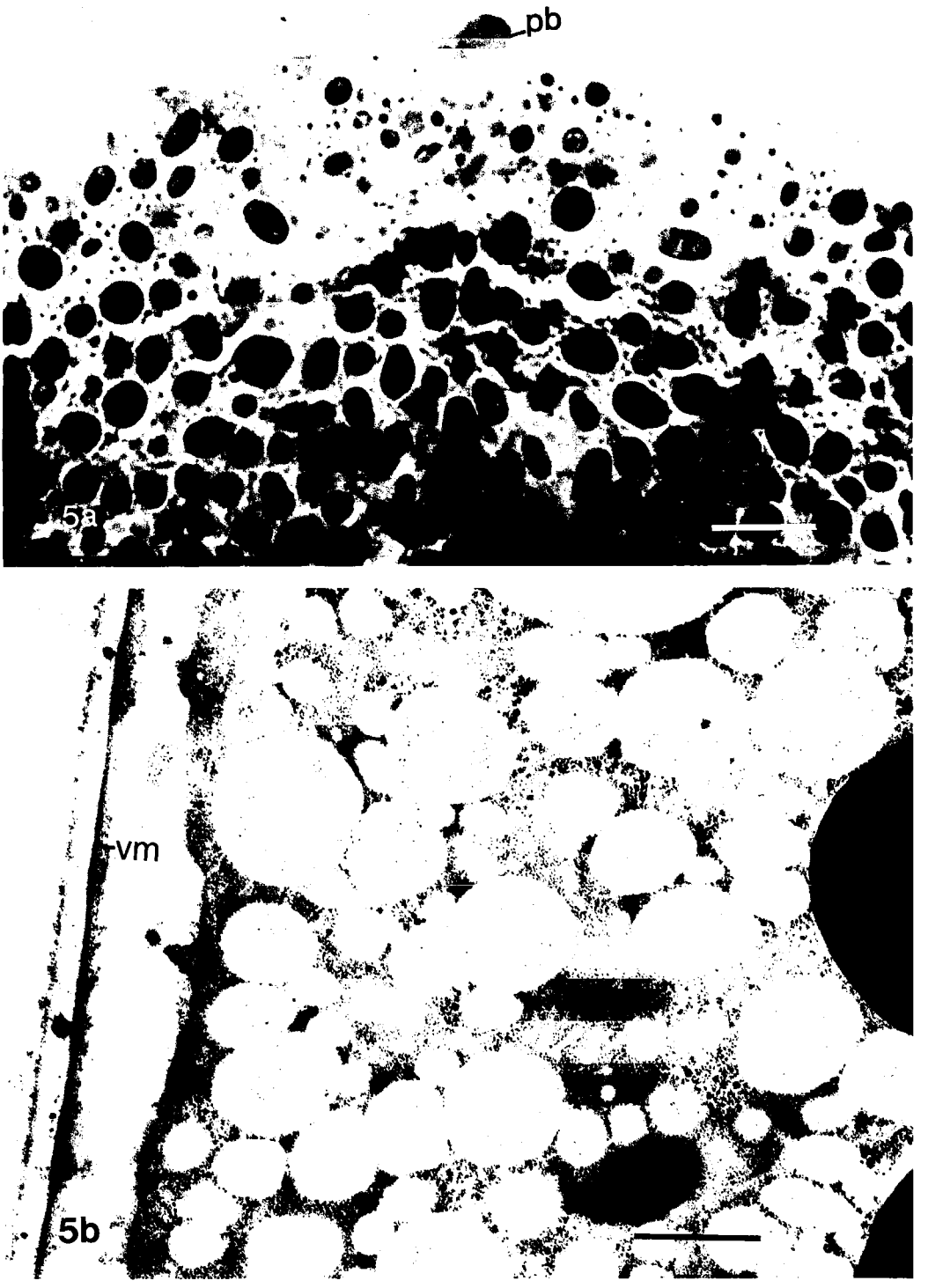


Fig. 5a, b. Light micrograph of fertilized oocyte of *Streptocephalus proboscideus* showing extrusion of polar body within 30 min of fertilization (bar = 10 μm) (a), and vitelline membrane after 5 h of fertilization (bar = 1 μm) (electron micrograph) (b). vm = vitelline membrane, pb = polar body.

within this group with regard to the fate of unfertilized oocytes, and (2) within the streptocephalids we found a uniform mechanism where copulation is required to trigger shell formation. This is not the case in *Artemia* and *Tanymastix*.

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