

The structure and development of the male internal reproductive organs in six European species of Ephemeroptera

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Anatomy, histology, testes, germinal cells, gonoducts, spermatogenesis, six European genera

Abstract. The anatomy, histology and development of testes and gonoducts were studied in six European genera (*Cloeon*, *Oligoneuriella*, *Ecdyonurus*, *Caenis*, *Paraleptophlebia* and *Ephemera*) which represent different morphological types within the order. They have paired testes situated along the gut in the dorsal, lateral or ventrolateral position, consisting of a great number of testicular follicles connected directly with the gonoduct. The testes extend from the thorax or the first abdominal segment to the sixth abdominal segment. The gonoducts are formed by the mesodermal seminal ducts with a pair of ducts openings. In young larvae (i.e. up to about 10th instar) the spermatogonia are encysted and dividing into spermatocytes. In older larvae cysts containing the spermatocytes II disintegrate, and the spermatocytes which have undergone meiosis develop into spermatids. Spermateliosis takes place only in the last 2–3 instars before moulting into subimago. The spermatogenesis occurs entirely in the larval stage. Descending of spermatozoa is terminated in the last larval instar or during the subimaginal stage and shrinking of testes takes place. No substantial changes were observed in subimagoes and adults; testes are completely disintegrated in all abdominal segments.

Our knowledge of the comparative anatomy of mayflies is relatively extensive. There are several organ systems which have been elaborated from this point of view: neuroreceptory organs (ARVY & GABE, 1953), abdominal muscles (GRANDI, 1962), tracheal system, malpighian tubes, nerve cord (LANDA, 1948, 1969) and thoracic muscles and integument (TSUI & PETERS, 1975). Less attention has been paid so far to the development and structure of mayfly gonads. The most useful general study on mayfly reproductive system still remains that by PALMEN (1884) published long before comparative anatomy and histology developed. The first histological data were obtained by NEEDHAM, TRAVER & HSU (1935) in the genus *Stenonema*. The anatomy of the male and female organs of reproduction and mating habits of eight Holarctic genera were investigated by BRINCK (1957) in his extensive comparative study which is of a general character, devoting only a little attention to the detailed histological structure. Some data are also given by several authors dealing with the descriptive anatomy of mayfly larvae and adults: DRENKELFORT (1910) — *Siphonurus lacustris* ETN.; HEINER (1915) — *Cloeon dipterum* (L.), *Baetis fuscatus* (L.) and *Habrophlebia fusca* (CURT.); VAYSSIÈRE (1934, 1937) — *Baetisca obesa* SAY and *Probosciplocia sikorai* (VAYSS.); LEVY (1948) — *Hexagenia limbata occulta* (WALKER) and also by certain earlier authors (Swammerdam, Eaton, Joly). Anatomy and histology of testes and seminal vesicles in adults of *Coloburiscus humeralis* (WALK.) are mentioned by WISELY (1965). BIRKET-SMITH (1971) studied the ectodermal part of mayfly reproductive system with regard to a general

abdominal morphology in *Povilla adusta* NAVÁS. The transfer of spermatozoa was investigated by GRIMM (1977). He discovered sperm-pump mechanism consisting of the posterior portion of vas deferens and a thick layer of circular muscles in *Habrophlebia lauta* ETN. Sperm-transfer in *Rhithrogena semicolorata* (CURT.) and *Cloeon* sp. is mentioned by SCHLEE (1969). Comparative anatomy of testes of 94 genera (164 species) of mayflies was studied by Sol-dán (in prep.).

This paper describes the anatomical and histological structure of the male gonads in six Palaearctic genera in details.

MATERIAL AND METHODS

The structure and development of testes were studied in larvae, subimagos and adults of the following species: *Cloeon dipterum* (LINNÉ, 1761) (Baetidae); *Ecdyonurus torrentis* KIMMINS, 1942 (Heptageniidae); *Oligoneuriella rhenana* (IMHOFF, 1852) (Oligoneuriidae); *Caenis robusta* EATON, 1884 (Caenidae); *Paraleptophlebia submarginata* (STEPHENS, 1835) (Leptophlebiidae); *Ephemera danica* (MÜLLER, 1764) (Ephemeridae). With the exception of *O. rhenana* all species investigated belong to the "winter species" (development of larvae during winter month — LANDA, 1968); *C. dipterum* and *C. robusta* may have two or even three generations a year. *O. rhenana* is typical "summer species". These species represent different phylogenetic lineages (LANDA, 1973) as well as different morphological, ecological and life cycle types within the order Ephemeroptera. All material studies was taken from two localities in Central Bohemia (Sázava river, Jílové; Zahofanský brook, Davle-Libřice). The total number of specimens was examined: 76 larvae, 23 subimagos, 17 adults (*C. dipterum*); 112 l., 34 s., 28 a. (*E. torrentis*), 19 l., 6 s., 9 a. (*O. rhenana*), 24 l., 10 s., 17 a. (*C. robusta*), 142 l., 64 s., 38 a. (*P. submarginata*), 185 l., 92 s., 120 a. (*E. danica*). Using Lemkuhl's classification of larvae (LEHMKUHL, 1970) there are five larval stages: newly hatched, younger, half grow, older, and ready for emergence (mature larvae).

The specimens examined were dissected in Pringle's physiological saline. The living material, namely testicular tubules and seminal vesicle, were observed under the phase (anoptal) and-interference contrast microscope. A fast green stain was used for staining of native preparations. The material of testes as well as the whole specimens used for histological treatment were fixed in Bouin (3–5 days) and Carnoy (5–10 minutes) fixatives, then transferred through alcohol and methylbenzoate and embedded in paraplast. The sections were cut on a microtome to thickness of 5–7 μ m and stained with Harris or Mayer haematoxylin. Cytoplasm was counter-stained with eosin or erythrosin. Pappenheim's modified method was also used for staining sections, smears and squashes. Sections and preparations were examined and microphotographs taken with a Zetopan microscope.

THE STRUCTURE OF THE TESTES AND GONODUCTS

The testes

There is a pair of testes situated along the alimentary canal in mayflies. The position of testes can be dorsal (*C. dipterum*), lateral (*E. torrentis*, *O. rhenana*, *P. submarginata*, *C. robusta*) or ventrolateral (*E. danica*) to the alimentary canal. The position of the testes and the structures described below (peritoneal structures) do not substantially change during the larval development. In the older larvae (growth of testis is finished) testes extend either from prothorax (*C. robusta*), mesothorax (*P. submarginata*), metathorax (*C. dipterum*, *E. torrentis*, *E. danica*) or from the anterior margin of the first abdominal segment (*O. rhenana*) to the posterior margin of the sixth segment. Testes are subcylindrical in *E. torrentis* and *O. rhenana*, spindle-shaped in *C. robusta* and *P. submarginata*, slightly flattened in *E. danica*. Apical part of testis in thorax or the first abdominal segment is rounded (*E. torrentis*, *O. rhenana*), bluntly pointed (*C. dipterum*, *E. danica*) or pro-

TABLE 1

Number of testicular follicles in *C. dipterum*, *E. torrentis* and *P. submarginata* (older larvae)

Species	No. of specimens investigated	Right testis		Left testis	
		range	mean	range	mean
<i>Cloeon dipterum</i> (1st generation)	7	250—311	276	271—307	289
<i>Cloeon dipterum</i> (2nd generation)	11	234—295	259	221—306	275
<i>Ecdyonurus torrentis</i> (population No. 1)	5	139—172	155	120—196	146
<i>Ecdyonurus torrentis</i> (population No. 2)	3	148—163	158	132—170	141
<i>Paraleptophlebia submarginata</i>	9	245—334	279	252—289	268

duced into a point (*C. robusta*, *P. submarginata*). Each testis consists of testicular follicles. The number of testicular follicles, as is apparent from Table 1, is not constant in individuals of the same species or even in individuals of the same population. Differences were also found in the right and left testis of one specimen (Table 1). Relatively higher number of testicular follicles was found in *C. dipterum* and *P. submarginata* (200—300); follicles of testis in *E. torrentis*, *O. rhenana* and *E. danica* are less numerous (approx. 100—200). In all specimens investigated testicular follicles are of the same length; very slight differences in the size of follicles were observed in *E. torrentis* and *O. rhenana*. Follicles are short, nearly spherical (*E. torrentis*, *O. rhenana*) or subcylindrical (*P. submarginata*, *E. danica*) connected directly with the vas deferens (seminal duct) and not forming vasa efferentia.

The outer membranous cover of the whole testis is formed by peritoneal membrane connecting all testicular follicles. This tissue is transparent, not pigmented and without nuclei or cell boundaries. In *C. dipterum* there are conspicuously light brown pigmented tracheae, but no pigmentation of tracheae was observed in other species investigated. The wall of testicular follicles is formed by peritoneal epithelium which is of the syncytial character. The nuclei are very small, flattened and very difficult to distinguish. The wall of neighbouring testicular follicles are closely adjacent but never fused, not containing any trophic material or reserves.

Germinal cells

The cavities of the testicular follicles are filled up by germinal cells. Apical (Verson's) cell is not developed in follicles in the species investigated. Spermatogonia was observed on the top of the follicle. Spermatogonia are of spherical shape and about 3—5 μm in size, nuclei with dense faintly granular chromatine and distinct nucleolus (Plate I, Figs. 2, 3).^{*} The mitotic activity has never been found in spermatogonia I. Spermatogonia are hardly distinguishable from the young cyst cells dispersed among spermatogonia. Spermatogonia II produced by the division of spermatogonia I are of the same shape and size with stainable nucleus, evident nucleolus and slightly

^{*} The Plates I—VI can be found at the end of this issue.

basophil cytoplasm (Pl. I, Fig. 3). Spermatogonia II are soon isolated by projections of cyst cells. Always only one spermatogonium is enclosed in one cyst (Pl. I, Fig. 2).

The zone of spermatocytes follows the zone of spermatogonia in the testicular follicle. Spermatocytes I are about 5–7 μm in size, the nuclei are smaller, less intensively stainable but with well stainable nucleoli. All spermatocytes are contained in cysts. Cysts are apparent on smears of testis (Pl. I, Fig. 3) but the cyst walls are hardly discernable on sections of testicular follicles (Pl. II, Figs. 5–8). Spermatocytes I were observed mostly in prediakinetic stage with spiralled chromosomes. It is very difficult to distinguish by staining the nucleus and by the amount of cytoplasm between spermatocytes I and II. Spermatocytes II are slightly smaller (4–6 μm) with smaller amount of cytoplasm. They are not distinctly separated from the spermatocytes I under the phase contrast. Judging from histological observations meiosis II starts as a rule immediately after the Ist one has been finished. Each cyst contains probably 16 spermatocytes.

The spermatids are about 3–6 μm in size with a large intensively stainable nucleolus, without evident nucleolus and with hardly any cytoplasm. The young spermatids are roundish with central nucleus. During further development they become elliptical and elongated with the nucleolus forming the semilunar meniscus (Pl. III, Fig. 11). The ripening spermatozoa are gradually prolonged and they are attached on the cyst walls in several layers. In this period the cysts usually disintegrate (Pl. III, Fig. 10) but the spermatids remain arranged in groups. The cysts can disintegrate earlier or later since sometimes free spermatocytes in follicles or presperms in cysts can be found. Empty cyst cells descend to the gonoduct or outflow ways together with mature spermatozoa. They were never observed in the basal portion of follicle.

The presperms are similar to spermatids in shape but have the foundation of a tail (Pl. III, Fig. 12). They are differentiated from the spermatids by condensation of the chromatine as well. The tail is usually coiled up into a little ball (Pl. VI, Fig. 21) but sometimes is not visible at all (*P. submarginata*).

While the spermatogonia, spermatocytes and spermatids of species investigated are approximately of the same shape and size, there are considerable differences in shape and size of mature spermatozoa (Pl. VI, Figs. 21–24). The mature spermatozoa are differentiated from presperms by further prolongation of head and by the tapered elongated tail. With the exception of *P. submarginata*, spermatozoa consists of a large head and tiny tail, and the cervical portion is not developed. They are rod-shaped with relatively very long tail (about twice longer than the head) (*C. dipterum*), rod-shaped with shorter tail (*O. rhenana*, *C. robusta*, *E. danica*), rod or spindle-shaped (*E. torrentis*) (Pl. VI, Fig. 22) or spherical, and under the light microscope show no discernable flagellum (*P. submarginata*) (Pl. VI, Fig. 23). Spermatozoa of mayflies are sometimes of two types: a) the more numerous (about 75%), smaller and easily stainable spermatozoa, and b) the less numerous (about 25%), larger and less stainable ones. Both types of spermatozoa are of the same shape. The less stainable spermatozoa are sometimes more rounded. These difference are conspicuous in some species, especially in *E. danica* (Pl. VI, Fig. 24). Similar differences occur also in

P. submarginata. There are only slight differences in *C. dipterum*, *O. rhenana* and *E. torrentis*. Mature spermatozoa are 2–10 μm in length (up to 15 to 25 μm in the less stainable type) and 1–4 μm (6–9 μm) wide. The tail is very thin, with the exception of *P. submarginata* well distinguishable in all species investigated.

The gonoducts

Gonoducts of the testes are formed by the mesodermal seminal ducts and ectodermal ejaculatory ducts. Paired ducts of testes are well separated and there is a pair of duct openings on the posterior wall of the penis lobes. Seminal ducts are not joined to form a common seminal duct (*vas deferens communis*), but lead directly to ejaculatory ducts.

The seminal ducts (*vasa deferentia laterales*) are simple and tubiform. They run from the apex of testis to the posterior margin of 9th abdominal segments. Seminal ducts directly connected with testicular follicles in abdominal segments I–VI are expanded several times, forming a large seminal vesicle (*vesicula seminalis*) in the area of segments VII–IX. The seminal vesicle (Pl. IV, Fig. 16; Pl. V, Figs. 17–20) is a simple roundish reservoir (*C. dipterum*, *C. robusta*, *O. rhenana*) or an elongated reservoir probably provided with lateral septa in the posterior portion (*E. torrentis*) (Pl. V, Figs. 19, 20). In some species the wall of the vesicle contains dark brown pigments (*P. submarginata*, *E. danica*) (Pl. V, Fig. 18), but the ducts and vesicles are usually whitish opaque or colourless (Pl. V, Fig. 17). The shape of the seminal vesicle and posterior portions of the ducts is rather variable, depending on the amount of spermatozoa present inside. Spermatozoa show no conspicuous motions in ducts *in vivo* but they move gradually caudad.

The walls of the ducts are formed by an inner layer of epithelium and an outer layer of circular muscles (Pl. II, Fig. 7), both layers being of equal width. Only hardly discernable, scattered muscle fibres were observed in the anterior portions. The cells of this epithelium are roundish or elliptical with large nuclei. Seminal vesicles are enclosed in an outer, very thin, circular muscle coat and are lined with an epithelium of syncytial character (Pl. IV, Fig. 16). The nuclei of these cells are much smaller than those of epithelium of the ducts. The epithelium of ejaculatory ducts is also of a syncytial character and deeply folded. Both anterior and posterior portions are similar. The posterior portion can be provided with several muscles assisting the sperm transfer in adults. These muscles appear in subimaginal stage.

THE DEVELOPMENT OF THE MALE REPRODUCTIVE SYSTEM

Newly hatched larvae

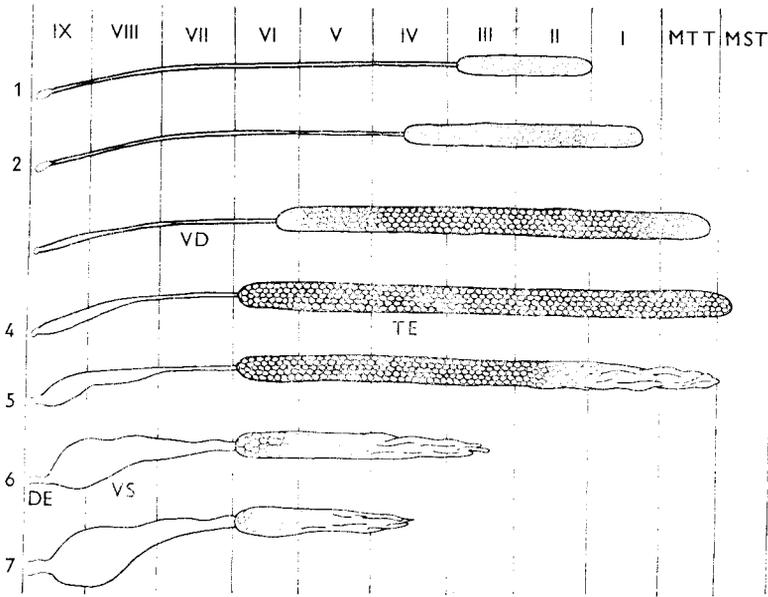
(Pl. I, Fig. 1)

Only the paired elongated groups of germinal cells were found in newly hatched larvae. These groups, consisting of several hundred cells, are situated in the second abdominal segment. The walls of follicles are not developed, no follicles are apparent. No histological evidence of outflow ways were observed.

Younger larvae

(Pl. I, Figs. 2, 3, 4; Figs. 1, 2)

In younger larvae (up to about tenth instar) the two testes are elongated, subcylindrical, hyaline whitish organs situated in the first three or four abdominal segments. The apex of the testis may intrude into the metathorax (*P. submarginata*, *C. robusta*). The surface of the testis is granular, and testicular follicles are not apparent in the first larval instars. They begin



Figs. 1—7: The development of the inner male reproductive system in Ephemeroptera (sketch): 1 — testis of younger larva (approx. 2nd—6th instar). 2 — testis of younger larva (approx. 5th—10th instar). 3 — testis of half grown larva (approx. 10th—15th instar). 4 — testis of older larva (about 15th instar). 5 — testis of older larva (about two instars before subimaginal moulting). 6 — testis of mature larva (several hours before subimaginal moulting). 7 — testis of subimago. MST — mesothorax. MTT — metathorax. I, II, III, IV, V, VI, VII, VIII, IX — abdominal segments I—IX. TE — testicular follicles. DE — ductus ejaculatorius. VD — vas deferens. VS — seminal vesicle. Testis and ducts in dorsal view.

to appear from the fifth to the seventh instars in abdominal segments II to III, I—III or in metathorax. With the exception of the apical portion of testis all follicles differentiated approximately up to the tenth instar. At this time the walls of follicles are formed by invaginations of peritoneal epithelium. The follicles are small and slender, and are apparent on the surface of testis in dorsal view (Fig. 2). The whole of the follicle is filled up with germarium containing spermatogonia I and cyst cells. These cells form a spermatogenetical region with a fully developed distal zone of free spermatogonia. In even younger larvae (in winter species at the beginning of autumn) cysts are being formed and in addition to the distal zone of sper-

matogonia a proximal zone of spermatocytes can be observed. This proximal zone contains both spermatocytes I and encysted spermatogonia (spermatogonia II).

Seminal ducts are formed by a pair of solid strands extending from the apex of testis to the posterior margin of abdominal sternum IX. Seminal ducts are connected with a pair of hollow ampules situated in segment IX. Seminal vesicles and ejaculatory ducts are not developed in younger larvae.

Half grown larvae

(Pl. II, Figs. 5, 8; Pl. V, Fig. 17; Fig. 3)

The testes are situated in the thorax and abdominal segments I—V or I—VI. Testicular follicles are differentiated in all abdominal segments. Large and short follicles of *E. torrentis*, *O. rhenana* and *E. danica* are well distinguishable in dorsal view. A more compact surface of the testis occurs in other species with smaller and longer follicles. The rest of the encysted spermatogonia divides into spermatocytes, and the cysts enlarge approximately in the middle of larval stage (from the 9th to 12th instar). At the same time the whole of the follicle increases in size as well. The proximal zone of spermatocytes is the only zone of the spermatogenetical region filled with follicles. The distal zone of spermatogonia (germarium) is reduced to a very thin apical layer. In even half grown larvae spermatocytes II are appearing at the basis of the follicle. The apical layer of free spermatogonia disappeared immediately after appearance of spermatocytes II in the most of specimens investigated. Meiosis takes place in half grown larvae (in winter species during winter).

Seminal ducts show only slight changes in half grown larvae. They are moderately enlarged in abdominal segments VIII and IX forming seminal vesicles. Although there are no mature spermatozoa present in the ducts seminal vesicles are apparent. Vesicles are connected with the anlagen of primary penial lobes situated in the intersegmental region behind abdominal sternum IX. The anlagen of penial lobes are paired and well separated. The ejaculatory ducts are not distinguishable.

Older larvae

(Pl. II, Figs. 6, 7; Pl. III, Figs. 9—12; Figs. 4—5)

The growth of testes is finished in older larvae (larvae from about the 13th instar with fully developed larval characters). All follicle of testes are well distinguishable. Follicles are filled with cysts containing spermatocytes (proximal zone of spermatogenetical region). The distal zone disappeared in all specimens investigated. Cysts containing spermatogonia I are restricted to a thin apical layer. In even older larvae the spermateleotical region is constituted. The spermatids which originate as daughter cells produced by the division of spermatocytes after meiosis appear in cysts at the basis of the follicle. The spermatids form the distal zone of spermateleotical region. The spermateleotical region is formed in all follicles of testis already several instars before subimaginal moulting (in winter species in early spring). The first encysted presperms forming the proximal zone of spermateleotical region appear in follicles approximately 2—3 instars before subimaginal

moulting. Only a thin apical layer of spermatocytes II remains. As mentioned above cysts containing presperms or spermatids are disintegrated by the increasing of inner pressure and exhaustion of trophic material by ripening germinal cells. The cysts break down into particles of different sizes in the basal region of the follicle. These particles are hardly distinguishable from presperms and spermatozoa and probably descend into seminal ducts together with germ cells. In *O. rhenana* and *C. robusta* (the species with very short subimaginal stage) the changes described above proceed very rapidly. The follicles are filled up with free or encysted presperms. Even in older larvae the first mature spermatozoa appear in seminal ducts.

Seminal ducts do not change in older larvae. Only solitary presperm or spermatids (mature spermatozoa in *O. rhenana* and *C. robusta*) were found in seminal ducts and vesicles. Seminal vesicles situated in abdominal segments VII—IX are three or four times broader than seminal ducts. The anlagen of the penis are united to form a single organ with two lobes. Ejaculatory ducts are apparent in segment IX.

Mature larvae

(Pl. IV, Figs. 12, 14, 15; Pl. VI, Fig. 21; Figs. 5, 6)

Shrinking of testes takes place in mature larvae (last instar before subimaginal moulting). Only the spermateleological region was observed in testicular follicles, and encysted or free spermatocytes are not present. Nearly all cysts are usually disintegrated. The testicular follicles are filled with a mixture of presperms, mature spermatozoa and the remaining spermatids. The distal and proximal zones of the spermateleological region are not separated. Mature spermatozoa descend into the seminal ducts and seminal vesicles. The follicles in the thorax and first abdominal segments are empty, converted into a hardly discernable membranous formation. Several hours before subimaginal moulting spermatids gradually fade away and the follicles as well as seminal ducts are filled with mature spermatozoa. In *O. rhenana* and *C. robusta* nearly all follicles are disintegrated and all spermatozoa are in ducts or seminal vesicle.

Seminal ducts are considerably enlarged before subimaginal moulting. Seminal vesicles filled with spermatozoa are 7—9 times broader than ducts. They extend from the anterior margin of abdominal segment VII to the posterior wall of the penis lobes. Ejaculatory ducts are filled with spermatozoa as well. Penis is basically of the same shape as in adults, with fully developed arms and well separated lobes.

Subimago and adults

(Pl. IV, Fig. 16; Pl. V, Figs. 18—20; Pl. VI, Figs. 22—24; Fig. 7)

No changes were observed in *O. rhenana* and *C. robusta*. Only slight changes take place in *E. torrentis* and *E. danica* (subimaginal stage 2—3 days). Testicular follicles are gradually disintegrated in all abdominal segments. Ejaculatory ducts are slightly enlarged. The spermatozoa stored in seminal vesicles are never completely exhausted during the mating flight of adults.

DISCUSSION AND CONCLUSIONS

The results obtained in the six European genera investigated fully agree with those obtained by NEEDHAM et al. (1935) in the genus *Stenomema* and BRINCK (1957) in the genera *Baetis*, *Ephemerella* and *Leptophlebia*. According to NEEDHAM et al. (1935) the epithelial cells of vasa deferentia and ejaculatory ducts are more or less glandular. There is no histological evidence that the epithelium of the outflow ways is an active secretory tissue in species investigated. Despite of histological conditions the sperm liquid (medium of spermatozoa) must be produced just by the epithelial cells of vasa deferentia or ejaculatory ducts.

No anastomosis or direct connections were observed between the left and right posterior portion of outflow ways in these species. On the other hand, this is contrary of the data of PALMEN (1884) who studied *Ephoron virgo*, as well as to those of CODREANU (1939) who described transversal anastomosis in front of the penial bases in larvae of *Ecdyonurus lateralis*. In the genera studied by the authors mentioned above anastomoses do not occur as well. The testes of mayflies are described to be the straight and elongated organs situated parallelly with the alimentary canal. BRINCK (1957) figured the testis of *Siphonurus* which is s-curved in abdominal segments II and III. This arrangement of testes was observed in a great number of genera of the Siphonuridae (subfamily Siphonurinae) (Soldán, in prep.) although the testes of species investigated are straight.

As already pointed out by BRINCK (1957) no accessory glands occur in the Ephemeroptera. The structures described and figured by earlier authors are probably malpighian tubules or parts of fat body. Ejaculatory ducts appearing in older larvae are evidently of the ectodermal origin. Chitinous elements were found in ejaculatory ducts of all species investigated with the exception of *Cloeon dipterum* where the duct and penis lobes are vestigial. There is no doubt that the ejaculatory ducts are ectodermal invaginations (cf. WHEELER, 1893; QUADRI, 1940).

The male inner reproductive organs of mayflies are of a very primitive character within the pterygotes. The paired testes and outflow ways are always separated and situated in the abdomen and also in the thorax and accessory glands are lacking. These characters are evidently synplesiomorphic within the Ephemeroptera. We can find a similar arrangement of male reproductive organs only in some families of the Thysanura or Diplura. Outflow ways are always separated and a pair of penis lobes are described in some families of earwings (Dermaptera) but this is probably only a secondary character. On the other hand there are apomorphic characters in mayfly male gonads as well, i.e. multiplying of the number of testicular tubules which have lost their metameric arrangement (synapomorphic character within the Ephemeroptera). A great number of testicular follicles approach those of the Odonata and Polyneoptera.

The study of spermatogenesis in six model species brought important results not only for the study of Ephemeroptera but also for other general problems of insect spermatogenesis. The spermatogenesis in Ephemeroptera takes place evidently only in one wave. In the follicles of the testis always only one type of germ cells predominates. In one follicle there are never found spermatogonia and spermatids simultaneously with the mature spermatozoa as is usual in insects that reproduce several times during the adult

stage. Otherwise spermatogenesis takes place in the usual way. The spermatozoa of mayflies are worth our attention. Although they are studied only in six species, considerable differences in the shape and size were observed. The spermatozoa of *Paraleptophlebia submarginata* are entirely atypical. In the light microscope the tail is not discernable. The spermatozoa of some species (*Ephemera danica*) are always of two types. The larger and the less numerous spermatozoa are probably of the apyrennic type and may serve for the nourishment of spermatozoa with nuclei. But no histological evidence of a typical two-folded spermatogenesis were found in the genera investigated. A great morphological diversity of spermatozoa seems to be a general character within the order Ephemeroptera. At least four principal groups of arrangement of spermatozoon were found in large sample of European species (SOLDÁN, 1979). The solving of structure and the problems of spermatozoal dimorphism require more detailed and specialized electron microscopic investigations. The latest research of mayfly spermatozoa shows a certain particularity of ultrastructure. In *Cloeon dipterum* central microfibrilae of the tail are not present (BACCETTI et al., 1969). A considerably great number of spermatozoa by which seminal vesicle and outflow ways are filled in adults is probably due to a very high fecundity of mayfly females (cf. CLIFFORD & BOERGER, 1974).

Another characteristic of spermatogenesis in Ephemeroptera is that it occurs entirely in the larval stage. The first spermatozoa develop already in older larvae, and in larvae of the last instar almost all male germ cells are in the stage of mature spermatozoa. In the last instar extensive degeneration of testicular follicles can even be observed. This degeneration is a very rare phenomenon in larvae of the other insects groups. The subimaginal stage is not necessary for the finishing of spermatogenesis. This fact brings the question of the function and biological significance of the subimaginal stage. Two possible explanations are: (i) protecting mayfly adults effectively from predation (SCHAEFER, 1975), (ii) the reduction of body weight by evaporation of water (BURKS, 1953). During the subimaginal stage already only slight changes take place. The descending of spermatozoa into the seminal vesicle is being terminated in species where this stage lasts longer (*Ecdyonurus torrentis*, *Paraleptophlebia submarginata*, *Ephemera danica*). It is generally regarded as a rule that the shorter the subimaginal stage the earlier spermatogenesis is terminated. Since in the ontogenetic development of the Ephemeroptera the trophic stage (larva) and the reproductive stage (subimago and adult that do not receive nourishment) alternate, the energetically demanding processes of spermatogenesis (and also oogenesis) were shifted to no other than the larval that is the trophic stage.

SUMMARY

1. The structure and development of the testes and gonoducts of larvae, subimagoes and adults of six European species were investigated. These are: *Cloeon dipterum*, *Ecdyonurus torrentis*, *Oligoneuriella rhenana*, *Paraleptophlebia submarginata*, *Caenis robusta* and *Ephemera danica*. These species represent different phylogenetic lines and different morphological, ecological and life cycle types within the order Ephemeroptera.

2. There are a pair of testes and always separated outflow ways situated along the gut in the dorsal, lateral or ventrolateral position. Testes consists

of a great number of rounded or elongated follicles connected directly with the seminal duct. The seminal vesicle is formed in the segments VIII—IX and connected with the ejaculatory ducts. Both seminal and ejaculatory ducts are formed by outer muscle coat and inner epithelium.

3. Germ cells are contained in the testicular follicles. No apical cells or clustres of spermatogonia were observed. The young cyst cells are dispersed among the spermatogonia. Cysts consist of 16 or 32 spermatocytes. Cysts are usually destroyed immediately after the starting of spermateliosis. Mature spermatozoa descend into seminal vesicle and seminal duct, and follicles are disintegrated in last larval instar. Two types of spermatozoa were observed in *E. danica*. Large and less numerous spermatozoa are probably apyrennic. Spermatozoa without tails were observed in *P. submarginata*.

4. There is only a pair of groups of germ cells situated in abdominal segments I and II in newly hatched larvae. Most of the follicles are differentiated and seminal ducts apparent in the young larvae. The follicles are filled with spermatogonia. Even in younger larvae the proximal zone of cysts with spermatocytes I appears. The growth of testes is finished in half grown larvae. Only an inconspicuous distal layer of spermatogonia remains and meiosis takes place in the proximal zone of cysts. Seminal ducts are enlarged in segments VIII and IX, forming seminal vesicles. The first spermatids appear in the follicles of older larvae, and the distal layers of spermatogonia disappear. Follicles are filled with spermatids and presperms several instars before moulting, only isolated groups of spermatocytes can be observed. In mature larvae nearly all germ cells are in the stage of mature spermatozoa. Follicles are disintegrated and spermatozoa descend into the seminal ducts and vesicles. Descending of spermatozoa is finished in the last larval instar or during the subimaginal stage. Follicles of adults are empty and membranous, all spermatozoa are contained in the seminal vesicle. The spermatogenesis as a whole is thus entirely shifted to the larval stage.

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Строение и развитие внутренних половых органов у шести европейских видов поденок

Анатомия, гистология, сперматогенез, 6 европейских видов, Ephemeroptera

Резюме. Изучалась анатомия, гистология и развитие семенников и половых протоков у видов из шести европейских родов поденок (*Cloeon*, *Oligoneuriella*, *Ecdyonurus*, *Caenis*, *Paraleptophlebia* и *Ephemerella*). Парные семенники расположены вдоль кишечника в дорсальном, латеральном и вентролатеральном положении и состоят из большого количества семенных фолликулов. У изучавшихся видов семенники расположены между грудью или 1-ым сегментом брюшка и 6-ым сегментом брюшка. Половые пути образованы мезодермальными семенопротоками с семенным пузырьком в 7-ом—8-ом сегментах брюшка и семеноизвергательными каналами с парой протоков наружу. У молодых личинок (т. е. до 10-ого возраста) сперматогонии находятся в цистах и делятся в сперматоциты. У старших личинок (свыше 15-ого возраста) цисты разрушаются и сперматоциты после мейоза развиваются в сперматиды. Сперматолиоз имеет место лишь в течение 2—3 последних возрастов. Зрелые спермии выходят в семенной пузырек и фолликулы дегенерируют. У имаго и субимаго семенники полностью разрушены.

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SOLDÁN T., 1979: The structure and development of the male internal reproductive organs in six European species of Ephemeroptera

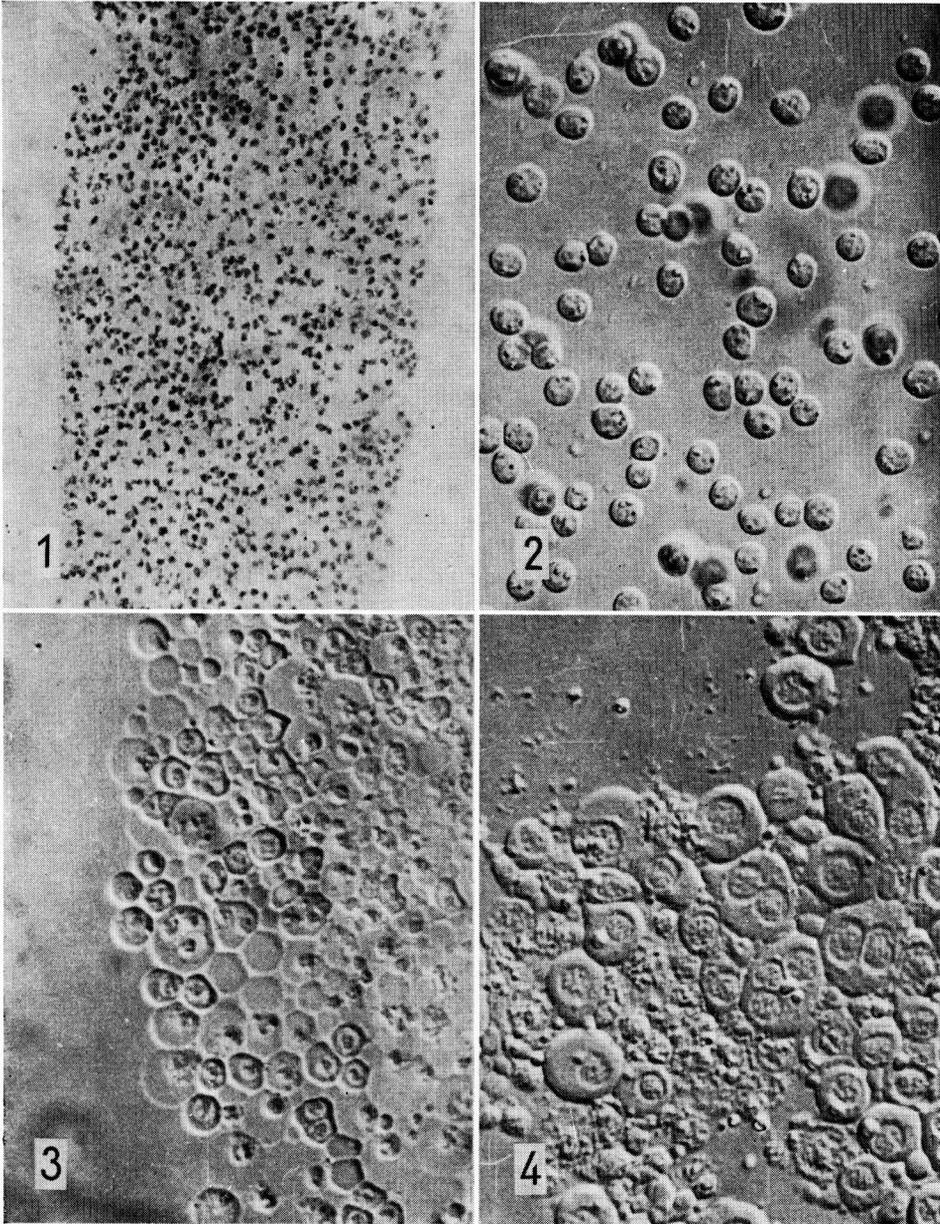


PLATE I, 1-4: 1 - *Ecdyonurus torrentis*, newly hatched larva, section through the testis. 2 - *Cloeon dipterum*, younger larva, spermatogonia I. 3 - *C. dipterum*, younger larva, spermatogonia I, encysted spermatogonia and cyst cells. 4 - *Ephemerella danica*, younger larva, cysts with spermatocytes I. 1 - Harris haematoxylin, eosin, objective 12.5, projection 10 \times . 2, 3, 4 - squash of testis, fast green, interference contrast, objective 25 \times , projection 8 \times .

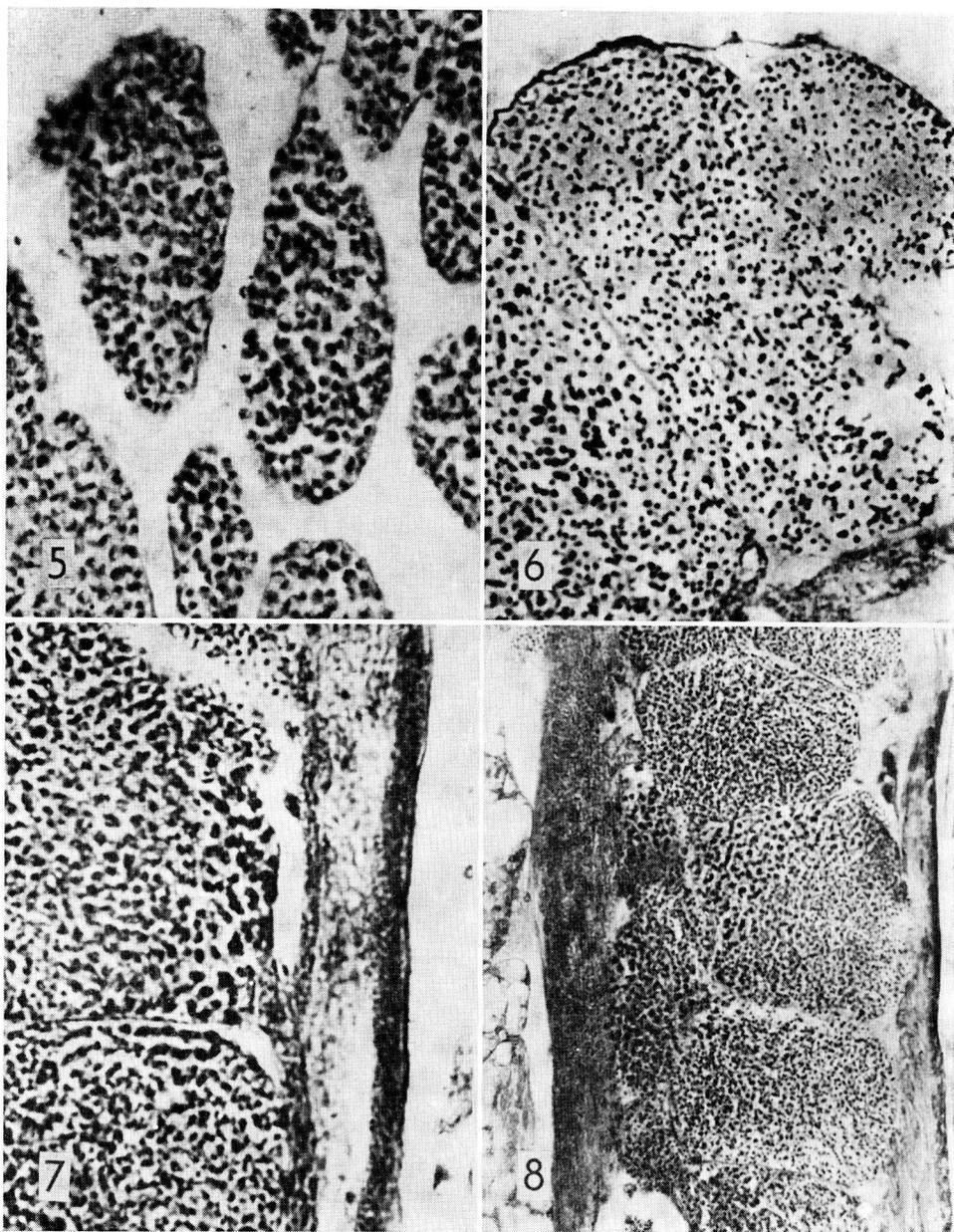


PLATE II, 5-8: 5 - *Caenis robusta*, half grown larva, section through follicles containing cysts with spermatocytes I. 6 - *Paraleptophlebia submarginata*, section through follicles containing spermatocytes I and II. 7 - *Oligoneuriella rhenana*, older larva, longitudinal section of seminal duct. 8 - *Ephemera danica*, half grown larva, longitudinal section through the testis. 5-8 - Harris hæmatoxylin, eosin, objective 25 \times , projection 12.5 \times .

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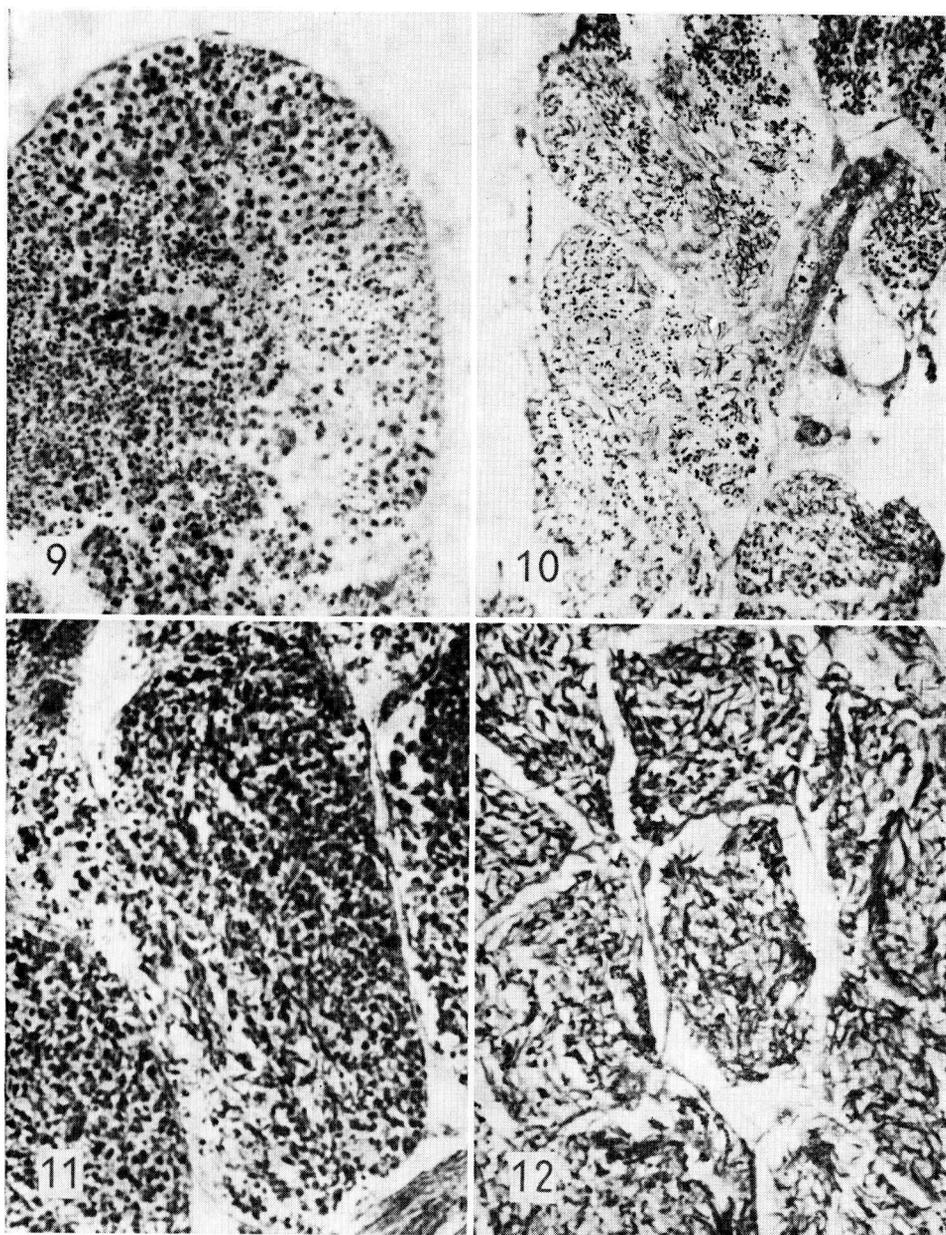


PLATE III, 9-12: 9 - *Ephmera danica*, older larva, section through follicle with apical layer of spermatocytes II and spermatids. 10 - *Ecdyonurus torrentis*, older larva, section through follicles with cysts with spermatids and presperms. 11 - the same, section of follicle after cyst disintegration. 12 - the same, section through follicles with presperms and mature spermatozoa. 9-12 - Harris haematoxylin, eosin, objective 25 \times , projection 8 \times .

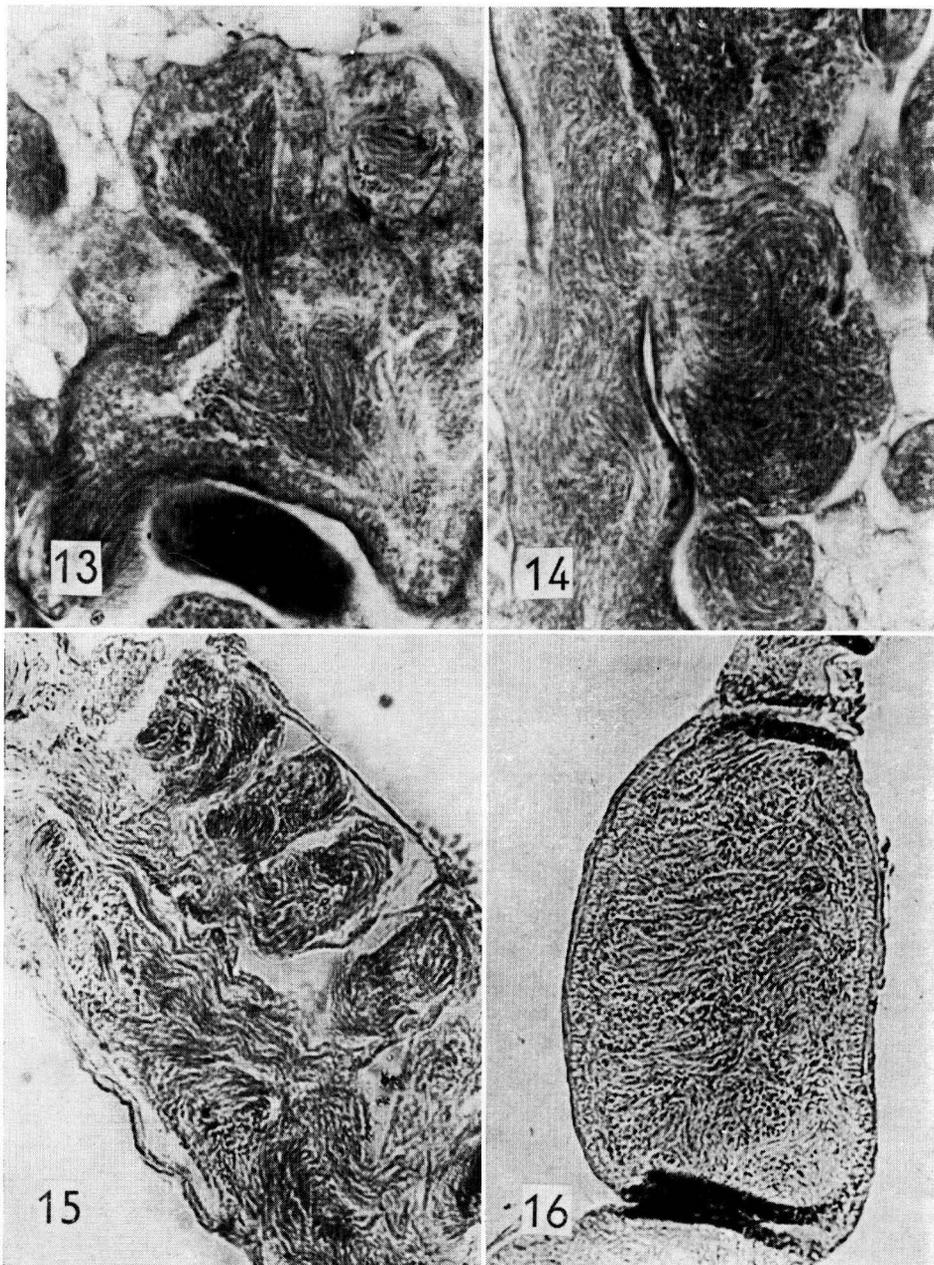


PLATE IV, 13-16: 13 - *Caenis robusta*, mature larva, section through disintegrated follicles with spermatozoa. 14 - *Oligoneuriella rhenana*, mature larva, section through follicle and seminal duct filled by spermatozoa. 15 - *Ephemera danica*, mature larva, longitudinal section through testis. 16 - *Paraleptophlebia submarginata*, subimago, section through seminal vesicle with mature spermatozoa. 13-16 - Harris haematoxylin, eosin, objective 25 \times , projection 8 \times .

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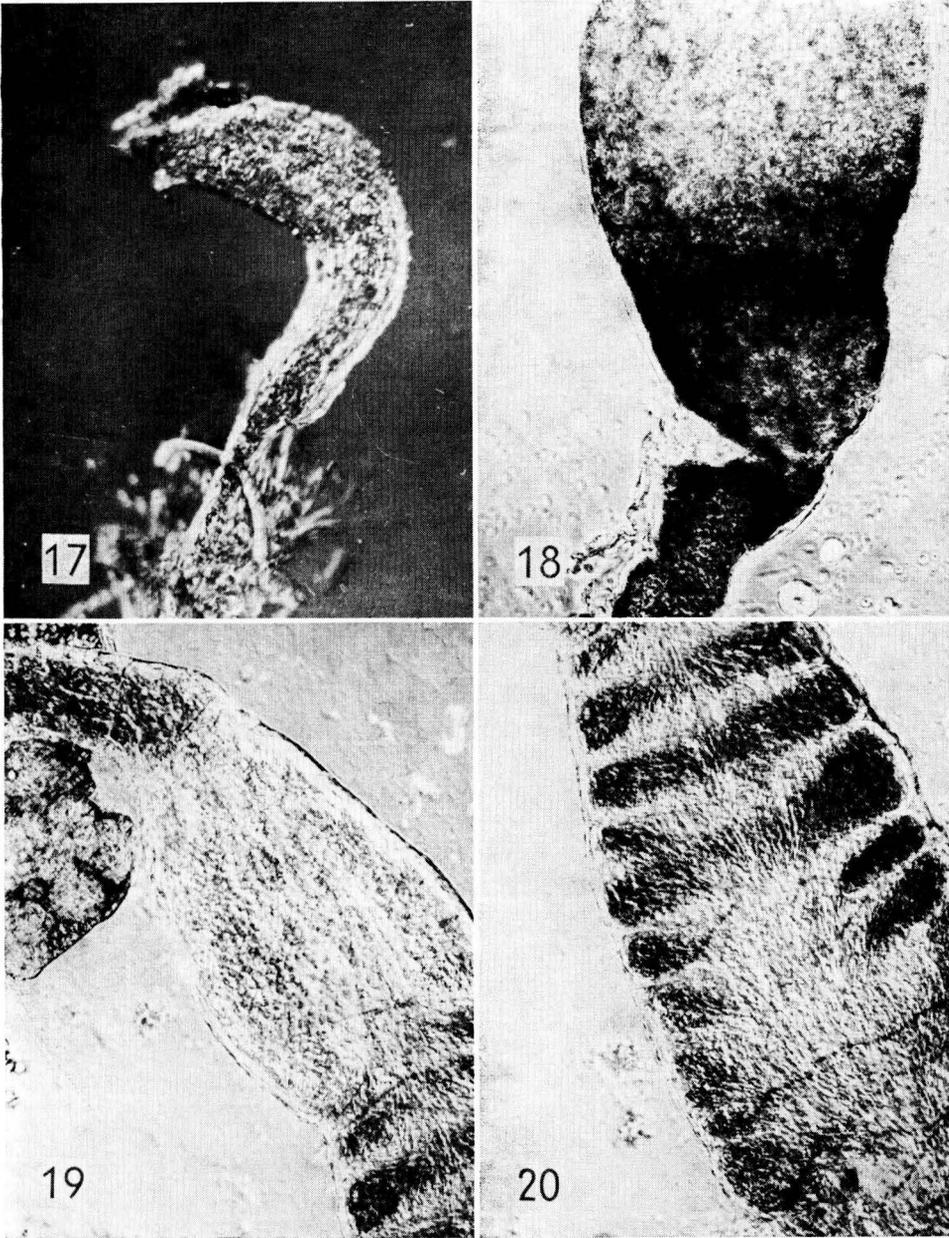


PLATE V, 17-20: 17 - *Caenis robusta*, half grown larva, seminal vesicle. 18 - *Ephemera danica*, subimago, seminal vesicle. 19 - *Ecdyonurus torrentis*, anterior portion of seminal vesicle. 20 - the same, posterior portion of seminal vesicle. 17 - anoptical phase contrast, objective 63 \times , projection 8 \times . 18, 19, 20 - interference contrast, objective 25 \times , projection 8 \times .

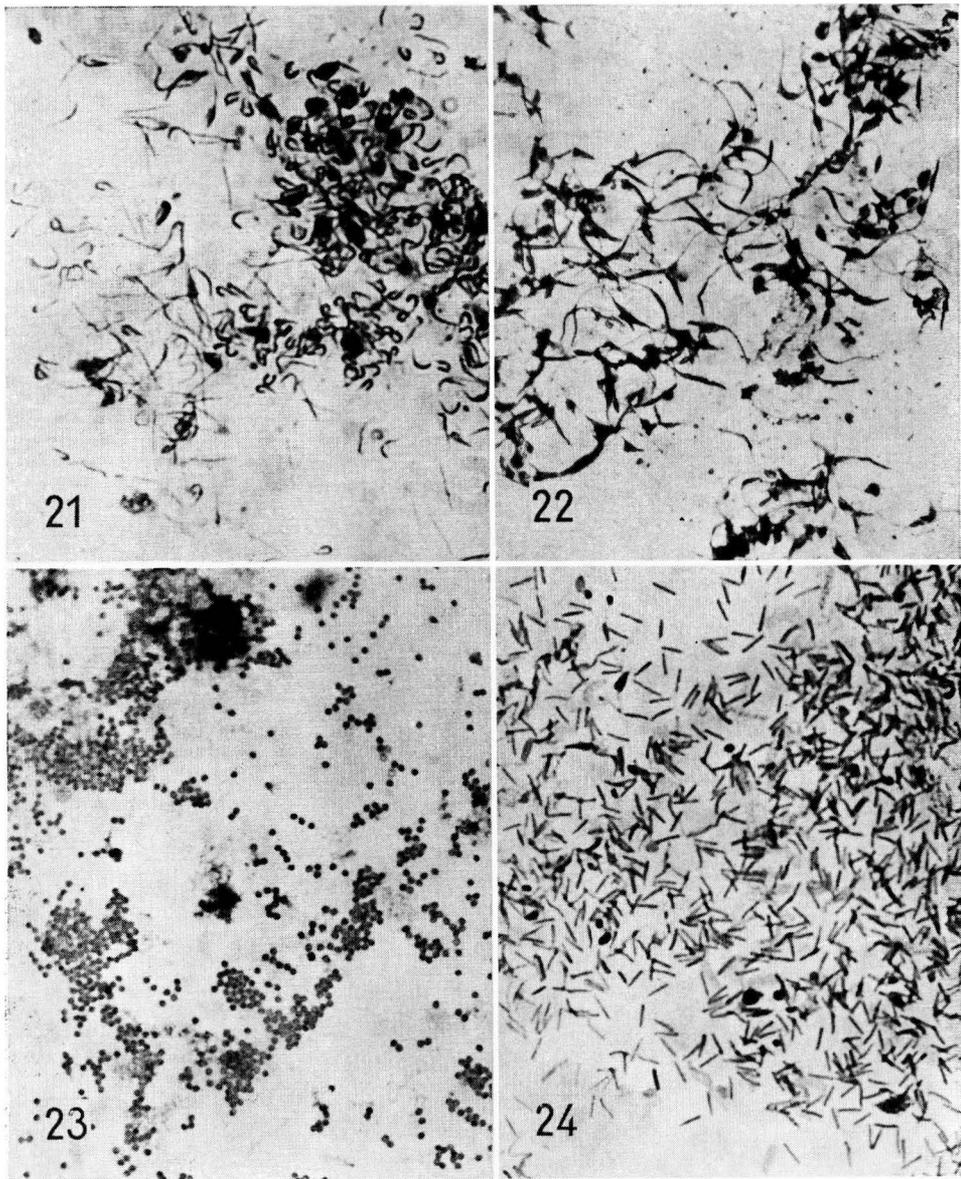


PLATE VI, 21-24: 21 - *Cloeon dipterum*, mature larva, spermateliosis, presperm and mature spermatozoa. 22 - *Ecdyonurus torrentis*, adult, spermatozoa. 23 - *Paraleptophlebia submarginata*, adult, spermatozoa. 24 - *Ephemera danica*, adult, spermatozoa. 21-24 - smears, Pappenheim, objective 100× (ol. im.), projection 8×.