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# Morphology of the reproductive systems of the Iranian species of *Hydrochara* BERTHOLD (Coleoptera: Hydrophilidae)

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## Abstract

Male and female reproductive systems as well as the shape of sperm and microscopic structure of testicles of *Hydrochara* BERTHOLD (Coleoptera: Hydrophilidae), *H. dichroma* (FAIRMAIRE) and *H. flavipes* (STEVEN) from Iran, are described and illustrated.

**Key words:** Coleoptera, Hydrophilidae, reproductive system, sperm, testicles, morphology, *Hydrochara*, Iran.

## Introduction

According to BAMEUL (1992), male and female reproductive systems of Hydrophilidae are very poorly known, and no complete study of their morphology is available.

The spermatheca provides good specific distinguishing characters in many female Coleoptera (see BAMEUL 1992, 1996, 1997a, 1997b; MILLER 2001a, 2001b). Comparing internal genitalic macro- or micro-structures may also play a valuable role in finding phylogenetic relationships of species groups (see DETTNER et al. 1986; SIMICZYJEW 2002).

In this paper a detailed study of the morphology of the reproductive systems of Iranian specimens of *Hydrochara* BERTHOLD, *H. dichroma* (FAIRMAIRE) and *H. flavipes* (STEVEN), is presented. In addition, histological studies on testicles in *H. dichroma* and on the shape of sperm in both species were carried out, aiming to distinguish the spermatheca, spermathecal gland and seminal vesicle in the reproductive system by tracing the sperm in these organs.

Details of the male and female internal genitalic structures of these two species have not been reported before.

## Material and Methods

Nineteen *Hydrochara dichroma* (8 ♂♂, 11 ♀♀) and five *H. flavipes* (2 ♂♂, 3 ♀♀) were dissected. All specimens of *H. dichroma* were collected from Fars province, and those of *H. flavipes* were collected from Khuzestan, Mazandaran and Sistan & Baluchestan provinces. Some specimens had been fixed in older and some in more recent fixative formulae (see below); each of the different formulae had its own advantages throughout this study and were therefore used for different purposes.

All specimens originated from the Insect Collection of the Department of Biology, Shiraz University (Shiraz, Iran). Some of the specimens had been fixed in a solution of 70% ethyl alcohol, 1% formaldehyde, 3.5% glycerine, and the rest up to 100% distilled water; others had been preserved in 70–80% alcohol; occasionally some glycerin (0.5%) was added. Sometimes,

tissues of those specimens which were fixed in the solution without formaldehyde, were found to be softer and more tender, which helped to find ducts and junctions under the stereo microscope in situ. Also, to study the bursa copulatrix and spermatheca, the softer specimens were superior. However, in specimens fixed with formaldehyde, the genitalia became harder and thus were much easier to be extracted than the softer ones. Apparently, formaldehyde helped to remove the whole abdominal membranous covering fat, and the ovarioles, testes, accessory glands and ducts could be pulled out easily without breaking (ovarioles are particularly brittle).

Terminology follows that of SNODGRASS (1935), IMMS (1964), DETTNER et al. (1986), HAPP et al. (1959–1995).

**Extracting male genitalia:** To extract the reproductive system (in both sexes), the abdomen was opened dorsally by lifting the wings and removing the whole abdominal tergites. The looped and folded hind gut was extracted, exposing the testes and accessory glands located among fat, muscles and tracheae of the third and fourth abdominal segments.

Occasionally, few drops of diluted methylen blue in and around the insect's abdomen were used during the operation to increase the contrast to distinguish the structures.

Connective tissues were cut and the genitalia were pulled out and put into distilled water. In the older specimens the genitalia could be extracted easier; however, to clean and clear them, they were put in 10% KOH (potassium hydroxide) for about 15–20 minutes to loosen excessive parts, relaxed them as well as made their ducts and junctions visible without unnecessary cutting or destroying the adjacent parts. The remaining fat and excessive tissues were then removed by use of a fine pin. During this procedure, care was taken that KOH did not harm the genitalic tissue. If this happened, the process was stopped and they were washed by distilled water, then placed in distilled water, again some methylen blue was added and cleaning continued. Finally, the cleaned and stained structures were washed with distilled water, kept in glycerine and studied.

**Preparing sperm smear:** A voluminous seminal vesicle of both species was chosen for sperm smear preparation. The vesicle was separated, masticated by a pin in one drop of distilled water and some smear slides were made. They were stained by Aniline Blue & Eosin (GURR 1962: 134) for 8–9 minutes. Permanent slides were made using Canada balsam.

**Tissue processing and dissecting of testis:** After making block paraffin of the testis of *H. dichroma*, the preparation was cut by a Leitz microtome in 5 µm thickness (BANCROFT & STEVENS 1990). The sections were stained by Hematoxilin & Eosin method (GURR 1962). Permanent slides were prepared using Canada balsam.

**Preparing whole mount (w.m.) slides of testicles:** Each testis was washed by distilled water carefully and then put into ethyl alcohol rinses of 50%, 75% and finally 96% (each about 2–3 minutes). Each testis was washed with distilled water and stained with Anilin blue & Eosin. Permanent slides were made using Canada balsam with the cover glass slightly pushed down to scatter the testicles.

**Separating female genitalia:** The method of separating female genitalia is the same as described for the male, except for cleaning the ovarioles, these were kept in a mixture of warm water, detergent powder and a small amount of bleaching agent for five minutes, and then cleaned carefully by use of a fine pin.

The freezing method for dissecting the fresh specimens was applied as usual (BAMEUL 1992; DETTNER et al. 1986).

**Separating bursa copulatrix and spermatheca:** Separation of the bursa copulatrix was easier than separation of ovaries. A few drops of 10% KOH were added to the dissected female's reproductive system and left for about 15 minutes to remove surrounding tissues. The bursa

copulatrix was cleaned of muscles and connective tissues, washing with distilled water, and stored in glycerine for study. Adding diluted methylen blue improved the contrast for examination of details.

More tender specimens, that were kept in alcohol only were preferred to those which were killed and fixed with alcohol and formaldehyde because the latter had been kept in warm water for 15–20 minutes before treating with KOH, the membranous wall of the bursa copulatrix as well as the common oviduct was always destroyed during the procedure.

To study the whole reproductive system, the entire abdomen had to be opened dorsally, but to dissect the bursa copulatrix and the spermatheca, it was sufficient to open only the last two or three abdominal tergites.

To clean the spermatheca and its duct, they were kept in 10% KOH for about 10 hours to permit the surrounding covering fat to dissolve.

### Descriptions and discussion

**Male reproductive system:** Male internal genitalia consist of a pair of testes, and each testis consists of short tubules or testicles. Each tubule leads to the vas deferens by a small stalk, the vas efferens (SNODGRASS 1935: 507–508). Each vas deferens dilates distally, called seminal vesicle, and the two seminal vesicles join to form the median ejaculatory duct. On top of each seminal vesicle there is a large accessory gland, a blind tube that folds circularly and finally continues posteriorly as a tube to join the ejaculatory duct.

According to IMMS (1964: 762), “two general types of reproductive organs are recognized, based on characters offered by the testes. In Adephaga, testes are simple and tubular and more or less closely coiled, each being enclosed in a membrane, in Polyphaga [for example *Hydrochara* species] they are compound and divided into a number of separate follicles”.

The accessory glands are very large (except in the immature stages or out of their reproductive cycle), and, according to SNODGRASS (1935: 573), have the function of secreting a mucous or viscid substance. This substance is either discharged as a liquid together with the spermatozoa, or, serves to form a hard cover or capsule known as a spermatophore. No work on distinguishing spermatophores or spermatozoa in *Hydrochara* species has been reported in the literature. HAPP et al. (1959–1995) described some secretory cells in *Tenebrio molitor* LINNAEUS (mealworm beetle) within their bean-shaped accessory gland producing cell-specific antigens that are integrated into a particular layer of the spermatophore secretion. The shape and volume of the accessory glands of this species are very similar to those of *Hydrochara*. This may represent a similar evolutionary path for both, but this would need further studies.

The male accessory glands generally arise from the short divergent anterior branches of the ejaculatory duct (SNODGRASS 1935: 573).

The ejaculatory duct is ectodermal in origin, and is formed as a median ventral invagination of the ectoderm at the posterior end of the ninth abdominal segment, therefore, it has a cuticular lining continued with that of the body wall (SNODGRASS 1935: 572) (Fig. 1b, cutl). About the middle of ejaculatory duct, there is a swelling which is more sclerotized than the rest of the duct (Fig. 1a). This seems to be a pumping site for sperm to be pumped through the median lobe into the bursa copulatrix of the female “by the force of a strong muscular sheath surrounding the epithelial wall of the duct” (SNODGRASS 1935: 572) (Fig. 1a, mscl).

In both species, the sperm are thin and elongate with a long flagellum (Figs. 3, 4).

Microscopic observation of the testicles (Figs. 5–7) reveals that they have a very typical cellular structure as in most other insects. Nomenclature as in MATHEWS (1982: 8–12) and CONN (1991: 151–163).

The structure and the shape of the male internal genitalia of the two species examined are identical (Figs. 1, 2).

Male external genitalia in insects are similar in evolutionary origin as those of females (SMITH 1969: 1056). In *Hydrochara*, male external genitalia (Figs. 8–11) are rather simple and primitive, of the trilobed type, and rest inside the abdomen in the primitive position, with the apical opening of the median lobe facing ventrally, stemming from segment IX (see SMETANA 1980; SNODGRASS 1935).

The male copulatory organ, or aedeagus, is of prime importance to distinguish *Hydrochara* species (SMETANA 1980). The aedeagus typically rests inside a genital capsule that represents the invaginated ninth and tenth abdominal segments. The capsule consists of: 1) a dorsal proctiger, tergite X, 2) two lateral paraprocts, the divided tergite IX, and 3) the spiculum gastrale, representing the modified sternite IX (MARSHALL 2001) (Figs. 1, 2).

The median lobe (Figs. 8–11) is membranous throughout the ventral surface and sclerotized on the dorsal surface; a fine longitudinal line arises from the apex and extends to the bases of the basal piece (WATANABE 1975). The parameres in *H. dichroma* and *H. flavipes* are significantly different (SMETANA 1980).

**Female reproductive system:** The general structure of female internal genitalia in both *Hydrochara* species examined (Figs. 12–17) agree well with those of other beetles (see SNODGRASS 1935; IMMS 1964; TUXEN 1970; DETTNER et al. 1986; BAMEUL 1992, 1996, 1997a, b; DEUVE 1993, 2001; MILLER 2001a, b). They consist of a pair of ovaries, each composed of some separated ovarioles. All ovarioles from each side join to form a lateral oviduct, which, converges posteriorly; left and right lateral oviducts join to form the common oviduct posteriorly. Several accessory glands on top and below each ovary join the oviduct on each side (clearly seen in Figs. 14, 15). The common oviduct ventrally leads into the middle of a large, sac-like bursa copulatrix. A long spermatheca opens into the bursa copulatrix anteriorly with a slender and long duct. A globular spermathecal gland is connected with the spermatheca.

The ovarioles consist of three parts (Fig. 14b): 1) a terminal filament; 2) an egg tube which contains the germ cells and their derivatives and at the end of some tubes there exist corpora lutea, and 3) the pedicel.

According to IMMS (1964: 763), female reproductive organs in Polyphaga are of the acrotrophic type. *Hydrochara* shows this type of ovarioles (Fig. 14b), but histological investigation is needed for confirmation. DETTNER et al. (1986: 348) mentions two theories with respect to the formation of corpora lutea: 1) follicular cell degenerates after egg deposition to remain as corpus luteum. The number of corpora lutea is correlated with the number of eggs deposited, 2) it may be due to egg resorption, in which no statement on completion of egg deposition would be possible.

According to WILLIAMS & FELTMATE (1994), egg cocoons in Hydrophilidae are made from silk secreted by Malpighian tubules, and the function of accessory glands must be in attaching egg cases to stems or leaves of plants or on the mud directly.

The spermatheca is primarily an invagination of the integument at the posterior end of the venter of the eighth abdominal segment (SNODGRASS 1935: 556). It is cylindrical, long and filled with spermatozoa (Fig. 16, spz); a globular spermathecal gland secretes a fluid into which the sperms

are discharged (SNODGRASS 1935); the spermathecal duct enters the anterior portion of the bursa copulatrix. It seems the duct gradually merges into the wall of the bursa copulatrix (Fig. 16, fsd).

The genital chamber, or bursa copulatrix, receives the common oviduct ventromedially. Just under the junction between the bursa copulatrix and common oviduct, the bursa copulatrix is slightly expanded forming a sac (= vagina) resembling the ventral pouch in Marsupials (Fig. 16, vg). On the wall of the bursa copulatrix four fine tube-like structures can be observed which seems to be the starting point for the entrance of the spermathecal duct, continuing along the wall of bursa copulatrix. Except in IMMS (1964), these fine tubes are not mentioned in the literature. IMMS (1964) describes “a second passage or ‘canal of fecundation’ leading from the spermatheca or its duct and opening into the vagina near the point of union of the two oviducts”, in *Oodes helopioides* (FABRICIUS, 1792) (Carabidae). The tissue structure of these tubes seems to be different from the wall tissue of the bursa copulatrix. Further histological studies are needed to clarify this question.

Female external genitalia (Figs. 18, 19) of *Hydrochara* are sclerotized and consist of: 1) laterotergite VIII (formerly ninth tergite), mediotergite VIII (formerly valvifer), coxostyle IX and gonostyle IX (formerly stylus) (BAMEUL 1992). The tenth segment (proctiger) appears as a sclerotized ring (Fig. 18, prg X) which bears the anus (SNODGRASS 1935: 596).

There is a very fine membranous part behind the gonostylus which we named “median membranous piece”. Under the compound microscope, a very fine hair-like cover can be observed, wrapping around the gonostylus on each side.

### Conclusions

The male and female internal reproductive systems and sperm of *Hydrochara dichroma* are quite similar to those of *H. flavipes* in regard to general shape and structure, with the exception of the parameres. Certainly, because of the size difference between these two species, all structures are smaller and finer in *H. flavipes*. The median lobe of *H. flavipes* is also smaller, narrower and more elongate (in relation to parameres) than the median lobe of *H. dichroma*; the parameres of *H. flavipes* are more slender, and with the external margins more smoothly curved (Figs. 8–11).

The external female genitalia of the two species show no significant differences, either, even for the spermatheca, just unlike other genera (BAMEUL 1992, 1996, 1997a, b). Although the genitalia of *H. flavipes* are generally slightly smaller, the size is not a very reliable distinguishing character.

This preliminary study could be improved with analysis of additional species, and use of biometric comparisons.

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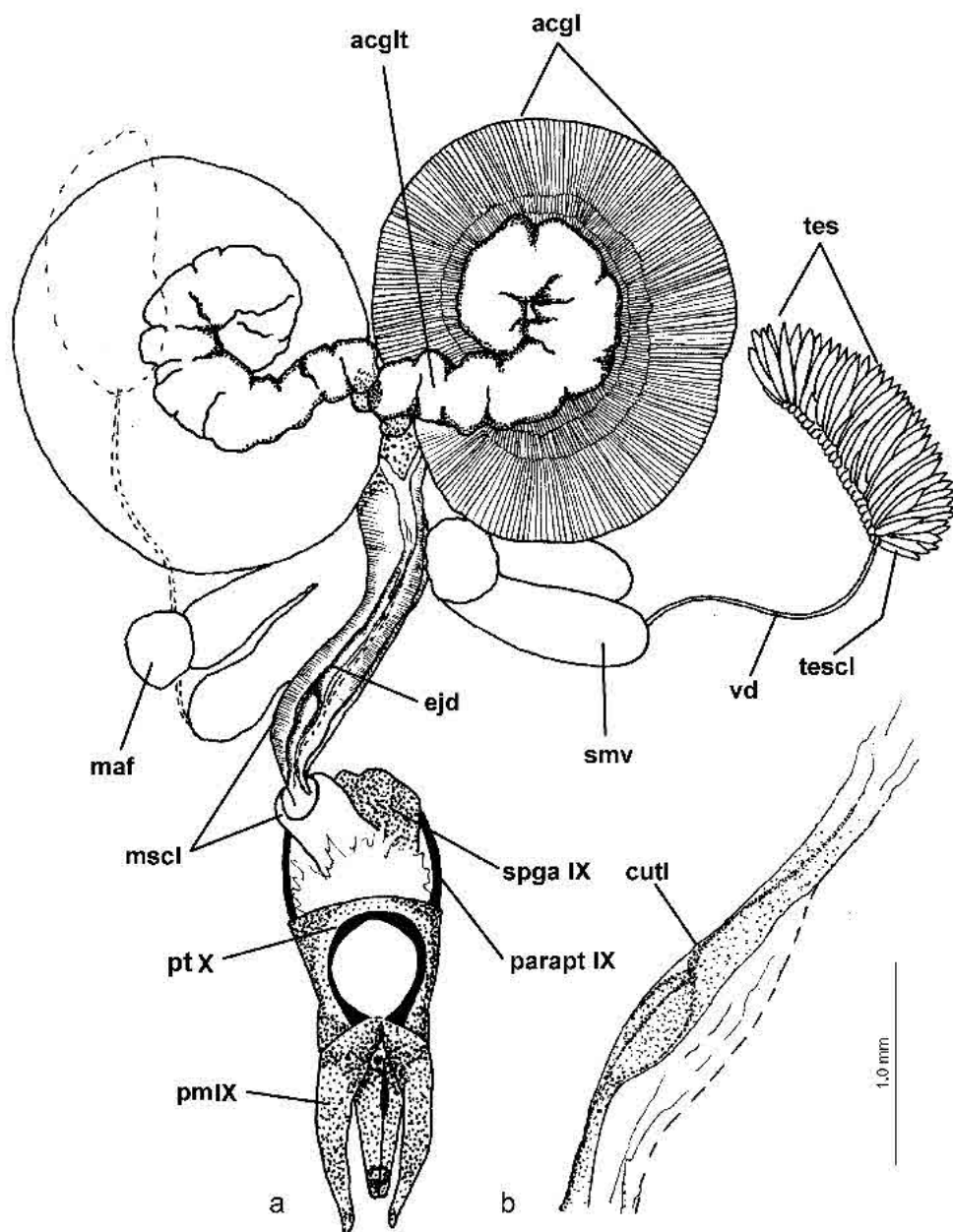


Fig. 1: *Hydrochara dichroma*; male reproductive system; a) dorsal view; b) ejaculatory duct, enlarged; acgl – accessory gland's terminal tube; cutl – cuticular line; ejd – ejaculatory duct; maf – mass of fat; mscl – covering muscle; parapt – paraproct (of seg. IX); spga – spiculum gastrale (of seg. IX), pt X – proctiger (of seg. X). Fars, Dasht-e-Arjan; coll. no. 453.

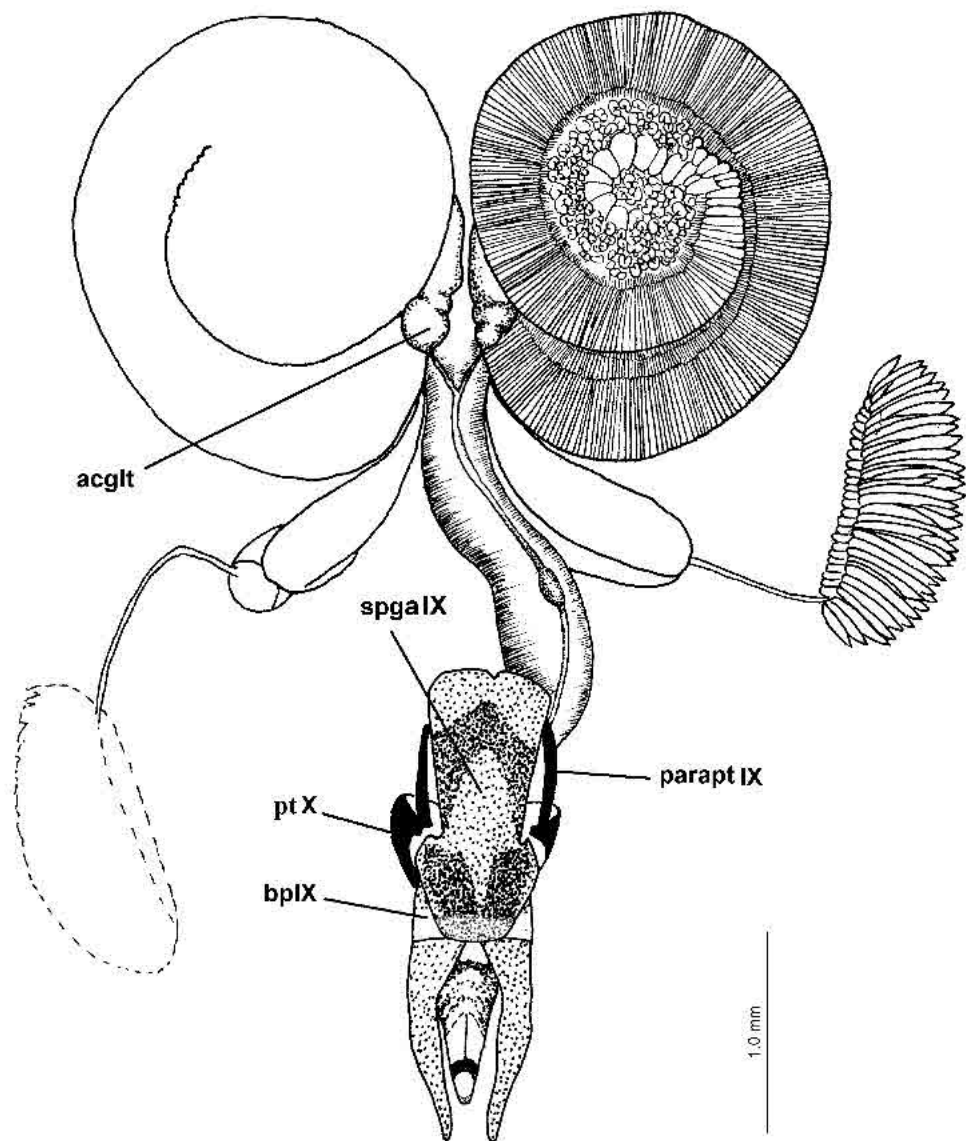
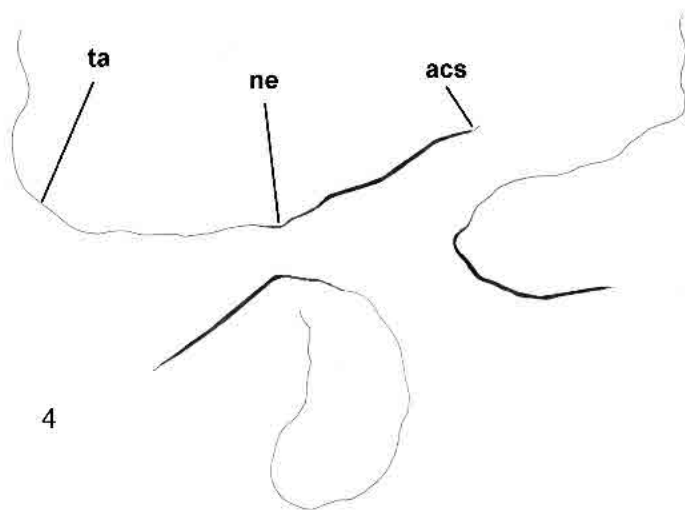
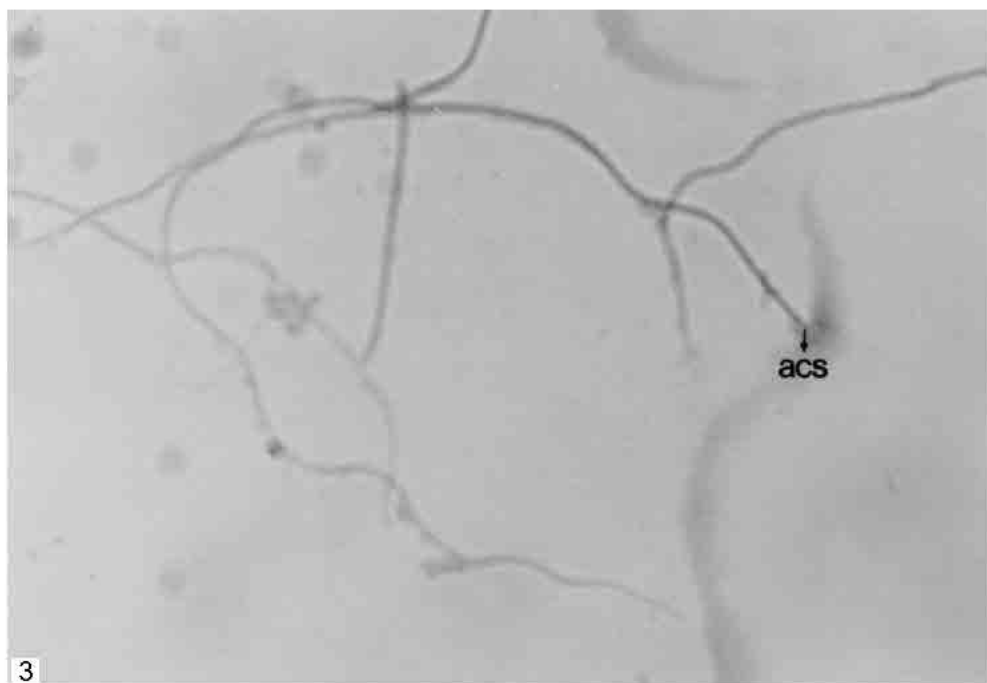


Fig. 2: *Hydrochara dichroma*; male reproductive system, ventral view. Fars, Dasht-e-Arjan; coll. no. 453.



Figs. 3–4: *Hydrochara dichroma*; 3) photo micrograph of smear of spermatozoa; acs – acrosome; 400 X; 4) spermatozoa. Fars, Bamoo, Darreh-Bisheh.



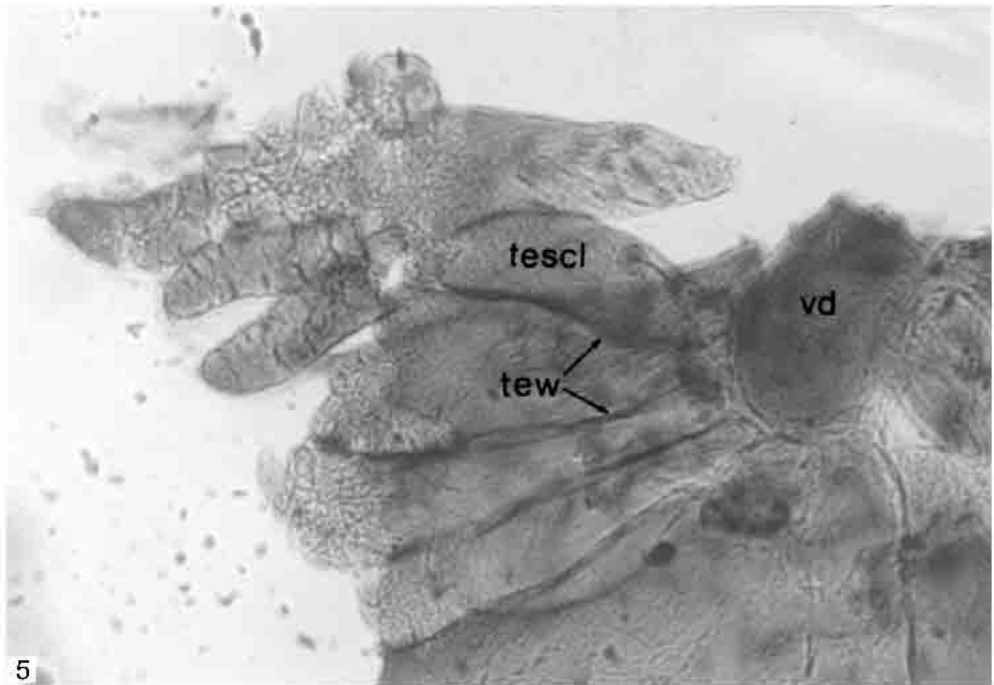


Fig. 5: *Hydrochara dichroma*, male reproductive system, whole mount, photomicrograph; tescl – testicle; tew – testicular wall; vd – vas deferens; 32 X.

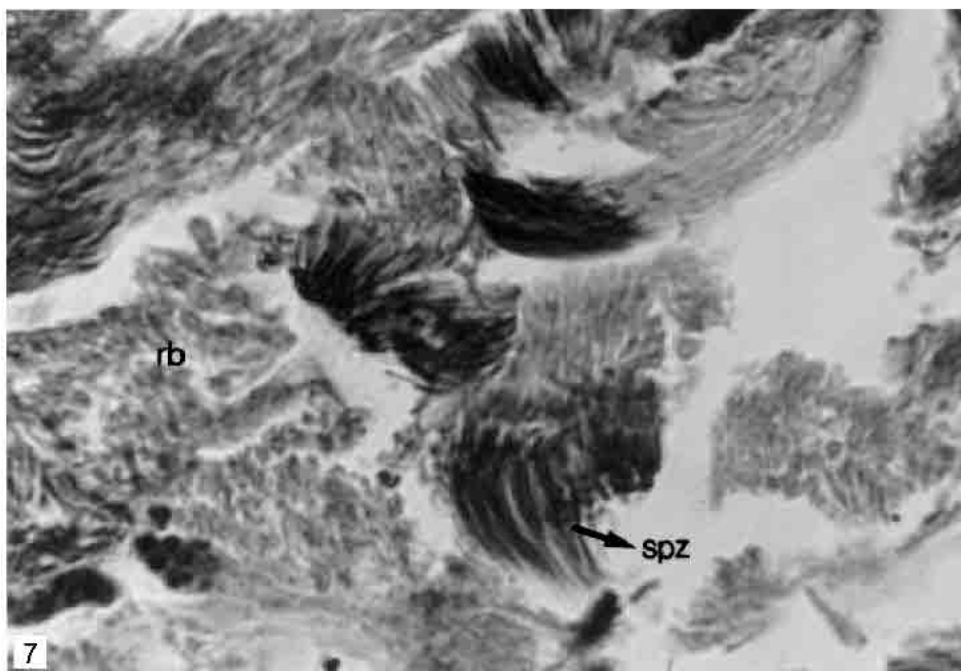
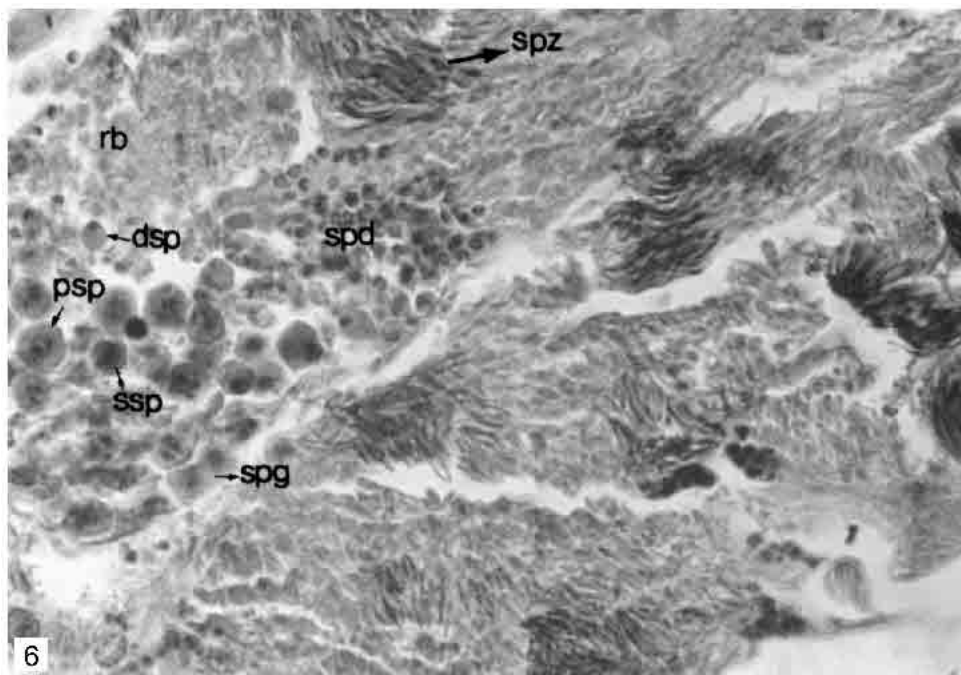
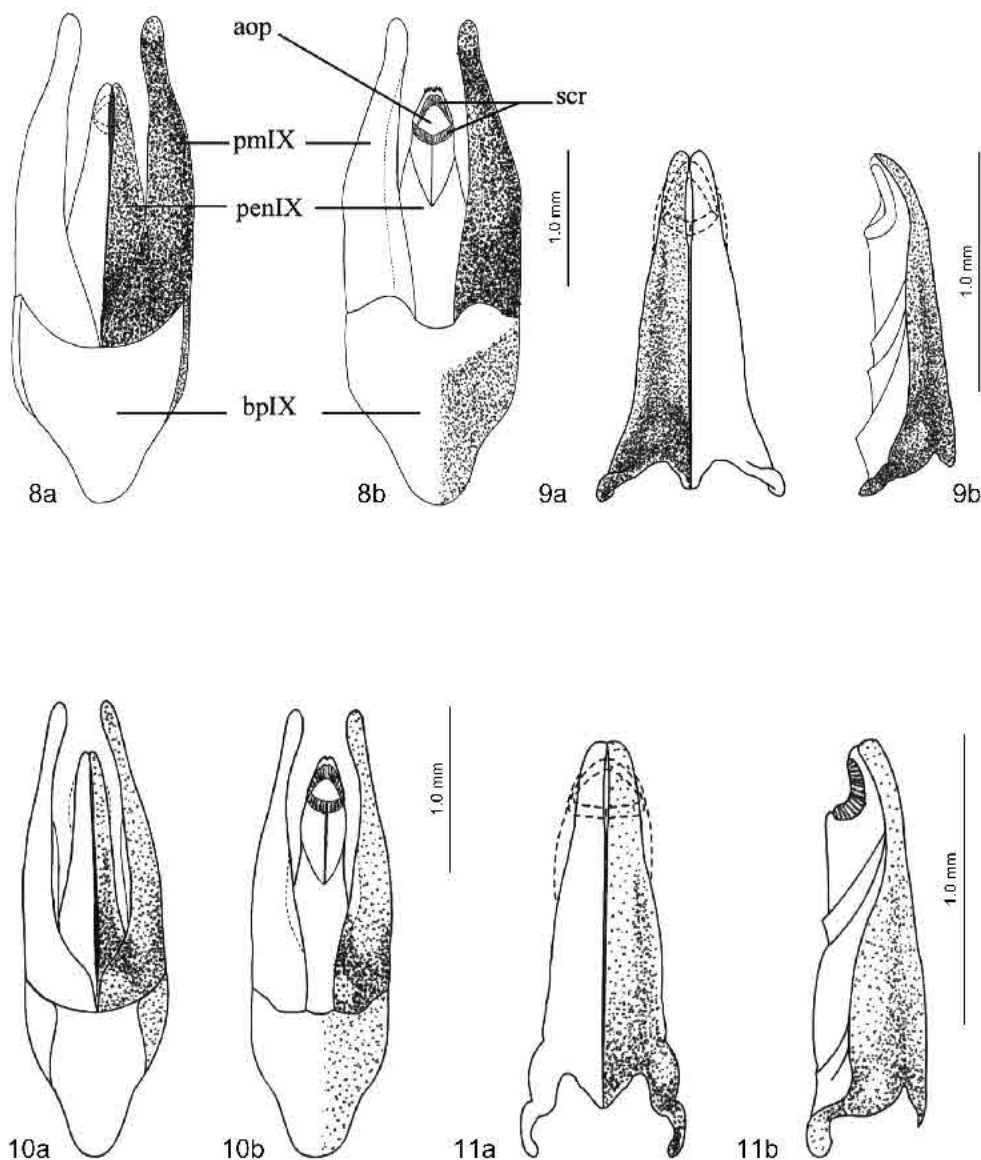


Fig. 6–7: *Hydrochara dichroma*; photomicrographs of 6) section of testicle; dsp – differentiated spermatid; rb – residual body; psp – primary spermatocyte; spd – spermatid; spg – spermatogonium; ssp – secondary spermatocyte; 160 X; 7) clusters of spermatozoa and residual bodies; 200 X.



Figs. 8–9: *Hydrochara dichroma*; 8) male external genitalia (aedeagus); a) dorsal view; b) ventral view; 9) penis; a) dorsal view; b) right lateral view

Figs. 10–11: *Hydrochara flavipes*; 10) male external genitalia (aedeagus); a) dorsal view; b) ventral view; 11) penis; a) dorsal view, b) right lateral view.

Abbreviations: aop – apical opening of internal sac; bp – basal piece (phallobase) (of seg. IX); pen – penis (of seg. IX); pm – paramere (periphalic organ) (of seg. IX); scr – sclerotized ring.

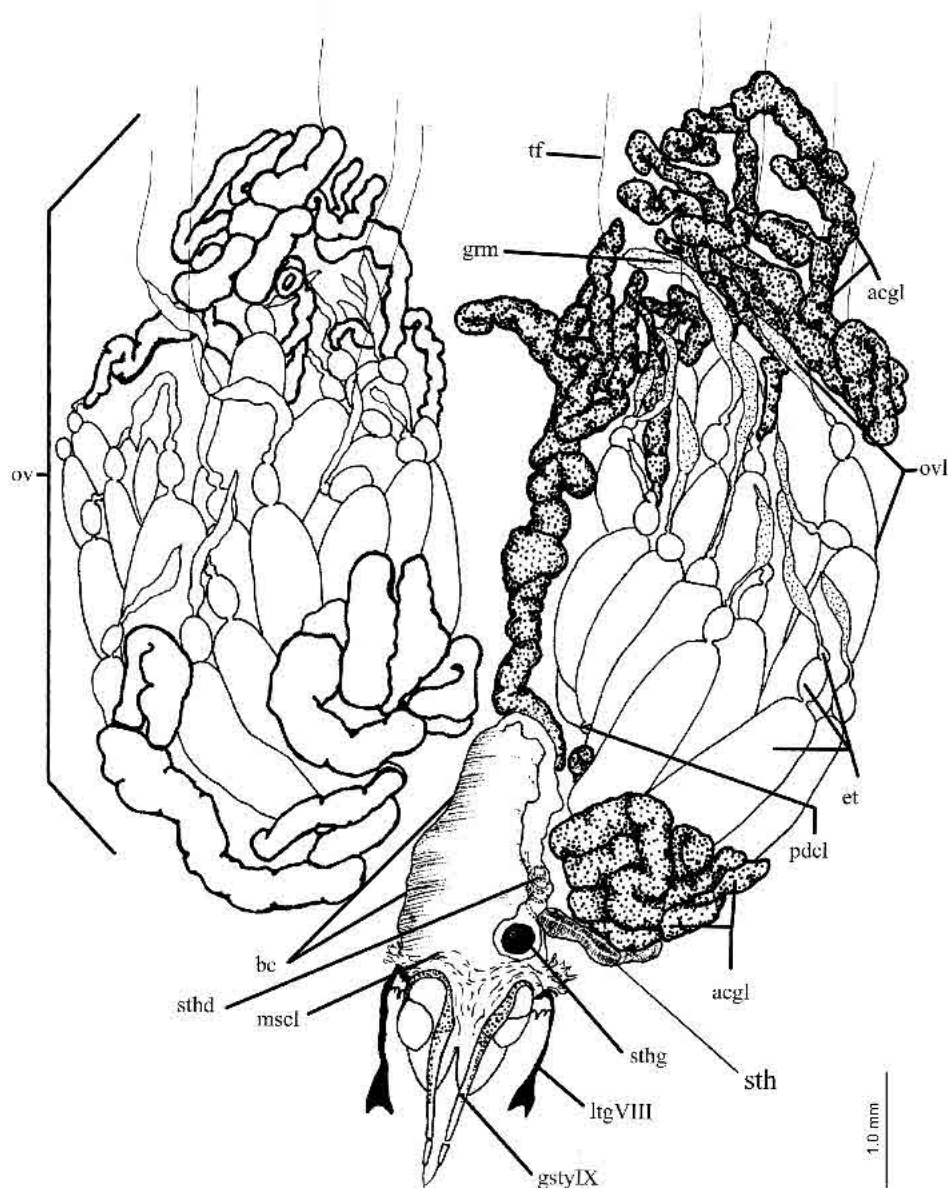


Fig. 12: *Hydrochara dichroma*; female reproductive system, dorsal view; acgl – accessory gland; bc – bursa copulatrix; et – egg tube; grm – germarium; gsty – gonostylus (of seg. IX); lrg – laterotergite (of seg. VIII); mscl – covering muscle; ov – ovary; ovl – ovariol; pdcl – pedicel; sth – spermatheca; sthd – spermathecal duct; sthg – spermathecal gland; tf – terminal filament. Fars, Bamoo, Cheshmeh-ye-Ghanbari.

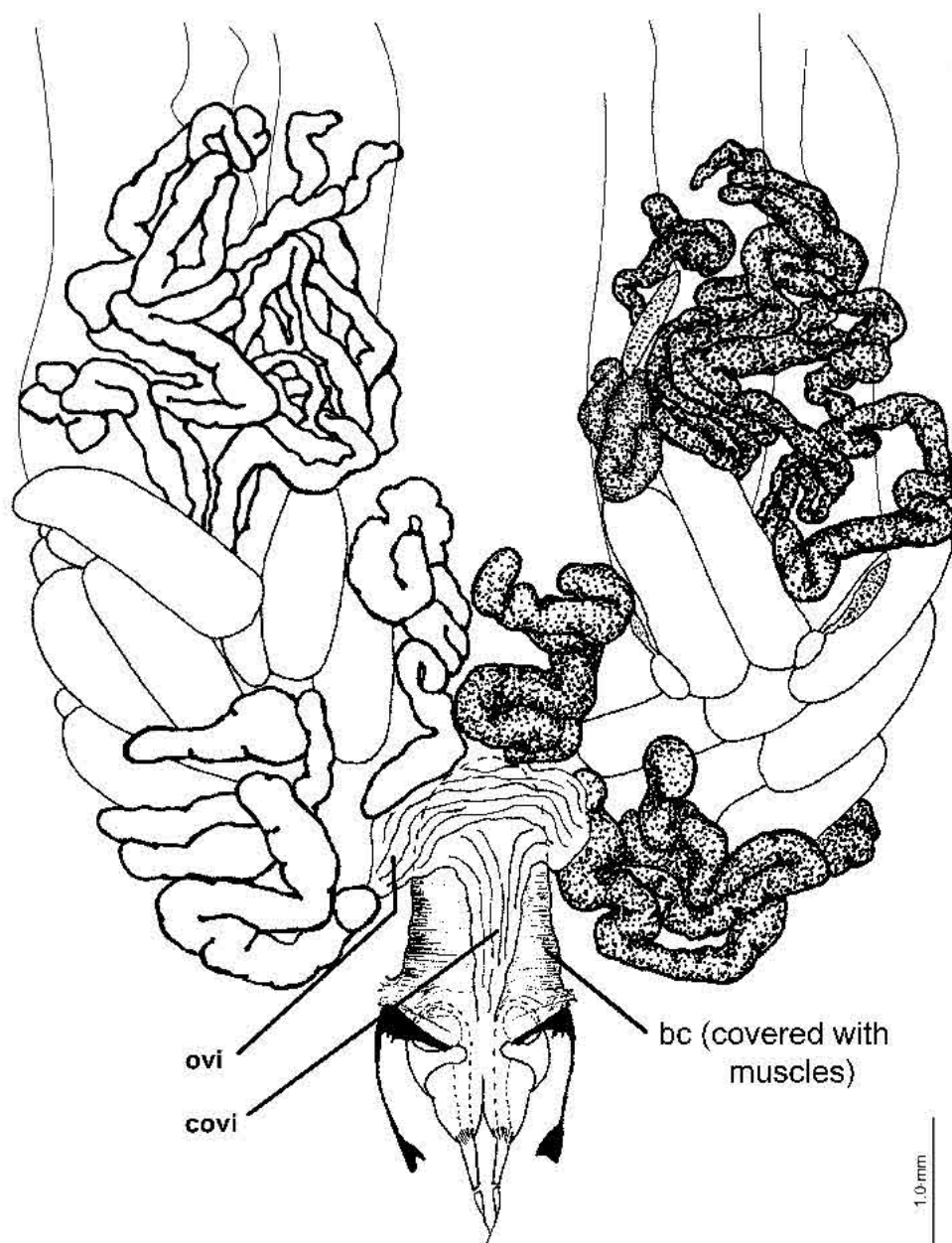


Fig. 13: *Hydrochara dichroma*; female reproductive system, ventral view; covi – common oviduct; ovi – oviduct. Fars, Bamoo, Cheshmeh-ye-Ghanbari.

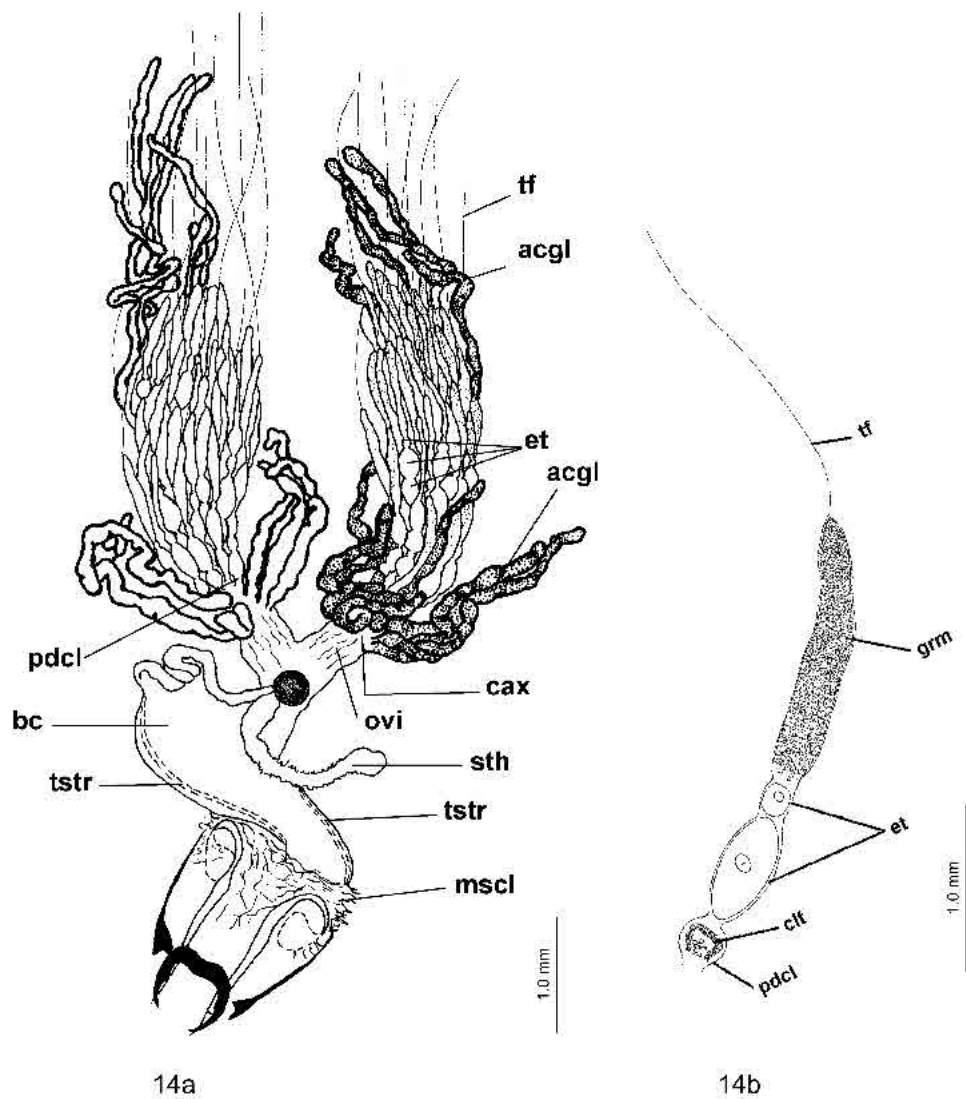


Fig. 14: *Hydrochara flavipes*; female reproductive system; a) dorsal view; b) ovariole, enlarged; cax – calyx; clt – corpus luteum. Khuzestan, Ahvaz to Khorramshahr; coll. no. 2245.

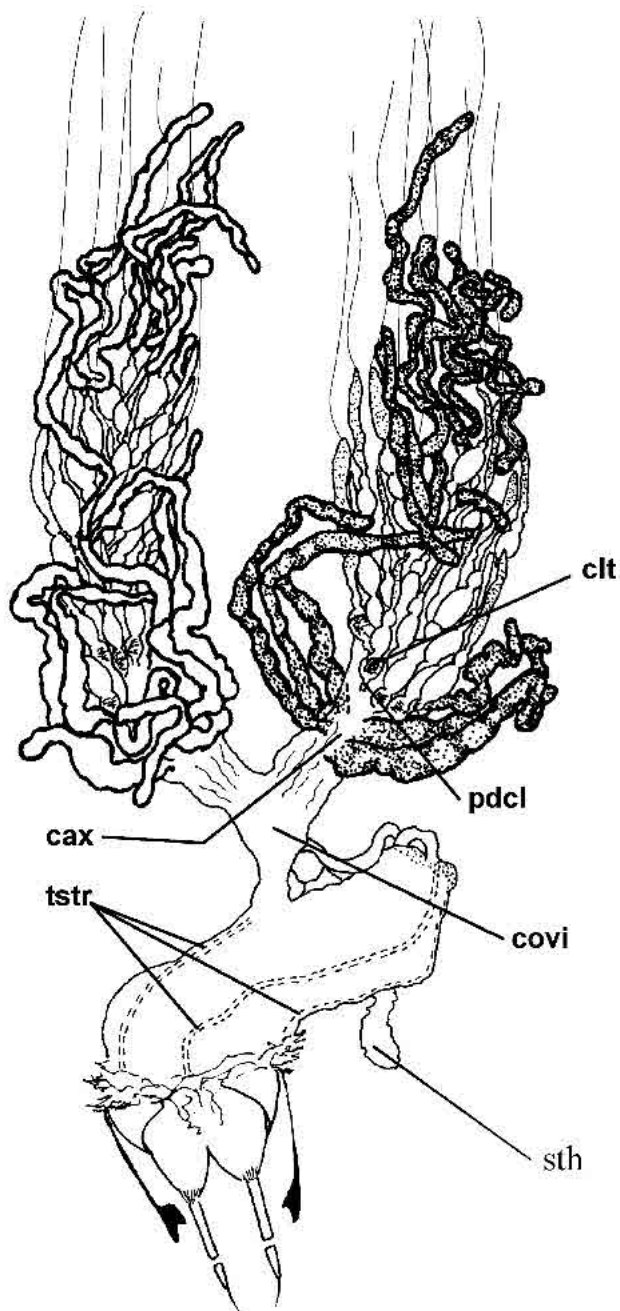
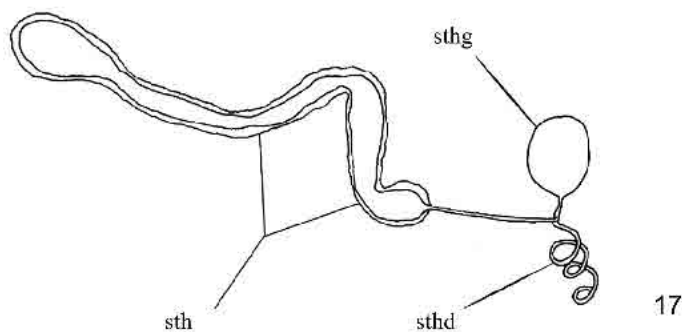
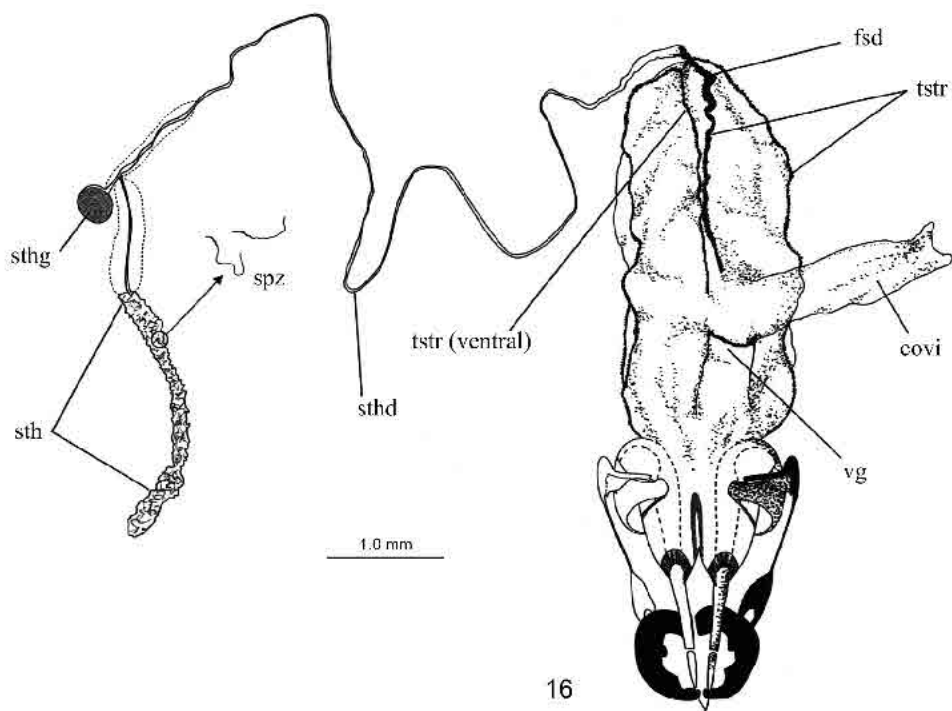


Fig. 15: *Hydrochara flavipes*; female reproductive system, ventral view. Khuzestan, Ahvaz to Khorramshahr; coll. no. 2245.



Figs. 16–17: *Hydrochara dichroma*; 16) external genitalia, bursa copulatrix and spermatheca, ventral view. Fars, Dasht-e-Arjan; 17) spermatheca and spermathecal gland.

Abbreviations: covi – common oviduct; fsd – fusing spermathecal duct; spz – spermatozoa; sth – spermatheca; sthd – spermathecal duct; sthg – spermathecal gland; tstr – tube-like structure (fecundation canal); vg – vagina.



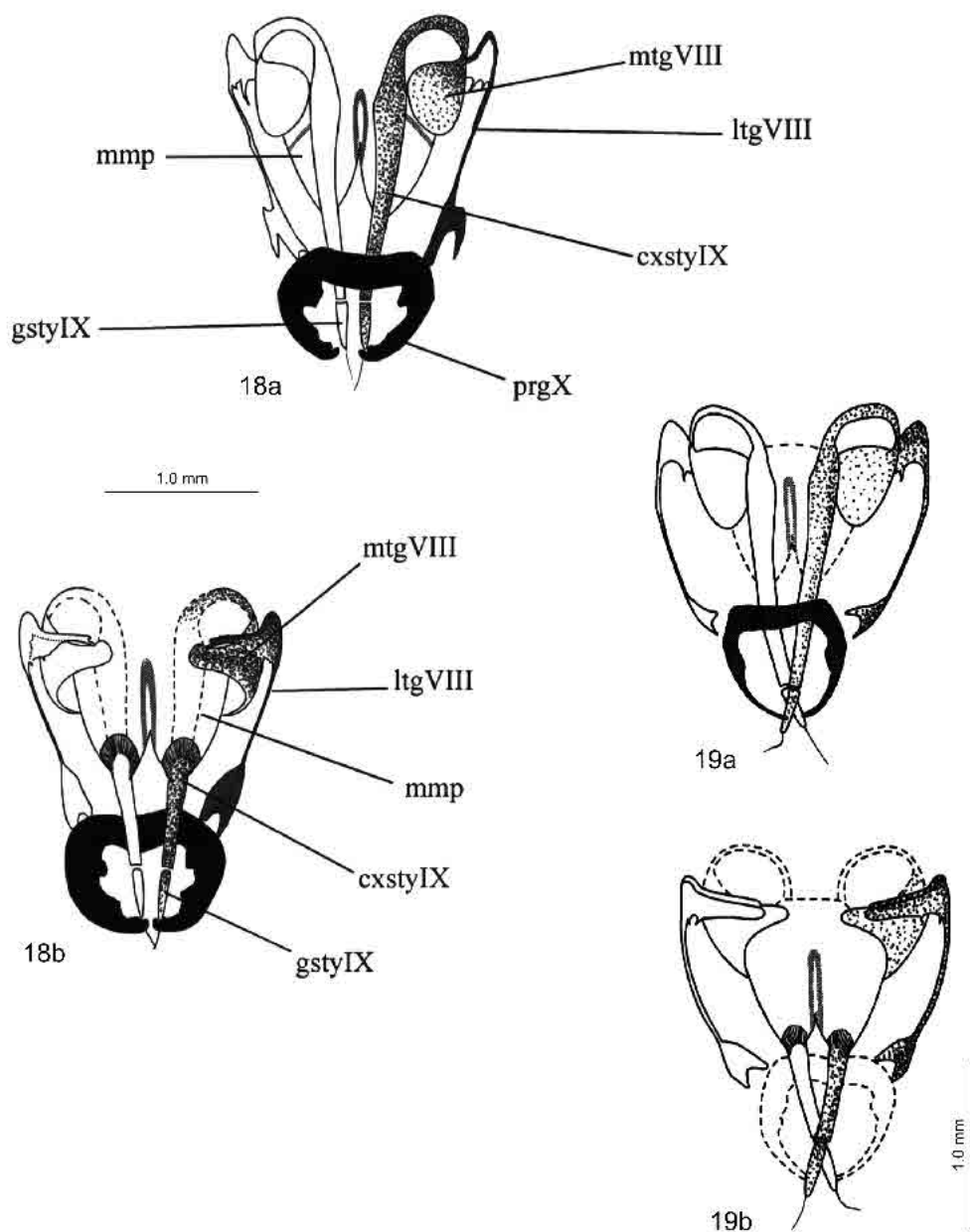


Fig. 18–19: Female external genitalia; a) dorsal view; b) ventral view of 18) *Hydrochara dichroma*. and 19) *H. flavipes*.

Abbreviations: cxsty – coxostylus (of seg. IX); gsty – gonostylus (of seg. IX); ltg – laterotergite (of seg. VIII); mmp – membranous median piece; mtg – mediotergite (of seg. VIII); prg – proctiger (sclerotized ring of seg. X).

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