

**Structure and development of the reproductive system
in *Aphelocheirus aestivalis* (Hemiptera: Heteroptera:
Nepomorpha: Aphelocheiridae)**

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Abstract. We study the histology, ultrastructure and morphological arrangement of male and female reproductive system of *Aphelocheirus aestivalis* (Fabricius, 1803) in the nymphs of instar 1-5 and variously aged apterous adults. The anlage of gonads becomes well apparent already in instar 1, testicular follicles and ovarioles start to differentiate in instar 2, and their entire differentiation is finished in instar 3. Paired accessory mesadenia, dichotomically branched in adult males, differentiate in instar 2 as well. The anlage of male ectodermal accessory gland is formed during instar 3, while spermatheca and bursa copulatrix start to invaginate in instar 4. Morphological differentiation of germ cell descendents as well as formation of the germarial trophic core from a membranous labyrinth in females occurs in late instar 4 and instar 5. Spermatogenesis shows a unique character within the whole Nepomorpha: it is a permanent, multi-waved process, as all developmental stages of male germ cells are found throughout the year. Vitellogenesis starts in adult females 3-4 weeks after the last moulting but apparently stops during winter. The timing of crucial reproductive processes in *A. aestivalis* seems to be rather different from most aquatic bugs; permanent, spring-to-autumn gradual spermatogenesis and physiological ability to copulate, and the presence of mature spermatozoa in the spermatheca of teneral, newly hatched females indicate possible semivoltinism in the life cycle. Three new apomorphies (type of spermatogenesis and secondary seminal vesicles in males, and a cuticular brush in bursa copulatrix in females) of the Aphelocheiridae are defined.

Key words. Heteroptera, Aphelocheiridae, *Aphelocheirus aestivalis*, nymphs, adults, gonads, efferent ways, development, morphology, histology, ultrastructure, spermatogenesis, oogenesis, reproductive strategy, anagenesis, Czech Republic

Introduction

Aphelocheirus aestivalis (Fabricius, 1803) and two endemic congeners from the Iberian Peninsula (NIESER & MILLÁN 1989) are the only European representatives of the family Aphelocheiridae. *Aphelocheirus aestivalis* is in many respects unique within aquatic Heteroptera (Nepomorpha). The plastron respiration and at least two-year-long life span in the northern parts of its distribution area separate this species from all other European aquatic bugs.

The distribution of *A. aestivalis* has a partially relict character (KANYUKOVA 1995). It is red-listed in some European countries and has shown dramatic declines during the last century in some parts of Europe (DAMGAARD 2005). This species was also mentioned in the list of endangered or protected species in the Czech Republic (see e.g. ŠTYS & ŠKAPEC 1992). On the other hand, the species has been recorded from numerous new localities in Austria, the Czech Republic, Germany, and Slovakia (BULÁNKOVÁ 1993, HOFFMANN 2004, PAPÁČEK 2006, RABITSCH 2007, ŠVAŇHALOVÁ 2006, ZETTLER 1998; D. S. Boukal, P. Hesoun, M. Mergl, pers. comm.) and also quite recently in south-eastern Europe (new records from Serbia – ŽIVIĆ et al. 2007; confirmed in Romania – COJOCARU 2005). KMENT & VILÍMOVÁ (2005), PAPÁČEK (2006) and PAPÁČEK & BAUER (2006) suggested that this species should only be treated as vulnerable, at least in the Czech Republic.

Our knowledge on its ecology and biology is rather fragmentary. Earlier and mostly controversial data were summarised, e.g., by SÆTTEM (1986) and PAPÁČEK (1989). Some field data apparently show its univoltinism in northern Europe (DAMGAARD 2005) while in central Europe the populations are semivoltine (PAPÁČEK & SOLDÁN 1996). In spite of the macroptery of the holotype (H. Zettel, pers. comm.) from France, and records of macropterous specimens in central Europe (AUKEMA et al. 2002), the Czech populations in the Labe river basin seem completely wingless. They are mostly found in somewhat isolated streamline stony bottom habitats of the epi- and mesopotamal segments of large rivers as well as small rivulets. In contrast, data by KANYUKOVA (1974) show that it inhabits different aquatic biotopes including lenitic habitats of stagnant waters; some populations of *A. aestivalis* are also able to migrate seasonally to other habitats like pools and backwaters (MESSNER et al. 1983).

Due to its considerable ecological range, *A. aestivalis* also represents a suitable model species for detailed studies of its morphology, respiration physiology and life cycle. Although the gross morphology and some histological aspects of both adult male and female gonads are relatively well known (LARSÉN 1938), the reproductive system and its development in nymphs and the reproductive strategy of this species have remained unknown. The male reproductive system consists of testes, each formed by four testicular follicles (4+4), vasa deferentia with seminal vesicle, a pair of long, dichotomically branched mesadenia (1+1), and unpaired simple ectadenium. The female reproductive system consists of ovaries formed by five ovarioles each (5+5), very short lateral oviducts, short common oviduct, and vagina with short and asymmetrical, dorsally placed spermatheca and dorsally evaginated bursa copulatrix provided with epithelial gland and thick intima (= Vaginaltasche sensu LARSÉN (1938)).

Generally, available information on the development of reproductive systems in most of families of aquatic bugs is still fragmentary (see, e.g., reviews by PAPÁČEK & SOLDÁN (1987, 1992)), PAPÁČEK & GELBIČ (1989), PAPÁČEK & SOLDÁN (1987, 1992), PAPÁČEK et al. (1997) and

RAWAT (1939) studied in detail the development of the reproductive system in *Notonecta glauca* Linnaeus, 1758 (Notonectidae), and *Ilyocoris cimicoides* (Linnaeus, 1758) (Naucoridae).

The aim of this study is to (i) describe the development of testes and ovaries and their efferent ways in detail, (ii) precisely correlate gametogenesis with the life cycle and reproductive events, and (iii) provide a comparison with other groups of aquatic Heteroptera based on literature data.

Material and methods

Nymphs and adults of *A. aestivalis* were collected at four localities in South Bohemia (Czech Republic): Nežárka River at Jemčina, Nová řeka River at Nový Řadov (both from April 1993 to May 1996, including winter months), and Dračice River at Františkov and Klikov (both 1999-2006). Regular winter samples were taken from February 1995 to May 1996 at three-week intervals. Specimens were collected by common hydrobiological methods ('kicking technique' using metal sieves or aquatic nets), fixed in the field with Bouin's fixative, and dissected under a stereoscopic microscope in physiological saline or 96% ethanol. Dissected reproductive organs and their total microscope slides (organs dehydrated in isopropanol, embedded in Euparal) were prepared for the study of gross morphology. Spermatogenesis, oogenesis and histology of outflow ways were studied on 4-6 μm paraplast sections stained with Harris hematoxylin-eosin (whole abdomens cut in nymphs of instars 1 and 2). To study the ultrastructure, dissected organs were fixed with 2.5% glutaraldehyde in 0.5 M phosphate buffer for 24 hours, rinsed three times in phosphate buffer containing 5.8% sucrose and postfixed for 1.5 h with 1% osmium tetroxide in 0.1 M phosphate buffer. Throughout fixation a pH of 7.4 was maintained. Organs were rinsed three times in distilled water, dehydrated in a graded series of alcohol and acetone, and embedded in Epon 812. Blocks were cut using a Tesla BS 490 A ultramicrotome. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined under a Tesla BS 500 transmission electron microscope (TEM). Semi-thin sections were stained with methylene blue. The terminology used here mostly follows those by LARSEN (1938, some aspects of gross morphology) and BÜNING (1994, ultrastructure of ovary).

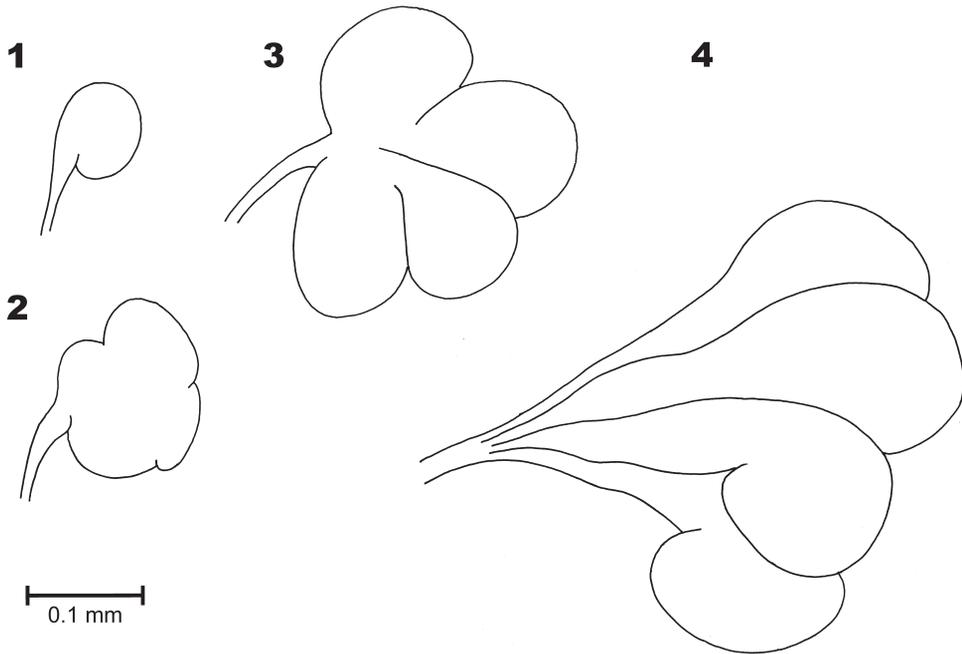
Results

Development of the reproductive system in males

Testes and spermatogenesis

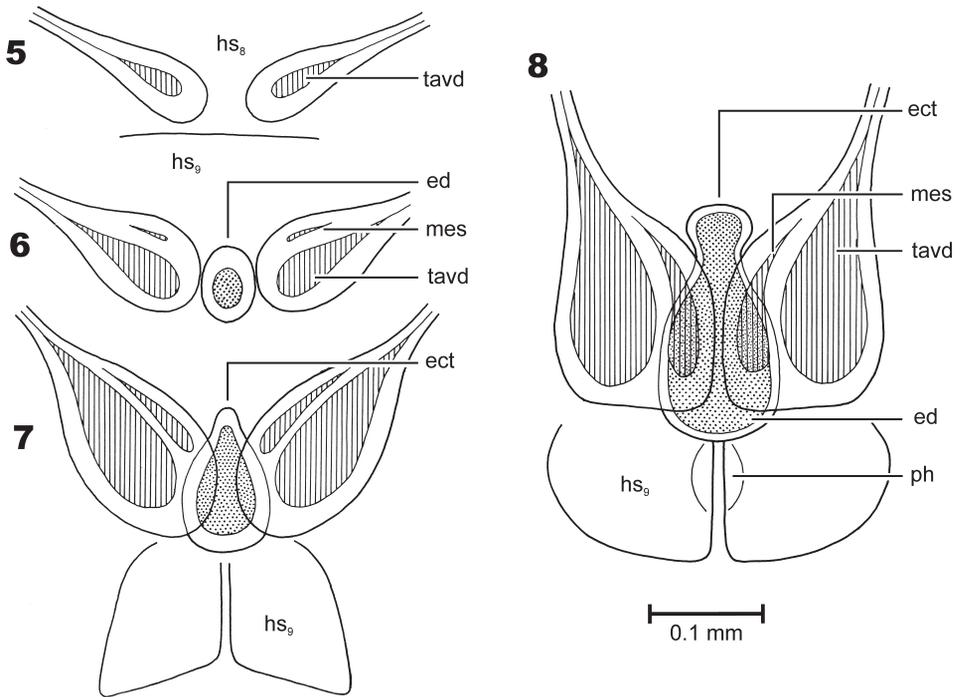
1. Nymphs

Testes of nymphal instar 1 are localised laterally at the anterior margin of the third abdominal segment. They are of spherical shape and without apparent testicular follicles, forming uniform mass of spermatogonia and follicular cells (Fig. 1). No specialised cells near the apices of future follicles were found. Each testis is already apparently connected with vas deferens. Individual follicles start to differentiate in nymphs of instar 2 (Fig. 2). However, no histological changes occur, although numerous mitoses of spermatogonia are apparent. Short, lobate follicles divided into two distinct groups (2+2) appear in nymphs of instar 3 (Fig. 3) and all spermatogonia are contained in cysts. Cysts with primary spermatocytes, although very



Figs. 1-4. Right testis of the first four nymphal instars (caudal view). 1 – nymph of instar 1, testicular follicles discernible; 2 – nymph of instar 2, beginning of testicular follicles differentiation; 3 – nymph of instar 3, testicular follicles distinguishable, divided into two groups (2+2); 4 – nymph of late instar 4, testicular follicles fully differentiated, period of rapid growth of their basal parts, apical parts slightly coiled.

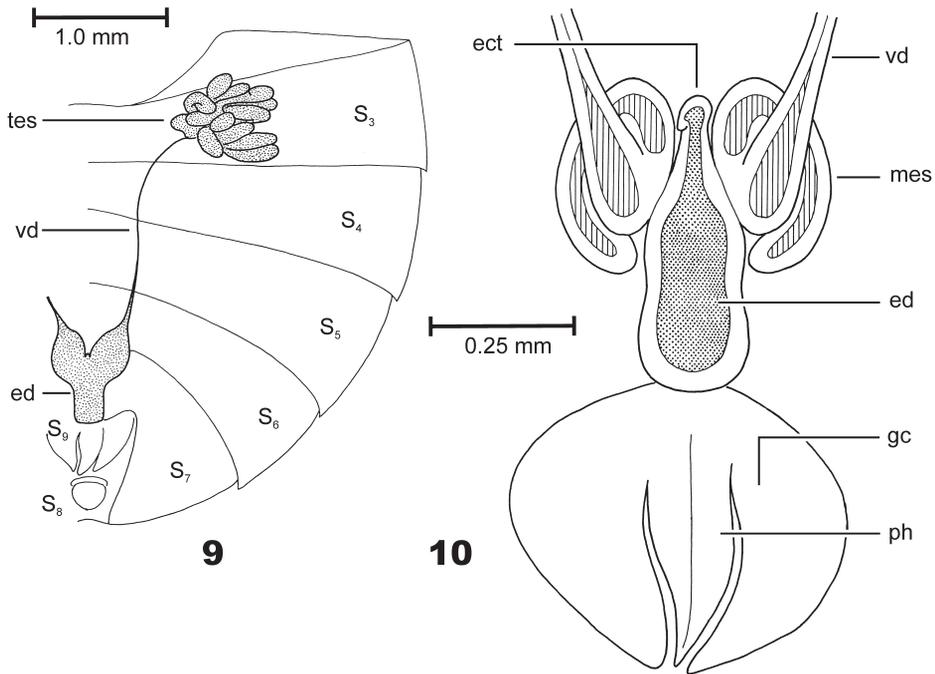
rare, start to appear near the base of testicular follicles. Gradual elongation of follicles takes place in nymphs of instar 4. In this instar, four equally sized testicular follicles are always present but the original 2+2 groups are not distinguishable. Follicles are evidently bent at the apex or even slightly spirally coiled (Fig. 4), and proximal parts of follicles become filled with cysts containing primary spermatogonia. Spermatocytes in the stage of late heterotypic (diplotene, diakinesis) or early homeotypic meiotic division can be observed in some of them, especially near vasa efferentia. Rapid longitudinal growth of follicles occurs in nymphs of instar 5. Before metamorphosis, growth in length is completed: male follicles, which are mostly irregularly coiled (Fig. 11), already measure about 7.5-8.5 mm as in adults. Owing to this conspicuous length growth, testes are contained in the third and fourth abdominal segments before ecdysis of instar 5, while they occupy only the third segment at the beginning of the instar. The growth of follicles is connected with rapidly proceeding spermatogenesis. Before imaginal moulting, follicles predominantly contain cysts with primary and secondary spermatocytes (about half length of the follicle is filled with them at the beginning of instar 5). Spermateliosis starts mostly in the last third of instar 5 when cysts with early spermatids start to occur. Early presperms are found rarely before moulting and mature spermatozoa never occur in instar 5.



Figs. 5-8. Differentiation of terminal portions of outflow ways in males (dorsal view, semischematic). 5 – nymph of instar 1, beginning of terminal ampullae formation; 6 – nymph of instar 2, beginning of ectodermal outflow ways and paired mesodermal accessory glands formation; 7 – nymph of instar 3, beginning of unpaired ectodermal accessory gland formation; 8 – nymph of instar 4, beginning of phallus formation. Abbreviations: *ect* – anlage of ectodermal accessory gland; *ed* – anlages of ejaculatory duct; hs_8 and hs_9 – hypodermis of abdominal sterna 8 and 9 (this structure forms the anlages of genital capsule of nymphal instars 3 and 4); *mes* – anlages of mesodermal accessory glands; *ph* – anlage of phallus; *tavd* – terminal ampullae of vasa deferentia. Vertical lines = cavities of mesodermal structures (localised ventrally); dots = cavities of ectodermal structures (localised dorsally).

2. Adults

Testes of teneral adults are of the same shape and size as in late nymphal instar 5. Spermateliosis proceeds relatively quickly and the first spermatozoa appear in follicles within two weeks after imaginal ecdysis. In mature and even in overwintering males, the zone of cysts with mature spermatozoa and presperms (Figs. 19, 23) occupies about one third or one half of the follicle. However, zones with primary and secondary spermatocytes (Figs. 19, 22) are well distinguishable and even a short zone of cysts with spermatogonia is apparent near the apex of the follicle. This situation changes very little during all imaginal stage of males. Consequently, spermatogenesis seems to be continual (Fig. 19) and mature spermatozoa are always present in follicles of males after more than about two weeks after the final moult (Figs. 24-25). Proximal parts of follicles and vasa efferentia are conspicuously expanded in mature males (Fig. 12, *ttl*) and filled with cysts containing bundles of mature spermatozoa.



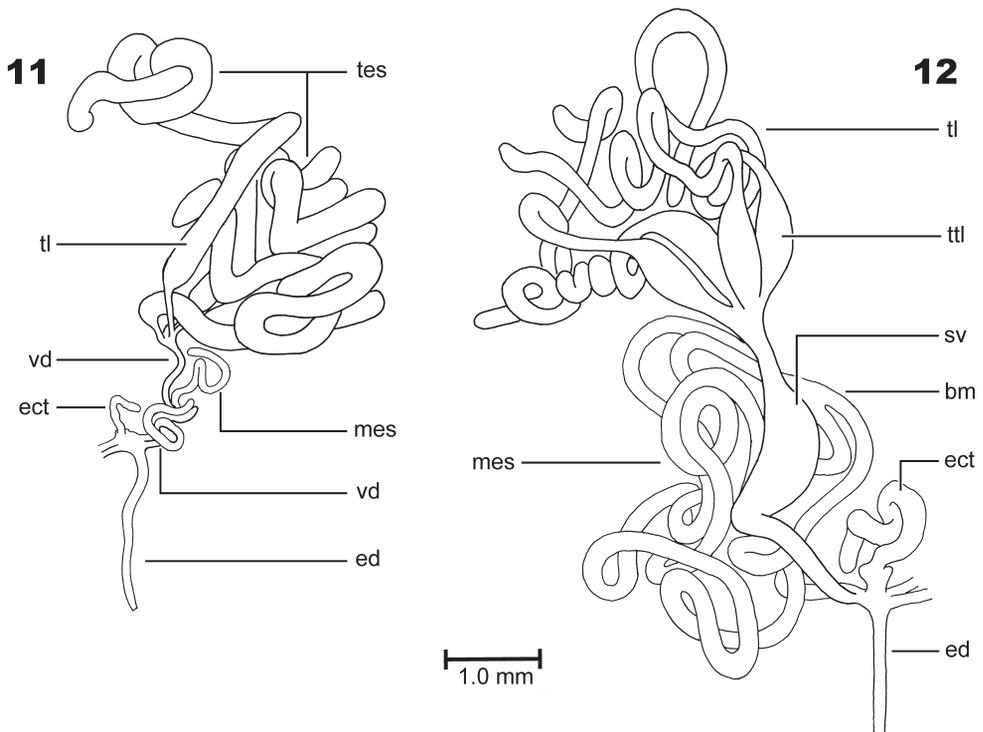
Figs. 9-10. Male internal reproductive system of a nymph of early instar 5 (dorsal view, semischematic). 9 – location of male reproductive system in abdominal cavity; 10 – terminal parts of outflow ways and hypodermal anlage of genital capsule. Abbreviations: ect – anlages of ectodermal accessory glands; ed – anlage of ejaculatory duct; gc – anlage of genital capsule; mes – anlages of mesodermal accessory glands; ph – anlage of phallus; S₃-S₉ – abdominal sterna 3-9; tes – testis; vd – vas deferens.

We have not observed disintegrated cysts or free spermatozoa in this area. These proximal portions of the follicles evidently form accessory seminal vesicles. Spermatogenesis is terminated in true seminal vesicles (expanded vasa deferentia), where most cysts disintegrate and remnants of cyst cells disappear (probably due to resorption). Free spermatozoa in gonoducts are slightly coiled; some of them remain in hardly distinguishable bundles resembling the original cysts. In most males these bundles are aggregated into large formations resembling spermatophores of some other insects. Spermatozoa are relatively long, measuring 750-800 μm in length including the flagellum.

Vasa deferentia, ejaculatory duct, and accessory glands

1. Nymphs

Only vasa deferentia are discernible in nymphs of instar 1. Terminal ampullae of their lumina are situated near the posterior margin of the eighth abdominal segment (Fig. 5). Mesodermal accessory glands (long and in adults dichotomically branched paired structures) start to



Figs. 11-12. Male internal reproductive system (dorsal view). 11 – nymphal instar 5 (pharate imago before moulting; left testis and outflow ways omitted); 12 – mature adult (right testis and outflow ways omitted). Abbreviations: bm – bifurcation of paired mesodermal accessory glands; ect – unpaired ectodermal accessory gland; ed – ejaculatory duct; mes – mesodermal accessory gland; sv – seminal vesicle; tes – testis; tl – testicular follicles; ttl – proximal part of testicular follicles (= expanded vasa efferentia forming ‘accessory seminal vesicles’); vd – vas deferens.

form in the epithelium of terminal ampullae of vasa deferentia in nymphs of instar 2. The invagination of ductus ejaculatorius anlage in the ectodermal portion appears at the same time (Fig. 6). The anlage of a short, unpaired ectodermal accessory gland is formed in nymphs of instar 3 in the anteromedial portion of the ejaculatory duct invagination (Fig. 7). Terminal ampullae of mesodermal outflow ways touch these ectodermal invaginations dorsally. We observed only slight morphological changes during nymphal instar 4 except for linear growth of structures formed earlier in instars 2 and 3 (Fig. 8). Mesodermal and ectodermal outflow ways in nymphs of early instar 5 remain fully separated (Fig. 10). Later on in late instar 5, cavities of vasa deferentia and mesodermal accessory glands connect with the future ejaculatory duct and other ectodermal structures. Apparent linear growth of both mesadenia and ectadenia take place during instar 5, when they measure about one fourth of their final length in adults. However, mesadenia remain unbranched. Other parts of outflow ways resemble those of adults in shape and size.

The histological structure of accessory glands is similar in both ectadenia and mesadenia in instars 3 and 4. We observed a unilayered secretory epithelium with nearly cuboidal or cylindrical cells. In instar 5, these cells are about twice as long, cylindrical with rounded distal portions. Most of them possess constricted or divided nucleus or even two nuclei. Slight secretion activity, especially in the proximal portion of long paired mesadenia can be observed even before imaginal moulting. Inner epithelial layer and outer layer of circular muscles form the walls of vasa deferentia. Epithelial cells are cuboidal in instars 3 and 4 and cylindrical in instar 5. Circular muscles were observed only in nymphs of instars 4 and 5.

2. Adults

Conspicuous linear growth of accessory glands is apparent during the period of maturation of males (about two or three weeks after imaginal ecdysis). While growth of unpaired ectodermal gland is not pronounced, growth of mesadenia is accompanied by a process of branching into two apical branches that ultimately reach the length of the common basal stem. Accessory glands measure about 3.5-4.5 mm (ectadenium) and 18-24 mm (mesadenia) at that stage. High secretion activity is apparent even before reaching maturity, i.e. before the emergence of mature spermatozoa. Lumina of both mesadenia and ectadenium are full of secretion, the structure of which is different in histological sections (Figs. 20-21). The secretion of ectadenium (Fig. 21) consists of mostly coarse, rounded, hardly stainable granulation. Secretion of mesadenia (Fig. 20) is faintly granulated with larger, intensively stainable spherical droplets and small vacuoles, in many respects resembling the ooplasm of previtelogenetic oocytes. Testicular follicles and the accessory glands fill the abdominal cavity from the third to the fifth abdominal segments in sexually active males. Proximal extensions of testicular follicles at the places of vasa efferentia ('accessory seminal vesicles') emerge in close connection with mature spermatozoa. They are evidently formed passively by internal pressure of generative cells. The structure of their walls formed by a thin layer of connective tissue is the same as that of follicular walls, while the structure of 'true seminal vesicles' does not differ from other portions of vas deferens. Seminal vesicles also appear towards the end of spermatogenesis and occupy about half the total length of vas deferens. Both these vesicles are apparent in males throughout their entire adult lifespan, their content being gradually supplemented with further mature spermatozoa.

Notes on the development of phallus and genital capsule

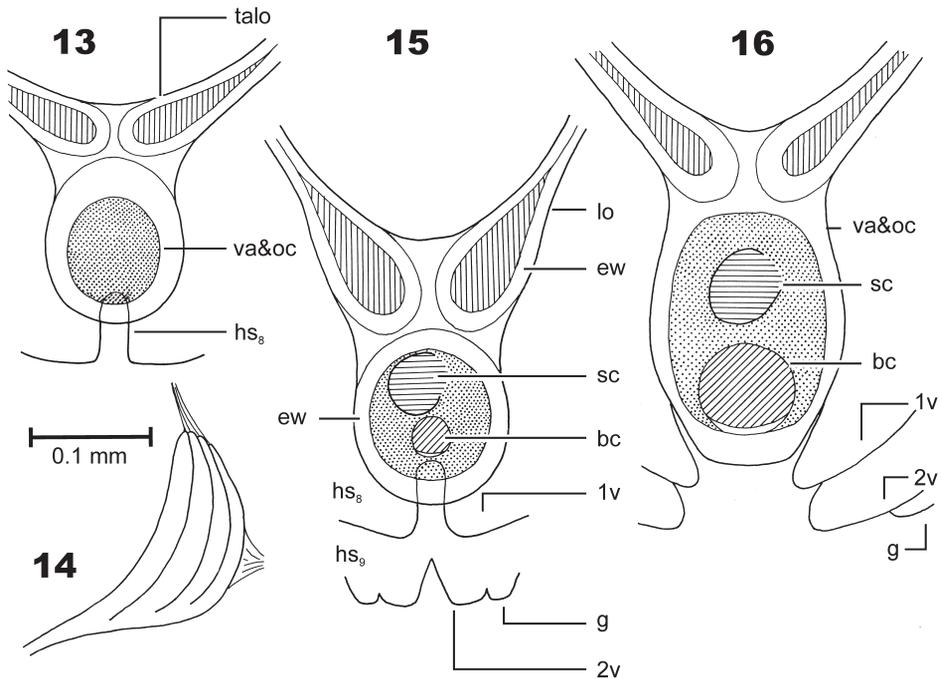
Medial division of hypodermis of the ninth abdominal sternum into two equal parts starts already in nymphal instar 2. A pair of alate formations is apparent in nymphs of instar 3 (Fig. 7). In instar 4, the anlage of phallus is formed as a paired symmetric semicircular structure in the area of the regularly subdivided ninth abdominal sternum (Fig. 8). Hypodermal anlage of phallus is apparent in nymphs of instar 5 as an unpaired and asymmetric structure (Fig. 10).

Development of the reproductive system in females

Ovaries and oogenesis

1. Nymphs

The ovaries of nymphs of instars 1 and 2 are discernible near the intersegmental membrane between the second and third abdominal segments as inconspicuous, rounded structures still not differentiated into ovarioles. Terminal filaments are not distinguishable. Partial or full differentiation of ovarioles takes place in instar 3 when Terminal filaments become apparent as well. While we observed only cells of equal shape and size (probably early oogonia) in the first two instars, germarial oocytes can be recognised in the proximal portion of ovarioles in instar 3. During further nymphal development the general shape and structure of ovaries

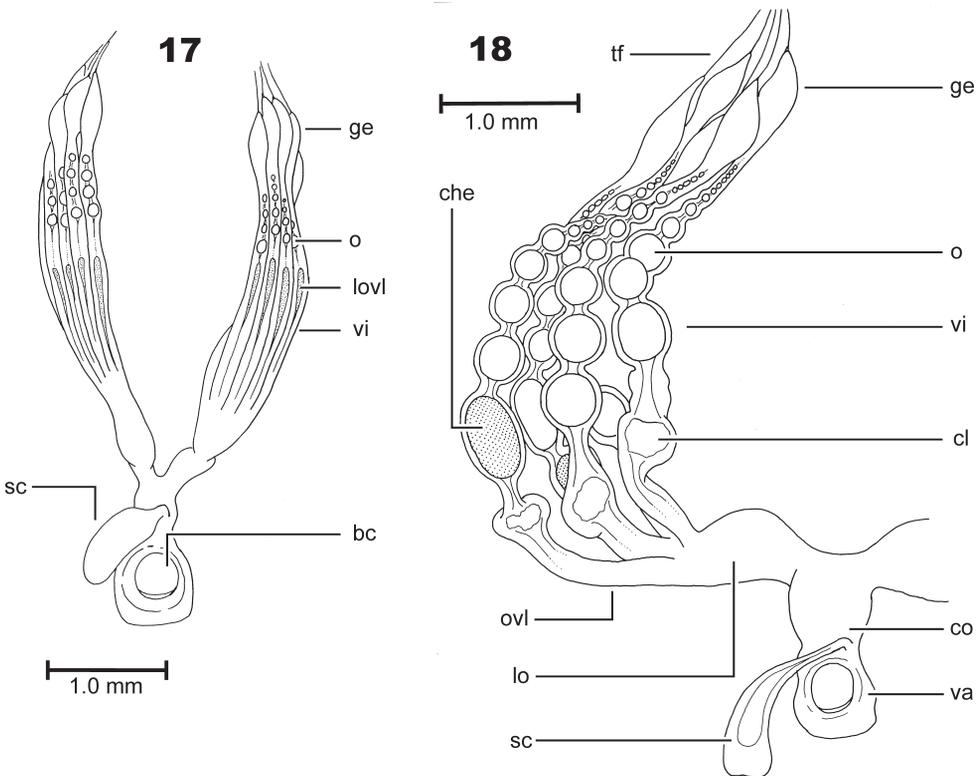


Figs. 13-16. Female internal reproductive system of nymphal stages, ovarium and terminal portion of outflow ways (dorsal view, semischematic). 13 – nymph of instar 3, beginning of ectodermal invagination of outflow ways; 14 – nymph of instar 3, right ovary; 15 – nymph of late instar 4, beginning of evagination of spermatheca and bursa copulatrix, hypodermis of abdominal sterna 8 and 9 forming anlagen of all three pairs of valvules; 16 – nymph of instar 5, same as in Fig. 15. Abbreviations: bc – evagination of bursa copulatrix; ew – wall of ectodermal invagination; g – gonapophyse; hs_8 and hs_9 – hypodermis of abdominal sterna 8 and 9; lo – lateral oviduct; sc – anlage of spermatheca; talo – terminal ampulla of lateral oviduct; 1v – first valvula; 2v – second valvula; va & oc – anlage of vagina and common oviduct. Vertical lines = cavities of the lateral oviducts; dots = anlagen of vagina and common oviduct; horizontal lines = cavity of anlage of spermatheca; oblique lines = cavity of anlage of bursa copulatrix.

change little except for linear growth of ovarioles. Trophic syncytium and the first egg chambers can be observed in the nymphs of late instar 4, while the follicular epithelium is still mostly multilayered and the oogonial and oocyte zones in germarium are well apparent. Definitive formation of trophic syncytium and previtellogenetic egg chambers with well apparent trophic cords occur during instar 5, during which conspicuously long lower (proximal) parts of the ovarioles are formed as well. The latter are filled with (inter)follicular cells with gradually formed, apparently empty lumen in late instar 5 (cf. Fig. 17) when about 3-5 previtellogenetic oocytes are contained in the distal part of vitellarium.

2. Adults

Extensive changes of shape and structural changes of ovarioles occur during the period of maturation, which lasts more than two months. The ovaries of teneral females do not differ from those of nymphs of instar 5. Vitellarium is well differentiated and contains about 4-6



Figs. 17-18. Adult female internal reproductive system (dorsal view). 17 – teneral female (immature female early after last moulting); 18 – fully mature, egg-laying female. Abbreviations: bc – bursa copulatrix; che – chorionated egg; cl – corpus luteum; co – common oviduct; ge – germarium; lo – lateral oviduct; lovl – lumen of ovariole; o – oocyte; ovl – ovariole; sc – spermatheca; tf – terminal filament; va – vagina; vi – vitellarium.

egg chambers with unilayered follicular epithelium of previtellogenetic oocytes. Empty proximal portion of vitellarium is very long, occupying more than a half of the total ovariole length (Fig. 17). Vitellogenesis starts in two or three proximal (oldest) egg chambers after 3-4 weeks after the final moult when the vitellarium contains about 7-10 follicles. The secretion of chorion starts simultaneously in two or three proximal follicles 5-7-weeks after the final moult. At that time about 6-8 vitellogenetic and previtellogenetic oocytes each are contained in each ovariole. Ovaries now fill nearly the entire abdominal cavity since the fat body is strongly reduced. Interfollicular zone separating individual vitellogenetic oocytes is very well apparent and forms funicle-like structures (Fig. 18). Interfollicular cells are accumulated in several layers superficially near the walls of ovarioles, and the central part of lumen is apparently empty. The same structures separate corpora lutea in the ovarioles of older females. After reaching sexual maturity, oogenesis seems to be continual but apparently stops in winter. Dissections of females collected at the end of November and in December, January, February, and early March showed their ovaries short of chorionated eggs although vitellogenetic oocytes were contained.

Lateral and common oviducts, vagina and spermatheca

1. Nymphs

Already in nymphal instars 1 and 2, the anlagen of ovaries are connected with lateral oviducts and the terminal ampullae, which are situated near intersegmental membrane between the seventh and eighth abdominal segments. Ectodermal invagination forming the anlage of the common oviduct and vagina starts to appear in nymphs of instar 3 (Fig. 13). In nymphal instar 4, further structures appear: invagination of the spermathecal anlage (symmetrical at first, asymmetrical in late instar 5) and evagination of the bursa copulatrix situated caudally. Circular sclerotized intima formation, well apparent in total microscope slides, is contained inside the bursa copulatrix. Only size changes of ectodermal structures of efferent ducts occur during instar 5. Shortly before the imaginal ecdysis, both mesodermal and ectodermal outflow ways are fused. Relative shortening of lateral oviducts caused by the onset of intensive ovariole linear growth is apparent at the same time. Both vaginal and spermathecal walls are differentiated in their final form during instars 4 and 5. Strong outer layer of longitudinal muscles and unilayered inner epithelium are formed in the last instar. Secretion cells of the spermathecal gland are apparent from instar 4. They are first nearly cuboidal and later on forming unilayered long cylindrical epithelium in instar 5. The secretion is most probably of the apocrine type.

2. Adults

Morphology and histology of vagina, spermatheca and bursa copulatrix with dorsal gland was described and illustrated in detail by LARSÉN (1938: 141-143, as 'Vaginaltasche'). We can add the following findings: Further relative shortening of lateral oviducts due to elongation of ovarioles occurs in immature females. On the contrary, spermatheca is slightly elongated (see Figs. 17-18). There are no shape or size changes of the spermathecal gland. Spermatheca of immature females with immature oocytes in vitellarium (Fig. 17) contains spermatozoa,

or their bundles, in most dissected specimens. Consequently, mature males copulate with immature females in this species.

Notes on the development of ovipositor

Hypodermis of the eighth abdominal sternum forms paired structures that constitute the anlagen of the first valvulae in nymphs of instar 3 (Fig. 13). Hypodermal anlagen of both second pair valvulae and epiphyses appear in the area of the ninth abdominal sternum during instar 4 (Fig. 15). These paired structures are medially completely separated in the last instar (Fig. 16) and occupy more or less the place of valvulae and gonapophyses of the 'imaginal ovipositor'.

Discussion and conclusions

1. Development of reproductive system

Aphelocheirus aestivalis is as a model species of the whole family Aphelocheiridae and is quite isolated among other families of the Nepomorpha as far as the development of reproductive organs and gametogenesis (especially spermatogenesis) is concerned. The key distinguishing characters can be summarised as follows.

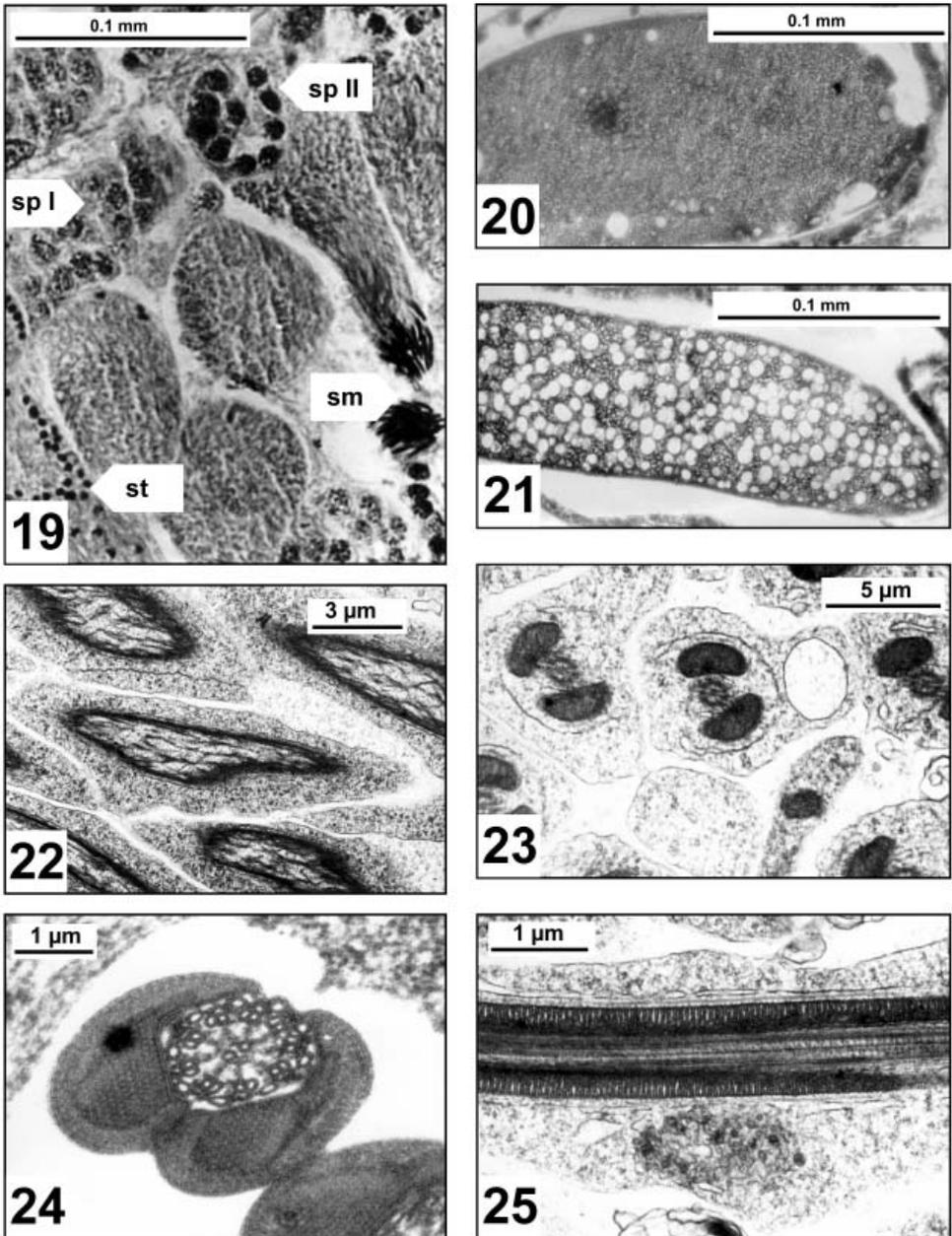
1.1. Males

Each testis contains only four testicular follicles (4+4). In our opinion, this is a reduced and derived character state within the Nepomorpha, equivalent to the state in some Naucoridae and in the Pleoidea. The common state is 7+7 or 5+5 follicles (cf. LARSÉN 1938, MAHNER 1993).

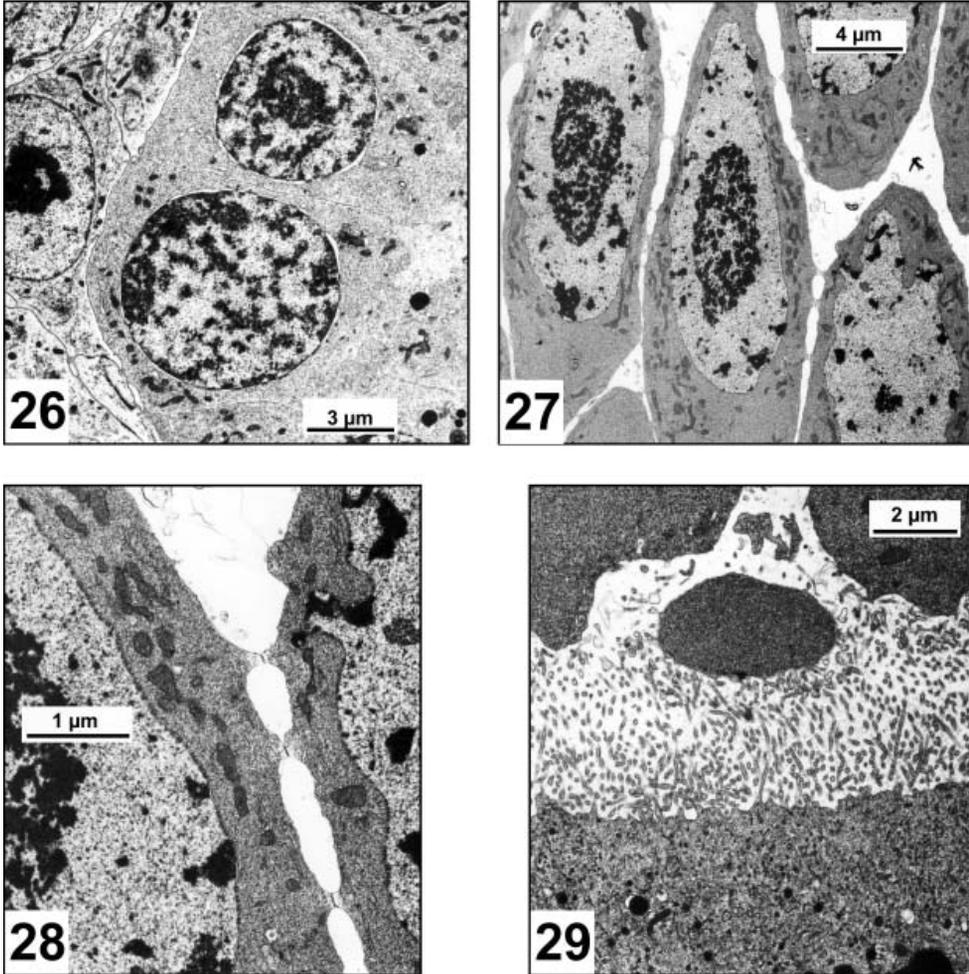
The spermatogenesis is quite unique in comparison with other aquatic bugs. Spermateliosis starts mostly in the last third of instar 5; cysts with early spermatids start to occur and early presperms are rarely found before imaginal moulting. Similar processes are observed in *Notonecta* (Notonectidae) and *Ilyocoris* (Naucoridae). However, spermatogenesis in adult *Aphelocheirus* is continual and multiwaved, and therefore quite different from any other nepomorphan species described so far. Proximal parts of testicular follicles in *Aphelocheirus* form accessory seminal vesicles in addition to 'normal' seminal vesicles that currently occur in other aquatic bugs. We suppose that this unique formation of accessory seminal vesicles is firmly connected to continual spermatogenesis. Storing capacity of 'normal' vesicles may not be sufficient to continual maturation of voluminous bundles of spermatozoa.

On the other hand, we have not found any substantial differences in ultrastructural arrangement of developing spermatocytes and spermatozoa (cf. Figs. 22-25). For instance, mature spermatozoa, although a little shorter (750-800 µm in length including the flagellum) than those of e.g. *Notonecta* (more than 1,200 µm), show the arrangement of head, acrosome, nucleus and array of microtubules in the 9+9+2 pattern not substantially different from other representatives of the Nepomorpha and other Heteroptera (cf. PHILLIPS 1970).

The paired mesodermal accessory glands (1+1) of *A. aestivalis* differentiating during instar 2 are homologous with mesadenia in *Ilyocoris* and with long mesadenia in *Notonecta*. These accessory glands in *A. aestivalis* develop in the same way as in the latter two genera. At least morphologically, the mesadenial secretion in *A. aestivalis* is very similar to secretion of long mesadenia in *Notonecta* (cf. PAPÁČEK & SOLDÁN 1992: 207 and Fig. 17; the figure erroneously referred to 'short accessory glands') and as mesadenial secretion in *Ilyocoris* (cf. PAPÁČEK

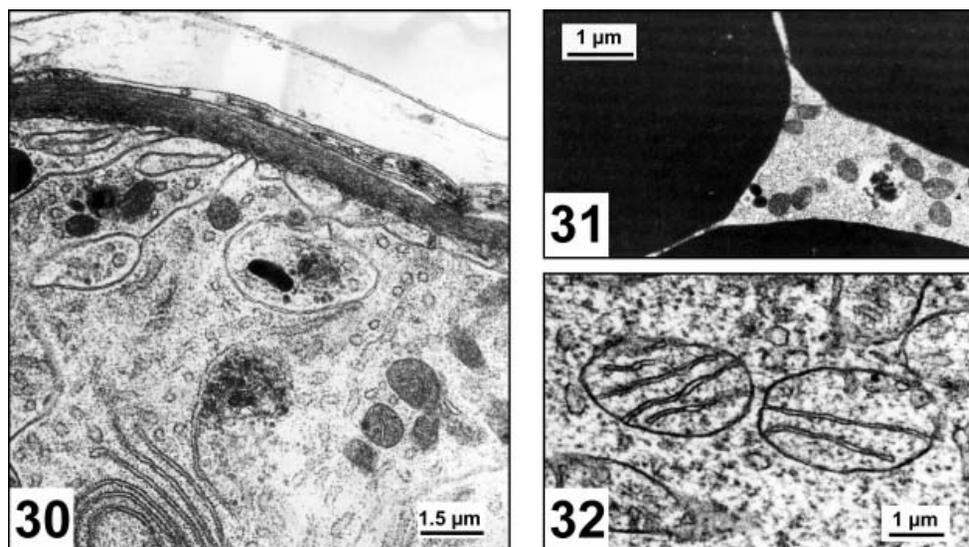


Figs. 19-25. Selected histological aspects of adult male internal reproductive system. 19-21 – light microscope photographs; 22-25 – TEM micrographs. 19 – continual multiwaved spermatogenesis in adult male, about four weeks after final moult, gonocytes; 20 – secretion of paired mesodermal accessory gland (transverse section of mesadenium); 21 – secretion of unpaired ectodermal accessory gland (transverse section of ectadenium); 22 – cysts of secondary spermatocytes; 23 – presperms, transverse section; 24 – spermatozoa, transverse section; 25 – spermatozoan flagellum, longitudinal section. Abbreviations: sp I – primary spermatocytes; sp II – secondary spermatocytes; st – spermatids; sm – spermatozoa.



Figs. 26-29. Selected histological and ultrastructural aspects of adult female internal reproductive system. Follicular cells and oocytes, TEM micrographs. 26 – follicular cells with large nuclei forming unilayered follicular epithelium of egg chambers; 27 – previtellogenic oocytes connected by cytoplasmic bridges; 28 – same, higher magnification; 29 – start of vitellogenesis, yolk granula forming in cytoplasm of vitellogenic oocyte.

& GELBIĆ 1989: 133). Ectodermal unpaired accessory gland in *A. aestivalis* differentiating during instars 3 and 4 is apparently homologous with the ectadenium of *Ilyocoris* (cf. LARSÉN 1938, PAPÁČEK & GELBIĆ 1989). Histological structures of efferent ways in *Aphelocheirus*, *Notonecta*, and *Ilyocoris* differ only in some details, mainly in the relative thickness of individual layers and size of epithelial cells.



Figs. 30-32. Selected ultrastructural aspects of adult female internal reproductive system. Ultrastructure of oocyte and cytoplasmic cord, TEM micrographs. 30 – surface and cortical cytoplasmic area of oocyte rich on membranes of endoplasmatic reticulum; 31 – late state of vitellogenesis – cytoplasm of vitellogenetic oocyte with large yolk granula (blackish); 32 – mitochondria of cytoplasmic trophic cord nourishing oocyte.

1.2. Females

Females of *A. aestivalis* possess five ovarioles in each ovary. The unequal number of testicular follicles in males (4+4) and ovarioles in females (5+5) is a relatively rare phenomenon within the Nepomorpha. Females of *Nepa* Linnaeus, 1758, and *Ranatra* Fabricius, 1790 (Nepidae) and some naucorids possess five ovarioles in each ovary, equal to the number of testicular follicles of males. The development of spermatheca and bursa copulatrix with dorsal gland (= ‘Vaginaltasche’ sensu LARSÉN (1938)) starts in nymphs of instar 4. Similar but relatively smaller bursa copulatrix with a gland is present in *Nepa* and *Ranatra* of the Nepidae but absent in *Naucoris* and *Ilyocoris* of the Naucoridae, a family with relatively close phylogenetic relationships to the Aphelocheiridae.

As in males, we have not found any substantial differences in the ultrastructural arrangement of developing oocytes and trophic tissues from the telotrophic (acrotrophic) ovaries of the Nepomorpha and other Heteroptera (Figs. 26-30). For instance, the vitellogenetic processes and the arrangement and number of mitochondria in trophic cords (Fig. 32) in *A. aestivalis* seem to be identical with *Notonecta* (density of about $175 \mu\text{m}^{-2}$), see BÜNING (1994) for details.

The function of the cuticular comb localised in the cavity of bursa copulatrix of *Aphelocheirus* is to fix and temporarily deposit the spermatophore (see LARSÉN 1938) and simultaneously assist in its partial break-up into individual spermatozoa. The ‘epithelial comb’ of spermatheca in *Ilyocoris* (see PAPAČEK et al. 1997) and the ‘membranal cuticular comb’ in *Notonecta* (see

LARSÉN 1938: 173-174) are functionally similar structures separating individual spermatozoa. On the contrary to the Notonectidae and Naucoridae, where only free aggregations of spermatozoa occur, *A. aestivalis* forms a compact secondary spermatophore. This structure most probably mechanically disintegrates in the cavity of bursa copulatrix before the transport of the spermatozoa to the spermatheca. The physiological (chemical) disintegration of spermatozoa in *Aphelocheirus* probably supports a mechanical one. The situation in other Nepomorpha (except for the Micronectidae, see LARSÉN (1938)) is simpler. They lack a complicated bursa copulatrix and/or spermatophore formation, and the spermatozoa are passed to spermatheca directly.

The cuticle comb in bursa copulatrix of *Aphelocheirus* represents a thick, large, and very spiny 'cuticle brush'. Its morphology is quite different from the same structure in *Ilyocoris* and *Notonecta*.

2. Notes on the life history and reproductive strategy

Many questions on the life history of *A. aestivalis* still remain to be answered reliably. Many authors tentatively considered this species univoltine, presenting different and often controversial data on its life cycle (e.g. LARSÉN 1927, 1932; KANYUKOVA 1974; KRAJEWSKI 1966; PAPÁČEK 1989; SÆTTEM 1986), but different types of life cycle may occur in populations from different areas of the Palaearctic region. For instance, the development of *A. aestivalis* in northern Europe lasts 2-3 years (SÆTTEM 1986). The life cycle of this species is therefore apparently very plastic and some authors (e.g. ŠTYS & ŠKAPEC 1992) suppose different development times even within the same population.

There is no doubt that the type of life cycle and voltinism in aquatic Heteroptera depend mostly on the overwintering stage(s) and diapause. We sampled *A. aestivalis* at regular intervals during winter to resolve this question. We found all developmental stages: eggs, nymphs of all instars, and adults (both males and females). Our findings are in close agreement with data obtained by MESSNER et al. (1983: 326-327) in north-eastern Germany. Consequently, this species can overwinter in an arbitrary life cycle stage and evidently has neither obligatory nor facultative diapause or quiescence in central Europe. All males reach sexual maturity quickly after the last moulting and are mature and possibly ready to mate when overwintering, judging from the presence of mature spermatozoa in their ducts. On the other hand, adults are inactive and deeply burrowed in the substrate at least during the period of low temperatures (marginal or total ice cover of the stream), even if they move when captured and their guts are not empty. On the other hand, the vitellogenesis in females of *A. aestivalis* is stopped during winter months as in some other aquatic bugs (*Corixa* Geoffroy, 1762, *Ilyocoris*, *Notonecta*, see PAPÁČEK & BOHONĚK (1989), PAPÁČEK & SOLDÁN (1987), PAPÁČEK et al. (1997)). Judging from the dissection of females after overwintering, all of them reach relatively quickly sexual maturity (chorionation of mature oocytes). This is a different life strategy from *Plea minutissima* Leach, 1817 (Pleidae), some females of which live for more than one year and overwinter only as adults. Females of *P. minutissima* can reproduce after overwintering dependent on the state of their dormancy (cf. KOVAC 1982). Non-dormant females of this species do not reproduce after overwintering and live more than one year. On the other hand, all females of

A. aestivalis are active during winter but their activity during overwintering is smaller than their activity during spring, summer, and autumn (MESSNER et al. 1983). At this moment we cannot resolve whether females of *A. aestivalis* live for more than one year in Central Europe. We could not find any characters indicating the age of overwintering females, and two overwintering generations cannot be excluded.

Males of insects (and animals in general) with continual spermatogenesis can always copulate repeatedly or continually. This reproductive strategy is quite unique within aquatic bugs and generally rare in insects. The finding of multiwaved continual spermatogenesis in adult males of *A. aestivalis* allows for several hypotheses on their mating strategy. We are in favour of the following one: both newly hatched and overwintering males continually copulate with some newly hatched immature females (specimens which overwinter in preimaginal stage) and females that reached sexual maturity in the previous spring and summer months (specimens that overwinter as adults). Taking into account the approximate length of development and other data (PAPÁČEK 1989), we believe that *A. aestivalis* is semivoltine in central Europe. The most likely population structure at the studied localities represents three overlapping age cohorts and a certain number of size cohorts within all of them. In this respect, a study of the plasticity of life cycle with the same area is urgently needed. The following important question is yet to be answered: how do different abiotic/biotic factors affect the life cycle type?

3. Apomorphies

In the traditional classification of the Nepomorpha, the superfamily Naucoroidea consists of the families Naucoridae, Aphelocheiridae, and Potamocoridae (ŠTYS & JANSSON 1988). MAHNER (1993), who used a traditional Hennigian cladistic method of phylogenetic reconstruction, found the Aphelocheiridae and the Naucoridae to be sister groups, placed them in the superfamily Naucoroidea and discussed possible (uncertain) phylogenetic positions of the family Potamocoridae. HEBGAARD et al. (2004) used combined quantitative phylogenetical analyzes of molecular and morphological datasets and presented a different phylogenetic concept: they removed the Aphelocheiridae from a close relationship with the Naucoridae and placed them as a sister group of the Potamocoridae, whereas the Aphelocheiridae + Potamocoridae formed the superfamily Aphelocheiroidea and the superfamily Naucoroidea became limited to the family Naucoridae.

MAHNER (1993) characterised the apomorphies of the Aphelocheiridae as follows:

(a) Synapomorphy of the Aphelocheiridae and the Naucoridae: crossing of terminal filaments of both ovaries in the cavity of metathorax.

(b) Autapomorphies of the Aphelocheiridae:

(i) Heteronomic number of testicular follicles and ovarioles.

(ii) Asymmetrical vagina with specialised bursa copulatrix.

(iii) Deposition of spermatophore in bursa copulatrix during copulation.

The question is whether the numbers of follicles or ovarioles and their heteronomy can be used to evaluate phylogenetic relationships within the Nepomorpha. Different nepomorphan species have 2-8 follicles/ovarioles (MAHNER 1993). Taking into account that seven follicles / ovarioles prevail in most taxa, it is obvious that the number has been reduced independently

in many species of different nepomorphan clades (e.g. MAHNER 1993: 117). These reductions seem to be a characteristic anagenetic tendency in numerous other groups of the Heteroptera (PAPÁČEK et al. 1990). Moreover, this evolutionary process seems to be still ongoing in some Nepomorpha. For example, *Cryphocricos barozzi* Signoret, 1850 (Naucoridae) exhibits a polymorphic number of follicles (five or six) within the same population. All remaining species of *Cryphocricos* always have six follicles, except for *C. vianai* De Carlo, 1951, which always has five (DE CARLO 1971, MAHNER 1993). The family Naucoridae is an example of a very broad spectrum of different reductions of follicle/ovariole number from seven down to two. Within the whole infraorder Nepomorpha, the same anagenetic tendency is most pronounced in the superfamilies Aphelocheiroidea, Naucoroidea and Pleoidea. Beside this trend in the reduction of follicle/ovariole numbers, heteronomy in follicle/ovariole number also occurs in one species of the Naucoridae: MAHNER (1993) found heteronomy (3/7 – follicles/ovarioles) in the naucorid *Laccocoris nervicus* Montadon, 1909.

Both the reduction and heteronomy found in *A. aestivalis* seems to be convergence rather than a true apomorphy (autapomorphy sensu MAHNER (1993)). We suppose this convergence to be due to general trends to reduce the size of different organs or structures within the whole infraorder. Nevertheless, we believe that the following are clearly defined autapomorphies of the Aphelocheiridae:

- (i) Continual multiwaved spermatogenesis.
- (ii) Development of secondary seminal vesicles.
- (iii) Thick and heavily spined 'cuticle brush' in bursa copulatrix.

These morphological characters support the hypothesis on the removal of the Aphelocheiridae from the Naucoroidea (HEBSGAARD et al. 2004). However, more comprehensive morphological data on the internal reproductive systems of both genera of the Potamocoridae and more species of the Naucoridae are required to confirm this conjecture.

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