JOURNAL OF ENTOMOLOGICAL SOCIETY OF IRAN
2020, 40 (2), 201-227
نامه انجمن حشره‌شناسی ایران ۲۲۷-۲۰۱ ,(۲) ۴۰ ,۱۳۹۹

JESI

DOI: 10.22117/JESI.2020.351817.1395

A comparative morphological study of the genital tube in some hydrophilid species (Coleoptera: Hydrophilidae) with a discussion on the importance of the internal genital characters in phylogenetic studies

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Abstract
The morphology of internal genital tubes in 52 species of water scavenger beetles (Col., Hydrophilidae) are investigated based on personal observation of 22 species and collecting data of formerly published studies of 30 species. In males, morphological differences are observed in generic level and in the structures of the testes. In females, morphological variations are detected in several features in different taxonomic levels: 1. connection position of spermathecal duct to bursa copulatrix, 2. length of spermathecal duct, 3. size and shape of spermathecal gland, 4. size and shape of spermathecal bulb, 5. connection of spermatheca and spermathecal gland with spermathecal bulb, and 6. presence or absence of sclerotized spine inside the membranous wall of the bursa. The length of the spermathecal duct is highly variable across the species, while, the connections of the spermathecal duct and gland with the bulb remain constant in tribe or subfamily level. The importance of using the female genital tube characters in phylogenetic analysis is discussed along with investigating the CI and RI of the genital characters of *Sternolophus* species in a data set of its morphological characters.
Key words: Hydrophilidae, comparative study, genital tube features, female genital morphology

مطالعه ریخت‌شناسی مقایسه‌ای اندام زادآوری داخلی در برخی گونه‌های هایدروفیلید (Coleoptera: Hydrophilidae) همراه با بحث کوتاهی بر روی اهمیت ویژگی اندام زادآوری داخلی در مطالعات فیلوژنتیکی

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چکیده
در این مقاله، ریخت شناسی اندام زادآوری داخلی ۵۲ گونه از قاب‌بالان آبزی خانوادهٔ Hydrophilidae بررسی شده است، که ۲۲ گونهٔ آن بر اساس مشاهدات شخصی و ۳۰ گونهٔ آن با جمع‌آوری مطالعات منتشر شده در مقالات مختلف است. در نرها تفاوت در سطح جنس و در ساختار بیضه‌ها می‌باشد. اما در ماده‌ها، این تفاوت‌ها در ویژگی‌های ساختارهای مختلف و در سطوح مختلف رده‌بندی مشاهده شده است که شامل: ۱- محل اتصال مجرای اسپرماتکی به کیسهٔ جفت گیری یا بورسا کاپولاتریکس، ۲- طول مجرای اسپرماتکی، ۳- اندازه و شکل غدهٔ اسپرماتکی، ٤- اندازه و شکل حباب اسپرماتکی، ٥- چگونگی اتصال اسپرماتک و غدهٔ اسپرماتکی با حباب اسپرماتیکایی و ٦- حضور یا عدم حضور خارهای شاخی در دیوارهٔ غشایی بورسا. در سطح گونه‌ها طول مجرای اسپرماتکی بسیار متنوع است. در حالی‌که وضعیت اتصال میان مجرا، غده و حباب اسپرماتکی ثابت‌تر بوده و در سطح زیرخانواده یا قبیله یکسان است. اهمیت استفاده از خصوصیات ریختی اندام زادآوری داخلی ماده در تجزیه و تحلیل فیلوژنتیک با نگاهی به CI و RI این خصوصیات در مجموعه داده‌های ریخت‌شناسی گونه‌های جنس *Sternolophus* مورد بحث قرار می‌گیرد.

Received: 19 July 2020, *Accepted:* 10 September 2020
Subject Editor: Shahab Manzari

واژه‌های کلیدی: Hydrophilidae، مطالعه مقایسه‌ای، ویژگی‌های اندام زادآوری داخلی، ریخت‌شناسی اندام زادآوری داخلی ماده

دریافت: ۱۳۹۹/۰٤/۲۹، پذیرش: ۱۳۹۹/۰٦/۲۰

Introduction

In Hydrophilidae, the morphology of the male external genitalia is a diagnostic character for identifying the species and also uses in systematics studies (e.g. Komarek & Beutel, 2007; Archangelsky, 2008; Fikáček & Short; 2010, Short, 2010). Unlikely, the morphology of female external genitalia rarely provides a valuable taxonomic character for determining the species (Hansen, 1987; Nasserzadeh *et al*., 2005).

In some studies on different taxa of Hydrophilidae with focus on the morphology of internal genital tube and tracts (e.g. Bameul, 1992; 1993a; 1993b; 1996; 1997; Nasserzadeh & Komarek, 2017), the observed variations provided diagnostic feature.

In addition to the taxonomic interest, several comparative studies on the structure of internal genitalia in different insects revealed phylogenetic value of the studied characters, mainly above species level (e.g. Dettner *et al*., 1986; Tschinkel & Doyen, 1980; Miller 2001; 2012; Katakura *et al*., 2007; Sasakawa & Kubota, 2007; Higginson *et al*., 2012a; 2012b; Prosvirov & Savitsky, 2011; Hünefeld *et al*., 2012; Liebherr, 2013). The current study was initiated after observing variations in female genital tube structure in *Sternolophus* species to estimate the amount of differences in other related taxa.

In this paper 52 species belonging to the subfamilies Hydrophilinae (25 species), Enochrinae (one species), Acidocerinae (one species) and Sphaeridiinae (23 species) are included. Because of high variation in female internal genital tube features and shortcomings in information on male genital morphology with restricted variabilities, this study mainly focuses on females.

Song & Bucheli (2009) studied 41 formerly published cladistic analyses of different groups of insects and compared individual CI and RI (Consistency and Retention Indices) between male and female characters. They claimed that CI and RI indices are measures of fit and can serve as reliable statistics for estimating the phylogenetic signal in the character of interest. In this paper, the strength of genital characters in phylogenetic aspects of the genus *Sternolophus* are investigated by estimating the CI and RI of the genital characters of *Sternolophus* species in a data set of morphological characters. The data set was provided by the author in Nasserzadeh *et al.* (2017).

Materials and methods

Alcoholic preserved material of *Hydrobius fuscipes* (Linnaeus, 1758) (n=14), *Limnoxenus niger* (Gmelin, 1790) (n=6), *Hydrophilus piceus* (Linnaeus, 1758) (n=8), *Sternolophus solieri* Castelnau, 1840 (n=13) and *S. decens* Zaitzev, 1909 (n=5) of CBSU

were dissected for studying the morphology of male and female gonads and glands. Sixty seven dried female specimens of the following 17 *Sternolophus* species, *Sternolophus acutipenis* Nasserzadeh & Komarek, 2017, *S. angolensis* (Erichson, 1843), *S. angustatus* (Boheman, 1851), *S. australis* Watts, 1989, *S. decens*, *S. elongatus* Schaufuss, 1883 , *S. immarginatus* Orchymon, 1911, *S. inconspicuus* (Nietner, 1856), *S. insulanus* Nasserzadeh & Komarek, 2017, *S. jaechi* Nasserzadeh & Komarek, 2017, *S. mandelai* Nasserzadeh & Komarek, 2017, *S. marginicollis* (Hope, 1841), *S. mundus* (Boheman, 1851), *S. prominolobus* Nasserzadeh & Komarek, 2017, *S. rufipes* (Fabricius, 1792), *S. solieri* and *S. solitarius* Nasserzadeh & Komarek, 2017, were dissected and their genital tubes and tracts studied. The examined *Sternolophus* specimens were selected of both alcoholic material from CBSU and dried material from AEZS, HMIM, ISBN, NMW and SMTD. The non-*Sternolophus* specimens were selected from the Iranian species available in CBSU and HMIM.

The material in alcohol was more suitable for internal genital dissection. The bursa copulatrix and its tracts were also dissected safely of well-preserved dried specimens after softening them by hot water but the gland and gonads were rarely preserved well enough to be extracted or observed accurately.

To extract the bursa copulatrix, spermatheca and spermathecal glands of dried specimens, specimens were put in hot water for 20–30 minutes for softening the tissues. Abdominal tergites 3–5 were opened and bursa, spermatheca and spermathecal gland pulled out by a forceps. The extracted organs were put in 5−10 % Potassium Hydroxide (KOH) to remove the fat. A few drops of diluted methylene blue were added during observation to increase the clearance of the ducts and membranes (for more details see Nasserzadeh *et al.* (2005)). The specimens were examined with stereomicroscope (Zeiss Stemi SV11). Measurements were taken using a micrometric eyepiece. Because of different conditions of samples with respect to collection time and fixation a range of variety in measuring the bursa and location of the connection position between spermathecal duct and bursa is considered. Pictures were taken with a digital camera (Cannon IXUS 3.2) and modified by Photoshop CS6. The dissected genital parts were preserved in Euparal on a transparent card or in glycerin in a small vial and pinned with the specimen.

Information of the species of *Hydrochara* Berthold, 1827 are taken from the author's former study on the Iranian species of this genus (Nasserzadeh *et al*., 2005).

Personal observation of two species, *Hydrochara* (*H. dichroma* (Fairmaire, 1892) and *H. flavipes* (Steven, 1808)) are taken from Nasserzadeh *et al.* (2005).

Morphological data of 30 female genital traits are taken from Bameul (1992; 1993a, 1993b; 1996; 1997), Gundevia & Ramamurty (1977) and De Marzo (2008) and marked by asterisk (*). Considering the brief descriptions of the morphology of female internal genitalia in the mentioned literatures, detail information is derived from measuring the line drawings

or schematic illustrations. Missing information because of failed dissection or uncertain information in the published articles, are not given in this paper. They are marked in Table 1 by question mark "?". To compare the distribution of character features throughout the species, they are arranged in Table 1 with the studied taxa. The arrangement of the species are based on Short & Fikáček (2013) in subfamilies and tribes level and genera is based on Hansen (1991). The cited Figures 7 and 10 are permitted by the publishers.

Data analysis

The consistency (CI) and retention (RI) indices (Kluge & Farris, 1969; Farris, 1989) were calculated by analysing the data set of the phylogenetic study of *Sternolophus* species by Nasserzadeh *et al.* (2017). Fifty-two discrete characters including four characters related to the internal genital tube (56–59) and 9 characters (46−55) related to the aedeagus were analyzed using PAUP version 4.0b10 (Swofford, 2002). The characters were equally weighted, and multistate characters treated as unordered. Heuristic searches were chosen with 20000 random additions, followed by branch swapping using tree bisection reconnection (TBR) holding a single tree (NCHUCK = 1, CHUCKSCORE = 1). The calculated CI and IR of individual characters are presented in Table 2.

Abbreviations. The following abbreviations are used in the text:

Anatomy
ad aedeagus
ag accessory gland (ag1 & ag2 in male)
bc bursa copulatrix
cmsl covering muscle
co common oviduct
cutl cuticular line
ejd ejaculatory duct
fc fecundation canal
feg female external genitalia
gc genital capsule
ov ovary
ovi oviduct
ovl ovariole
pm paramere
sb spermathecal bulb
sd spermathecal duct
sf secreted fluid from spermathecal bulb
sg spermathecal gland
sgd duct of spermathecal gland
sph spermatophore extended from ostium
ss sclerotized spines
sv seminal vesicle
ts testis
tscl testicle
vd vas deferens
vg vagina

Collections

AEZS – coll. A. Short, University of Kansas, Lawrence, KS, USA (A. Short)

CBSU – Collection of Department of Biology, Shiraz University, Iran (S. Hosseinie & S. Sadeghi)

HMIM – Hayk Mirzayans Insect Museum, Tehran, Iran (H. Nasserzadeh)

ISNB – Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgique (D. Drugmand)

NMW – Naturhistorisches Museum Wien, Vienna, Austria (M. A. Jäch)

SMTD – Staatliches Museum für Tierkunde, Dresden, Germany (O. Jäger)

Results

General morphology of male and female reproductive systems in Hydrophilidae.

Male (Figs 1, 3, 7A, 8)**:**

It generally comprises of a pair of testes, each contains many testicles. Testes lead to seminal vesicles via vas deferens. Vas deferens may not be recognizable in the specimens that are out of reproductive period. The median ejaculatory duct has a cuticular lining along the body wall (Figs 8A, B). The ejaculatory duct is surrounded by a strong muscular coat and ends in the ostium or apical opening of the median lobe of the aedeagus (Nasserzadeh *et al*., 2005). The large part of the reproductive system consists of accessory glands (ag) that based on Gundevia & Ramamurty (1977), includes ag1 and ag2. During the reproductive period the glands (ag1 and ag2) are larger and visible but out of the reproductive period, they are smaller and the tubes are degenerated (Dailey *et al*., 1980; personal observation). Because of the fragility, only a few dissections were successful for extracting ag1 (Figs 3; 8: ag1).

The aedeagus comprises two parameres, a median lobe and a basal piece, rests inside the genital capsule (3; 8A, B).

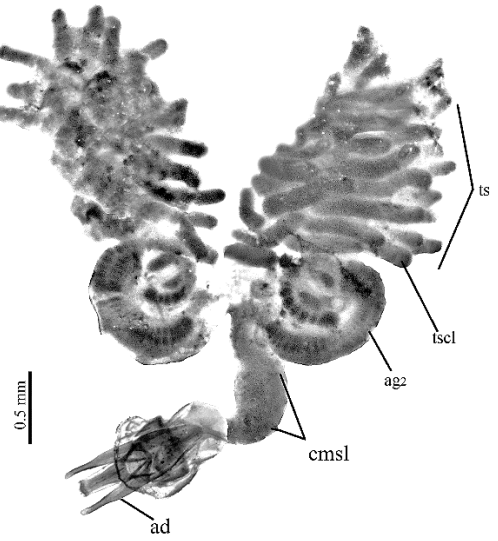


Fig. 1. *Hydrobius fuscipes*, male reproductive system, dorsal view. Abbreviations: ad – aedeagus; ag2– accessory gland 2; cmsl – covering muscle; ts – testis; tscl – testicle.

Female (Figs 2, 4−6, 7B, 9, 10)**:**

It generally consists of two ovaries, each comprises of several telotrophic (acrotrophic) ovarioles (Nasserzadeh *et al*., 2005). Lateral oviducts join and form a common oviduct. Accessory glands are on top and below of each ovary. They join to the oviduct of their own side. The common oviduct connects ventrally to a chamber near the anterior end of the vagina called bursa copulatrix that forms a sac anteriorly to receive the spermatophores.

The bursa copulatrix is membranous with many villi-like folds. In copulated females the sac expands and the folds open. Four longitudinal crease-like lines on the membranous wall of the bursa are observed. Nasserzadeh *et al.* (2005) in agreement with Imms (1964), nominated the creased-like lines as'fecundation canal' in *Hydrochara* species. In this study, particles of the covering muscles were observed attached to the bursa over the crease-like lines. Based on this observation, it can be imagined that the longitudinal creases on the membranous wall of the bursa copulatrix capture the secretion of the spermathecal gland in the creases and serve as passages or canals for transferring the secretion fluid in the bursa. The fluid can also be flown by the help of contractions of the mentioned muscles. In a few species, sclerotized teeth-like spines lay inside the membranous wall of the bursa (Figs 5B, 9). Based on Chapman (2013) and Klowden (2013), one of the functions of the spines is assumed to speed up the releasing of spermatozoa by helping in rupturing the spermatophore. Spermathecal duct connects bursa to spermatheca and the spermathecal gland. The connection to the bursa is from the ventral side, anteriorly or medially. Gundevia & Ramamurty (1977) chose the term spermathecal bulb instead of spermatheca which is preferred here.

External genitalia have no specific morphological value. They generally consist of laterotergite, mediotergite, coxostyle and gonostyle (see Nasserzadeh *et al*., 2005).

Spermatophores:

The spermatophore formation in the bursa copulatrix was observed in the extracted bursa of the newly copulated hydrophilids (e.g. Figs 5B, D, E). In virgin females or those which copulated several days ago (e.g. Figs 2; 5C; 6), the bursa appears like a collapsed and folded membranous sac. Gundevia & Ramamurty (1977) mentioned that the deposition of the sperm mass in spermatophores is shaped in a helically coiled column. Gadzama & Happ (1974) also described the spermatophore in *Tenebrio molitor* Linnaeus as a blind tube. In few specimens of *Sternolophus* as in Figure 5B, the twisted column formation are observed inside the expanded bursa. The pumped spermatophore streams in the ejaculatory duct to the ostium. It transfers into the female genitalia via the everted internal sac from the ostium of the median lobe (Fig. 8C) (Williams & Feltmate, 1994).

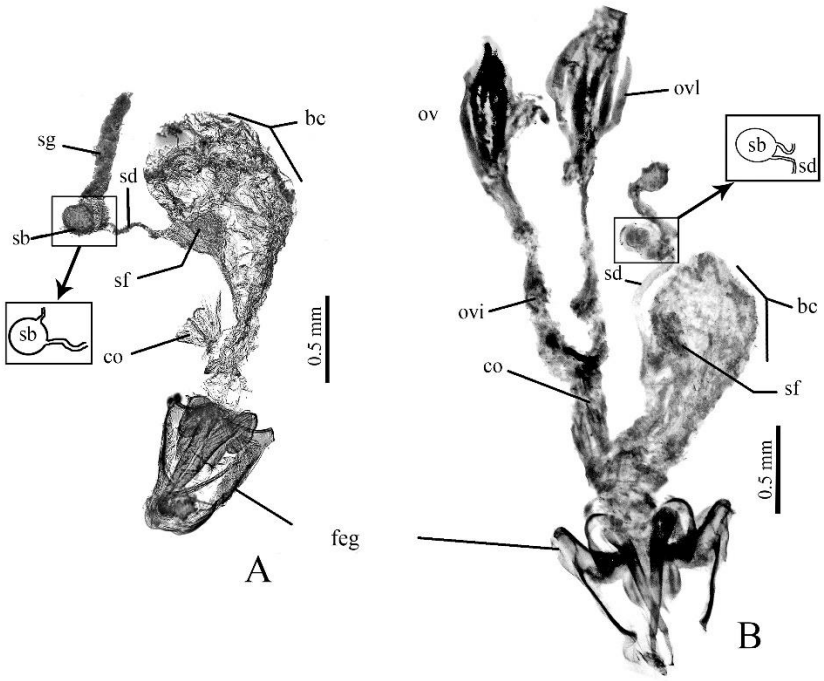


Fig. 2. Female reproductive system, dorsal view. **A.** *Hydrobius fuscipes*; **B.** *Limnoxenus niger*. Abbreviations: bc – bursa copulatrix; co – common oviduc; feg – female external genitalia; ov – ovaries; ovi – oviducts; sb – spermathecal bulb; sd – spermathecal duct; sf – secreted fluid from spermathecal bulb; sg – spermathecal gland; sgd – duct of spermathecal gland.

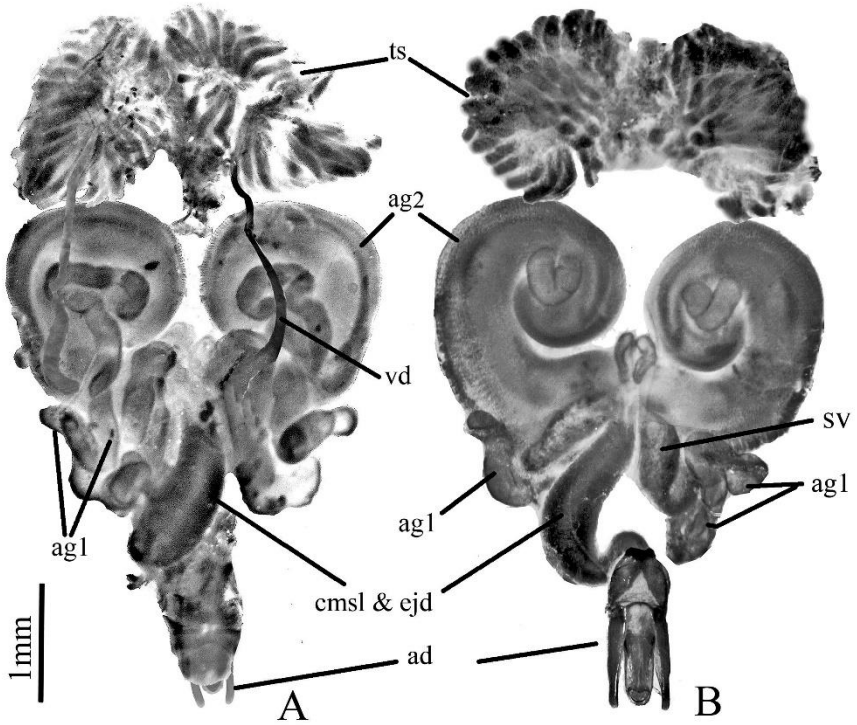


Fig. 3. *Sternolophus decens*, male reproductive system morphology. **A.** dorsal view; **B.** ventral view. Abbreviations: ag1 – accessory gland 1; ejd – ejaculatory duct; sv – seminal vesicle; vd – vas deferens.

Definition of features in variable structures

The morphological changes of the variable structures are defined as follows:

Males: Morphology of testes change in generic level. The variations in testes are divided into, large, as large as ag2 or larger and, small, smaller than the half size of the ag2; testicles are divided to tick and tubular that clustered loosely (Figs 1, 3), thin and delicate that clustered compact (Fig. 7A) or very compact in which the testicles are not visible (Fig. 8).

Females (Fig. 11): Variation of connection between spermathecal duct and bursa copulatrix is divided generally into anterior and medial positions. The anterior connection of the spermathecal duct is considered from the apex (e.g. Figs 4, 5D, 7B) to the anterior quarter of the length of the bursa (e.g. Figs 6B,C,E,F, 9). Medial connection is defined for the connection approximately in half-length to anterior third of the distance between the apex of the bursa and the common oviduct (e.g. Figs 2; 5A, C, E; 6A, D).

Variations of the length of the spermathecal duct are divided to short, moderate, long and very long. The length defines as short, when the length of the duct is shorter than the half-length of the bursa from the apex to the common oviduct (e.g. Figs 4; 5D; 6C, E, F; 9); moderate, when the duct is longer than the half-length of the bursa to approximately equal size (Figs 2; 5A, C, E; 6A, B, D); long, when the duct is approximately twice as long as the bursa (Fig. 7B); very long, when the duct is more than three times longer than bursa and coiled (Figs 10A−C).

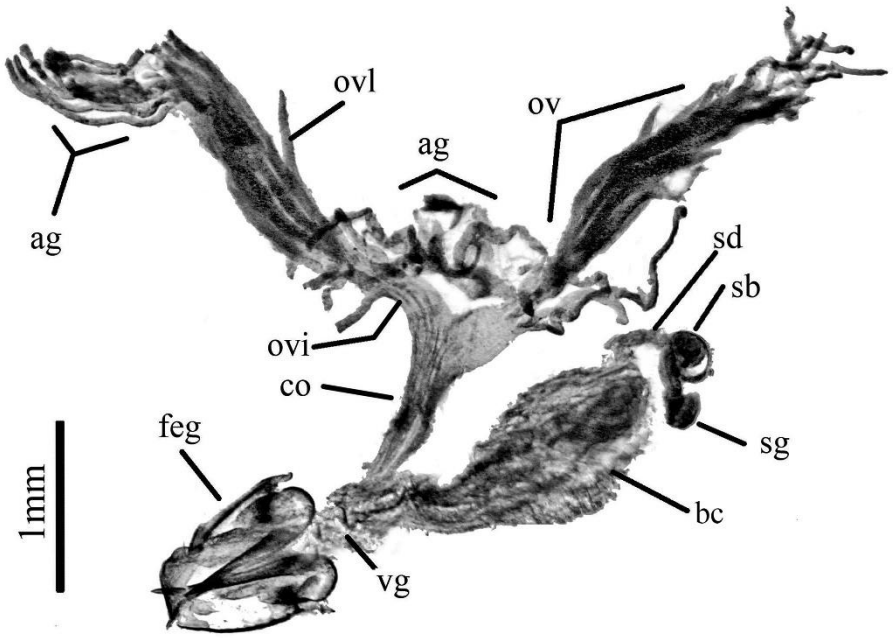


Fig. 4. *Sternolophus solieri*, female reproductive system, dorsal view. Abbreviations: ag – accessory gland; vg – vagina.

Spermathecal bulb is variable in size and shape. The size divided as small (Figs 2, 5–7, 9), smaller than half-length of the spermathecal gland and large (Fig. 10), larger than spermathecal gland. The shape is divided to regular or irregular, oval or spherical.

The variation of spermathecal gland is divided to four types, irregular globular, irregular elongated, oval and tubular. The duct of spermathecal gland is shorter than its half-length or longer.

Spermathecal gland and spermathecal ducts connect to spermathecal bulb separately (Fig. 2) or spermathecal gland joins spermathecal duct before connecting to the spermathecal bulb (Fig. 7B).

Description of internal genital tubes and tracts: Descriptions taken from formerly published data are marked with an asterisk (*).

Subfamily Hydrophilinae

Tribe Berosini

(Fig. 11, Table 1)

Genus *Berosus* Leach, 1817

* *Berosus signaticollis* (Charpentier, 1825) (Fig. 11, Table 1). Description is based on schematic illustration in De Marzo (2008).

Male: Unknown.

Female: Connection between spermathecal duct and bursa copulatrix medially; length of spermathecal duct moderate; spermathecal bulb small, rather regular oval shape; spermathecal gland oval, at least two times larger than spermathecal bulb; duct of spermathecal gland short, one third of the length of the spermathecal gland; spermathecal gland and spermathecal ducts join before connecting spermathecal bulb.

Tribe Hydrophilini

(Fig. 11, Table 1)

Genus *Sternolophus* Solier, 1834 (Figs 3−6)

General descriptions that shares in all *Sternolophus* species:

Male: Testes as large as accessory gland or slightly smaller; testicles large, thick and visible, rather loosely clustered above accessory glands (Fig. 3).

Female: Length of bursa between 1.6−3.5 mm, sclerotized spine inside the membranous wall of bursa (except in *S. acutipenis*) absent; connection between spermathecal duct and bursa copulatrix anteriorly or medially; length of spermathecal duct short to moderate; spermathecal bulb small, with regular spherical shape; spermathecal gland elongated tubular; duct of spermathecal gland very short, shorter than one third of the length of spermathecal gland; spermathecal gland and spermathecal duct connect to spermathecal bulb separately.

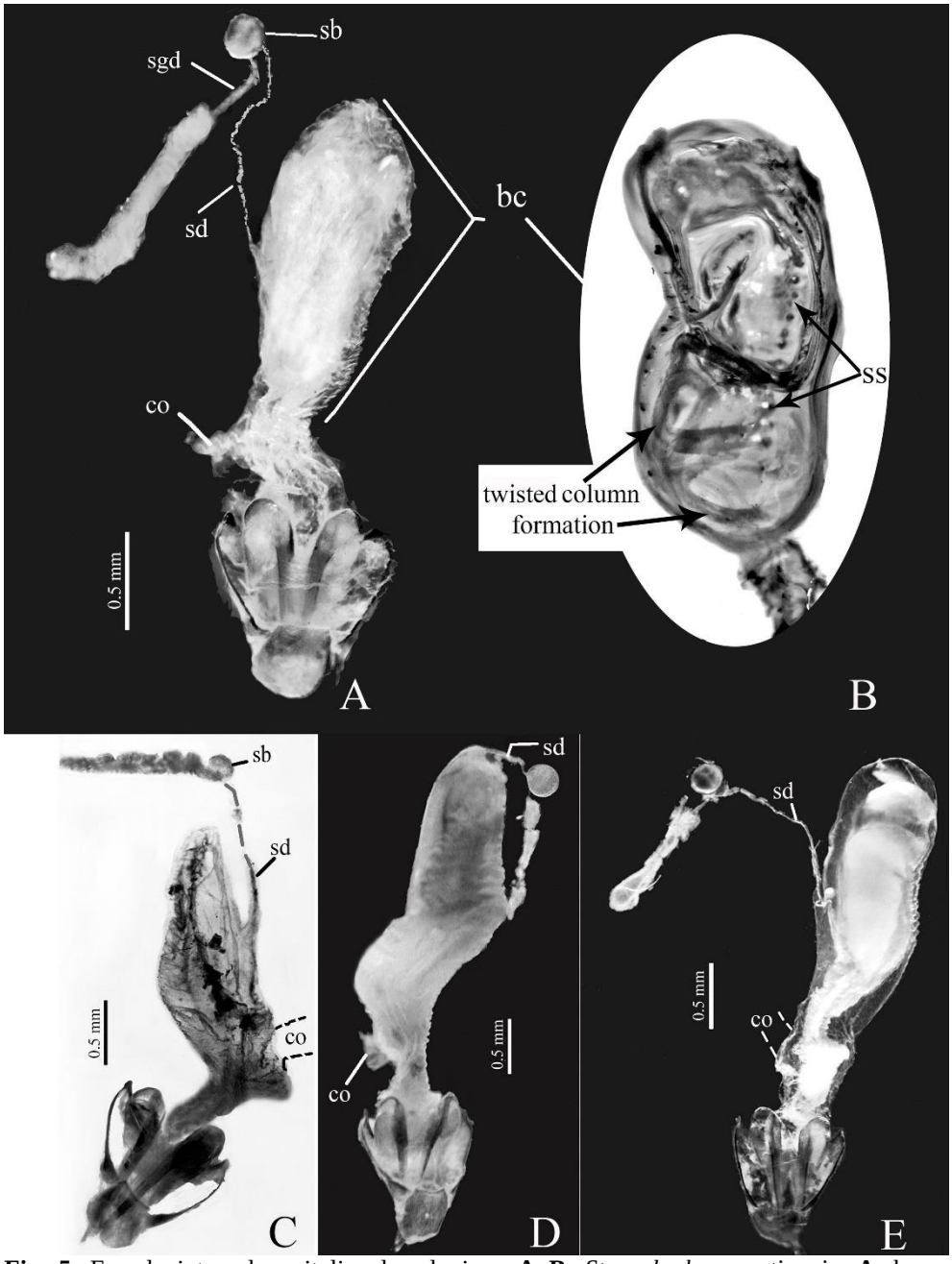


Fig. 5. Female internal genitalia, dorsal view. **A**–**B.** *Sternolophus acutipenis*: **A.** bursa copulatrix (bc) without spermatophore; **B.** bursa copulatrix filled with spermatophore, sclerotized spines (ss) and a twisted column formation inside the spermatophore is visible; **C.** *S. angolensis*; **D.** *S. angustatus*. **E.** *S. decens.*

Description of interspecific variable features:

Sternolophus acutipenis (Figs 5A, B). Length of bursa from anterior top to common oviduct 1.6−1.9 mm; longitudinal rows of sclerotized spines inside the membranous wall of bursa copulatrix present (Fig. 5B); connection between spermathecal duct and bursa copulatrix medially, 0.6−0.8 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus angolensis (Fig. 5C). Length of bursa from anterior top to common oviduct 1.8 mm; connection between spermathecal duct and bursa copulatrix medially, 1.1 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus angustatus (Fig. 5D). Length of bursa from anterior top to common oviduct 2.5 mm; connection between spermathecal duct and bursa copulatrix anteriorly, apically; length of spermathecal duct short.

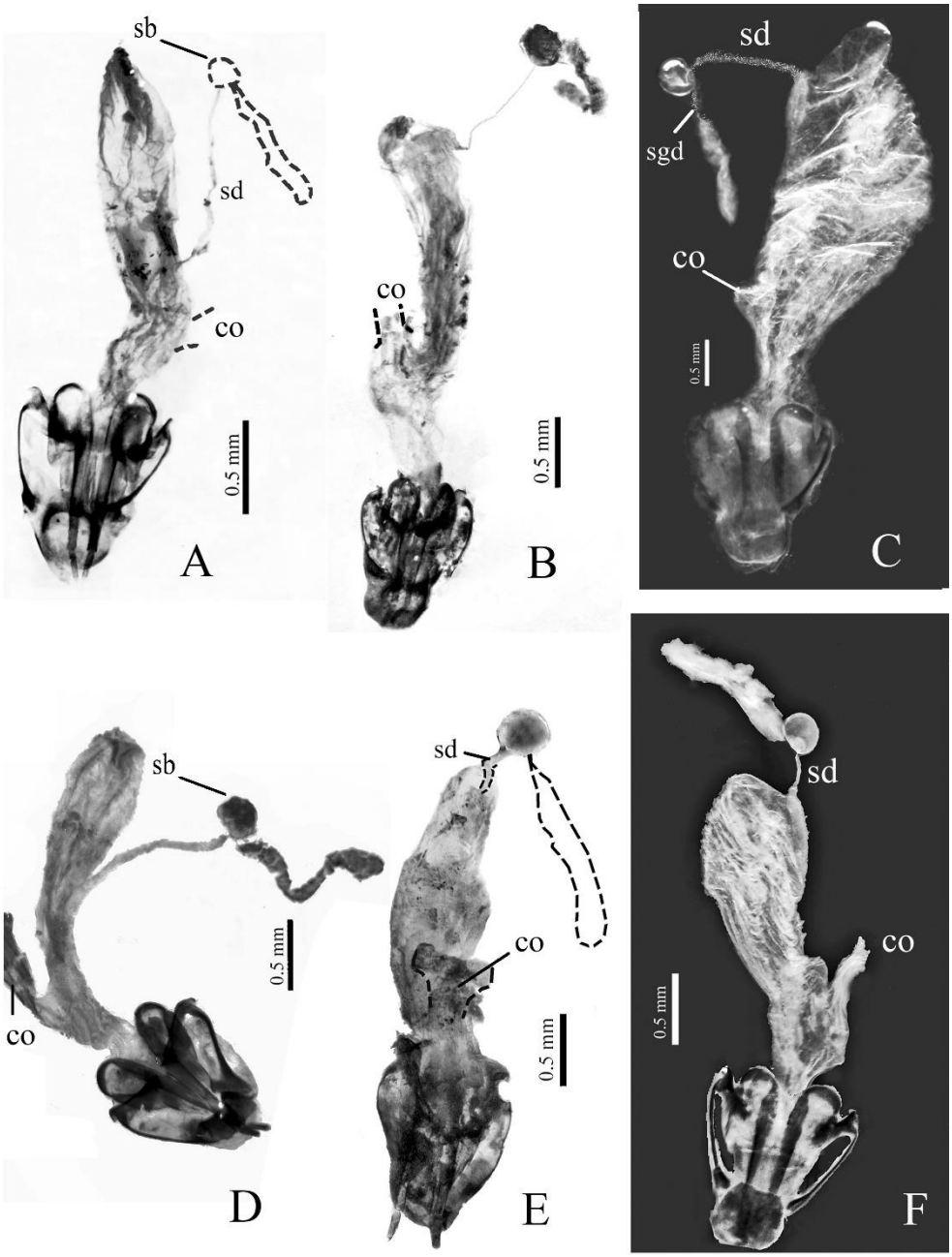


Fig. 6. Female internal genitalia, dorsal view. **A.** *Sternolophus inconspicuus*; **B.** *S. insulanus*; **C.** *S. mandelai*; **D.** *S. marginicollis*; **E.** *S. prominolobus*; **F.** *S. rufipes*. Abbreviations: fc – fecundation canal.

Sternolophus australis. Length of bursa from anterior top to common oviduct about 2.1−2.5 mm; connection between spermathecal duct and bursa copulatrix medially, 1.0−1.2 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus decens (Fig. 5E). Length of bursa from anterior top to common oviduct 2.5 mm; connection between spermathecal duct and bursa copulatrix medially, 1.4 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus elongatus. Length of bursa from anterior top to common oviduct 2.0−2.5 mm; connection between spermathecal duct and bursa copulatrix anteriorly, apically; length of spermathecal duct short.

Sternolophus immarginatus. Length of bursa from anterior top to common oviduct 2.0 mm; connection between spermathecal duct and bursa copulatrix medially, 1.1 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus inconspicuus (Fig. 6A). Length of bursa from anterior top to common oviduct 1.6 mm; connection between spermathecal duct and bursa copulatrix medially, 1.0 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus insulanus (Fig. 6B). Length of bursa from anterior top to common oviduct 1.7 mm; connection between spermathecal duct and bursa copulatrix anteriorly, 0.4 mm near apex of bursa; length of spermathecal duct moderate.

Sternolophus jaechi. Length of bursa from anterior top to common oviduct 3.1 mm; connection between spermathecal duct and bursa copulatrix anteriorly, apically; length of spermathecal duct short.

Sternolophus mandelai (Fig. 6C). Length of bursa from anterior top to common oviduct 3.5 mm; connection between spermathecal duct and bursa copulatrix anteriorly, 0.9 mm from apex of bursa; length of spermathecal duct short.

Sternolophus marginicollis (Fig. 6D). Length of bursa from anterior top to common oviduct 2.1 mm; connection between spermathecal duct and bursa copulatrix medially, 1.5 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus mundus. Length of bursa from anterior top to common oviduct 3.5 mm; connection between spermathecal duct and bursa copulatrix medially, 1.5 mm from apex of bursa; length of spermathecal duct moderate.

Sternolophus prominolobus (Fig. 6E). Length of bursa from anterior top to common oviduct about 2.0 mm; connection between spermathecal duct and bursa copulatrix anteriorly, 0.3 mm near apex of bursa; length of spermathecal duct short.

Sternolophus rufipes (Fig. 6F). Length of bursa from anterior top to common oviduct 1.9 mm; connection between spermathecal duct and bursa copulatrix anteriorly, 0.3−0.5 mm near apex of bursa; length of spermathecal duct short.

Sternolophus solieri (Fig. 4). Length of bursa from anterior top to common oviduct 2.0−2.3 mm; connection between spermathecal duct and bursa copulatrix anteriorly, apically; length of spermathecal duct short.

Sternolophus solitarius. Length of bursa from anterior top to common oviduct 2.7 mm; connection between spermathecal duct and bursa copulatrix medially, 1.2 mm from apex of bursa; length of spermathecal duct moderate.

Genus *Hydrochara* Berthold, 1827 (Fig. 7, Table 1)

* *Hydrochara dichroma* & *H. flavipes*. Description is based on previous study by author (Nasserzadeh *et al*., 2005).

Male: Testis smaller than half size of accessory gland; testicles small but visible, cluster form a compact testis above accessory gland (Fig. 7A).

Female (Fig. 7B): Length of bursa from anterior top to common oviduct 3.3 mm and 2.6 mm in *H. dichroma* and *H. flavipes* respectively; connection between spermathecal duct and bursa copulatrix anteriorly, apically; length of spermathecal duct long; spermathecal bulb small and regular spherical; spermathecal gland elongated tubular; duct of spermathecal gland short, shorter than half-length of spermathecal gland (Fig. 7A); spermathecal gland and spermathecal ducts join before connecting to spermathecal bulb.

* *Hydrochara caraboides* (Linnaeus, 1758), * *H. vicina* Bameul 1996 and * *H. semenovi* (Zaitzev, 1908).

Descriptions of *H. caraboides* are based on a schematic illustration by De Marzo (2008), *H. vicina* and *H. semenovi* are based on Bameul (1996).

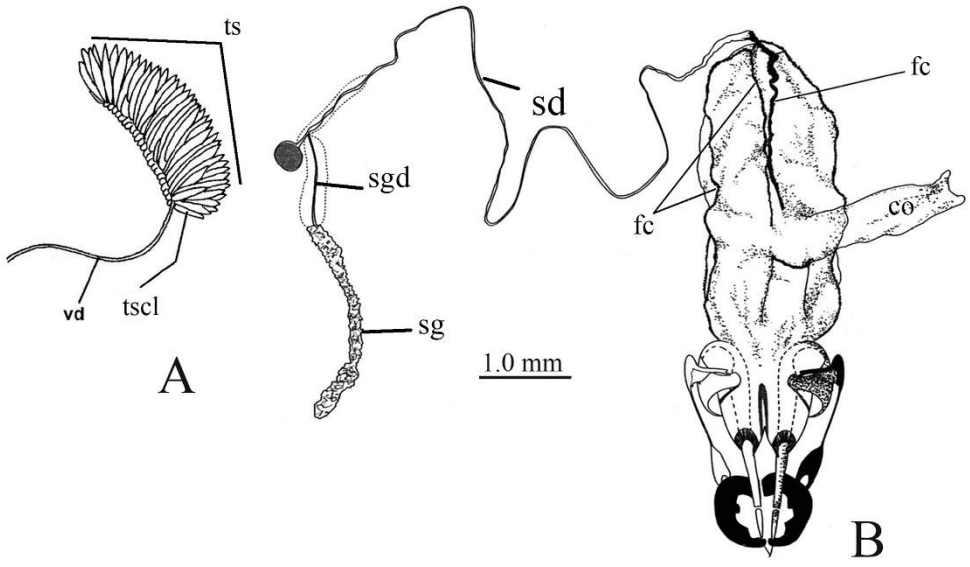


Fig. 7. *Hydrochara dichroma*. **A.** testis, testiculs and vas deferens (taken from Nasserzadeh *et al*., 2005: 232). **B.** female internal genitalia (taken from Nasserzadeh *et al*., 2005: 242).

Male: Unknown

Female: Connection between spermathecal duct and bursa copulatrix anteriorly, apically; length of spermathecal duct long; spermathecal bulb small and regular spherical; spermathecal gland elongated and tubular, in *H. semenovi* two diverticules present in bent region before apex while in *H. vicina* and *H. caraboides* diverticules absent; duct of spermathecal gland short, shorter than half-length of spermathecal gland; spermathecal gland and spermathecal ducts join before connecting to spermathecal bulb.

Remarks: Bameul (1996) mentioned two diverticules of spermathecal gland in *H. semenovi* as a diagnostic character between *H. semenovi* and *H. vicina*.

Genus *Hydrophilus* Geoffroy, 1762 (Table 1)

Hydrophilus piceus (Linnaeus, 1758) (Figs 8, 9).

Male: Testis distinctly larger than accessory gland; testicles large, tubular and visible separately, loosely clustered and form expanded testes above accessory glands.

Female: length of bursa from anterior top to common oviduct 1.6−1.8 mm, connection between spermathecal duct and bursa copulatrix medially; length of spermathecal duct moderate, connected to bursa approximately 0.8 mm from anterior top of bursa; sclerotized spine inside membranous wall of bursa absent; spermathecal bulb small with regular spherical shape; spermathecal gland elongated and slender; duct of spermathecal gland very short, one third of the length of spermathecal gland or shorter; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

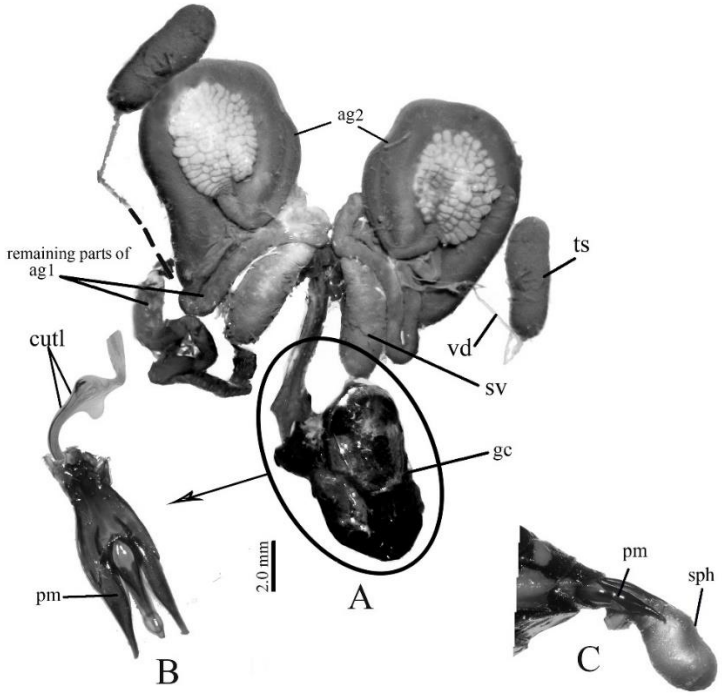


Fig. 8. *Hydrophilus piceus*. **A.** male reproductive system with aedeagus in genital capsule (gc), dorsal view; **B.** aedeagus exposed, cuticular line (cutl) of ejaculatory duct visible; **C.** extended spermatophore (sph) from ostium, lateral view.

Remarks: The studied material (males and females) were collected in the first half of summer. They were all out of the mating period with residual accessory glands and gonads. There was a small yellowish transparent mass inside the bursa near the entrance of the spermathecal duct into the sac (2A, sf). It is similar to the retention material in the spermathecal bulb.

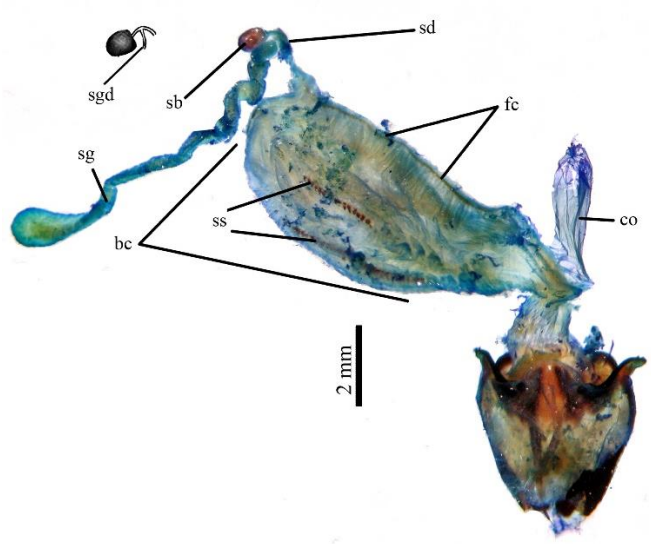


Fig. 9. *Hydrophilus piceus*, female internal genitalia, ventral view.

Genus *Limnoxenus* Motschulsky, 1853 (Table 1)

Limnoxenus niger (Gmelin, 1790) (Fig. 2B).

Male: Unknown. Extraction failed.

Female: Length of bursa from anterior top to common oviduct about 1.7 mm; connection between spermathecal duct and bursa copulatrix medially; length of spermathecal duct moderate, connected to bursa about 0.7 mm from anterior top of bursa; spermathecal bulb small and regular spherical; spermathecal gland elongated and slender, duct of spermathecal gland very short, one third of the length of spermathecal gland or shorter; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

Remarks: Few male and female specimens collected in early summer with residual reproductive glands and gonads. The yellowish transparent mass similar to the retention material in spermathecal bulb (see Remarks of *Hydrobius fuscipes*) was observed in the bursa (Fig. 2B: sf).

Subfamily Enochrinae

Genus *Enochrus* Thomson, 1859 (Fig. 11, Table 1)

* *Enochrus quadripunctatus* (Herbst, 1791). Description is based on a schematic illustration in De Marzo (2008).

Male: Unknown.

Female: Connection between spermathecal duct and bursa copulatrix anteriorly; length of spermathecal duct short; spermathecal gland rather oval and almost as large as spermathecal bulb; duct of spermathecal gland short; connection of spermathecal gland and spermathecal ducts to spermathecal bulb not clear.

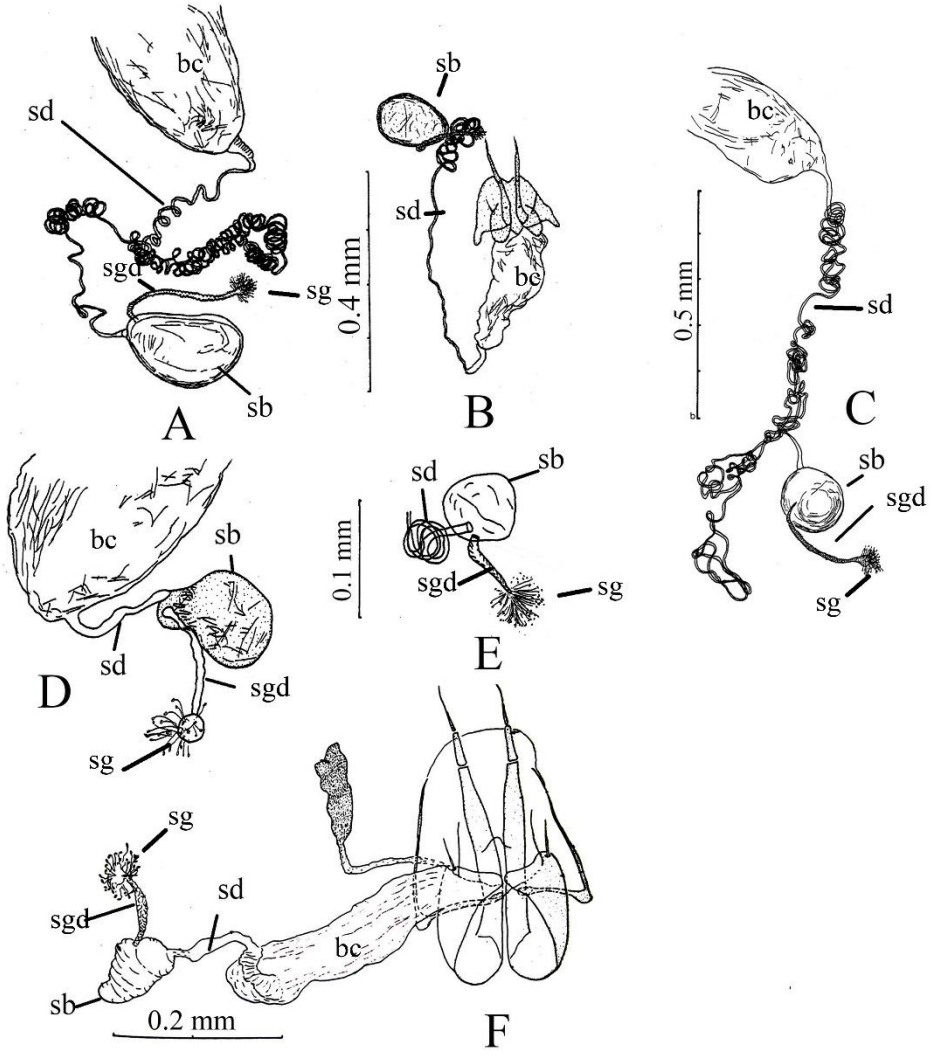


Fig. 10. Female internal genitalia of Spheridiinae. **A.** *Paromicrus bicarinatus*; **B.** *P. scotti*; **C.** *Stanmalcolmia sulawesiensis*; **D.** *Aculomicrus brendelli*; **E.** *A. minimus*. (taken from Bameul, 1993a); **F.** *Psalitrus serendibensis* (taken from Bameul 1992).

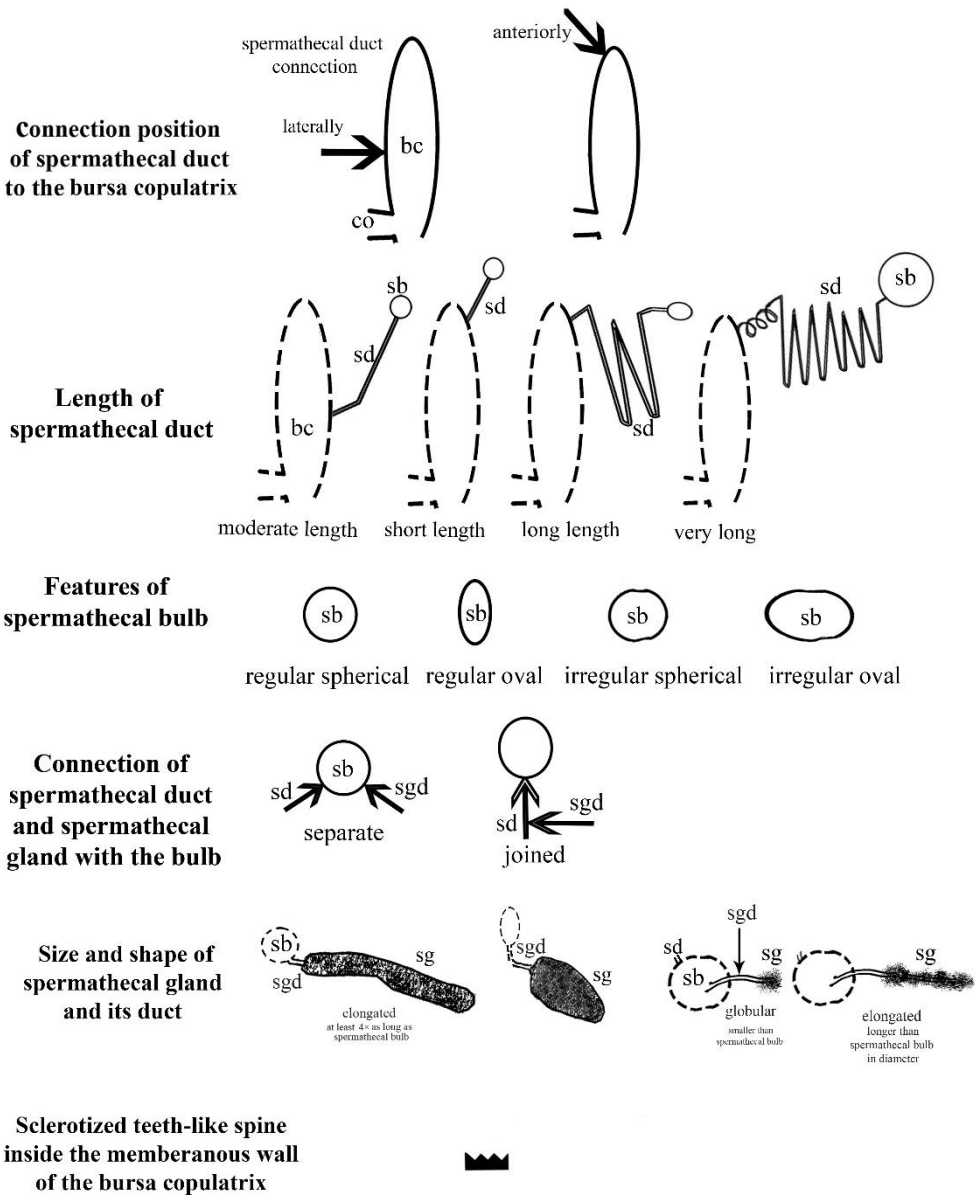


Fig. 11. Schematic representation of the features of the variable characters in female internal genitalia.

Table 1. Features of male and female internal genitalia in Hydrophilinae.

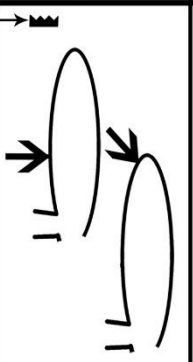
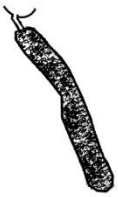
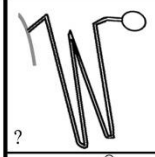
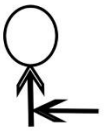
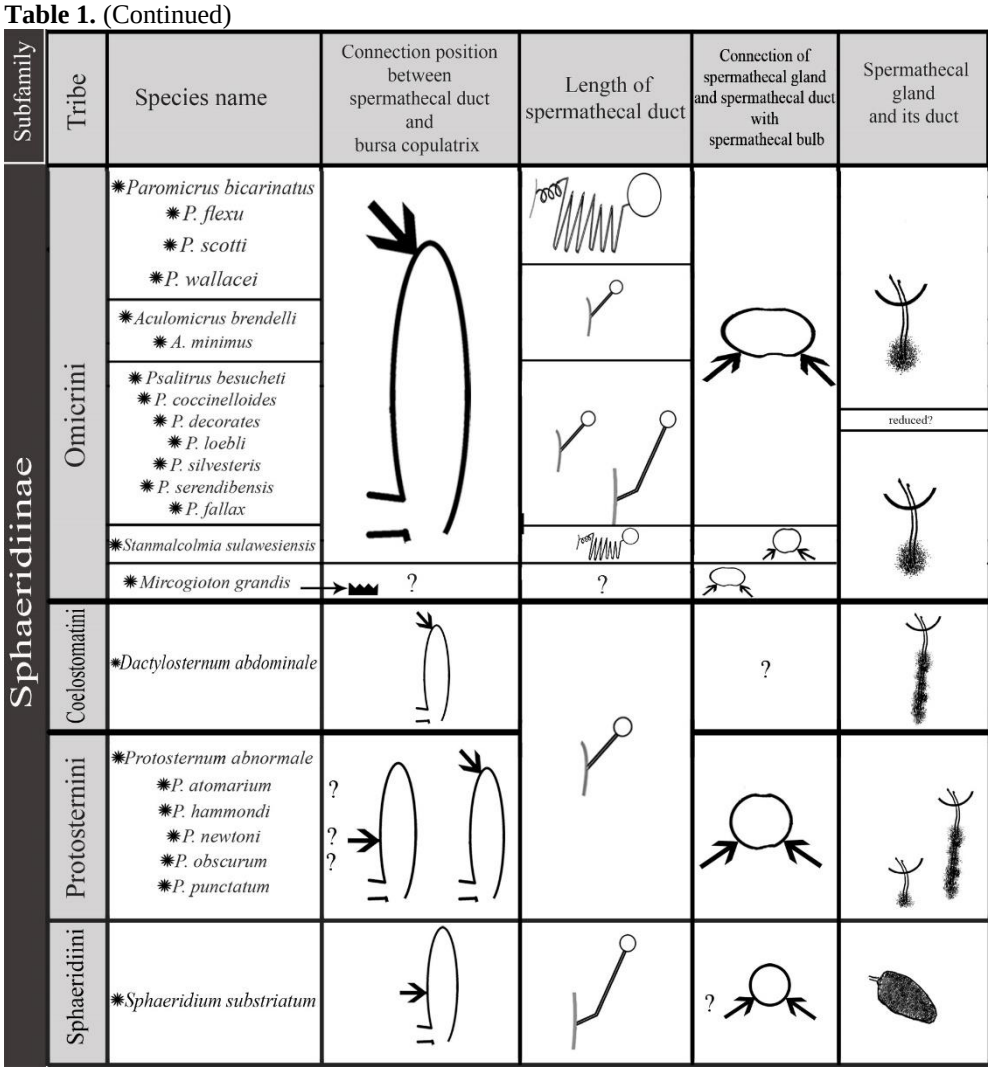
Subfamily	Tribe	Species name	Connection position between spermathecal duct and bursa copulatrix	Length of spermathecal duct	Connection of spermathecal gland and spermathecal duct with spermathecal bulb	Spermathecal gland and its duct
Hydrophilinae	Berosini	✷*Berosus signaticollis*	? 			? 
	Hydrophilini	*Sternolophus acutipenis* *S. angolensis* *S. angustatus* *S. australis* *S. decens* *S. elongatus* *S. immarginatus* *S. inconspicuus* *S. insulanus* *S. jaechi* *S. mandelai* *S. marginicollis* *S. mundus* *S. prominolobus* *S. rufipes* *S. solieri* *S. solitarius*				
		✷*Hydrochara caraboides* *H. flavipes* ✷*H. semenovi* *H. dichroma* ✷*H. vicina*		 ?		
		✷*Hydrophilus olivaceus* *H. piceus* →				
	Hydrobiusini	*Hydrobius fuscipes* *Limnoxenus niger*				
Enochrinae		✷*Enochrus quadripunctatus*		? 	?	? 
Acidocerinae		✷*Helochares lividus*		? 	? 	

Table 1. (Continued)



Subfamily Acidocerinae

Genus *Helochares* Mulsant, 1844 (Fig. 11, Table 1)

* *Helochares lividus* (Forster, 1771). Description is based on a schematic illustration in De Marzo (2008).

Male: Unknown.

Female: Connection between spermathecal duct and bursa copulatrix anteriorly; length of spermathecal duct moderate; spermathecal bulb small with regular spherical shape; spermathecal gland elongated and slender; duct of spermathecal gland short; connection of spermathecal gland and of spermathecal ducts to spermathecal bulb separated.

Subfamily Sphaeridiinae

The following information is based on the Bameul (1992; 1993a; 1993b; 1997) and De Marzo (2008).

Tribe Omicrini

(Fig. 11, Table 1)

Genus *Paromicrus* Scott, 1913 (Table 1)

* *Paromicrus bicarinatus*, * *P. flexus*, * *P. scotti* & * *P. wallacei* Bameul, 1993a (Figs 10A, B)**.** Length of bursa estimated less than 0.2 mm in *Paromicrus scotti* (body length 1.2−1.3 mm), connection between spermathecal duct and bursa anteriorly, spermathecal duct very long and coiled; spermathecal bulb rather regular oval and large; spermathecal gland globular, smaller than spermathecal bulb, duct of spermathecal gland as long as the diameter of spermathecal bulb or slightly longer; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

Genus *Aculomicrus* Smetana, 1990 (Table 1)

* *Aculomicrus brendelli* Bameul, 1993a & * *A. minimus* Smetana, 1975 (cited in Bameul 1993a) (Figs 10D, E)**.** Connection between spermathecal duct and bursa anteriorly; spermathecal duct short; spermathecal bulb large with irregular oval shape; spermathecal gland globular, smaller than spermathecal bulb, duct of spermathecal gland as long as spermathecal duct or longer; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

Remarks: In *A. brendelli* (Fig. 10D), the connection sites of spermathecal duct and gland to spermathecal bulb are very close but no specific explanation is mentioned in Bameul 1993a.

Genus *Psalitrus* Orchymont, 1919 (Table 1)

* *Psalitrus besucheti*,* *P. silvesteris*, * *P. serendibensis*, * *P. loebli*, * *P. decoratus*, * *P. coccinelloides* Bameul, 1992 and * *P. fallax* Balfour-Brown, 1948 (taken from Bameul 1992)**,** (Fig. 10F)**.** Length of bursa estimated 0.3 mm; connection between bursa and spermathecal duct anteriorly; spermathecal duct in *P. decorates*, *P. fallax*, *P. silvesteris* short and in *P. besucheti P. loebli*, *P. serendibensis* moderate; spermathecal bulb with irregular oval shape; duct of spermathecal gland as long as diameter of spermathecal bulb or slightly shorter; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

Genus *Stanmalcolmia* Bameul, 1993a (Table 1)

* *Stanmalcolmia sulawesiensis* Bameul 1993a (Fig. 10C)**.** Spermathecal duct connected to the bursa anteriorly; length of spermathecal duct very long and coiled; spermathecal bulb regular spherical; duct of spermathecal gland as long as diameter of spermathecal bulb or slightly longer; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

Genus *Mircogioton* Orchymont, 1937 (Table 1)

* *Mircogioton grandis* Bameul, 1993b. Bursa with sclerotized spines; spermathecal bulb irregular oval. Other character features are not mentioned in original description or not clear in line drawing.

Tribe Coelostomatini

(Fig. 11, Table 1)

Genus *Dactylosternum* Wollaston, 1854 (Table 1)

* *Dactylosternum abdominale* (Fabricius, 1792)**.** Description is based on a schematic illustration cited in De Marzo (2008).

Male: Unknown.

Female: Connection between spermathecal duct and bursa copulatrix apically; length of spermathecal duct short; spermathecal bulb irregular oval, large; spermathecal gland elongated, approximately as long as the diameter of spermathecal bulb; connection of spermathecal gland and spermathecal ducts to spermathecal bulb unclear in schematic illustration.

Tribe Protosternini

(Fig. 11, Table 1)

Genus *Protosternum* Sharp, 1890 (Table 1)

* *Protosternum abnormale* (Orchymont, 1913), * *P. atomarium* Sharp, 1890 (both cited in Bameul, 1997) and * *P. hammondi*, * *P. newtoni*, * *P. obscurum*, * *P. punctatum* Bameul, 1997. Length of bursa in *P. atomarium* estimated 0.5 mm (body length 1.8 mm) (Bameul, 1997, p. 31); connection between spermathecal duct and bursa in *P. abnormale*, *P. hammondi* and *P. punctatum* anteriorly, in *P. atomarium*, *P. newtoni* and *P. obscurum* medially; length of spermathecal duct short, spermathecal bulb rather spherical, larger than spermathecal gland in *P. punctatum*, rather smaller than that in other species; spermathecal gland in *P. abnormale*, *P. obscurum* and *P. punctatum* elongated, in three other species globular; length of the duct of spermathecal gland as long as the spermathecal bulb in diameter to two times longer; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

Remarks: Bameul (1997) mentioned to chitinous structure of the ectodermal origin of the spermathecal lumen, but no further detail was included.

Tribe Sphaeridiini

(Fig. 11, Table 1)

Genus *Sphaeridium* Fabricius, 1775 (Table 1)

* *Sphaeridium substriatum* Faldermann, 1838. Description is based on a schematic illustration cited in De Marzo (2008).

Male: Unknown.

Female: Connection between spermathecal duct and bursa copulatrix medially; length of spermathecal duct moderate; spermathecal bulb rather spherical and small (no further information is recognizable); spermathecal gland rather irregular oval shape, more than two times longer than the diameter of spermathecal bulb; spermathecal gland and spermathecal ducts connect to spermathecal bulb separately.

Conclusion

Morphological differences of the character features across the studied taxa

In male genital tubes (Figs 1, 3, 7A, 8) (Table 1), the most recognizable morphological variation is the size and structure of the testes and testicles that change through the genera. In *Hydrobius fusciceps* (Fig. 1) testis is larger than the accessory gland (ag2) with thick and tubular testicles loosely clustered; in *Sternolophus* testis is as large as accessory gland (ag2), consist of tubular testicules forming a cluster; in *Hydrochara*, testis is half size of the accessory gland (ag2), testicles small and thin but visible, form a compact cluster; and in *Hydrophilus* testis is almost quarter size of the accessory gland (ag2), testicles are not visible and testis is a compact bean-like structure.

In female genital tubes (Fig. 11, Table 1), the character features are variable in subfamilies down to species level. The connection position between spermathecal duct and bursa is variable at species level. In the tribes with higher number of studied representatives (e.g. Omicrini & Hydrophilini) the anterior connection is more frequent than the medial position. The presence of sclerotized spines inside the membranous wall of the bursa is only observed in *Sternolophus acutipenis*, *Hydrophilus piceus* and *Mircogioton grandis*. The length of the spermathecal duct is diverse at species level. The three features of the length of the spermathecal duct, short, long and very long, do not appear when the duct is connected to the bursa medially whereas all of the mentioned features for the length of the spermathecal duct (short, moderate, long, very long and coiled) appear in anterior position connection.

The morphology of the spermathecal bulb and spermathecal gland and their size ratio remain constant through the species of a subfamily. The exception is Protosternini with globular and elongated spermathecal glands. Regular spherical or oval spermathecal bulbs with small sizes is developed similar in Hydrophilinae, Enochrinae and Acidocerinae (Table 1, Figs 5,6,7, 9, 11); it is more irregular and larger in Sphaeridiinae (Table 1, Fig. 10). Spermathecal gland with tubular and elongated shape remains constant in Hydrophilinae and Acidocerinae while it is more diverse in Sphaeridiinae. The duct of the spermathecal gland in proportion to the size of spermathecal bulb is shorter in Hydrophilinae, Enochrinae and Acidocerinae than Sphaeridiinae and more or less longer in Sphaeridiinae. The separate connection of the spermathecal duct and spermathecal gland with the spermathecal bulb is widely developed feature in studied hydrophilids. The exceptions are *Berosus signaticollis* (Berosisni), *Hydrochara* and *Hydrophilus* species (Hydrophilini) with joined spermathecal gland and spermathecal ducts before connecting to the spermathecal bulb.

CI and RI calculation of *Sternolophus* characters (Table 2).

In phylogenetic study on *Sternolophus* species based on morphologic characters (Nasserzadeh *et al*., 2017), four characters of female internal genitalia were included in a data set consisting of 60 (0–59) morphological characters (52 discrete and 8 continuous). The diagnostic character analysis for the most supported parsimonious tree using PAUP on 52

discrete characters was performed, and the consistency and retention indices were calculated (Table 2). The four female internal genital tube characters were: connection position between bursa copulatrix and spermathecal duct (ch. 56) (CI=0.333, RI=0.750); connection of spermathecal duct and spermathecal gland to the spermathecal bulb (ch. 57) (CI=1.000, RI=0/0); length of spermathecal duct (ch. 58) (CI= 0.500, RI=0.714); and small spines on the membranous wall of the bursa (ch. 59) (CI=1.000, RI=0/0). Contrary to the uninformative characters 57 and 59, characters 56 and 58 strongly supported the best tree. The characters 46–55 are related to the morphology of the aedeagus. Character 53 (ch. 53) with RI=0.857 and 54 (ch. 54) with RI=0.667 are calculated over 0.500 the other eight characters were uninformative or poorly estimated.

Table 2. Consistency and retention indices (CI and RI) calculated for the most supported parsimonious tree of 52 discrete characters in genus *Sternolophus* (Nasserzadeh *et al.*, (2017)) using PAUP. Ten characters (46–55) are related to the morphology of the aedeagus and four characters (56–59) to the female internal genital tube.

Character	CI	RI	Character	CI	RI	Character	CI	RI
8	0.333	0.000	26	1.000	0/0	44	1.000	1.000
9	0.143	0.000	27	1.000	1.000	45	0.500	0.857
10	1.000	0/0	28	0.500	0.750	46	1.000	0/0
11	1.000	1.000	29	1.000	0/0	47	0.250	0.250
12	0.250	0.000	30	0.333	0.667	48	1.000	0/0
13	0.333	0.000	31	0.250	0.250	49	0.250	0.000
14	1.000	0/0	32	0.500	0.000	50	1.000	0/0
15	0.500	0.857	33	1.000	0/0	51	0.333	0.500
16	0.500	0.857	34	1.000	0/0	52	0.333	0.000
17	0.333	0.500	35	0.400	0.500	53	0.667	0.857
18	1.000	1.000	36	0.500	0.000	54	0.500	0.667
19	0.500	0.833	37	0.500	0.800	55	0.333	0.000
20	1.000	1.000	38	0.500	0.000	56	0.333	0.750
21	1.000	1.000	39	0.333	0.000	57	1.000	0/0
22	0.500	0.667	40	0.333	0.333	58	0.500	0.714
23	1.000	0/0	41	0.750	0.000	59	1.000	0/0
24	0.667	0.833	42	1.000	1.000			
25	1.000	0/0	43	1.000	0/0			

Aedeagus characters

Female internal genital tube characters

Discussion

Contrary to the rapid evolve in aedeagus structure and its species-specific features, the study of male internal genital structure suggests a constant similarity at least in generic level. This is in agree with the previous studies (e.g. Dettner *et al.*, 1986; Opitz, 2014), in which the morphological variation of the male internal genital morphology was considered for interpreting evolutionary trends in higher taxa.

In females, the diversification of features is different through the taxa in subfamilies down to species level (Table 1).

The separate connection of the spermathecal duct and spermathecal gland with the spermathecal bulb is highly developed feature (Table 1). Based on the data analysis (Table 2), this is an informative character (ch. 57) because it is the invariant character in *Sternolophus* species. Tubular elongated spermathecal gland with short duct and small regular spherical spermathecal bulb is highly developed in Hydrophilinae and globular small spermathecal gland with long duct in Omicrini both are highly-developed features in their respective species. The irregular shape and large spermathecal bulbs combined with small spermathecal glands with long ducts in Sphaeridiinae within terrestrial species (Hansen, 1991) are significantly different to the other studied groups showing small regular shapes of the spermathecal bulbs connected to mainly elongated and tubular spermathecal glands. Considering Short & Fikáček (2013), the terrestrial lifestyle can be assumed as a reason for such distinct differences when compared to other aquatic subfamilies. The connection position between the spermathecal duct and the bursa copulatrix; and the length of the spermathecal duct are both variable in species level. The latter shows the most variable feature through the studied taxa (Fig. 11, Table 1). Based on the presented data analysis of *Sternolophus* species (Table 2), the last two mentioned characters are also strongly fitted on the most parsimonious tree (characters 56 and 58).

The sclerotized spine inside the membranous wall of the bursa rarely appeared in the studied species and is an autapomorphic character in *Sternolophus* species (RI=0/0; Table 2, ch. 59). Song & Bucheli (2009) by comparing the individual CI and RI as phylogenetic signal indicated that there is no difference between male and female characters in different groups of insects while they also found similar phylogenetic signal between genital and non-genital characters.

The CI and RI values of the *Sternolophus* data set in Table 2 can reveal the relative strong phylogenetic signal in female internal genital character in comparison with male genital (aedeagus) characters. Two out of four female internal genital tube characters (ch. 56 and ch. 58) show strong phylogenetic signals while it is two out of ten characters in males (ch. 53) with RI over 0.50. In both males and females half of the characters were uninformative. The current data analysis yielded stronger phylogenetic signals for the female characters in the data set than the male characters.

The morphological diversity of the female internal genital tubes and tracts across the taxa and analyzing the individual characters in the *Sternolophus* data set highlights the importance of studying the female genital tube morphology in phylogenetic analysis in higher taxa as well as species level in hydrophilid species.

Acknowledgements

My thanks go to the curators of the mentioned museums for their kind cooperation and loaning of material. I heartily thank Drs. A. Komarek (Austria), J. Diaz (Spain), M. Fikáček

(Czech Republic), F. Bameul and O. Montreuil (France) for their precious advises and help when reviewing the paper. Thanks are also due to the Invertebrate Systematic Journal, CSIRO Publishing, Systematic Entomology, John Wiley and Sons Inc., Koleopterologische Rundschau Journal and Dr. F. Bameul (the Author) for granting permission to use material from their publication.

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