**The structure of the male reproductive system
of the genus *Psallus* FIEBER (Hemiptera: Heteroptera: Miridae)**

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ABSTRACT. The paper presents information on the structure of the male reproductive system in representatives of six subgenera of the genus *Psallus*. The research demonstrated that the morphological layout of this system is much the same in all the species examined. There are no differences at the subgeneric level.

KEY WORDS: true bug, male reproductive system, taxonomy, comparative anatomy.

INTRODUCTION

The genus *Psallus* was established by FIEBER (1858) and so far includes around 150 species (KERZHNER & JOSIFOV 1999, YASUNAGA & VINOKUROV 2000).

In Poland 20 species have been identified (GORCZYCA 2007). The species belonging to the genus *Psallus* are traditionally classified into six subgenera (*Apocreminus* FIEBER, *Hylopsallus* WAGNER, *Mesopsallus* WAGNER, *Phylidea* REUTER, *Pityopsallus* WAGNER and *Psallus* FIEBER), distinguished on the basis of the morphological differences between their representatives. The features distinguishing particular subgenera are the following: the different length of particular metatarsal segments, the colouring of the hairs covering the body, the colour of some segments of the antennae and legs. The subgenera also differ in the structure of the male genitalia, but no clear scheme can be established. The taxonomy of these subgenera used to be a subject of much dispute, and there are still a few species in the genus *Psallus* with an unclear subgeneric position (KERZHNER & JOSIFOV 1999). There are many examples of sibling species in the genus *Psallus*; only the males of these are distinguishable, however, on the basis of the aedeagus.

Representatives of the subgenus *Pityopsallus* feed on coniferous trees and are often encountered in the boreal and mountainous parts of the Palaearctic (VINOKUROV 1998). The representatives of the other subgenera are ecologically associated mostly with deciduous trees and bushes.

The comparative anatomy of true bugs has been the subject of numerous papers, which have made a valuable contribution to modern taxonomic analyses (KULLENBERG 1947, CARAYON 1950, WOODWARD 1950, PENDERGRAST 1957, LESTON 1961, KUMAR 1967, MALIPATIL 1978, AKINGBOHUNGBE 1983, GROZEVA & KUZNETSOVA 1992, MRÓZ 2007, MRÓZ & WOJCIECHOWSKI 2011).

Research based on the features of reproductive system morphology useful for taxonomic analyses has been conducted in Heteroptera by PENDERGRAST (1957), LESTON (1961), AKINGBOHUNGBE (1983), GROZEVA & KUZNETSOVA (1992), MRÓZ (2007) and MRÓZ & WOJCIECHOWSKI (2011). These studies have shown that, on the basis of reproductive system morphology, some evolutionary tendencies within individual phylogenetic lineages of Heteroptera can be perceived. The number and arrangement of follicles, as well as the number, origin and structure of accessory glands are the features most commonly taken into consideration. However, this kind of anatomical research has not been carried out in order to solve the taxonomic status of subgenera and evolutionary tendencies within the genus *Psallus*.

The aim of this research was to determine whether, on the basis of an examination of anatomical structures, it would be possible to re-evaluate the relationships within the genus *Psallus*. For this purpose the structures of the male reproductive system were thoroughly analysed.

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MATERIALS AND METHODS

Male individuals of eight species, representatives of all six subgenera in the genus *Psallus*, were analysed.

Material examined

Apocremnus:

P. (A.) betuleti (FALLÉN, 1807) – Sosnowiec-Zagórze, 29.05.2006, 3 males, Sosnowiec-Środula, 14.06.2007, 3 males, 10.06. 2009, 4 males, leg. E. Mróz. Collected from *Betula* sp.

***Hylopsallus*:**

P. (H.) perrisi (MULSANT et REY, 1852) – Dąbrowa Górnicza – Park Zielona, 2.06.2005, 3 males, 20.05.2006, 3 males, 10.06.2006, 3 males, leg. D. Chłond and E. Mróz. Collected from *Quercus* sp.

P. (H.) wagneri (OSSIANNILSSON, 1953) – Dąbrowa Górnicza – Park Zielona, 10.06.2006, 2 males, leg. D. Chłond and E. Mróz. Collected from *Quercus* sp.

P. (H.) variabilis (FALLÉN, 1807) – Dąbrowa Górnicza – Park Zielona, 2.06.2005, 4 males, leg. D. Chłond and E. Mróz, Ojców National Park, 14.06.2005, 2 males, leg. D. Chłond, Sosnowiec-Zagórze, 14.06.2006 (5), leg. E. Mróz. Collected from *Quercus* sp.

***Mesopsallus*:**

P. (M.) ambiguus (FALLÉN, 1807) – Dąbrowa Górnicza – Park Zielona, 2.06.2005, 2 males, 3.07.2009, 2 males, leg. D. Chłond and E. Mróz, Ojców National Park, 14.06.2005, 1 males, 27.06.2006, 3 males, leg. D. Chłond. Collected from *Alnus incana* MOENCH.

***Phylidea*:**

P. (Ph.) quercus (KIRSCHBAUM, 1856) – Dąbrowa Górnicza – Park Zielona, 2.06.2005, 2 males, leg. D. Chłond and E. Mróz. Collected from *Quercus robur* L.

***Pityopsallus*:**

P. (P.) vittatus REUTER, 1883 – Sosnowiec-Zagórze, 3.08.2006, 2 males, 15.08.2007, 3 males, leg. E. Mróz, Zakopane – Kościelisko 2.07.2007, 3 males, leg. E. Mróz, Katowice – Park Kościuszki 29.08.2009, 1 males, leg. D. Chłond and E. Mróz. Collected from *Larix decidua* MILL.

***Psallus*:**

P. (P.) falleni REUTER, 1883 – Sosnowiec-Śródula 29.07.2006, 4 males, 14.06.2007, 5 males, and 10.06.2009, 4 males, leg. E. Mróz. Collected from *Betula* sp.

The entire male reproductive system of each of these specimens were mounted in a drop of glycerine; in addition, histological slides were prepared for 5 species: *P. (M.) ambiguus* (FALLÉN, 1807), *P. (H.) variabilis* (FALLÉN, 1807), *P. (P.) falleni* REUTER, 1883, *P. (A.) betuleti* (FALLÉN, 1807), *P. (P.) vittatus* REUTER, 1883).

In order to facilitate the anatomical analysis, the material was preserved in Carnoy liquid (glacial acetic acid + absolute alcohol) for 20-30 minutes and then transferred to 70% ethyl alcohol. Together with standard histological methods, the paraffin method was used to prepare the specimens. These were stained using Erlich acid haematoxylin, oxygenated under running water and differentiated with 0.5% xyridine ponceau (in 1% acetic acid) (BAGIŃSKI 1969).

Flat reconstructions (cross-sections) of the reproductive system were prepared from the microscopic slides. Longitudinal sections and total specimens of the male reproductive system of the species were also prepared.

Digital images were obtained with a DN-100 camera installed in a Nikon Eclipse-E600

light microscope. The measurements were performed using Lucia net software, and the drawings of the male reproductive system were made with the aid of an Olympus SZX9 stereo microscope with a 7x zoom.

RESULTS

The anatomical analysis showed that the structure of the male reproductive system in all the species of the genus under study represented a common scheme. The measurements of the various structures are listed in the Table.

Table. Measurements of the male reproductive system structures in some species of *Psallus* (all values in mm).

		<i>P. betuleti</i>	<i>P. variabilis</i>	<i>P. perrisi</i>	<i>P. wagneri</i>	<i>P. ambiguus</i>	<i>P. quercus</i>	<i>P. vittatus</i>	<i>P. falleni</i>
Testis	Length of testis	0.48 – 0.68	0.44 – 0.63	0.45 – 0.58	0.45 – 0.52	0.47 – 0.64	0.42 – 0.46	0.4 – 0.6	0.45 – 0.58
	Width of testis	0.3 – 0.45	0.28 – 0.42	0.29 – 0.39	0.28 – 0.36	0.3 – 0.46	0.27 – 0.38	0.27 – 0.39	0.28 – 0.4
	Diameter of testicular follicles	0.12 – 0.14	0.12 – 0.14	0.12 – 0.13	0.12 – 0.13	0.1 – 0.13	0.1 – 0.12	0.11 – 0.12	0.09 – 0.11
Vas deferens	Length of vas deferens	0.7 – 0.8	0.6 – 0.7	0.59 – 0.7	0.6 – 0.68	0.6 – 0.71	0.6 – 0.7	0.58 – 0.7	0.6 – 0.7
	Width of vas deferens	~0.03	~0.03	~0.03	~0.03	~0.03	~0.03	~0.03	~0.025
	Diameter of seminal vesicles	0.07 – 0.08	0.07	0.06 – 0.07	0.06 – 0.07	0.06 – 0.07	0.07	0.06 – 0.07	0.05 – 0.7
	Diameter of common vas deferens	0.07 – 0.09	0.07 – 0.09	0.07 – 0.09	0.07 – 0.09	0.07 – 0.09	0.07 – 0.08	0.07 – 0.09	0.07 – 0.09
Ectodermal internal lateral glands	Total length	1.5 – 1.85	1.16 – 1.6	1.4 – 1.7	1.2 – 1.43	1.3 – 1.68	1.32 – 1.41	1.1 – 1.6	1.2 – 1.52
	Length of ascending arm	1.15 – 1.4	0.96 – 1.3	1.13 – 1.32	0.95 – 1.1	1.03 – 1.35	1.1 – 1.15	0.8 – 1.22	0.9 – 1.32

	Width of narrow part of ascending arm	0.06 – 0.08	0.05 – 0.07	0.05 – 0.06	0.05	0.05 – 0.07	0.06	0.05 – 0.06	0.05 – 0.07
	Length of expanded part of ascending arm	0.37 – 0.5	0.29 – 0.4	0.3 – 0.4	0.27 – 0.32	0.32 – 0.42	0.33 – 0.35	0.3 – 0.33	0.28 – 0.41
	Width of expanded part of ascending arm	0.09 – 0.12	0.08 – 0.1	0.09 – 0.1	0.07 – 0.08	0.08 – 0.11	0.09 – 0.1	0.08 – 0.09	0.09 – 0.1
	Length of expanded part of descending arm	0.3 – 0.4	0.2 – 0.3	0.23 – 0.34	0.2 – 0.24	0.23 – 0.3	0.22 – 0.25	0.27 – 0.35	0.19 – 0.28
	Width of expanded part of descending arm	0.05 – 0.65	0.04 – 0.06	0.03 – 0.05	0.2 – 0.25	0.04 – 0.06	0.05	0.05 – 0.06	0.05 – 0.06
Ectodermal external lateral glands	Total length	1.8 – 2.6	1.5 – 2.32	1.71 – 2.06	1.65 – 1.8	1.8 – 2.4	1.86 – 2.16	1.4 – 2.1	1.4 – 2.4
	Length of ascending arm	1.05 – 1.03	0.8 – 1.24	1.01 – 1.19	0.9 – 1.05	0.96 – 1.3	1.03 – 1.01	0.75 – 1.18	0.7 – 1.13
	Width of ascending arm	0.14 – 0.18	0.12 – 0.15	0.13 – 0.15	0.11 – 0.12	0.12 – 0.16	0.14 – 0.15	0.12 – 0.14	0.13 – 0.15
	Length of descending arm	0.8 – 0.98	0.6 – 0.9	0.68 – 0.8	0.67 – 0.73	0.68 – 0.9	0.69 – 0.75	0.53 – 0.76	0.55 – 0.96
	Width of descending arm	0.1 – 0.15	0.1 – 0.12	0.09 – 0.12	0.09 – 0.11	0.1 – 0.13	0.1 – 0.12	0.09 – 0.11	0.1 – 0.12

	Length of the part of descending arm additionally curved upwards	0.18–0.3	0.13–0.18	0.14–0.17	0.1–0.11	0.16–0.2	0.16–0.17	0.09–0.15	0.15–0.18
Mesodermal dorsal medial gland	Diameter	0.04 x 0.02	0.04 x 0.01	-	-	0.04 x 0.02	-	0.04 x 0.01	0.04 x 0.02
Mesodermal ventral medial gland	Diameter	0.05 x 0.06	0.03 x 0.04	-	-	0.05 x 0.06	-	0.03 x 0.04	0.05 x 0.06
Ejaculatory bulb	Length	0.25	0.22	0.23	0.22	0.23	0.24	0.23	0.2
	Width	0.26	0.24	0.24	0.24	0.22	0.23	0.24	0.21

The whole reproductive system is contained in the genital capsule.

The testes are situated on the sides, outside the lateral glands. They consist of testicular follicles, which are internally divided into three regions, according to the development of the germ cells.

The three testicular follicles are identical in all species (Figs 3-7). The wall of each follicle is derived from peritoneal epithelium. Each follicle tapers in its basal part to form a short vas efferens. The vasa efferentia of all the testicular follicles of the same testis lead into the widened part of the vas deferens. The follicles are elongate and run rosette-like into the vas deferens, which leads out of the middle part of the testis. Although the individual follicles are appressed, they are never joined. All the follicles are enclosed in a common peritoneal membrane.

The peritoneal membrane is thin and translucent: the follicles can be seen through it. The borders between individual follicles are clearly visible in the external view.

The independently running vasa deferentia expand in the proximal part to form seminal vesicles, providing a protective space for mature spermatozoa. The diameter of this part of the vas deferens varies, depending on the physiological state of the insect. The vasa deferentia of newly moulted adult males are always straight and the seminal vesicles are invisible. In the distal part the vasa deferentia expand, forming seminal vesicles (Fig. 10).

The distal seminal reservoirs are interconnected to form the common vas deferens, i.e. the so-called seminal duct (Figs 11-12) opening into the ejaculatory bulb.

Great diversity is typical of the genus *Psallus* as far as the number and kind of accessory glands are concerned. Within the accessory glands there are two single mesodermal glands and two pairs of ectodermal ones.

The ectodermal accessory glands are of a considerable size and constitute the largest part of the reproductive system of this species (Figs 1-2, 8-13). They are described below in detail.

The pair of external lateral glands: their size varies and depends on the age and the physiological condition of the insect. In freshly eclosed specimens they are rather small and empty. These glands are never divided into a number of separate parts. They run along the longitudinal axis of the body, and their proximal parts, bent at an acute angle, point towards the end of the abdomen. Thus, their ascending and descending arms can be distinguished. The descending arm of the gland is additionally bent upwards, forming loops (Figs 1-2). The diameter of the descending arm is smaller than that of the ascending arm.

The pair of internal lateral glands: their length is less variable than that of the external glands. In the species analysed here, these glands are divided into 3 parts, a characteristic of the genus under investigation (Figs 1-2). The two external parts – proximal and distal – are rather short. The long middle part is bent, changing from an ascending arm into a descending one. Both glands open into the anterior lateral part of the ejaculatory bulb; the internal glands are positioned proximally in relation to the external glands (Fig. 13).

The glands, which are small in size and therefore become visible only after the preparation of the histological slides, are mesodermal glands (Fig. 13).

The ventral medial gland is situated between the internal lateral glands (Fig. 11).

The dorsal medial gland is located between the vasa deferentia at the point where they become fused (Fig. 12). It opens into the ejaculatory bulb in its frontal part, above the outlet of the joint vasa deferentia.

Over their entire length the vasa deferentia and accessory glands run independently and their endings open into the ejaculatory bulb. This is the terminal section of the male reproductive system and is formed by the ducts that open into it. The ducts opening into the ejaculatory reservoir constitute parts of its wall (Fig. 13). The bulb is connected with the seminal duct of the aedeagus.

Figs 1-7. Male reproductive system of *Psallus*. 1-2. *P. (H.) variabilis*: 1 – Diagram of the male reproductive system, 2 – Total preparation, light microscopy (40x). 3-7. Fragments of cross-sections through the testis: 3 – *P. (P.) falleni*, 4 – *P. (H.) variabilis*, 5 – *P. (M.) ambiguus*, 6 – *P. (A.) betuleti*, 7 – *P. (P.) vittatus*. The testicular follicles (f.t.) making up the testis are visible. Haematoxylin + xylydine ponceau light microscopy (100x).

t. – testis, v.d. – vas deferens, v.s. – seminal vesicles, g.l.e. – external lateral gland, g.l.i. – internal lateral gland, b.e. – ejaculatory bulb.

Figs 8-13. Fragments of cross-sections through the male reproductive system in some representatives of *Psallus*: 8 – *P. (P.) falleni* – fragment of cross-section through proximal part of ectodermal accessory glands (g.l.e., g.l.i.) (100x), 9 – *P. (P.) falleni* – fragment of cross-section through proximal part of vasa deferentia (v.d.) and ectodermal accessory glands (g.l.e., g.l.i.), (100x), 10-12 – *P. (P.) falleni* – fragment of cross-section through distal part of vasa deferentia (v.d.) and accessory glands (g.l.e., g.l.i., g.m.d., g.m.v.). Cross-section of seminal vesicles (v.s.) visible (100x), 13 – *P. (P.) vittatus* – cross-section through male reproductive system at the point where the distal parts of the system come together to form the walls of the ejaculatory bulb (b.e.) (100x).

v.d. – vas deferens, v.s. – seminal vesicles, d.s. – seminal duct, g.l.e. – external lateral gland, g.l.i. – internal lateral gland, g.m.d. – dorsal medial gland, g.m.v. – ventral medial gland, b.e. – ejaculatory bulb. Haematoxylin + xylydine ponceau, light microscopy.

DISCUSSION

The whole male reproductive system of the *Psallus* species analysed here is included in the genital capsule. In contrast, in many other studied species, e.g. *Leptopterna dolabrata* (LINNAEUS, 1758), *Adelphocoris lineolatus* (GOEZE, 1778) and *Phytocoris ulmi* (LINNAEUS, 1758) it is much longer and extends to the first abdominal segments (BONHAG & WICK 1953, KULLENBERG 1947, MRÓZ 2007, MRÓZ & WOJCIECHOWSKI 2011).

The testes are located on the outside of the external lateral glands, as in species belonging to such genera as *Adelphocoris* (MASNER 1965) and *Callocoris* (MRÓZ 2007), but unlike *Oncopeltus* (BONHAG & WICK 1953), *Phytocoris* (KULLENBERG 1947), *Stenodema*, *Leptopterna* and *Notostira* (MRÓZ 2007, MRÓZ & WOJCIECHOWSKI 2011), where the testes are located above the ectodermal glands.

The vasa deferentia, running independently in their proximal part, expand to form seminal vesicles, as in the species of the Stenodemini and Mirini tribes already described (KULLENBERG 1947, MASNER 1965, MRÓZ 2007, MRÓZ & WOJCIECHOWSKI 2011).

Two pairs of ectodermal glands of a considerable size, constituting the main part of the reproductive system, together with two small mesodermal glands, were observed in the *Psallus* species studied here, as in other representatives of Miridae (KULLENBERG 1947, MASNER 1965).

Two pairs of ectodermal glands were found in these *Psallus* species, as in other representatives of Miridae (KULLENBERG 1947, MASNER 1965). These glands are large and make up the main part of the reproductive system. Two smaller mesodermal glands were also observed.

The internal ectodermal glands have a very characteristic morphology, as they are divided into three distinctive parts. The first and third parts are relatively short. In comparison to other representatives of the Miridae family, these glands may be uniform, as in *Notostira* (MRÓZ 2007, MRÓZ & WOJCIECHOWSKI 2011), or clearly subdivided into two parts, as in *Adelphocoris* (MASNER 1965), *Phytocoris* (KULLENBERG 1947) or *Trigonotylus* (MRÓZ 2007, MRÓZ & WOJCIECHOWSKI 2011). Within the genus *Psallus* the proximal parts of the internal glands are situated above the external glands.

It can be inferred from the results that the morphology of the male reproductive system in the genus *Psallus* has a uniform set of characteristics and that there are similarities among the species of this genus. The differences concern mainly the placement of individual elements, which ensues from the shape of the abdomen. Therefore, for example, the elements of the reproductive system of *Psallus falleni* are slimmer than their counterparts in that of *Psallus ambiguus*, which extend farther to the sides.

No essential differences have been observed in the morphology of this system among species belonging to different subgenera within the genus *Psallus*, but there are significant

differences in the structure of the male reproductive system in the genus *Stenodema*, which corresponds to the division of this taxon into two subgenera (MRÓZ 2007, MRÓZ & WOJCIECHOWSKI 2011).

All the species analysed have the same number of testicular follicles, identically built accessory glands, as well as very characteristic internal lateral glands. The results of this analysis confirm that the inclusion of all the species analysed here in the same genus is justified. However, the anatomical structure of the male reproductive system cannot serve to resolve problems concerning the taxonomic status of subgenera within the genus *Psallus*.

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