

ORIGINAL ARTICLE

Comparative morphological study of female internal reproductive system among nine species in the genus *Tipula* (Diptera: Tipulidae)

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Abstract Female internal reproductive systems of nine species in the genus *Tipula* are described and illustrated, namely *T. (Beringotipula) amurensis* Alexander, 1925, *T. (Lunatipula) lunata* Linnaeus, 1758, *T. (Lunatipula) submanca* Savchenko, 1964, *T. (Pterelachisus) biaciculifera* Alexander, 1937, *T. (Sivatipula) parvaauricula* Alexander, 1941, *T. (Vestiplex) longitudinalis* Nielsen, 1929, *T. (Vestiplex) sternotuberculata* Alexander, 1935, *T. (Yamatotipula) couckeii* Tonnoir, 1921 and *T. (Yamatotipula) marginella* Theowald, 1980. The morphological differences of internal reproductive organs for these species are listed. Additionally, taxonomic potential of some internal reproductive structures are analyzed and discussed.

Key words *Tipula*, bursa copulatrix, spermatheca, accessory gland, anatomical study.

1 Introduction

The Tipulidae is a big family of the order Diptera with about 4413 species, distributed in all zoogeographical regions and survived in various habits (Oosterbroek, 2019). In China, the family includes about 570 species based on plenty of taxonomic researches over the past century (Oosterbroek, 2019).

Anatomical studies of the insect genitalia are the basic prerequisite to understand their function and the foundation for further physiological research (Wensler & Rempel, 1962). They can answer the questions about the co-evolution of anatomy, mating the behavior and the fertilization mechanisms (Wortham-Neal, 2002), and offer an important tool for the phylogenetic analysis in different taxonomic levels (Quicke *et al.*, 1992; Chaboo, 2007). However, contrasting with the constant attention to external morphological differences of genital structures, the internal reproductive systems were frequently ignored, even though they displayed great morphological diversity.

The internal reproductive system was poorly studied in the family Tipulidae, except several genera, such as *Dolichopeza* Curtis, 1825, *Holorusia* Loew, 1863, *Nephrotoma* Meigen, 1803, *Tipula* Linnaeus, 1758 and *Tipulodina* Enderlein, 1912 (Neuman, 1958; Byers, 1961; Frommer, 1963; Tjeder, 1979a, b; Tangelder, 1985; Ji *et al.*, 2014; Men *et al.*, 2015, 2018a, b; Xue *et al.*, 2019). The amount of researches have been done poorly reveals the morphological diversity of internal reproductive system.

In this study, nine species of the genus *Tipula* were dissected for their female internal reproductive systems: *T. (Beringotipula) amurensis* Alexander, 1925, *T. (Lunatipula) lunata* Linnaeus, 1758, *T. (Lunatipula) submanca* Savchenko, 1964, *T. (Pterelachisus) biaciculifera* Alexander, 1937, *T. (Sivatipula) parvaauricula* Alexander, 1941, *T. (Vestiplex)*

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longitudinalis Nielsen, 1929, *T. (Vestiplex) sternotuberculata* Alexander, 1935, *T. (Yamatotipula) couckeii* Tonnoir, 1921 and *T. (Yamatotipula) marginella* Theowald, 1980. Their morphological differences are compared and listed.

2 Materials and methods

2.1 Insects

Samples used in present study and their information are listed in the Table 1. All fresh samples were stored in 80% ethyl alcohol.

Table 1. Species of *Tipula* used in the present study and their collecting information.

Species	Collecting information	NOS*
<i>T. (Beringotipula) amurensis</i>	MONGOLIA: Hovsgol Aimag Turaggot-River, 2011.07.17. coll. Sigita Podenas	2
<i>T. (Lunatipula) submanca</i>	MONGOLIA: Khentu Aimag, Binder Soum Khurkh Gol, 2011.07.17. coll. Sigita Podenas	2
<i>T. (Lunatipula) lunata</i>	MONGOLIA: Hovsgol Aimag Turaggot-River, 2011.07.17. coll. Sigita Podenas	3
<i>T. (Pterelachisus) biaciculifera</i>	CHINA: Yaoluoping National Nature Reserve, Anhui, 2016.08.22. coll. Qiulei Men	3
<i>T. (Sivatipula) parvauricula</i>	CHINA: Wuyishan National Nature Reserve, Fujian, 2017.05.19. coll. Qiulei Men	3
<i>T. (Vestiplex) sternotuberculata</i>	CHINA: Leigongshan National Nature Reserve, Guizhou, 2015.06.01. coll. Guoxi Xue	4
<i>T. (Vestiplex) longitudinalis</i>	MONGOLIA: Khentu Aimag, Binder Soum Khurkh Gol, 2011.07.17. coll. Sigita Podenas	2
<i>T. (Yamatotipula) marginella</i>	SWITZERLAND: Rottenschwil, 379m, 1980.05.24. coll. C. Dufour	2
<i>T. (Yamatotipula) couckeii</i>	LITHUANIA: Varena district, Sktublus river delta, 1994.05.15. coll. L. Vaiciulyte	2

*Number of samples

2.2 Dissection

The internal reproductive systems were dissected in water, and then examined and drawn using Leica MZ125 stereomicroscope (Leica, Germany). Dissection was made with the aid of two very fine needles, scissors and fine-tipped tweezers. For the description of joints between spermathecal duct and bursa copulatrix, the direction of head was defined as front. The terminology and methods of description and illustration follow that of Frommer (1963) and Alexander & Byers (1981).

The abbreviations of the internal reproductive systems are used as following.

- AG—accessory gland;
- BC—bursa copulatrix;
- S—sternite;
- SP—spermatheca;
- SPD—spermathecal duct;
- VA—vaginal apodeme.

3 Result

3.1 The female internal reproductive system

3.1.1 *Tipula (Beringotipula) Savchenko, 1961*

Tipula (Beringotipula) amurensis Alexander, 1925 (Fig. 1)

Description. Bursa copulatrix stout, basal half distinctly broader than apical half. Spermatheca three, spherical, 0.20 mm in diameter, with a lateral cap. Three spermathecal ducts distinctly longer and narrower than bursa copulatrix, arising from same level, lateral ducts distinctly longer than central one. Accessory gland irregular sac-like, swollen, connected to each other by a short stem which is connected to base of bursa copulatrix (Fig. 1).

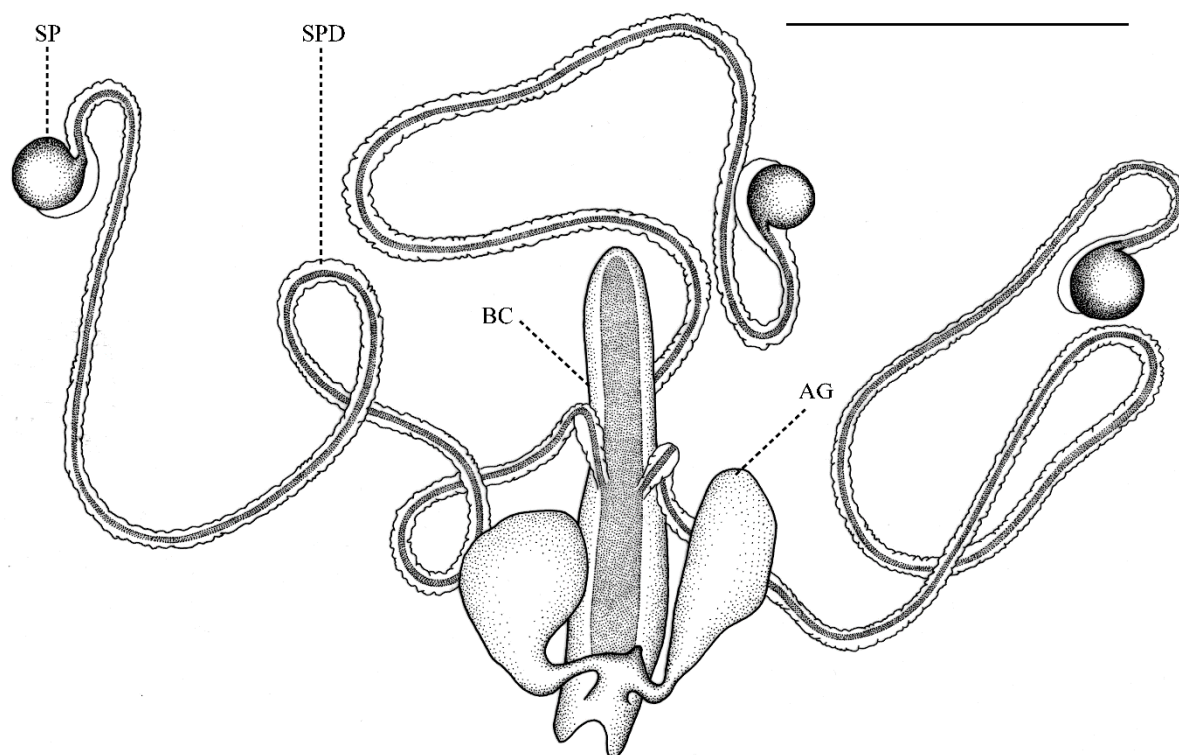


Figure 1. Female internal reproductive system of *T. (B.) amurensis*. Scale bar = 1.0 mm.

3.1.2 *Tipula (Lunatipula)* Edwards, 1931

Tipula (Lunatipula) lunata Linnaeus, 1758 (Fig. 2)

Description. Bursa copulatrix very narrow and elongated, rounded apically (Fig. 2A). Spermatheca three, pear-shaped, 0.20 mm at widest point. Spermathecal ducts distinctly shorter than bursa copulatrix, not arising from same level, lateral ducts arising front of central duct (Figs 2A–B). Accessory gland being elongated sac, swollen, connected to each other by a long stem (Fig. 2C). A special structure generated from base of bursa copulatrix, characterized as follows: swollen in general, terminating cephalad in a thin lobe which truncated apically, with a small hole medially, in which common stem of accessory gland generated, with a concaved region medially in lateral view (Figs 2A, D–F). Vaginal apodeme broad basally, narrowed to a sharp terminal (Figs 2A, F).

Tipula (Lunatipula) submanca Savchenko, 1964 (Fig. 3)

Description. Bursa copulatrix narrow, base slightly broad, gradually narrowed to tip, rounded apically. Spermatheca three, pear-shaped, width 0.25 mm, length 0.30 mm. Spermathecal ducts not arising from same level, with lateral pair distinctly longer than bursa copulatrix, with central duct distinctly shorter than bursa copulatrix, central duct arising front of lateral ducts. Accessory gland being round sac, swollen, connected to each other by a short stem which connected to base of bursa copulatrix (Fig. 3).

Remarks. In the two species of the subgenus *Tipula (Lunatipula)*, their bursas copulatrix are generally long and narrow despite of their various lengths in different species, and the three spermathecal ducts of both species are not arising from the same level of bursa copulatrix. Additionally, a special structure is present at the base of bursa copulatrix in *Tipula (Lunatipula) lunata*, which is not described before in the family Tipulidae.

3.1.3 *Tipula (Pterelachisus)* Rondani, 1842

Tipula (Pterelachisus) biaciculifera Alexander, 1937 (Fig. 4)

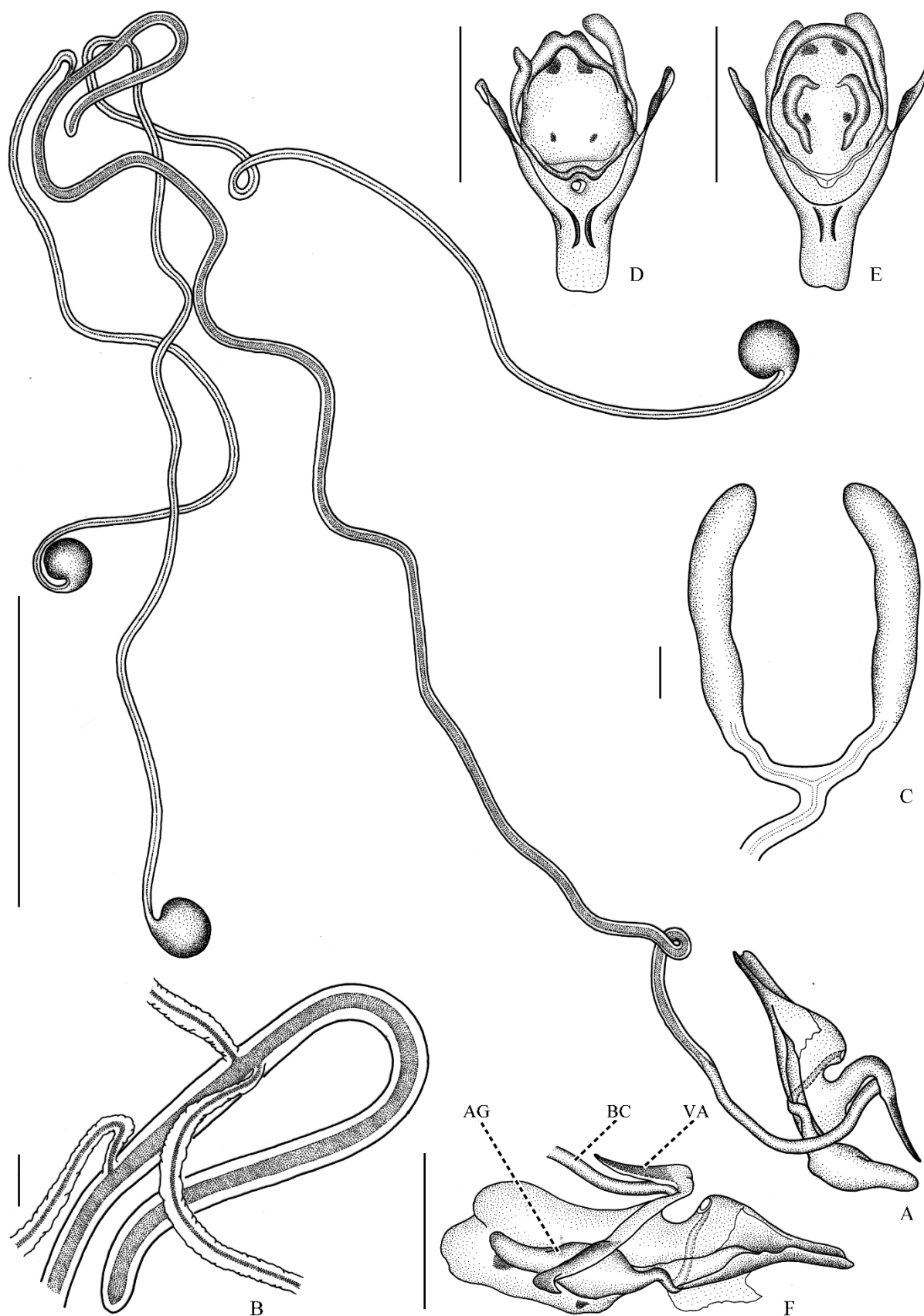


Figure 2. Female internal reproductive system of *T. (L.) lunata*. A. Internal reproductive system. B. Joints between spermatheca and bursa copulatrix. C. Accessory glands. D. Special structure of bursa copulatrix, dorsal view. E. Special structure of bursa copulatrix, ventral view. F. Special structure of bursa copulatrix, lateral view. Scale bars: A, D–F=1.0 mm; B–C=0.5 mm.

Description. Bursa copulatrix straight and stout, basal 1/5 slightly broadened. Spermatheca three, spherical, 0.25 mm in diameter. Spermathecal ducts distinctly longer and narrower than bursa copulatrix, not arising from same level, lateral ducts same length to central duct, lateral ducts arising front of central one. Accessory gland spindle-shaped, swollen, connected to each other by an elongated tube, terminated to a long stem which connected to base of bursa copulatrix (Fig. 4).

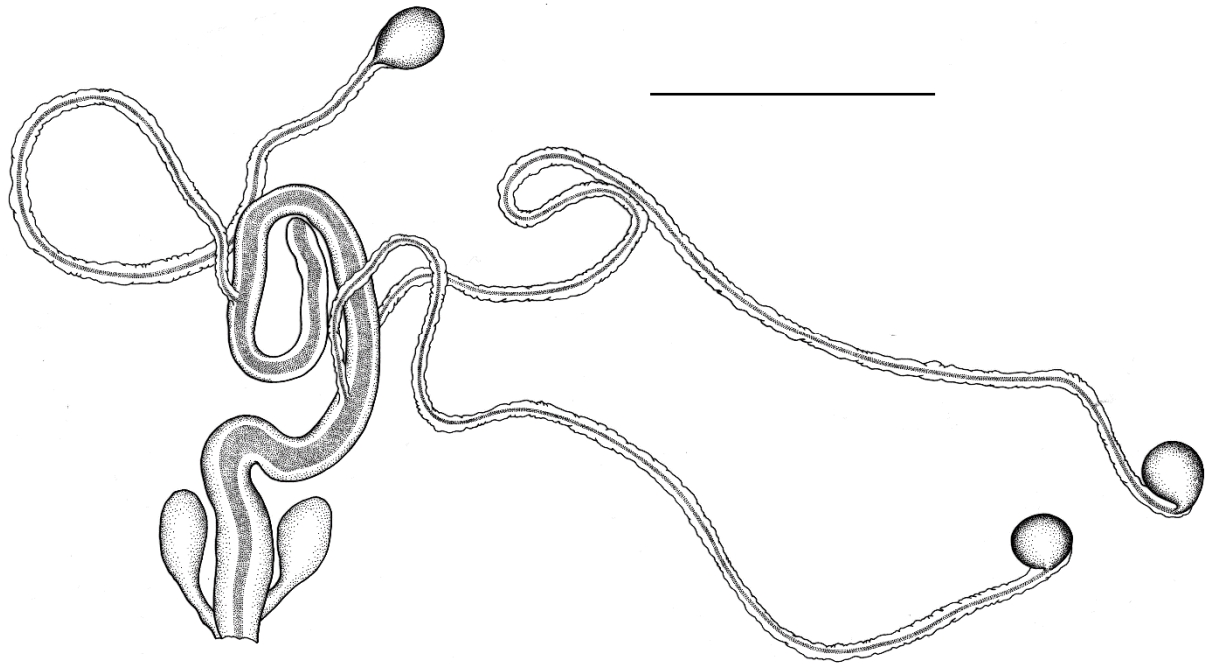


Figure 3. Female internal reproductive system of *T. (L.) submanca*. Scale bar=1.0 mm.

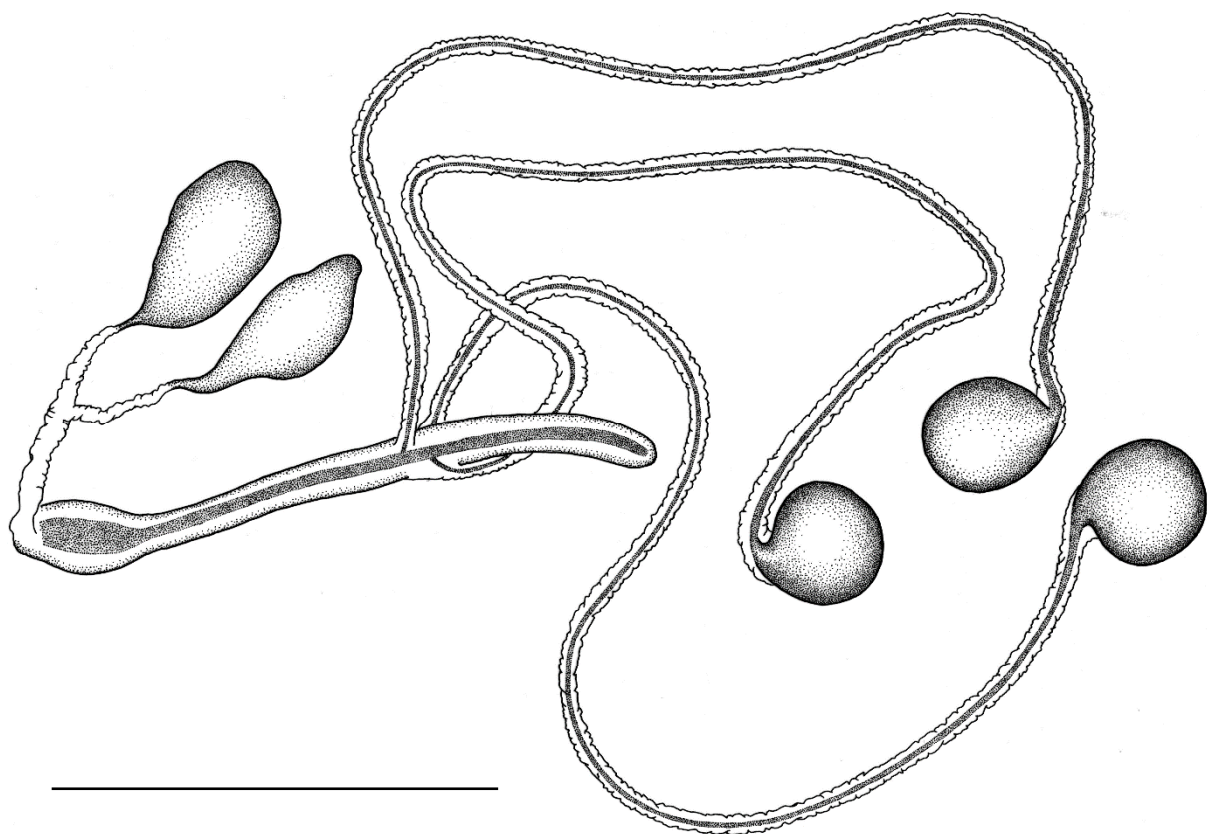


Figure 4. Female internal reproductive system of *T. (P.) biaciculifera*. Scale bar=1.0 mm.

3.1.4 *Tipula (Sivatipula) Alexander, 1964*

Tipula (Sivatipula) parvaurecula Alexander, 1941 (Fig. 5)

Description. Bursa copulatrix flexible, narrow and elongated, slightly broadened at base. Spermatheca three, spherical, 0.40 mm in diameter. Spermathecal ducts distinctly shorter than bursa copulatrix, same width to bursa copulatrix, not arising from same level, lateral ducts distinctly longer than central one, lateral ducts arising front of central one. Spermathecal ducts without outer layer near apex, inner tube exposed apically. Accessory gland spindle-shaped, swollen, connected to each other by a short stem (Fig. 5).

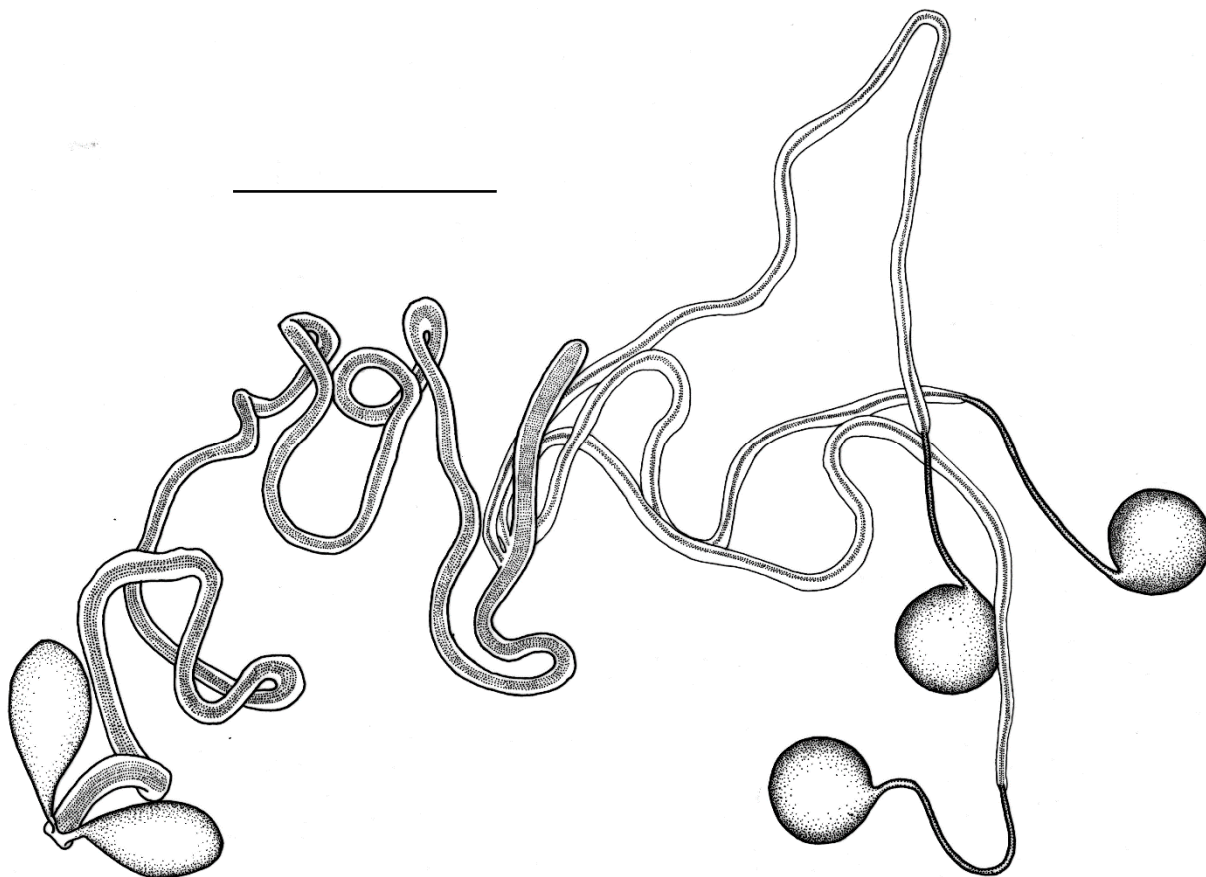


Figure 5. Female internal reproductive system of *T. (S.) parvaurecula*. Scale bar = 1.0 mm.

3.1.5 *Tipula (Vestiplex) Bezzi, 1924*

Tipula (Vestiplex) longitudinalis Nielsen, 1929 (Fig. 6)

Description. Bursa copulatrix stout and relatively short. Spermatheca three, pear-shaped, laterals (width 0.20 mm, length 0.25 mm) smaller than central one (width 0.35 mm, length 0.45 mm). Spermathecal ducts significantly narrower than bursa copulatrix, arising from same level, lateral ducts distinctly longer than central one which almost same length as bursa copulatrix. Accessory gland being irregular long sac, subequal in length to bursa copulatrix, narrowed basally, swollen apically, connected to each other by a short stem (Fig. 6).

Tipula (Vestiplex) sternotuberculata Alexander, 1935 (Fig. 7)

Description. Bursa copulatrix straight and elongated, narrowed medially. Spermatheca three, pear-shaped, laterals (width 0.20 mm, length 0.30 mm) smaller than central one (width 0.25 mm, length 0.35 mm). Spermathecal ducts slightly narrower than bursa copulatrix, not arising from same level, lateral ducts distinctly longer than central one. Lateral ducts

same length as bursa copulatrix, but central duct shorter, lateral ducts arising front of a single tube. Accessory gland being elongated sac, swollen, connected to each other by narrow tube, terminating in an elongated stem which broad basally and gradually narrowed to apex, subequal in length to accessory gland, connected to base of bursa copulatrix (Fig. 7).

Remarks. In the two species of the subgenus *Tipula* (*Vestiplex*), their accessory glands are sac-like and elongated. We also observed that the middle spermatheca is bigger than the lateral spermathecae in both species.

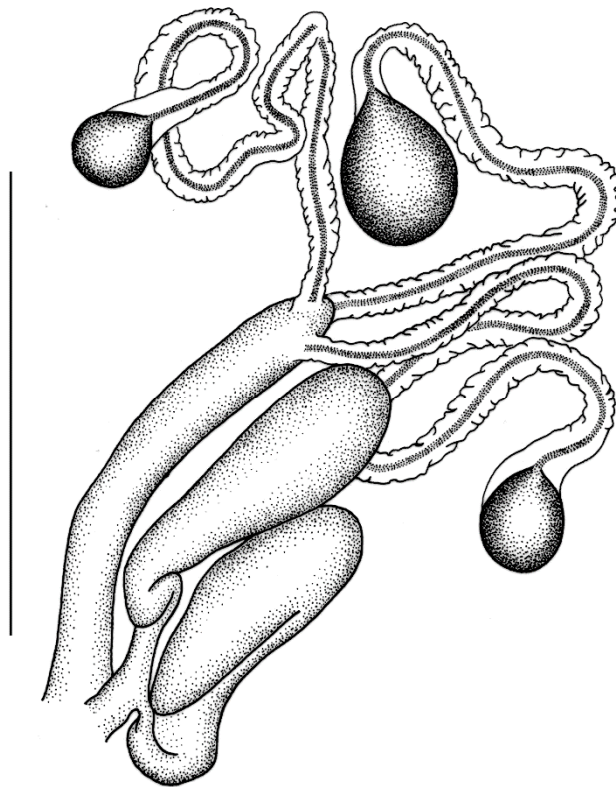


Figure 6. Female internal reproductive system of *T. (V.) longitudinalis*. Scale bar = 1.0 mm.

3.1.6 *Tipula* (*Yamatotipula*) Matsumura, 1916

Tipula (*Yamatotipula*) *couckeii* Tonnoir, 1921 (Fig. 8)

Description. Bursa copulatrix relatively narrow and short, gradually broadened at apical third, terminal blunt. Spermathecal ducts narrowed at basal half, swollen at apical half. Spermatheca three, spherical, relatively big, 0.45 mm in diameter. Spermathecal ducts arising from same level, with lateral pair distinctly shorter than bursa copulatrix, with central duct subequal in length to bursa copulatrix. Accessory gland being elongated sac, almost same length as bursa copulatrix, swollen, without common stem or very short if stem present (Fig. 8).

Tipula (*Yamatotipula*) *marginella* Theowald, 1980 (Fig. 9)

Description. Bursa copulatrix sinuate and elongated, basal one fourth distinctly broad, gradually narrowed to tip. Spermatheca three, pear-shaped, width 0.25 mm, length 0.35 mm. Spermathecal ducts same length, distinctly shorter and narrower than bursa copulatrix, not arising from same level, central duct arising front of lateral ducts. Accessory gland spindle-shaped, swollen, strongly curved, connected to each other by a short stem (Fig. 9A). Sternite nine trapeziform, with two pairs of arms extended cephalad and caudalad respectively (Fig. 9A). Vaginal apodeme broad basally to form a triangular plate, and then abruptly narrowed to form a narrowed rod (Fig. 9B), tip sharply acute which is curved directed ventrad in lateral view (Fig. 9C).

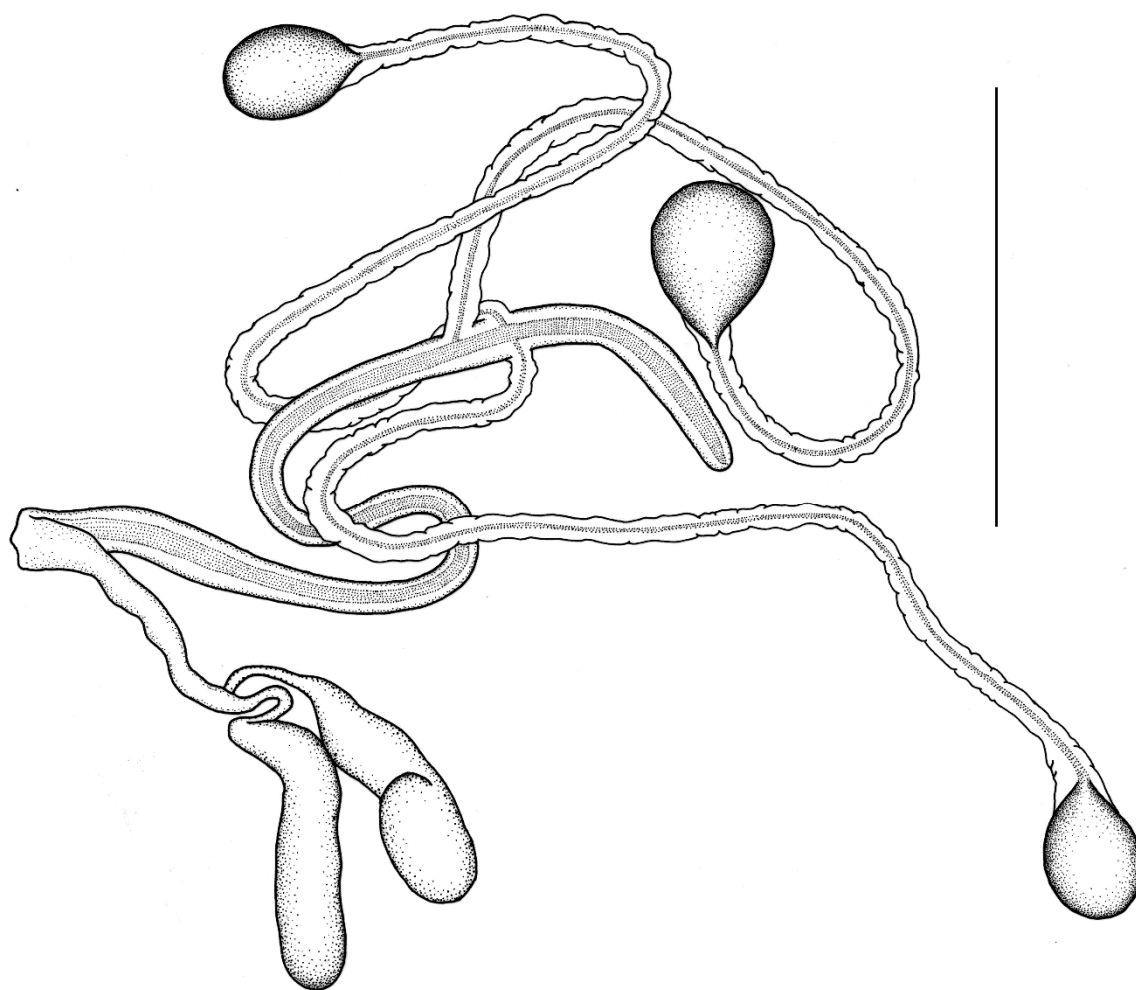


Figure 7. Female internal reproductive system of *T. (V.) sternotuberculata*. Scale bar = 1.0 mm.

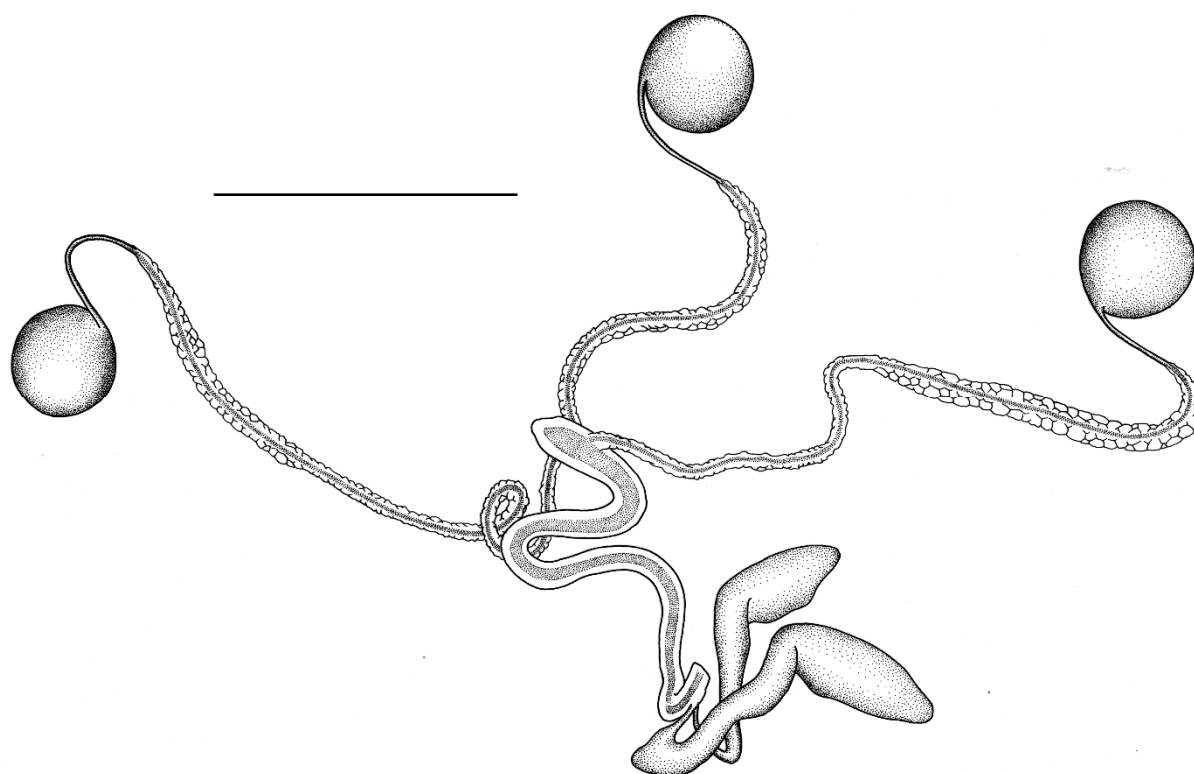


Figure 8. Female internal reproductive system of *T. (Y.) couckei*. Scale bar = 1.0 mm.

Remarks. In the two species of the subgenus *Tipula* (*Yamatotipula*), their accessory glands are generally elongated and irregular in shape, and their spermathecae are relatively big.

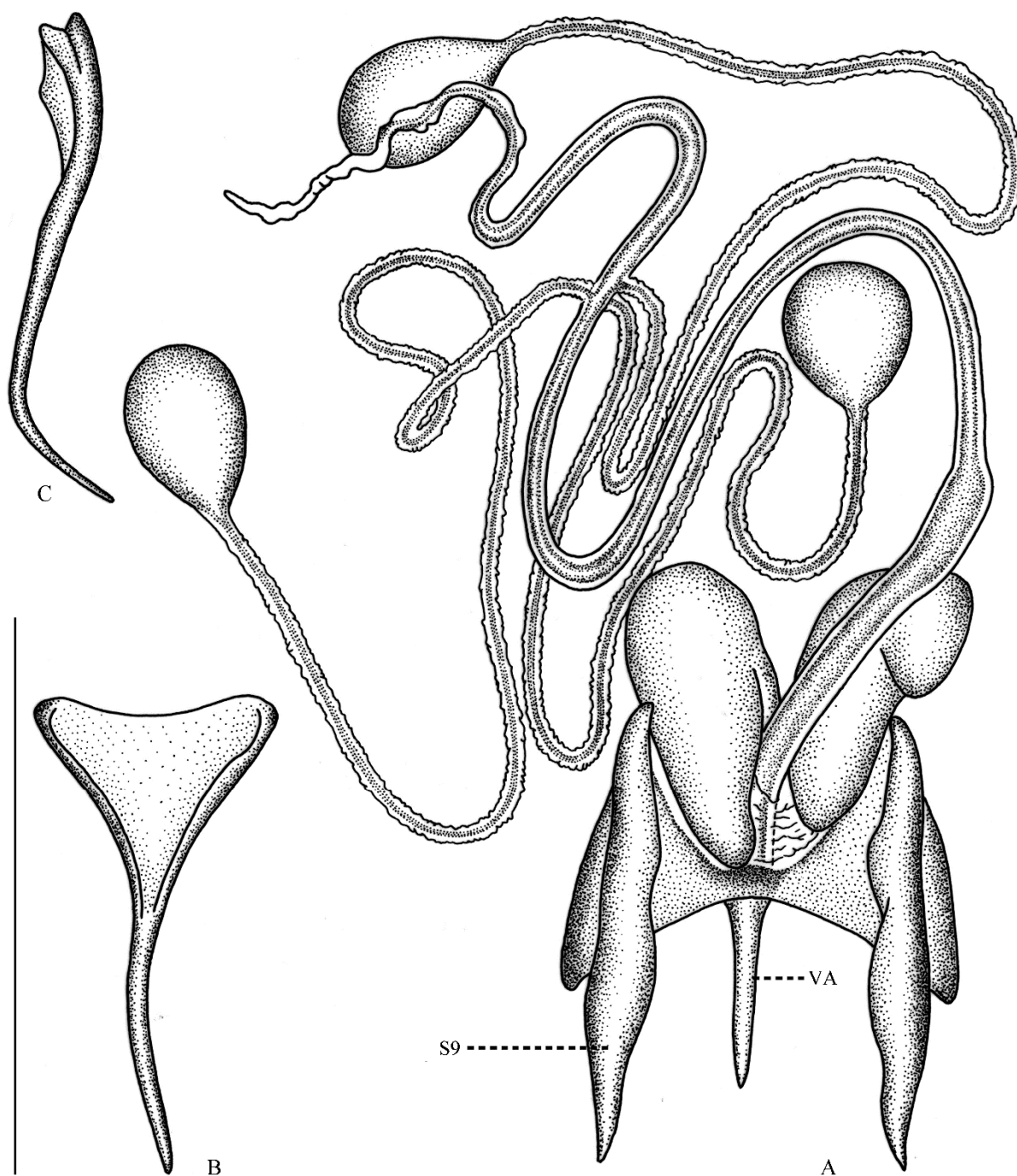


Figure 9. Female internal reproductive system of *T. (Y.) marginella*. A. Sternite nine, vaginal apodeme and internal reproductive system, dorsal view. B. Vaginal apodeme, dorsal view. C. Vaginal apodeme, lateral view. Scale bar = 1.0 mm.

3.2 Morphological comparisons of female internal reproductive system

Based on the morphological comparisons (Table 2) of nine species in the genus *Tipula*, the features of their female internal reproductive systems can be preliminary summarized as follows: (1) spermatheca is three in number; (2) sizes of spermatheca and accessory gland show regularity at specific level; (3) shape of bursa copulatrix is greatly varied in different species; (4) thicknesses and lengths of spermathecal duct and bursa copulatrix are significantly variable in different species,

which occurs in the absence of clear rules at specific level; (5) three connection types between spermathecal duct and bursa copulatrix can be divided into: type I, arising from the same level; type II, the lateral ducts arising front of the central one; type III, the lateral ducts arising behind of the central one.

Table 2. Morphological comparisons of female internal reproductive systems among six species of *Tipula*.*

Species	Nmber, shape and size of SP	Stem of AG	Shape of AG	Arising position from SPD
<i>T. (B.) amurensis</i>	3, spherical, 0.20 mm in diameter, with a lateral cap	Short	Irregular sac	At same level
<i>T. (L.) lunata</i>	3, pear-shaped, 0.20 mm at widest point	Elongated	Long sac	Lateral ducts in front of central duct
<i>T. (L.) submanca</i>	3, pear-shaped, width 0.25 mm, length 0.30 mm	Short	Rounded sac	Central duct in front of lateral ducts
<i>T. (P.) biaciculifera</i>	3, spherical, 0.25 mm in diameter	Elongated	Spindle-shaped	Lateral ducts in front of central duct
<i>T. (S.) parvaauricula</i>	3, spherical, 0.40 mm in diameter	Short	Spindle-shaped	Lateral ducts in front of central duct
<i>T. (V.) longitudinalis</i>	3, pear-shaped, laterals with width 0.20 mm, length 0.25 mm, middle with width 0.35 mm, length 0.45 mm	Short	Long sac	At same level
<i>T. (V.) sternotuberculata</i>	3, pear-shaped, the lateral spermatheca with width 0.18 mm, length 0.30 mm, the middle one with width 0.25 mm, length 0.35 mm	Elongated	Long sac	Lateral ducts in front of central tube
<i>T. (Y.) couckeii</i>	0.45 mm in diameter	Without or short	Long sac	At same level
<i>T. (Y.) marginella</i>	3, pear-shaped, width 0.25 mm, length 0.35 mm	Short	Spindle-shaped	Central duct in front of lateral ducts

*Abbreviation: AG—accessory gland; SP—spermatheca; SPD—spermathecal duct.

4 Discussion

4.1 Spermatheca

The base of spermathecal duct is enclosed in the genital chamber, or lies in the dorsal wall of genital chamber (bursa copulatrix) (Snodgrass, 1935). In crane flies, the origin of spermatheca should be assigned to the latter. At family level in Tipuloidea, numbers of spermatheca are variable, such as, one, two or three spermathecae in Limoniidae, and two or three spermathecae in Pediciidae (Tjeder, 1963a, b). In family Tipulidae, spermatheca generally remains three, but paired spermathecae were observed in genus *Nephrotoma* (unpublished data). The shape and structure of spermatheca are highly variable at supraspecific level, which may provide us a useful tool to separate some taxa and to analyze their phylogenetic relationships.

4.2 Length of bursa copulatrix

In copulation, the aedeagus of male is inserted into the bursa copulatrix of female, and the spermatozoa are presumably stored in spermatheca until the eggs mature (Snodgrass, 1935). For species in the genus *Tipula*, their bursa copulatrix are simple tubes in general with different lengths, widths and shapes. Correspondingly, their aedeagi are slender tubes which has the same length as the female bursa copulatrix. This may be a mode of the lock-and-key configuration, which needs further research in future. An exceptional sample was observed in *Nephrotoma repanda* (Alexander, 1914) that its female bursa copulatrix is only one third to half length of the male aedeagus, which might mean the aedeagus partially insert into the bursa copulatrix during copulation (unpublished data).

4.3 Position of the arising of spermathecal ducts

The arising position of spermathecal ducts is various in the genus *Tipula*. Three distinct types of connection arrangements between spermathecal duct and bursa copulatrix were found. For type I, the arising position is at the same

level, such as *T. (Beringotipula) amurensis*, which was also observed in *Tipulodina xyris* (Alexander, 1949), *Dolichopeza (Nesopeza) multidentata* Men, 2018, *D. (Nesopeza) incisuraloides* Men, 2018 and *Tipulodina bifurcata* Xue & Men, 2019 (Men *et al.*, 2018a; Xue *et al.*, 2019). For type II, the lateral ducts are arising front of the central one, as in *T. (Pterelachisus) biaciculifera*, *T. (Sivatipula) parvaauricula* and *T. (Vestiplex) sternotuberculata*. For type III, in contrast to type II, the lateral ducts are arising behind of the central one, such as in *T. (Lunatipula) submanca* and *T. (Yamatotipula) marginella*, and as in *Holorusia* sp. (Men *et al.*, 2018b). For better understanding the evolutionary sense and the phylogenetic usefulness of these modes, more anatomical studies are undoubtedly necessary in future.

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