

ORIGINAL ARTICLE

A morphological study on reproductive system of *Tipula* (*Yamatotipula*) *nova* Walker (Diptera: Tipulidae)

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Abstract The paper provides descriptions and illustrations of the internal reproductive systems in both sexes of a wide-spread species, *Tipula* (*Yamatotipula*) *nova*, and analyzes the possible usefulness of some internal reproductive structures for taxonomy in subgenus *Tipula* (*Yamatotipula*). The female ovipositor is described and illustrated for the first time. The original description of the male hypopygium is expanded upon the description of new morphological characters and detailed illustrations.

Key words Nematocera, crane flies, ovipositor, hypopygium, taxonomy.

1 Introduction

Tipula is a large genus in the family Tipulidae and comprises more than 2 400 species. It is extremely rich in Palaearctic Region with about 1 010 species. The subgenus *Tipula* (*Yamatotipula*) includes 125 species, which are mainly distributed in Palaearctic Region with 65 species and Nearctic Region with 56 species (Oosterbroek, 2014).

The species of subgenus *T.* (*Y.*) *nova* in China remains inadequately studied and only ten species have been reported, of which *T.* (*Y.*) *nova* is the only species widely distributed in both Oriental and Palaearctic Regions, including China (Shanxi, Henan, Anhui, Hubei, Jiangxi, Zhejiang, Fujian, Sichuan, Yunnan, Guizhou, Guangdong, Taiwan, Hong Kong), India, Japan and Korea (Oosterbroek, 2014). It can be easily collected by light trap.

T. (*Y.*) *nova* was originally described by Walker in 1848 based on a single female specimen collected from Hong Kong. However, his original description was very rough and lack of the descriptions and illustrations of genitalia. In subsequent studies, the male genitalia of *T.* (*Y.*) *nova* received more attention than the female genitalia. Savchenko (1961) redescribed this species in his monograph of crane flies of the USSR fauna. As the first detailed morphological study, the illustrations of male genitalia and the habitus of female were provided. Yang and Yang (1997) redescribed *T.* (*Y.*) *nova* in a monograph of insect fauna, *Insects of the Three Gorge Reservoir of Yangtze River*, based on one male specimen. They provided an illustration of hypopygium viewed laterally, but the description and illustration of female genitalia were absent. Until now, there is still no report on the morphological characters of female genitalia of *T.* (*Y.*) *nova*.

Studies of reproductive morphology under phylogenetic framework play an important role in answering questions about the co-evolution of anatomy, mating behaviors and fertilization mechanisms (Wortham-Neal, 2002). Reproductive systems are traditional source of phylogenetic characters in entomology (Chaboo, 2007). In crane flies species, some internal reproductive organs, such as male semen pump and female vaginal apodeme, can be used in phylogenetic analysis (de Jong, 1993, 1994a, b, 1995a, b). Many researchers dealt with studies of reproductive system in the genus *Tipula*,

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including Frommer (1963) on *T. (Triplictipula) triplex*, Erhan-Dina (1976) on subgenus *T. (Lunatipula)*, Afanaseva (1980) on male *T. (Tipula) czizeki*, Simova-Tosic and Vukovic (1983) on *T. (Lunatipula) fasciculata* and *T. (Lunatipula) falcate*, Savchenko (1983) on male *T. (Tipula) paludosa*, Vermoolen (1983) and de Jong (1993) on *T. (Acutipula) maxima* species group, Dufour and Oosterbroek (1990) on *T. (Vestiplex) vailanti*, de Jong (1995b) on subgenus *T. (Mediotipula)* and Gelhaus (2005) on subgenus *T. (Eremotipula)*. However, features of reproductive system and characteristic variations of reproductive structures between relative species were still largely unknown in subgenus *T. (Yamatotipula)*. This induced us to carry out this study which may contribute to a better understanding of the diversity of reproductive structures and taxonomic potential of some internal reproductive structures in subgenus *T. (Yamatotipula)*.

In the present paper, we provide the morphological description of female ovipositor for the first time, including detailed drawings of each part. We expand on the original description of male hypopygium by adding additional morphological characters and detailed illustrations. The internal reproductive systems of *T. (Y.) nova* in both sexes are also first described in Chinese *T. (Yamatotipula)* species. Additionally, taxonomic potential of some internal reproductive structures in the subgenus *T. (Yamatotipula)*, including male semen pump and female vaginal apodeme, are analyzed and discussed by comparing with these structures of congeneric species.

2 Materials and methods

The specimens examined in this study were respectively collected from Huangshan, Yaoluoping National Nature Reserve, and campus of Anqing Normal University, Anhui Province, by the authors and undergraduates of the School of Life Sciences, Anqing Normal University. All the fresh specimens were preserved in 80% ethyl alcohol. The genitalia, including male hypopygium and female ovipositor, were removed and soaked in 10% NaOH for 12 hours to clear the muscle, and then immersed in glycerin jelly for drawing using Leica MZ125 (Leica, Germany) stereomicroscope. The internal reproductive systems were dissected in water, and then examined and drawn using the same stereomicroscope. All measurements were made with the aid of a digital caliper in millimeters (mm). The terminology and methods of description and illustration follow that of Frommer (1963) and Alexander and Byers (1981).

List of abbreviations

A	adminiculum
ab	apical beak
Ae	aedeagus
AG	accessory gland
AIA	anterior immovable apodeme
BC	bursa copulatrix
CA	compressor apodeme
Ce	cercus
Co	copulatory opening
E	egg
ED	ejaculatory duct
Enph	endophallus
Go	gonopore
Hy	hyovalva
IG	inner gonostylus
LDS	lateral duct of spermatheca
O	ovary
OG	outer gonostylus
PIA	posterior immovable apodeme
S	sternite
SP	spermatheca
SPD	spermatheca duct
SV	seminal vesicle

T	tergite
TS	testes
VA	vaginal apodeme
VD	vas deferens
vp	ventral process

3 Results

3.1 Male reproductive system (Figs 1–8)

3.1.1 Hypopygium

Description. Tergite IX (Fig. 1) distinctly larger than sternite IX, separated from sternite IX by membrane, many long hairs arising from the base of tergite IX in caudal view (Fig. 2); tergite IX (Figs 1–3) with dorsal margin bilobed, divided by a V-shaped notch, with a finger-shaped process at dorsal side; lateral margin of tergite IX concaved, with a hook-shaped process at dorsal angle. Outer gonostylus (Figs 1, 4) almost elliptical, distinctly smaller than inner gonostylus, with an angular process at dorsal margin. Inner gonostylus (Figs 1, 5) with apical beak black, terminating into four or five teeth, with ventral process having hyaline membrane at apex. Adminiculum (Figs 1–2) long, slender. Aedeagus (Fig. 1) elongate, extending into genital chamber, tubular and very slender.

3.1.2 Internal system

Description. Male internal reproductive system consists of semen pump, ejaculatory duct, seminal vesicle, accessory gland, vas deferens and testes.

Posterior immovable apodeme elongate, S-shaped, gradually tapered to terminus in lateral view (Figs 1, 6). Compressor apodeme black, fan-shaped in dorsal view (Fig. 7), medially divided by a V-shaped notch, in a 30° angle with posterior immovable apodeme (Figs 1, 6). Anterior immovable apodeme elongate, gradually narrowed proximally, almost connected in dorsal view (Figs 1, 6–7).

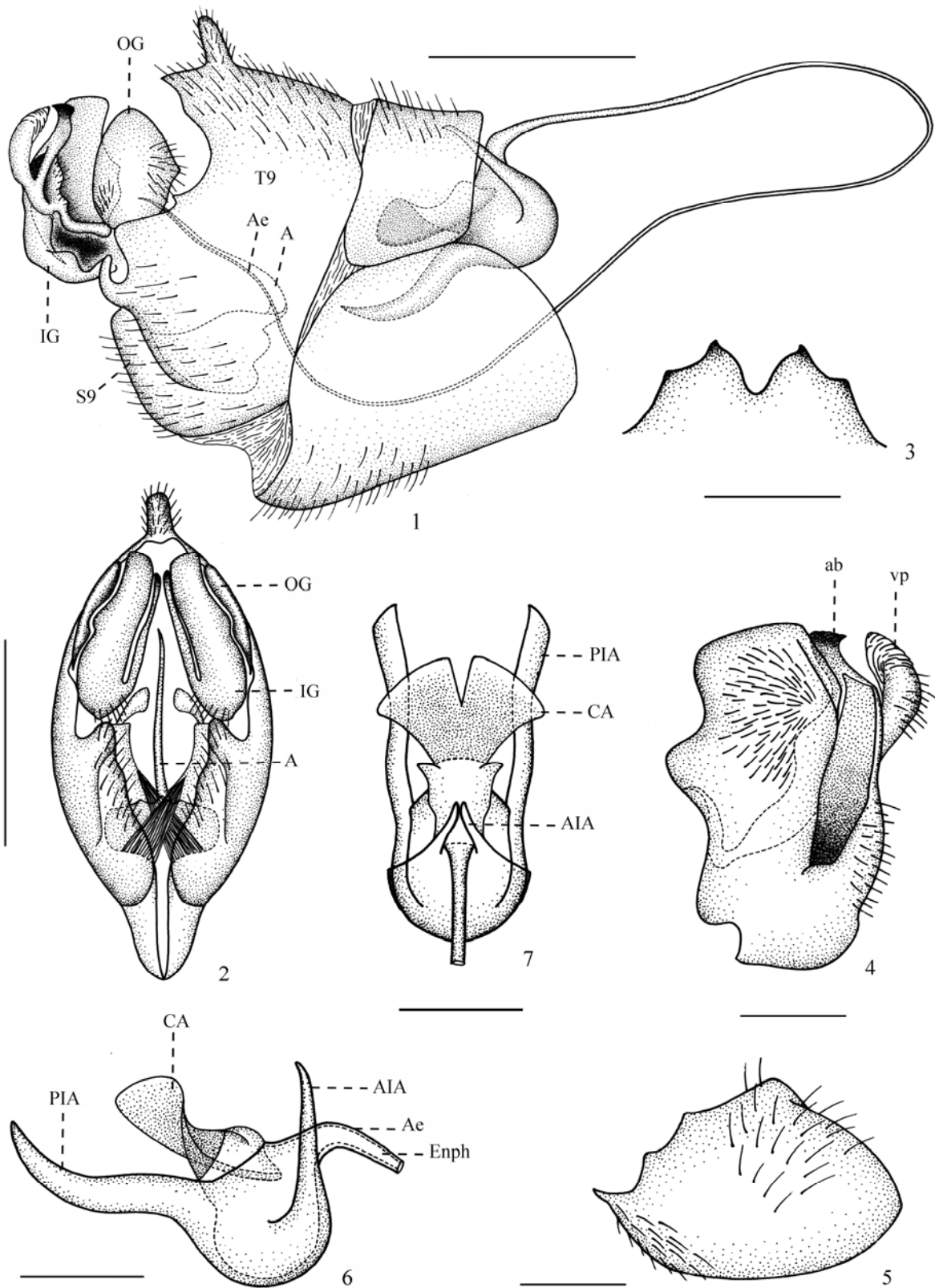
Ejaculatory duct (Fig. 8) slender and flexible, opening into ventral side of semen pump, attachment point below endophallus. Seminal vesicle (Fig. 8) relatively thick, tough and retractable, forming uniform set of characters in different individuals. Accessory glands (Fig. 8) being a pair of slender filaments, simple, elongated and somewhat convoluted, arising from base of seminal vesicle. Vas deferens (Fig. 8) paired, elongated, swollen at basal half, slender at apical half, forked at end of seminal vesicle, extending laterally to either side of rectum to join testes. Testes (Fig. 8) an elliptical structure arising at termination of vas deferens.

Remarks. Semen pump of *T. (Y.) nova* (Figs 1, 6–7) belongs to the type III, which proposed by Frommer (1963) based on the anatomical study of male reproductive system of tipuline crane-flies species in North American. The type III of semen pump is the most common type, which is characterized by the strongly bowed intromittent organ (Frommer, 1963).

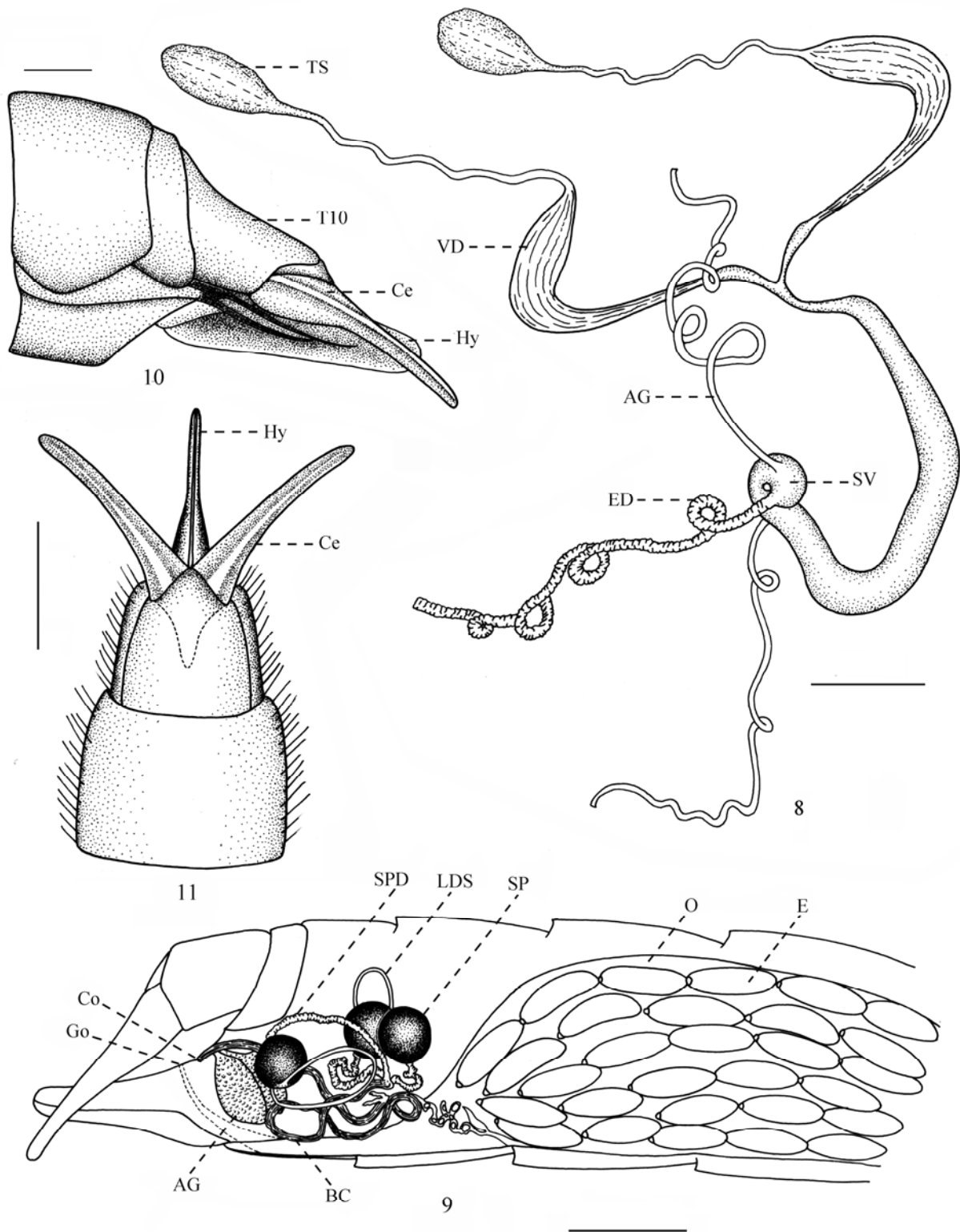
3.2 Female reproductive system (Figs 9–16)

3.2.1 Ovipositor

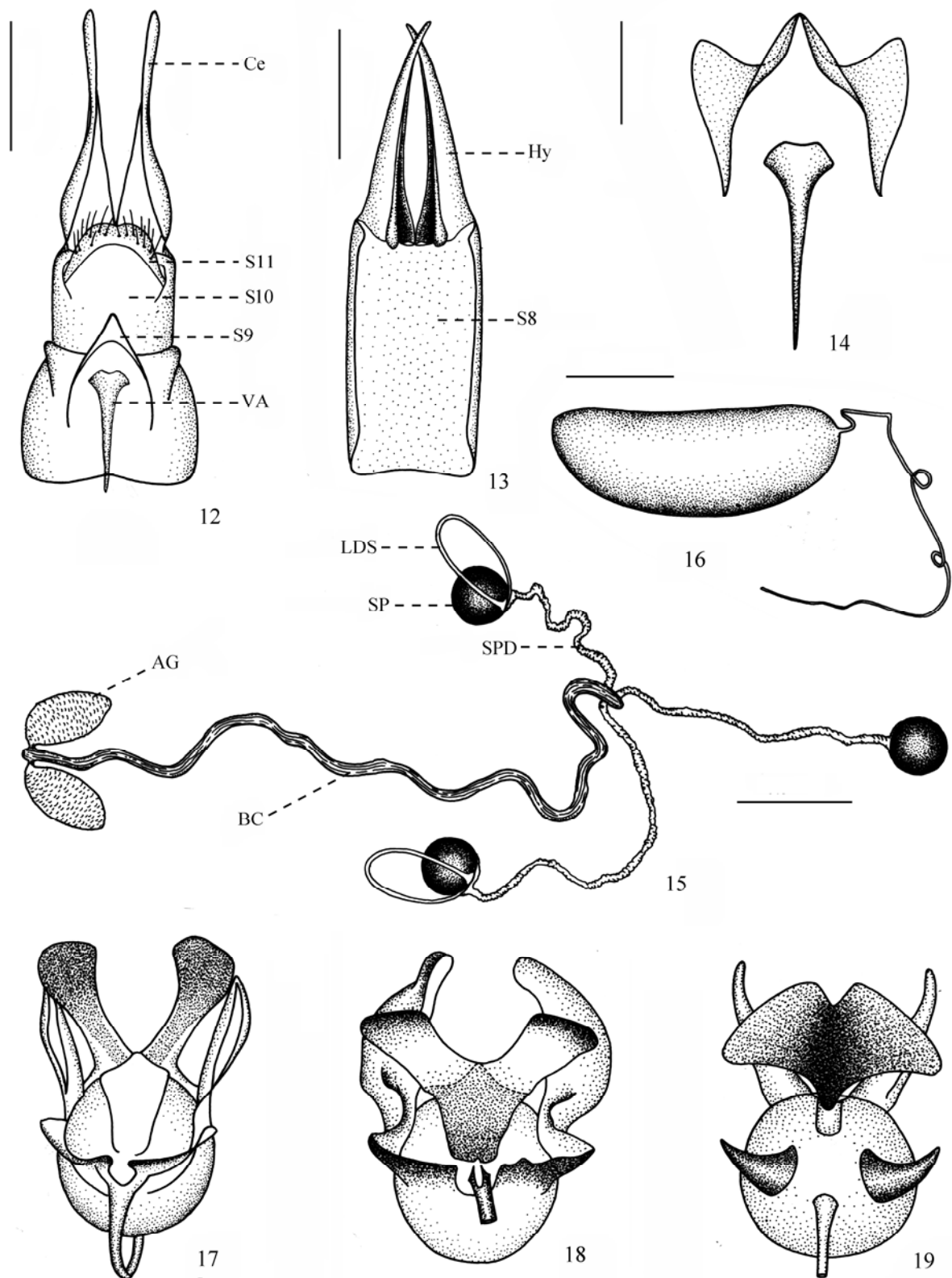
Description. Sternite IX (Figs 12, 14) with median membrane over a narrow zone, lateral with triangular sclerotized lobes, extension produced into a sclerotized elongate and pointed process. Sternite X (Figs 9–12) slightly shorter than cercus, parallel in lateral margins in dorsal view. Sternite XI (Fig. 12) densely covered with long setae, with anterior margin concaved caudad, with posterior margin terminating into two rounded lobes. Cercus (Figs 9–12) long, acinacifoliate, widened at basal two fifths, parallel at apical three fifths, surpassing end of hypovalva. A median ridge arising in middle of cercus in lateral view (Figs 10–11). Hypovalva (Figs 9–11, 13) simple, extending to nearly three quarters length of cercus.



Figs 1–7. *Tipula (Yamatotipula) nova*. 1. Male hypopygium, lateral view. 2. Male hypopygium, caudal view. 3. Male tergite IX, dorsal view. 4. Inner gonostylus, lateral view. 5. Outer gonostylus, lateral view. 6. Semen pump, lateral view. 7. Semen pump, dorsal view. Scale bars: 1–2=1.0 mm; 3, 6–7=0.5 mm; 4–5=0.25 mm.



Figs 8–11. *Tipula (Yamatotipula) nova*. 8. Male internal reproductive system. 9. Female internal reproductive system, lateral view. 10. Ovipositor, lateral view. 11. Ovipositor, dorsal view. Scale bars: 8, 10=0.5 mm; 9, 11=1.0 mm.



Figs 12–19. Reproductive System. 12–16. *Tipula (Yamatotipula) nova*. 17–19. Semen pump, dorsal view. 12. Cercus, caudal view. 13. Hypovalva, dorsal view. 14. Vaginal apodeme and sternite IX, dorsal view. 15. Female internal reproductive system. 16. Egg. 17. *T. stackelbergi* (after Salmela, 2012). 18. *T. pruinosa* (after Salmela, 2012). 19. *T. pierrei* (after Stary and Martinovsky, 1969). Scale bars: 12–13=1.0 mm, 14–15=0.5 mm, 16=0.25 mm.

3.2.2 Internal system

Description. Female internal reproductive system consists of bursa copulatrix, accessory gland, spermatheca, spermatheca duct and vaginal apodeme.

Bursa copulatrix (Figs 9, 15) relatively thick, curved and tough, anterior end of bursa copulatrix straight. Accessory gland (Figs 9, 15) spindle-shaped, swollen, densely covered with small sockets, terminating to base of bursa copulatrix. Spermatheca (Figs 9, 15) spherical, black, well-sclerotized. Lateral spermatheca with a slender duct arising from lateral side, median spermatheca lacking of such duct (Figs 9, 15). Spermatheca duct (Figs 9, 15) slender and flexible, arising from end of bursa copulatrix and leading to spermatheca, with membrane surrounding attachment point of spermatheca and spermatheca duct. Vaginal apodeme (Figs 12, 14) widened at base and gradually tapered, very acute apically. Female internal reproductive system with two openings between eighth and ninth sternite, copulatory opening and gonopore (Fig. 9).

Remarks. Frommer (1963) found the anterior terminus of bursa copulatrix varies in shape. It is a crook in *Nephrotoma*, but becomes swollen in *Dolichozeza* while remains straight in *Tipula*. In this study, the bursa copulatrix of *T. (Y.) nova* shows a straight end as Frommer's description.

3.3 Egg

Egg (Figs 9, 16) black, with overall appearance kidney-shaped or elongated-oval, length 0.8 ± 0.2 mm, width 0.5 ± 0.1 mm ($n=20$). Surface smooth, densely covered with small rounded sockets, apically with a long filament. The numbers of eggs varied from 295 to 342, with an average of 311 in 10 examined individuals.

3.4 Potential taxonomic characters

3.4.1 Semen pump

After comparing the structure of semen pump in four species (Figs 6–7, 17–22), *T. stackelbergi*, *T. pruinosa*, *T. pierrei* and *T. nova*, significant differences were observed in their morphological features, which can be effectively used as means for distinguishing species in subgenus *Tipula* (*Yamatotipula*). Except *T. nova*, characterizations and illustrations of the other three species are from literatures. *T. stackelbergi* and *T. pruinosa* are from Salmela (2012); *T. pierrei* are from Stary and Martinovsky (1969). The differences of semen pumps are summarized in Table 1.

3.4.2 Vaginal apodeme

Vaginal apodemes of ten species are compared in this study (Figs 14, 23–31). Except *T. nova*, the illustrations of vaginal apodeme of the other nine species are cited from literatures. *T. stackelbergi*, *T. chonsaniana*, *T. freyana*, *T. pruinosa* and *T. moesta* are from Salmela (2012); *T. tephrocephala*, *T. spernax*, *T. furca* and *T. tricolor* are from Frommer (1963). The structure of vaginal apodeme in the ten species analyzed here showed distinct variations in shape, which are listed in Table 2. The result suggests great taxonomic potential of vaginal apodeme for distinguishing species in subgenus *Tipula* (*Yamatotipula*).

4 Discussion

4.1 Openings of female genital segment and swollen portion of male vas deferens

In female *T. (Y.) nova*, two openings, the copulatory opening and gonopore, occur between the eighth and ninth sternite. However, another opening, the pore of accessory gland, was observed in *T. (Lunatipula) uncinata* illustrated by Frommer (1963). Whether the pore of accessory gland presents is decided by the position of terminus of accessory gland, which situates at the base of bursa copulatrix in former, but situates on intersegmental membrane between the eighth and ninth sternite in latter.

Table 1. Characters of semen pump in four species of *Tipula* (*Yamatotipula*).

Species	CA	PIA	AIA
<i>T. stackelbergi</i> (Figs 17, 20)	V-shaped, dark brown in apical half, entirely divided by a V-shaped notch in the middle	Shorter than CA, narrow, acute apically, in a 60° angle with CA	Very small, lateral margins almost parallel, not connected apically
<i>T. pruinosa</i> (Figs 18, 21)	Y-shaped, dark brown at apex, medially divided by a U-shaped notch, which extending to half of length	Slightly longer than CA, broad medially and acute apically in dorsal view, bent upward at apical one third, in a 90° angle with CA	Gradually narrowed, not connected apically
<i>T. pierrei</i> (Figs 19, 22)	Fan-shaped, deeper in coloration in median region, medially divided by a small V-shaped notch in apical one fifths	Distinctly longer than CA, basally broad and gradually tapered to the apex, bent upward at apical half, in a 70° angle with CA	Horn-shaped, not connected apically
<i>T. nova</i> (Figs 6–7)	Fan-shaped, entirely dark gray, medially divided by a V-shaped notch, which extending to half of length	Distinctly longer than CA, S-shaped, gradually narrowed to apex, in a 30° angle with CA	Very narrow, apically sharp, connected at apex

In the studies of Keuchenius (1913) and Bodenheimer (1923), the vas deferens was not swollen at some point along their lengths. But in the present study, the swollen portion was present, the same as the result of Frommer (1963). Therefore, the research on taxonomic value of vas deferens as well as correlation between phylogeny and structure should be carried out in the future.

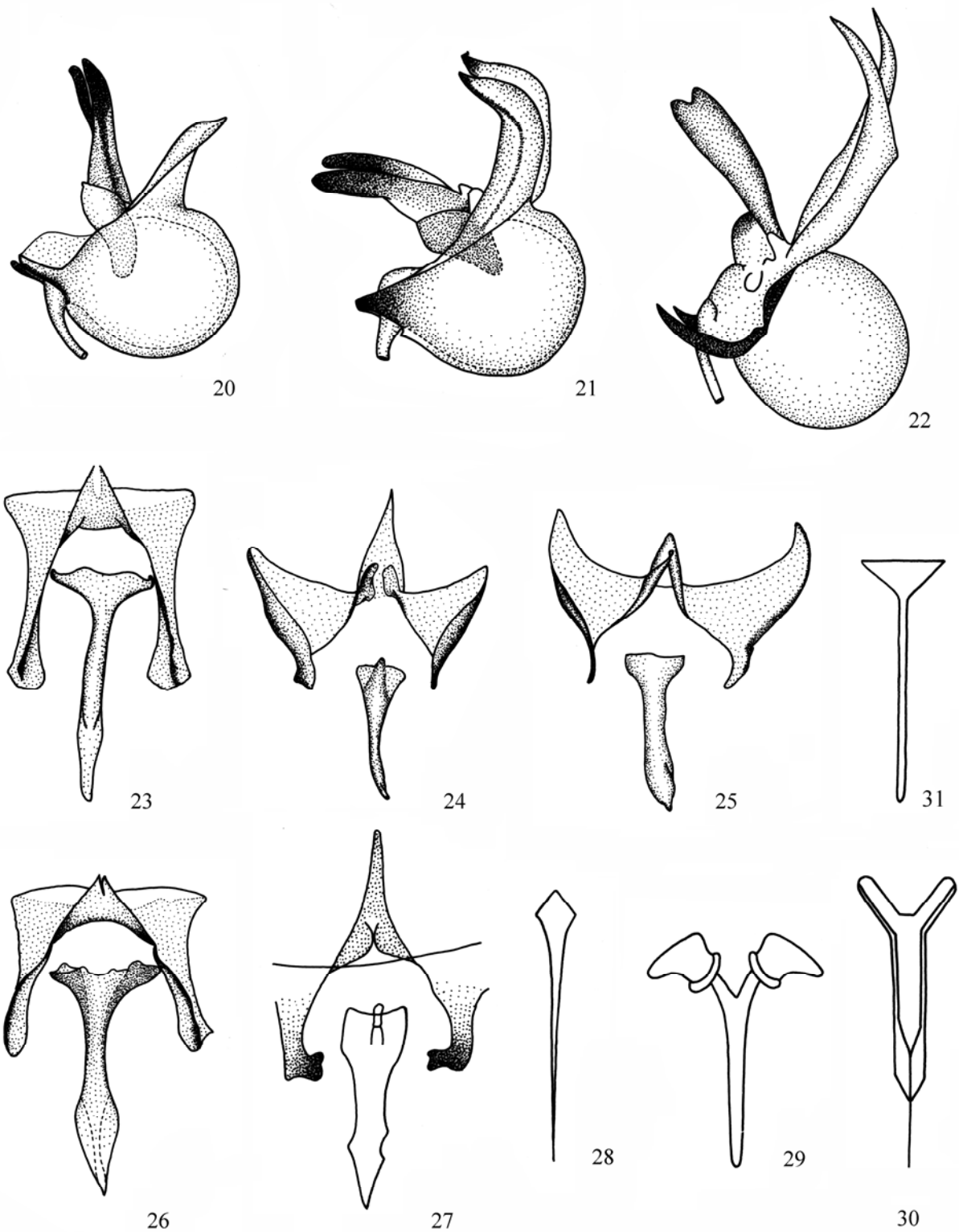
4.2 Semen pump and vaginal apodeme

In previous studies, various terms were applied to designate the sclerotizations of vaginal wall. Snodgrass (1933) considered that this portion represents fused first gonapophyses, which was agreed by Crampton (1942). Rees and Ferris (1939) used term apodeme to define this portion. Tuxen (1956) employed term vaginal apodeme, which was accepted by Frommer (1963) and Salmela (2012). Byers (1961) adopted term furca due to the forked shape in some fly species. Young (2013) and de Jong (1994a, b) followed Byers although the latter used a synonym, genital fork. However, Frommer (1963) observed that the structure is not forked in many tipuline crane flies examined. After dissecting and observing ten individuals of *T. (Y.) nova* during this study, we found the vaginal apodeme was not forked as described by Frommer.

Although the characters of vaginal apodeme are uniform in different individuals of *T. (Y.) nova*, this structure showed noticeable differences among species within the same subgenus. Therefore, the characters of vaginal apodeme can be used to identify species. For taxonomic working on this subgenus, it could be more effective to distinguish species based on female vaginal apodeme when males are difficult to collect, despite the fact that the characters of female genitalia are used less frequently at present. Additional research needs to be done to investigate the variation of vaginal apodeme among relative species in other genera.

Table 2. Characters of vaginal apodeme in ten species of *Tipula* (*Yamatotipula*).

Species	Vaginal apodeme
<i>T. nova</i>	Broad basally, gradually narrowed to apex, very slender (Fig. 14)
<i>T. stackelbergi</i>	Broad at basal one third, cylindrical, slightly expanded near the apex (Fig. 23)
<i>T. chonsaniana</i>	Broad at base, gradually tapered to apex (Fig. 24)
<i>T. freyana</i>	Base slightly wide, cylindrical, relatively thick (Fig. 25)
<i>T. pruinosa</i>	Broad at base, gradually widening toward caudal and proximal ends, narrowest around midpoint (Fig. 26)
<i>T. moesta</i>	Base slightly wide, relatively thick (Fig. 27)
<i>T. tephrocephala</i>	Broad basally, very slender (Fig. 28)
<i>T. spernax</i>	Bilobed basally, boot-shaped, cylindrical, rounded apically (Fig. 29)
<i>T. furca</i>	Forked basally, cylindrical, apically with a needle like process (Fig. 30)
<i>T. tricolor</i>	Triangular at base, cylindrical, rounded apically (Fig. 31)



Figs 20–31. Reproductive System. 20–22, Semen pump, lateral view. 23–27. Vaginal apodeme and sternite IX. 28–31. Vaginal apodeme. 20. *T. stackelbergi* (after Salmela, 2012). 21. *T. pruinosa* (after Salmela, 2012). 22. *T. pierrei* (after Sary and Martinovsky, 1969). 23. *T. stackelbergi* (after Salmela, 2012). 24. *T. chonsaniana* (after Salmela, 2012). 25. *T. freyana* (after Salmela, 2012). 26. *T. pruinosa* (after Salmela, 2012). 27. *T. moesta* (after Salmela, 2012). 28. *T. tephrocephala* (after Frommer, 1963). 29. *T. spernax* (after Frommer, 1963). 30. *T. furca* (after Frommer, 1963). 31. *T. tricolor* (after Frommer, 1963).

The structure of semen pump in the four species of subgenus *Tipula* (*Yamatotipula*) showed substantial variation in shape and color, which suggests that the characters of semen pump are useful in distinguishing relative species. More detailed comparative studies of these structures with more sophisticated techniques, such as electron microscope, will provide us information not only with respect to their taxonomic value, but also to their phylogenetic significance and functional morphology.

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