

Comparative morphology of ovipositor in cicadas (Hemiptera: Cicadidae), with considerations on their taxonomic significance

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Abstract Although previous studies have documented the morphology of ovipositor in Cicadidae, little is known about the morphology and ultrastructure of their gonapophyses, which may be closely related to the divergence of related groups and the host plant selection in oviposition. The morphology and ultrastructure of gonapophyses of 13 representative cicadas, belonging to the three subfamilies of Cicadidae, is investigated using scanning electron microscopy. The results show that the morphology of gonapophyses can be adopted in cicada taxonomy at both higher and species levels, and some characters may be promising for phylogenetic analysis of Cicadidae. With regard to the genus *Karenia*, whose systematic status remains controversial, it resembles members of Cicadinae more than those of Cicadettinae (=Tibicininae *auct.*) in morphology of gonapophyses. This indicates that it is more reasonable to place this genus into Cicadinae instead of Cicadettinae. The various types of sensilla on the gonapophyses and their distribution are described in detail, and their functions were discussed. Our results supply

information not only for future study of taxonomy and phylogeny of the Cicadoidea, but also for researches of oviposition behavior and oviposition-site selection of cicadas.

Keywords Cicadomorpha · Female · Genitalia · Ultrastructure · Sensilla · Taxonomy

Introduction

Ovipositor of insects is a complex structure usually modified from appendages of the 8th and 9th abdominal segments, consisting of a pair of gonapophyses VIII on the eighth segment, and a pair of gonapophyses IX and gonoplacs on the ninth segment (Emeljanov 2014). Female insects use their ovipositor to lay eggs in specific sites, such as in crevices, soil, tissues of plants, on the surface of plants or animals, or even within bodies of other arthropod hosts (Emeljanov 2014). The ovipositor in different insect groups is modified for various purposes, e.g., the ovipositor of hymenopterans may be used for boring, piercing, sawing, or stinging (Gillott 2005). The selection of host plant is vital for offspring to survive, and the sensilla on the ovipositor play major roles in the assessment of potential host selection sites (Chadha and Roome 1980; Hallberg and Ahman 1987).

Species in the Cicadoidea (Hemiptera: Cicadomorpha) feed exclusively on xylem fluid from host plants in both nymphal and adult stages (Lloyd and White 1987). Injuries caused by the feeding of cicadas usually go undetected as their nymphs are long-lived underground. However, cicadas cause great harm including twig dieback in host plants when large numbers of certain cicada species insert eggs into the stems of trees and shrubs (Lloyd and White 1987).

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For example, the long-term impact of oviposition by certain species results in change of branch architecture, causing breakage in young trees and great loss of growth (Miller and Crowley 1998; Williams and Simon 1995). Moreover, the oviposition wounds can likely lead to pathogenic infections of trees and shrubs (Smith and Linderman 1974; Ostry and Anderson 1983). Oviposition of adult cicadas determines the long-term habitats of their underground nymphal stages on larger scales (Oberdorster and Grant 2006). Their nymphs are relatively sessile underground due to the close association with their host plants, generally traveling only a few centimeters per year (Maier 1980), using their legs to transport the anal liquid for facilitating the digging process by softening the substrate (Kudryasheva 1979). Female cicadas use multiple indirect cues at multiple scales to select oviposition sites (Oberdorster and Grant 2006). Morphological features and sensilla of ovipositors may serve as important signals conveying information about the oviposition-site selection of cicadas.

The morphology of the ovipositor has provided useful taxonomic characters (Nielson 1965; Saxena et al. 1985; Mejdalani 1998; Rodrigues and Mejdalani 2009; Mejdalani and Silva 2010; Meng and Yang 2012). To date, there are some documents on the ovipositor of cicadas. Pioneering descriptions of the morphology of the ovipositor were made by Hilsman (1921), who found some structural taxonomic characteristics of ovipositor among genera and within the genus. Later, studies successively depicted some aspects of the cicada ovipositor (Radio 1922; Snodgrass 1926; Myers 1928; Weber 1930; Müller 1942; Kramer 1950; Scudder 1957, 1961a, b, 1964; Boulard 1965, 1996). As the least species-rich superfamily in the Cicadomorpha (Hemiptera: Auchenorrhyncha), the Cicadoidea comprises approximately 3200 described species classified into two

families: Tettigarctidae and Cicadidae (Sanborn 2013). Currently, the Tettigarctidae comprises only one extant genus, *Tettigarcta* (including two species confined to Australia), and five extinct genera known from fossils; the Cicadidae contains three subfamilies, i.e., Cicadinae, Tettigadinae, and Cicadettinae (Moulds 2005). Detailed comparative investigation about the morphology of cicada ovipositor is desirable.

Herein, for the first time, the morphology and ultrastructure of the ovipositor of 13 representative cicada species from three subfamilies of Cicadidae are investigated, aiming to explore useful taxonomic characters of the Cicadidae, and to reveal signals conveying information about the oviposition-site selection of cicadas.

Materials and methods

Sampling of taxa

Females of cicadas were captured from different regions across China. Their identity and detailed collecting information are shown in Table 1. The subfamily classification of Cicadidae follows that of Moulds (2005).

Sample preparation and scanning electron microscopy observation

Under the Motic SMZ168, live females (at least five individuals for each species) were anesthetized, and their gonapophyses and gonopods were cut off and detached separately, then fixed in 2.5% glutaraldehyde (0.2 M PBS, pH 7.2) for 12 h at 4 °C. The materials were rinsed four times with phosphate-buffered saline (PBS, 0.1 M, pH 7.2), followed by dehydration in a graded series of ethanol

Table 1 Taxonomic status and collecting information for 13 species investigated in this study

Subfamily	Species	Collecting sites	Date
Cicadinae	<i>Cryptotympana atrata</i> (Fabricius, 1775)	Yangling, Shaanxi, China	vii. 2010
	<i>Platyleura kaempferi</i> (Fabricius, 1794)	Yangling, Shaanxi, China	vii. 2010
	<i>Platyleura hilpa</i> Walker, 1850	Jianfeng, Hainan, China	v. 2011
	<i>Meimuna mongolica</i> (Distant, 1881)	Yangling, Shaanxi, China	vii. 2010
	<i>Meimuna opalifera</i> (Walker, 1850)	Xiajiang, Jiangxi, China	viii. 2015
	<i>Hyalessa maculaticollis</i> (Motschulsky, 1866)	Zhouzhi, Shaanxi, China	vii. 2012
	<i>Pomponia linearis</i> (Walker, 1850)	Ya'an, Sichuan, China	vii. 2009
	<i>Tanna japonensis</i> (Distant, 1892)	Hongya, Sichuan, China	vii. 2009
	Tettigadinae	<i>Subsalstria yangi</i> Chen, 1943	Helan Mountains, Ningxia, China
Cicadettinae	<i>Kosemia yezoensis</i> (Matsumura, 1898)	Helan Mountains, Inner Mongolia	viii. 2010
	<i>Karenia caelatata</i> Distant, 1890	Ningshan, Shaanxi, China	viii. 2010
	<i>Katoa neokanagana</i> (Liu, 1940)	Pingwu, Sichuan, China	vii. 2013
	<i>Huechys sanguinea</i> (Degeer, 1773)	Qiongzong, Hainan, China	v. 2011

[30–100% (v/v)]. After dehydration, the samples were dried in a critical point-drier, mounted on stubs, coated with gold, and examined under a scanning electron microscope (JEOL JSM-6360 LV) at 15 kV.

Terminology of ovipositor follows that of Boulard (1996), with exception of the term gonoplacs which is adopted from Mejdalani (1998). Terminology of sensilla is adopted from Dietrich (1989), Hummel et al. (2006) and Merivee et al. (2002).

Results

Structure and comparative morphology of ovipositor

The female abdomen consists of 11 segments including a trochiformis pygofer. The pygofer is formed by a coalescence of pleurite and tergite of the ninth abdominal segment. The elongate ovipositor is composed of three pairs of gonapophyses and two pairs of valvifers. The gonapophyses are surrounded externally by the pygofer. Each gonapophysis possesses an articulation point with a valvifer on its corresponding side: the gonapophyses VIII are borne by the anterior margins of the first valvifers; the gonapophyses IX are borne by the anterior margins of the second valvifers, while their posterior margins give rise to the gonoplacs. The concave gonoplacs are a pair of flaplike structures which envelope the gonapophyses (VIII and IX), serving as a protective sheath. The gonapophyses VIII slide along the gonapophyses IX, comprising two morphologically distinct parts: a pair of tongue-shaped slices and a pair of tooth-bearing portions. The tongue-shaped slices attached laterally to the tooth-bearing portions, with tips being free. The gonapophyses IX mostly unite along their dorsal edges but grooved ventrally, and separate from each other for a short space at the apical region (Fig. 1).

The gonoplacs exhibit a significant similarity among examined species. Similarly, the gonapophyses also possess some common features, but evident differences exist among different taxa, for details, see below and Table 2.

Gonapophyses in Cicadinae

Cryptotympana atrata (Fabricius, 1775)

Each gonapophysis VIII bears 13 teeth. The first three ones are ridge-shaped (Fig. 2a, b). The first and second teeth overlap the broad apically rounded tongue-shaped slice. Mesal margin of the slice is straight. Distal parts of the slices are slightly separated from each other. Tooth four is large and rounded marginally. Tooth five, the largest one, is rounded marginally, standing out and widely separated from

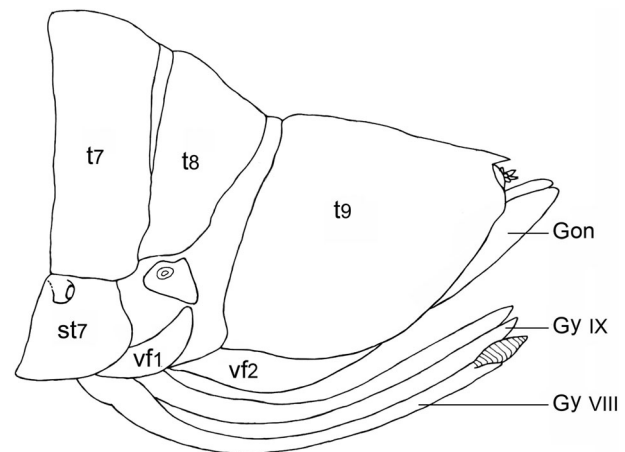


Fig. 1 Schematic illustration of the female terminalia of cicada *Platyleura kaempferi*. *t7* tergite 7, *t8* tergite 8, *t9* tergite 9, *vf1* valvifer 1, *vf2* valvifer 2, *st7* sternite 7, *Gon*, gonoplacs; *Gy VIII*, gonapophysis VIII; *Gy IX*, gonapophysis IX

teeth four and six. Tooth six is rounded, but smaller than tooth five. Teeth five to eight possess the same shape, decreasing gradually in size. Teeth nine to 13 slanted towards the tip, decreasing gradually in size. The tongue-shaped slice (~0.70 mm in length) is approximately 0.37 times the length of the tooth-bearing portion (~1.90 mm long). Ventral outer margin of the teeth contains many polygonal (especially quadrangular and hexagonal) cells with high depression. Each two adjoining cells share a common ridge (~1.30 μm wide) (Fig. 2c, d). A pair of modestly obvious, hump-like protuberances are observed adjacent to the tips of the gonapophyses IX (Fig. 2e). Regularly arranged ridges distributed along the midline are vestigial inward at the basal and distal regions (Fig. 2f, g).

Platyleura kaempferi (Fabricius, 1794)

Each gonapophysis VIII possesses 17 teeth. The first four ones overlap the narrow apically rounded tongue-shaped slice (Fig. 2h). Teeth one to six are ridge-shaped and morphologically similar. Tooth seven is slightly larger than the first six ones. Teeth seven to 11 decrease in size, rectangular in shape, standing out, and widely separated from each other (Figs. 2h, 3a). Morphologically similar teeth ranging from 12 to 17 are decreased gradually in size. The tongue-shaped slice (about 0.48 mm in length) is wide basally (about 0.13 mm wide) and narrow distally (~0.05 mm wide). It is approximately 0.40 times the length of the tooth-bearing part (~1.16 mm long). Distal parts of the slices are highly separated from each other. Mesal margin of the slice is straight. Ventral outer margin of the teeth has many hexagonal cells containing campanulate protuberances (~2.5 μm in diameter) (Fig. 3a, b). Common ridge shared by each two adjoining cells is about 1.3 μm in width. The

Table 2 A summary of ultramorphology of the gonapophyses

Subfamily	Species	TN		TS		NR		OM		Pr-T	Pr-D	R
		MM	TS	Ap	Sp	Ce	Ce					
Cicadinae	<i>C. atrata</i>	13	Straight	Broad, rounded	Slightly separated	3	Quadrangular, and hexagonal	Absent	A pair, hump-like	Absent		
	<i>Pl. kaempferi</i>	17	Straight	Narrow, rounded	Highly separated	6	Hexagonal	Campanulate	Two pairs; hump-like	Absent		
	<i>Pl. hilpa</i>	13	Straight	Narrow, pointed	Closely meet	5	Absent	Absent	A pair; slightly raised	Absent		
	<i>M. mongolica</i>	15	Straight	Narrow, rounded	Slightly separated	5	Quadrangular	Spherical	A pair; separated	Cordillera-like		
	<i>M. opalifera</i>	15	Straight	Narrow, rounded	Slightly separated	5	Quadrangular	Campanulate	A pair; meet	Absent		
	<i>Kar. caelata</i>	15	Straight	Broad, rounded	Slightly separated	3	Quadrangular	Oval-/spherical-/rod-shaped	Absent	Cordillera-like		
	<i>Hy. maculaticollis</i>	14	Straight	Narrow, rounded	Highly separated	4	Irregularly quadrangular	Oval	A pair; slightly raised	Cordillera-like		
	<i>Po. linearis</i>	12	Straight	Narrow, pointed	Modestly separated	–	Absent	Absent	Two pairs; ovoid and ridge-like	Absent		
	<i>T. japonensis</i>	9	Straight	Narrow, pointed	Slightly separated	–	Irregularly quadrangular/hexagonal	Absent	Two pairs; ovoid and ridge-like	Absent		
Tettigadinae	<i>S. yangi</i>	13	Straight	Narrow, pointed	Slightly separated	3	Absent	Slightly raised	Two pairs; spherical, and flake-like	Herringbone pattern		
Cicadettinae	<i>Ko. yezoensis</i>	17	Straight	Broad, rounded	Slightly separated	–	Absent	Absent	Absent	Pectinate		
	<i>Kat. neokanagana</i>	15	Highly curved	Broad, pointed	Highly separated	–	Absent	Absent	Absent	Pectinate		
	<i>Hu. sanguinea</i>	15	Slightly curved	Narrow, pointed	Highly separated	–	Absent	Absent	Absent	Pectinate		

NT number of teeth, MM mesal margins, TS tongue-shaped slices, Ap apex of the tongue-shaped slices, Sp separation at the distal parts of the tongue-shaped slices, NR number of ridge-like teeth, OM near the ventral outer margin of teeth, Ce cells exist near the ventral outer margin of the teeth, Pr-T protuberances near or inside the cells on the ventral outer margin of teeth, Pr-D protuberances on the distal dorsal surface of the gonapophysis IX, R ridges along the midline on the gonapophysis IX

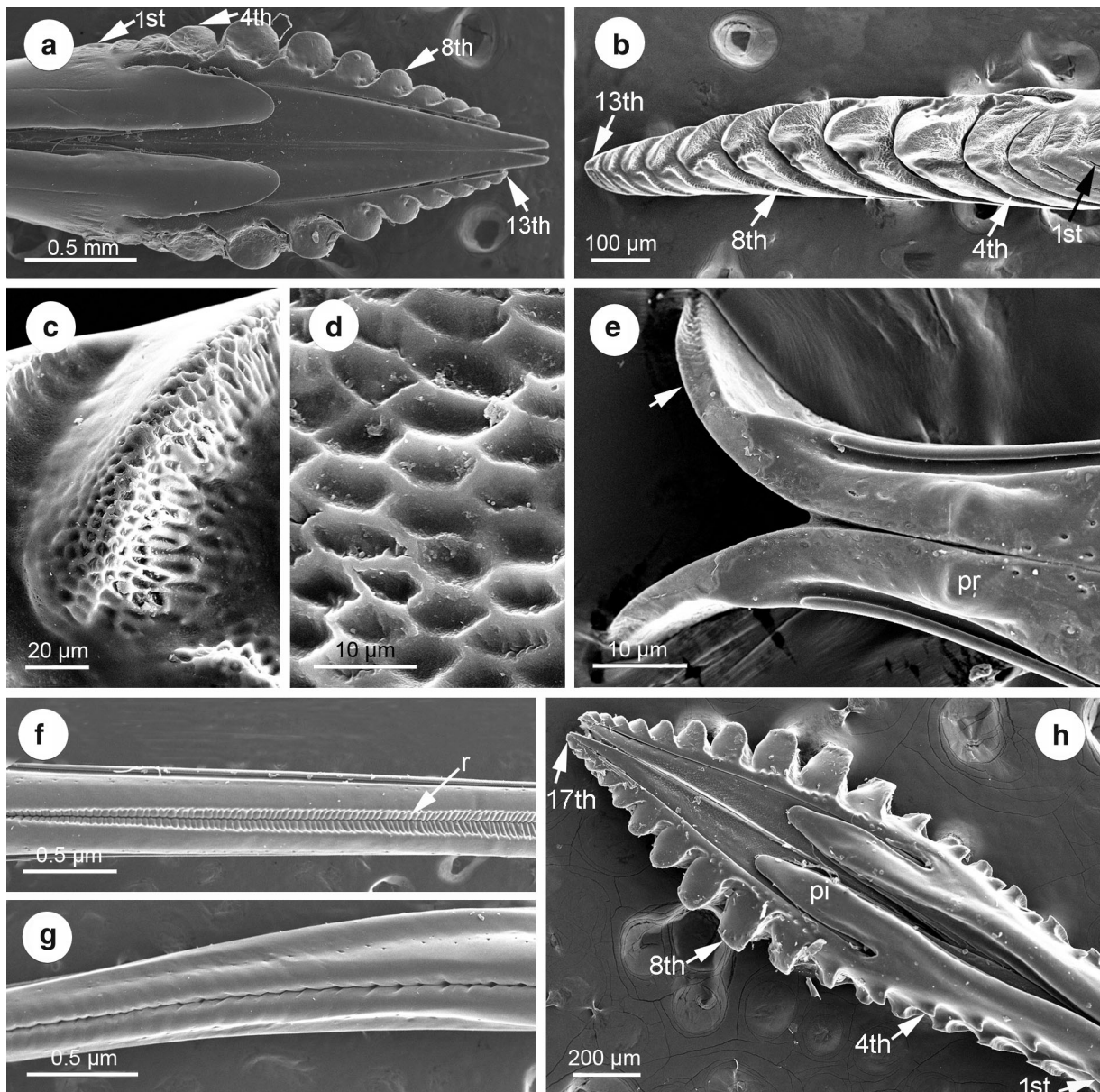


Fig. 2 The gonapophyses of *Cryptotympana atrata* and *Platyleura kaempferi*. **a–g** *C. atrata*, **h** *Pl. kaempferi*. **a** Ventral view of the gonapophyses. **b** Lateral view of the gonapophysis VIII. **c** Lateral view of the three teeth of the gonapophysis VIII, showing polygonal cells at the outer margin; each two adjoining 1 cells share a common ridge. **d** Close-up of polygonal cells of a tooth. **e** Dorsal view of the distal gonapophyses IX. *Arrow* indicates the apical part of the

gonapophysis IX. **f** Dorsal view of the distal and middle parts of the gonapophysis IX, showing ridges along the midline. **g** Dorsal view of basal region of the gonapophysis IX. **h** Ventral view of the gonapophyses of *P. kaempferi*. *pi* tongue-like slice, *r* ridge, *pr* protuberance, *1st* the first tooth, *4th* the fourth tooth, *8th* the eighth tooth, *13th* the thirteenth tooth, *17th* the seventeenth tooth

gonapophyses IX possess two pairs of protuberances: a pair of vestigial hump-like protuberances (~ 0.03 mm wide and 0.05 mm long) located at the convergent point of inner apical margins, and a pair of large hump-like protuberances (~ 0.08 mm wide and 0.18 mm long) located about 0.30 mm far away from the former pair (Fig. 3c). Each large protuberance possesses an acute postero-lateral angle. No ridges are present along the midline.

Platyleura hilpa Walker, 1850

The gonapophysis VIII possesses 13 teeth. The first four ones overlap the narrow, apically pointed tongue-shaped slice (Fig. 3d, e). Mesal margin of the slice is straight. Distal parts of the slices meet each other closely. Teeth ranging from one to five are ridge-shaped and morphologically similar. Tooth six is slightly larger than the

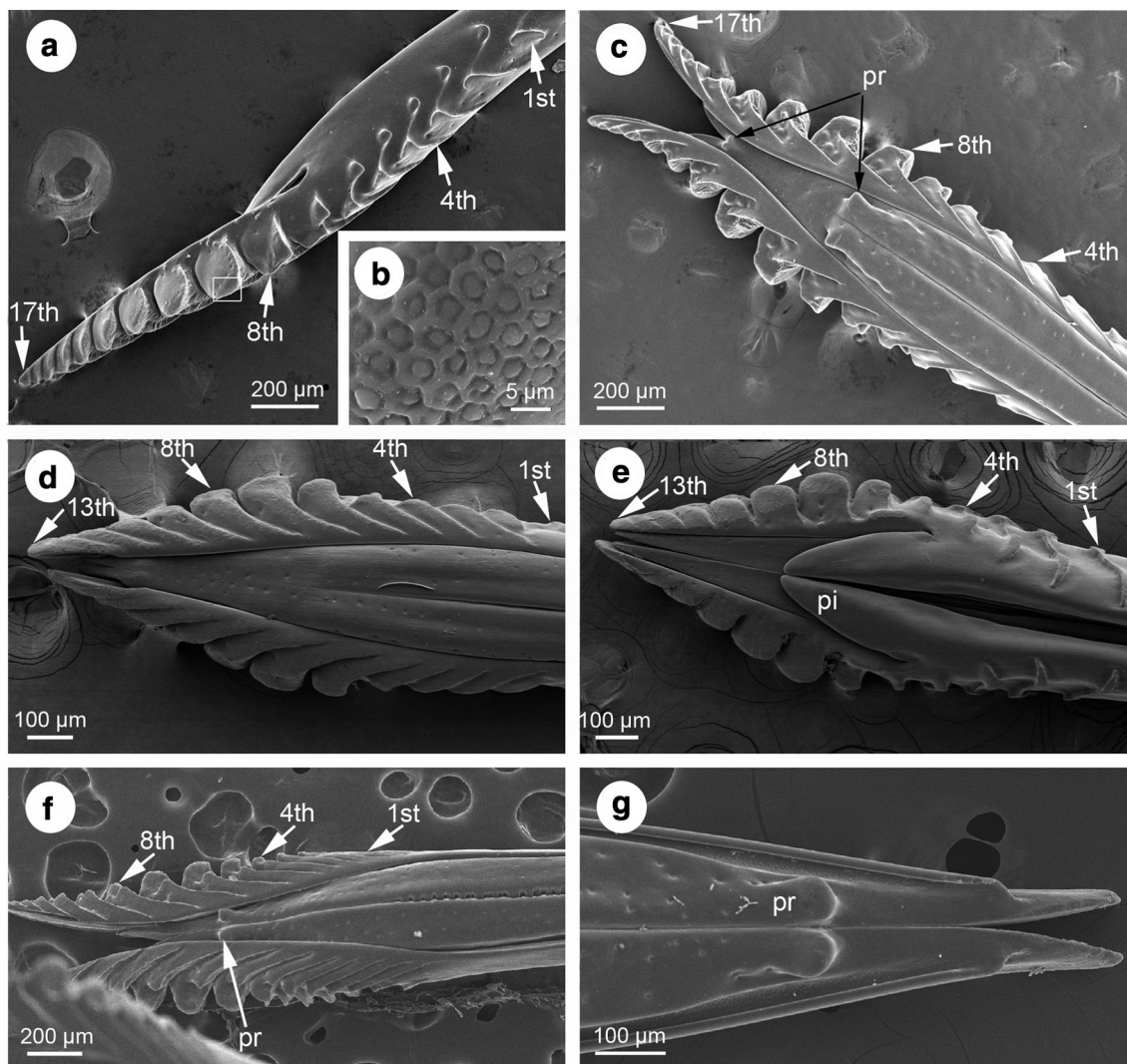


Fig. 3 The gonapophyses of *Platypleura kaempferi*, *Pl. hilpa*, and *Meimuna mongolica*. **a–c** *Pl. kaempferi*, **d, e** *Pl. hilpa*, **f, g** *M. mongolica*. **a** Lateral view of the gonapophysis VIII. White rectangular inset, polygonal cells magnified in **b**. **b** Polygonal cells with campanulate protuberances inserted from **a**. **c** Dorsal view of the gonapophyses. **d** Dorsal view of the gonapophyses. **e** Ventral view of

the gonapophyses. **f** Dorsal view of the gonapophyses. **g** Dorsal view of distal region of the gonapophyses IX, showing a pair of protuberances on surface. *pi* tongue-like slice, *pr* protuberance, *1st* the first tooth, *4th* the fourth tooth, *8th* the eighth tooth, *13th* the thirteenth tooth, *17th* the seventeenth tooth

first five ones. Rectangular teeth seven to nine decrease in size, standing out, and widely separated. Teeth ten to 13 are morphologically similar, and decreased gradually in size. The tongue-shaped slice (~ 0.35 mm in length) is wide basally (~ 0.13 mm wide) and narrow distally (~ 0.03 mm wide). It is approximately 0.28 times the length of the tooth-bearing part (~ 1.25 mm long). Neither polygonal cells nor protuberances are observed on the ventral outer margin of the teeth. The gonapophyses IX that are devoid of any ridges along the midline, possess a pair of slightly raised protuberances (~ 50.0 μm in diameter) located about 100.0 μm far away from the tips (Fig. 3d).

Meimuna mongolica (Distant, 1881)

Each gonapophysis VIII has 15 teeth, of which the first five teeth are ridge-like; the teeth ranging from six to ten are rounded (Figs. 3f, 4a). The first three ones overlap the narrow and apically rounded tongue-shaped slice. The slice is about 0.53 mm long, and approximately 0.37 times the length of the tooth-bearing part (~ 1.43 mm long). The slices with a straight mesal margin are slightly separated at their distal parts. Many quadrangular cells with spherical protuberances (~ 3.0 μm in diameter) are visible on the ventral outer margin of the teeth (Fig. 4b, c). Common ridge shared by each two adjoining cells is about 1.30 μm in width. The gonapophyses

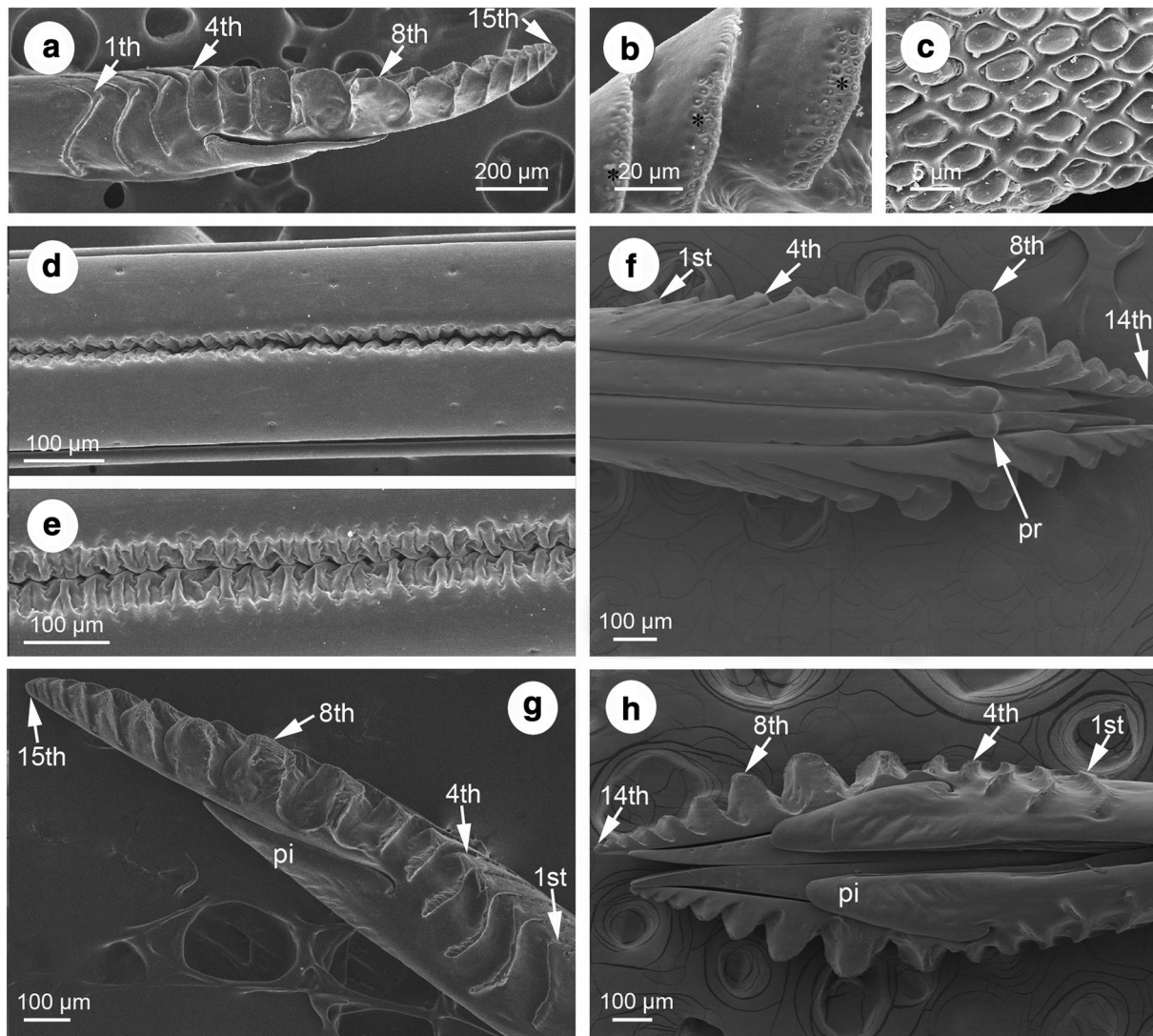


Fig. 4 The gonapophyses of *Meimuna mongolica* and *M. opalifera*. **a–e** *M. mongolica*, **f–h** *M. opalifera*. **a** Lateral view of the gonapophysis IX. **b** Lateral view of the distal five teeth of the gonapophysis VIII, showing special regions containing polygonal cells (asterisks). **c** Close-up of the polygonal cells with spherical and raised protuberances of a tooth. **d** Dorsal view of the basal region of

the gonapophysis IX, showing ridges along the midline. **e** Dorsal view of the middle region of the gonapophysis IX, showing ridges along the midline. **f** Dorsal view of the gonapophyses. **g** Lateral view of the gonapophysis VIII. **h** Ventral view of the gonapophyses. *pi* tongue-like slice, *pr* protuberance, *1st* the first tooth, *4th* the fourth tooth, *8th* the eighth tooth, *14th* the fourteenth tooth, *15th* the fifteenth tooth

IX possess dorsally a pair of large, elongated protuberances (~ 0.10 mm long) (Fig. 3f, g); bear cordillera-like ridges with varying gullies on the inner margin (Fig. 4d, e).

Meimuna opalifera (Walker, 1850)

Each gonapophysis VIII possesses 15 teeth, and the first four ones overlap the narrow, apically rounded tongue-shaped slice (Fig. 4f, g). Teeth ranging from one to five are ridge-shaped. Teeth from seven (the largest one) to 15 are rectangular in shape, widely separated from each other, and decreased gradually in size (Fig. 4h). The tongue-shaped slice (~ 0.48 mm in length) is wide basally (~ 0.18 mm

wide) and rounded distally (~ 50.0 μm wide), approximately 0.36 times the length of the tooth-bearing part (~ 1.35 mm long). Mesal margin of the slice is straight, with apex slightly separated from the other one. Ventral outer margin of each tooth possesses many quadrangular cells containing campanulate protuberances (~ 3.0 μm in diameter). The arrangement and shape of the cells and protuberances are similar to those of *M. mongolica*. Ridge shared by each two neighboring cells is about 1.3 μm wide. The gonapophyses IX are devoid of ridges along the midline. This gonapophyses possess a pair of large, hump-like protuberances (~ 55.0 μm both in width and length) located about 0.38 mm far away from the apices (Fig. 4f).

Hyalessa maculaticollis (Motschulsky, 1866)

Each gonapophysis VIII contains 14 teeth (Fig. 5a, b). Teeth one to four are ridge-like, of which the first three ones overlap the narrow and apically rounded tongue-shaped slice. Teeth ranging from five to nine are rounded, standing out and widely separated from each other. Teeth from four to six increase in size successively. The last five teeth are similar in shape, slant towards the tip increasingly. The tongue-shaped slice (~ 0.52 mm long) is wide basally and narrowed apically, approximately 0.44 times the length of tooth-bearing part (~ 1.18 mm long) (Fig. 5a). The slices with straight mesal margin are widely separated from each other at their distal parts. Many irregular, quadrangular cells with raised oval protuberances (about $4.0 \mu\text{m}$ in diameter) are observed on the ventral outer margin of the teeth (Fig. 5b, c). A common ridge shared by each two neighboring cells is about $0.85 \mu\text{m}$ in width. The gonapophyses IX possess a pair of slightly raised protuberances near the tips (Fig. 5d). Cordillera-like ridges with varying gullies distribute on the inner side of

the middle region; and vestigial grooves are only located at the basal part of the gonapophyses. The ridges are simpler than those of *M. mongolica*.

Pomponia linearis (Walker, 1850)

Each gonapophysis VIII contains 12 teeth (Figs. 5e, 6a), of which the first tooth is ridge-like and overlaps the narrow and apically pointed tongue-shaped slice (Fig. 6b). Teeth ranging from five to nine are rectangular marginally, standing out and slightly separated from each other. Teeth from three to seven increase in size successively (Fig. 5e). The last five teeth are similar in shape, and slanted towards the tip increasingly. The tongue-shaped slice (~ 0.35 mm long) is wide basally and narrowed apically, approximately 0.44 times the length of the tooth-bearing part (~ 0.80 mm long) (Fig. 5e). The slice with straight mesal margin are modestly separated from each other at their distal parts. Ventral outer margin of each teeth is devoid of polygonal cells and protuberances. The gonapophyses IX possess two pairs of morphologically distinct protuberances: a pair of

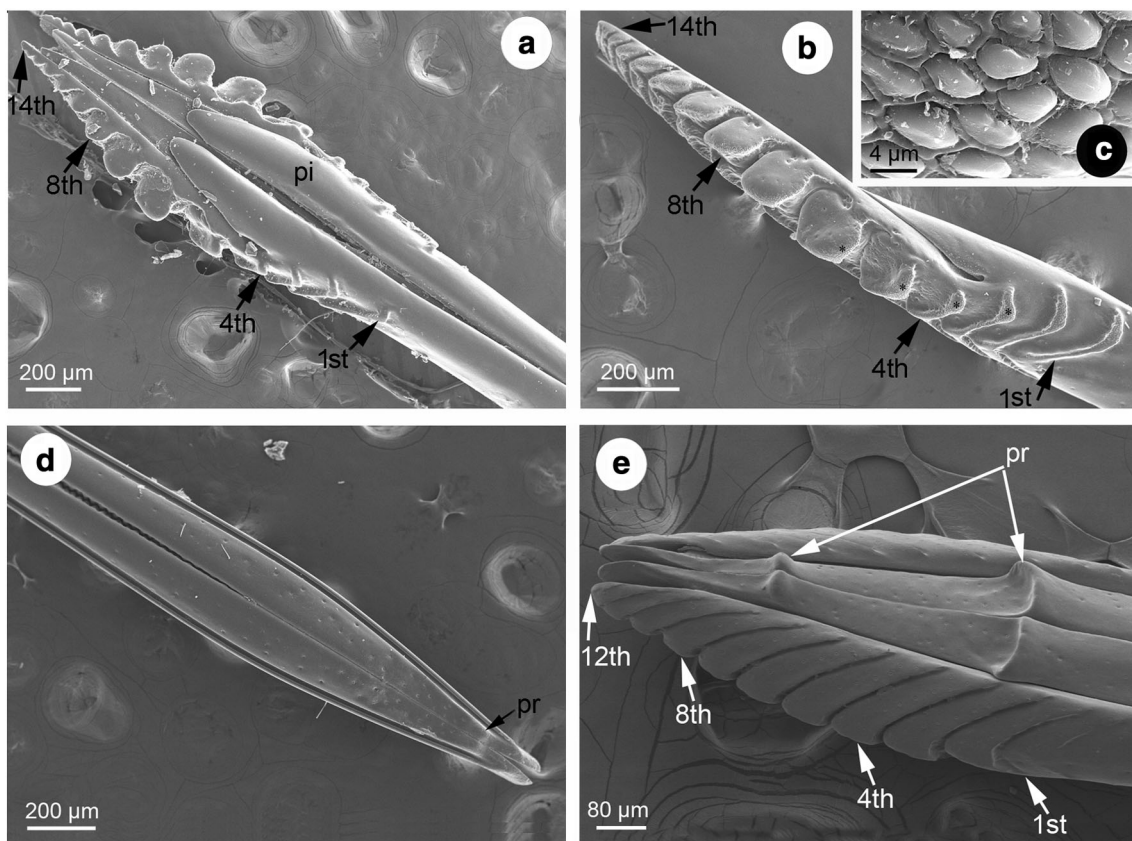


Fig. 5 The gonapophyses of *Hyalessa maculaticollis* and *Pomponia linearis*. **a–d** *Hy. maculaticollis*, **e** *Po. linearis*. **a** Ventral view of the gonapophyses. **b** Lateral view of the gonapophysis VIII. Asterisks indicate special region containing polygonal cells. **c** High magnification of oval protuberances filling the polygonal cells of the

gonapophysis VIII. **d** Dorsal view of the gonapophysis IX. **e** Lateral view of the gonapophyses. *pi* tongue-like slice, *pr* protuberance, *1st* the first tooth, *4th* the fourth tooth, *8th* the eighth tooth, *12th* the twelfth tooth, *14th* the fourteenth tooth

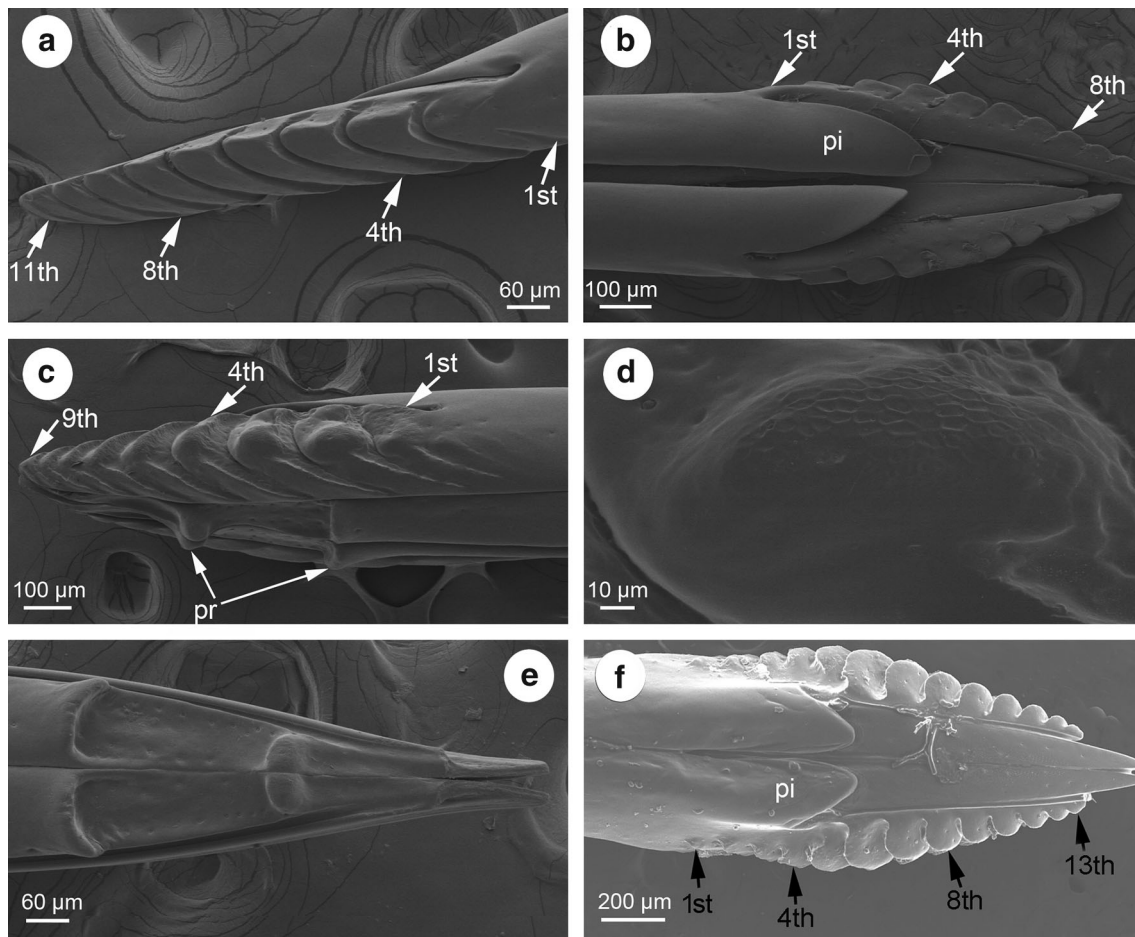


Fig. 6 The gonapophyses of *Pomponia linearis*, *Tanna japonensis*, and *Subpsaltria yangi*. **a**, **b** *P. linearis*, **c–e** *T. japonensis*, **f** *S. yangi*. **a** Lateral view of the gonapophysis VIII. **b** Ventral view of the gonapophyses. **c** Lateral view of the gonapophyses. **d** Close-up of

polygonal cells of a tooth. **e** Dorsal view of the gonapophyses IX. **f** Ventral view of the gonapophyses. *pi* tongue-like slice, *pr* protuberance, *1st* the first tooth, *4th* the fourth tooth, *8th* the eighth tooth, *11th* the eleventh tooth, *13th* the thirteenth tooth

ovoid protuberances ($\sim 0.04 \mu\text{m}$ in diameter) located very closely ($\sim 0.28 \text{ mm}$) to the tips, and a pair of ridge-like protuberances ($\sim 70.0 \mu\text{m}$ long) positioned relatively far away ($\sim 0.68 \text{ mm}$) from the apex. Distance between the two pairs of protuberances is about 0.36 mm (Fig. 5e). No ridges are found along the midline.

Tanna japonensis (Distant, 1892)

Each gonapophysis VIII bears only nine teeth, without ridge-like teeth overlapping the narrow, apically pointed tongue-shaped slice (Fig. 6c). Teeth ranging from three (the largest one) to five are rounded marginally, standing out and widely separated. Tooth six is rounded, but smaller than tooth five. Teeth from six to nine, slanted towards the tip, possess the same shape but decrease gradually in size. The tongue-shaped slice ($\sim 0.43 \text{ mm}$ long) with straight mesal margin is approximately 0.41 times the length of the tooth-bearing portion ($\sim 1.05 \text{ mm}$ long). Distal parts of the

slices are slightly separated. Irregularly quadrangular or hexagonal cells are observed on the ventral outer margin of the teeth (Fig. 6d). The cells possess shallow depressions that are devoid of protuberances inside. Ridge shared by each two adjoining cells is about $0.6 \mu\text{m}$ in width. The gonapophyses IX possess two pairs of protuberances (Fig. 6e): a pair of ovoid protuberances ($\sim 45.0 \mu\text{m}$ in diameter) located very closely ($\sim 0.36 \text{ mm}$ far away) to the tips, and a pair of ridge-like protuberances ($\sim 0.12 \text{ mm}$ long) positioned relatively far away ($\sim 0.69 \text{ mm}$) from the apex. Distance between the two pairs of protuberances is about 0.27 mm . No ridges are observed along the midline.

Gonapophyses in Tettigadinae

Subpsaltria yangi Chen, 1943

Each gonapophysis VIII bears 13 teeth, of which the first three ones are ridge-like, overlapping the narrow, apically

pointed tongue-shaped slice. Teeth ranging from four to eleven are greatly rounded (Fig. 6f). Teeth from four to six increase successively in size. Teeth from six to 13 decrease in size gradually. Teeth 12 and 13 are very small. The tongue-shaped slice (~0.20 mm long) possesses the following characters: (1) tip pointed, about 0.05 mm wide distally and 0.23 mm wide basally; (2) approximately 0.16 times the length of the tooth-bearing part (~1.28 mm long); and (3) outer margin is far away from the tooth-bearing part (Fig. 6f). The slices with straight mesal margin are slightly separated at their distal parts. Ventral outer margin of the teeth is devoid of polygonal cells, with the exception for slightly raised protuberances (Fig. 7a, b). Irregular, developed ridges are visible between the outer and inner margins of each tooth (Fig. 7c, d). The gonapophyses IX possess two pairs of distinct protuberances: a pair of spherical protuberances (~0.04 mm in diameter) located far away (~19.30 mm) from the tips; and a pair of flake-like protuberances (~0.09 mm wide, 0.06 mm long) positioned relatively closely (~15.30 mm far away) to the apex (Fig. 7e). Distance between the two pairs of protuberances is about 3.30 mm. Regularly arranged ridges exhibit a herringbone pattern (Fig. 7e, f), which arise along the midline of the flake-like protuberances, reaching to the middle region of the gonapophyses IX. The ridges exhibit regularly arranged sulci at the middle area, and become vestigial at the basal region (Fig. 7f).

Gonapophyses in Cicadettinae

Kosemia yezoensis (Matsumura, 1898)

Each gonapophysis VIII bears 16 teeth that are lack of ridge-like ones (Fig. 7g, h). The first tooth, smaller than the second one, is tongue-shaped and closely attached to the latter. Teeth two and three which are slanted toward the apex, are small and pointed (Fig. 7h). Teeth four and five possess a rounded margin, and the latter is larger than the former. Teeth six and seven with similar size and shape are the largest ones among all the teeth. Teeth ranging from eight to 14 have more rounded margins and decrease successively in size. The broad, apically rounded tongue-shaped slice (~0.60 mm long) is overlapped by tooth one. The slice is approximately 0.46 times the length of the tooth-bearing part (~1.30 mm long). The slice with straight mesal margin is slightly separated at their distal parts (Fig. 7h). Ventral outer margin of the teeth is devoid of protuberances (Fig. 7g), instead, wrinkles form longitudinally parallel ridges with serrated distal are observed (Fig. 8a, b). The gonapophyses IX are devoid of protuberances (Fig. 7g). Regularly arranged pectinate ridges of this gonapophysis exhibit the following distinct characters (Fig. 8c–e): (1) the surface between each two neighboring ridges is smooth; ridges near

the distal region are short and arranged interlacedly; (2) long ridges with interlaced arrangement at the middle region is slanted towards the apex; and (3) long ridges near the basal region are parallel. Paralleled sulci distribute at the basal region of the gonapophyses IX are similar to those of *Hy. maculaticollis* and *S. yangi*.

Katoa neokanagana (Liu, 1940)

Similar to *T. japonensis* and *Ko. yezoensis*, no ridge-like teeth are observed on gonapophysis VIII. Each gonapophysis VIII bears 15 teeth (Fig. 8f), of which the first two teeth are tongue-shaped and closely attached to each other. Small pointed teeth three and four are slanted towards the apex (Fig. 8g). Tooth five has a rounded margin. Margin-rounded teeth ranging from six to 11 decrease successively in size. Teeth from 12 to 15 are similar in shape, slanted towards the tip and decrease gradually in size. The tongue-shaped slices (~0.70 mm long) overlapped by the tooth one are broad and apically pointed, approximately 0.57 times the length of the tooth-bearing part (~1.23 mm long). The slices with notably curved mesal margin are remarkably separated at their distal parts. Ventral outer margin of the teeth is devoid of protuberances, instead, undeveloped longitudinal ridges are distributed irregularly (Fig. 8h). The gonapophyses IX are devoid of protuberances. Pectinate ridges with regular arrangement are distributed along the midline at the middle region (Fig. 8i). The pectinate ridges between distal and middle regions are arranged interlacedly, whereas at the medial-posterior area they are arranged the opposite. Similar to *S. yangi*, ridges at the basal and distal regions of the gonapophyses IX are vestigial inside.

Huechys sanguinea (Degeer, 1773)

Each gonapophysis VIII bears up to 15 teeth (Fig. 9a, b), however, no ridge-like teeth are observed. The first tooth is smaller than the second one and closely attached to the latter. Small apically pointed teeth two and three are slanted towards the apex. Tooth four possesses a rounded margin. Tooth five with a rounded margin is larger than tooth four. Teeth six and seven, similar in size and shape, are the largest ones among the teeth. Teeth ranging from eight to 14 are similar in shape, having a rounded margin and decreasing successively in size. Narrow, apically pointed tongue-shaped slices (~0.25 mm long) are overlapped by tooth one. The slices are approximately 0.24 times the length of the tooth-bearing parts (~1.05 mm long). The slices with slightly curved mesal margin are widely separated at their distal parts. Ventral outer margin of the teeth is devoid of protuberances, instead, wrinkles form longitudinal ridges observed (Fig. 9c; the wrinkle are

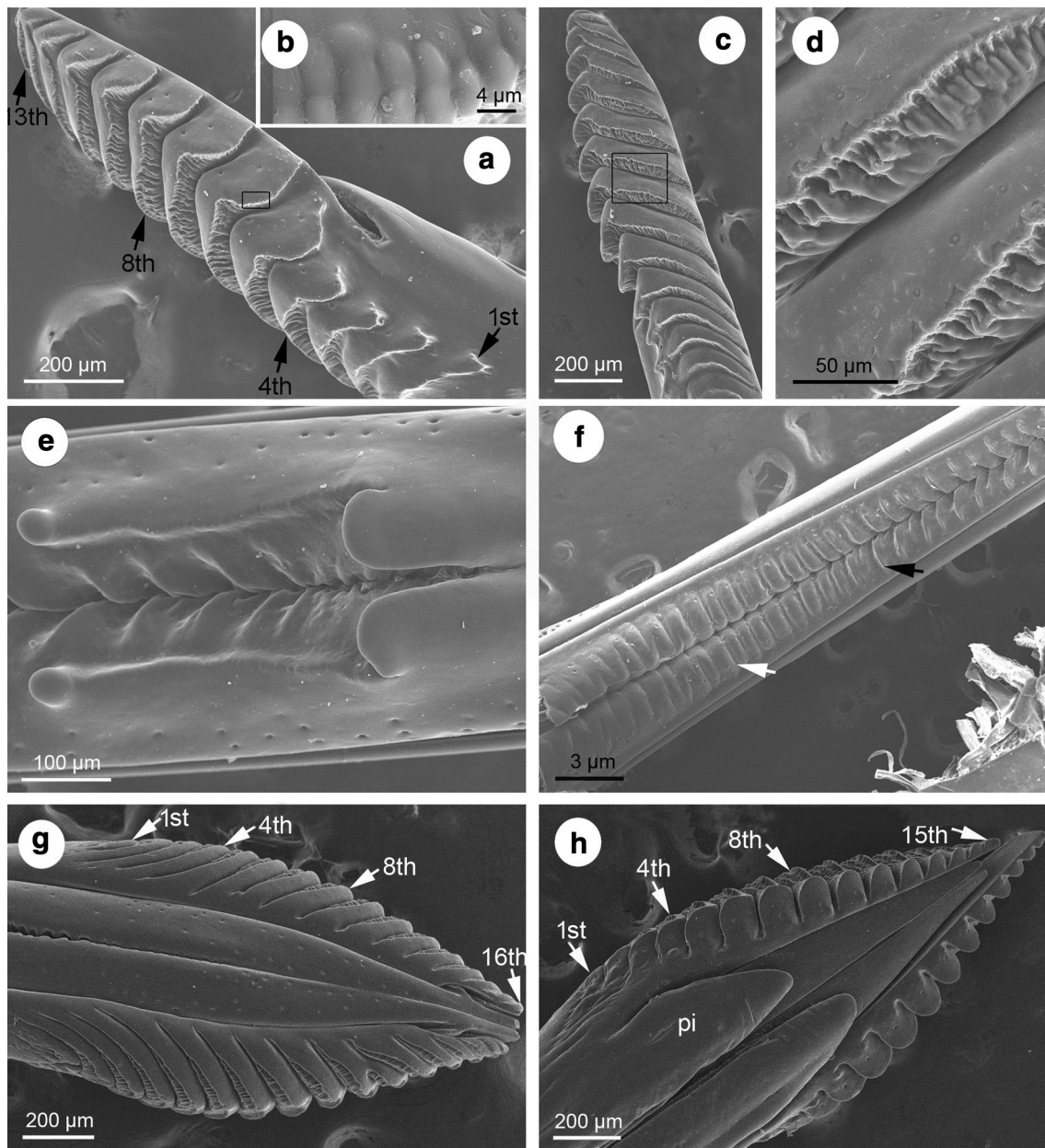


Fig. 7 The gonapophyses of *Subpsaltria yangi* and *Kosemia yezoensis*. **a–f** *S. yangi*, **g–h** *Ko. yezoensis*. **a** Ventral-lateral view of the gonapophysis VIII. *Inset* slightly raised protuberances, magnified in **b**. **b** *Inseted* from **a**. Close-up of the slightly raised protuberances on the surfaces of teeth. **c** Dorsal inner margin of the teeth. *Inset*, developed ridges magnified in **d**. **d** Close-up of the developed ridges, *inseted* from **c**. **e** High magnification of a pair of spherical

protuberances and a pair of flake-shaped protuberances on the gonapophyses IX. **f** Dorsal view of the basal and middle regions of the gonapophyses IX showing regularly arranged ridges (as indicated by *black arrow*) and sulci (indicated at *white arrow*) along the midline. **g** Dorsal view of the gonapophyses. **h** Ventral view of the gonapophyses. *pi* tongue-like slice

more developed than those of *Kat. neokanagana*). Regularly arranged pectinate ridges of gonapophyses IX exhibit the following distinct characters (Fig. 9d): (1) the surface between each two neighboring ridges is smooth; undeveloped ridges near the distal region are short and arranged interlacedly; (2) long ridges at the middle region with the opposite arrangement are slightly slanted towards the apex; and (3) long ridges near the basal region are parallel.

Protuberances are lacked on the gonapophyses IX. Paralleled sulci observed at the basal region are similar to those of *Ko. yezoensis*, *Hy. maculaticollis* and *S. yangi*.

Karenia caelatata Distant, 1890

Each gonapophysis VIII bears 15 teeth, and the first three ridge-shaped teeth overlap the broad, apically rounded

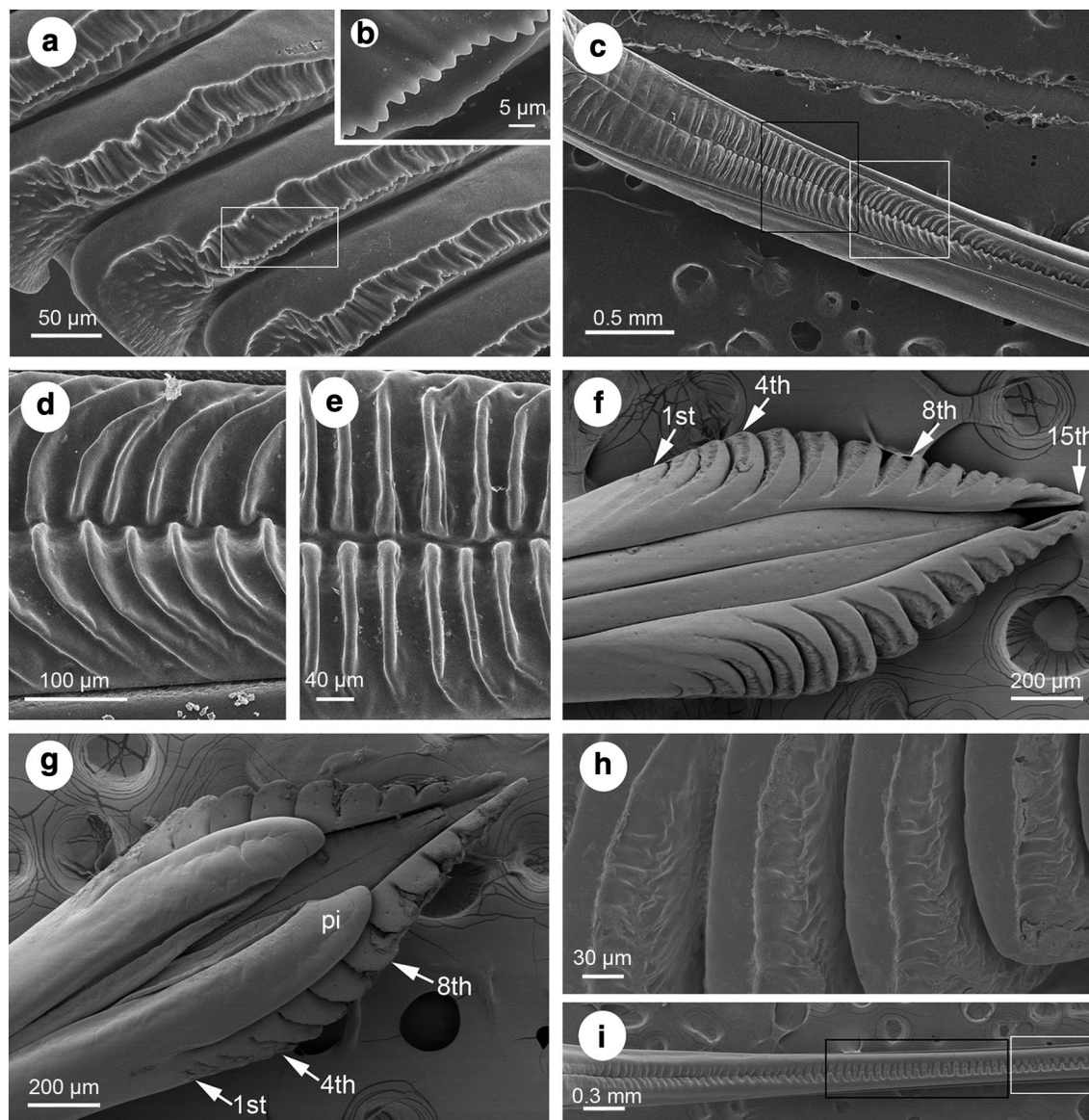


Fig. 8 The gonapophyses of *Kosemia yezoensis* and *Katoa neokanagana*. **a–e** *Ko. yezoensis*, **f–i** *Kat. neokanagana*. **a** High magnification of the middle partition of the gonapophysis VIII. *Inset* serrated distal ends of longitudinal parallel ridges on the outer margin of the teeth, magnified in **b**. **b** Close-up of the serrated distal ends of longitudinal parallel ridges *inseted* from **a**. **c** Dorsal view of the middle and basal parts of the gonapophyses IX. *White inset*, ridges along the midline of the middle region, magnified in **d**. *Black inset*, ridges along the middle line adjacent to the basal region, magnified in **e**. **d** Dorsal view of the gonapophyses IX, *inseted* from **c**. Close-up of the ridges of the

tongue-shaped slice (Fig. 9e). Teeth ranging from four to eight gradually increase in size (Fig. 9f). Teeth from seven to ten are rounded marginally, standing out and separated from each other. Teeth from eight (the largest one) to eleven are similar in shape, and decreased successively in size. The last four teeth are same shaped, slanted towards the tip, and decreased successively in size. The tongue-shaped slice (~0.65 mm long) is narrow distally and wide

basally. **e** Dorsal view of the gonapophyses IX, *inseted* from **c**. Close-up of the ridges near the basal region. **f** Dorsal view of the gonapophyses. **g** Ventral view of the gonapophyses. **h** Close-up of the undeveloped ridges on the outer margin of teeth. **i** Dorsal view of the middle and basal parts of the gonapophyses IX. *White rectangular inset*, pectinate ridges which are arranged interlacedly. *Black inset*, pectinate ridges which are arranged in opposite. *pi* tongue-like slice, *1st* the first tooth, *4th* the fourth tooth, *8th* the eighth tooth, *15th* the fifteenth tooth

basally. It is approximately 0.3 times the length of the tooth-bearing part (~2.0 mm long) (Fig. 9e). The slices with straight mesal margin are slightly separated at their distal parts. Oval-, spherical-, or rod-shaped protuberances (~3.8 µm in diameter) of various sizes are irregularly exposed on the ventral outer margin of the teeth (Fig. 9g). A few quadrangular cells are found near the protuberances. The gonapophyses IX are devoid of any protuberances,

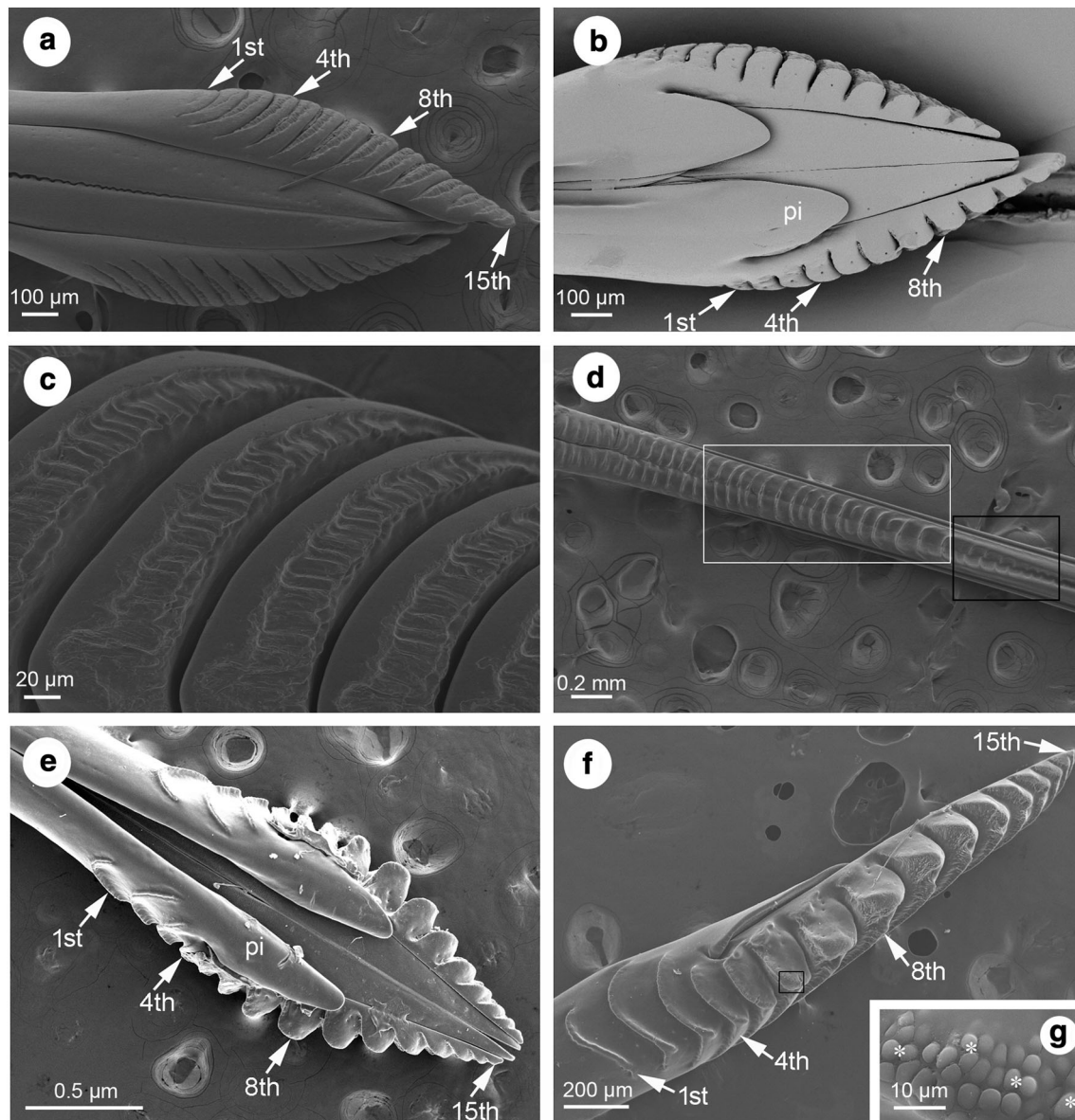


Fig. 9 The gonapophyses of *Huechys sanguinea* and *Karenia caelata*. **a–d** *Hu. sanguinea*, **e–g** *Kar. caelata*. **a** Dorsal view. **b** Ventral view. **c** Close-up of the undeveloped ridges on the outer margin of teeth. **d** Dorsal view of the gonapophyses IX. *Black inset*, pectinate ridges which are arranged interlacedly near the distal region. *White rectangular inset*, pectinate ridges which are arranged in

with shape and arrangement of ridges being very similar to those observed in *Hy. maculaticollis*.

Sensilla on the gonapophyses and gonoplags

Based on morphological features, eight types of campaniform sensilla, two types of basiconica sensilla, five types of trichoid sensilla, one type of coeloconic sensilla, and cuticular depressions are identified from the two pairs of gonapophyses and one pair of gonoplags. The

opposite at the middle region. *pi* tongue-like slice. **e** Ventral view of the gonapophyses. **f** Lateral view of the gonapophysis IX. *Inset*, raised protuberances, magnified in **g**. **g** Raised protuberances on the cell-less area of a tooth of the gonapophysis VIII, *inseted* from **f**. *15th* the fifteenth tooth

morphological characters and distribution of these sensilla are described below and summarized in Table 3.

Campaniform sensilla (SCa)

Dome-like or round-shaped campaniform sensilla are located in a pit. They are distributed dorsally on the gonapophyses IX of *Pl. kaempferi*, *Pl. hilpa*, *M. mongolica*, *M. opalifera*, *Hy. maculaticollis*, *C. atrata*, *S. yangi*, *Kar. caelata*, and *Ko. yezoensis* (Fig. 10a, b), and

Table 3 A summary of sensilla type and distribution on the gonapophyses and gonoplaacs in Cicadidae

Sensilla type	Subtypes	Shape	Sensilla on gonapophyses VIII			Sensilla on gonapophyses IX			Sensilla on gonoplaacs		
			Location	D/S	Species	Location	D/S	Species	OS	D/S	Species
Campaniform sensilla	Sca I	Protuberant center	Dorsal surface	Dense	<i>S1, Pl. hilpa</i> and <i>T. japonensis</i>	Dorsal surface	Scattered	<i>S1, Pl. hilpa</i> and <i>T. japonensis</i>	-	-	-
	Sca II	Tightly inserted into sockets	-	-	-	Dorsal surface	Dense	<i>S1</i> and <i>Hu. sanguinea</i>	-	-	-
	SCa III	Located in a shallow pit	-	Scattered	<i>T. japonensis</i>	Dorsal surface	Dense	<i>S1, Ko. yezoensis, M. opalifera, Kat. neokanagana, Po. linearis</i> and <i>Hu. sanguinea</i>	-	-	-
	Sca IV	Inserted in loose sockets	Ventral surface	Scattered	<i>Hy. maculaticollis, Po. linearis, M. opalifera</i> and <i>T. japonensis</i>	Ventral surface	Scattered	<i>Pl. kaempferi, Pl. hilpa, M. opalifera</i> and <i>M. mongolica</i>	-	-	-
	Sca V	Center with a molting pore	Dorsal surface	Scattered	<i>S. yangi</i>	Dorsal and ventral surfaces	Scattered	<i>Pl. Kaempferi</i> and <i>Kar. caelatata</i>	-	-	-
Basiconica sensilla	Sca VI	Inserted deeply in tight sockets	-	-	-	Dorsal surface	Scattered	<i>Pl. kaempferi, Pl. hilpa,</i> and <i>Hu. sanguinea</i>	-	-	-
	Sca VII	Located in deep depressions	Lateral surface	Dense	<i>M. mongolica</i> and <i>M. opalifera</i>	-	-	-	-	-	-
	Sca VIII	Apical portion with several pegs	-	-	-	Ventral surface	Scattered	<i>C. atrata, Pl. kaempferi, Hy. maculaticollis</i> and <i>Kar. caelatata</i>	-	-	-
	Sba I	Conical pegs insert shallowly	Outer surface	-	<i>M. mongolica</i> and <i>M. opalifera</i>	-	-	-	-	-	-
Trichoid sensilla	Sba II	Conical pegs insert deeply	Outer surface; ventral center	Scattered	<i>Ko. yezoensis</i> and <i>Kat. neokanagana</i>	Ventral surface	Scattered	<i>S. yangi</i>	-	-	-
	Str I	Extremely long, narrow pointed	Outer surface	-	-	-	-	-	+	Dense	<i>S2</i> and <i>Hy. maculaticollis</i>
	Str II	Slightly long, narrow pointed	-	-	-	-	-	-	+	Dense	<i>S2</i>
	Str III	Short, wide pointed, having longitudinal grooves	-	-	-	-	-	-	+	Dense	<i>Pl. kaempferi, Pl. hilpa,</i> and <i>M. opalifera</i>
	Str IV	Short, wide pointed, having smooth walls	-	-	-	-	-	-	+	Dense	<i>Pl. kaempferi</i> and <i>S. yangi</i>

Table 3 continued

Sensilla type	Subtypes	Shape	Sensilla on gonapophyses VIII			Sensilla on gonapophyses IX			Sensilla on gonoplics		
			Location	D/S	Species	Location	D/S	Species	OS	D/S	Species
Coelomic sensilla	–	Contain pegs, devoid of pit orifices centrally	–	–	–	–	–	–	+	Scattered	<i>C. atrata</i>
Cuticular depressions	–	Oval cuticular depressions	Outer surface	Scattered	<i>Hy. maculaticollis</i> , <i>Pl. kaempferi</i> and <i>T. japonensis</i>	Outer surface	Scattered	<i>M. mongolica</i> , <i>Hu. sanguinea</i> and <i>M. opalifera</i>	–	–	–

D/S dense/scattered, OS outer surface, SI species including: *C. atrata*, *Pl. kaempferi*, *M. mongolica*, *Hy. maculaticollis*, *Kar. caelatata*, *S. yangi*; S2 species including: *C. atrata*, *Pl. kaempferi*, *M. mongolica*, *Kar. caelatata*, *Pl. hilpa*, *M. opalifera*, *Hu. sanguinea*, *T. japonensis* and *Po. linearis*; “–” indicates no distribution; “+” indicates distribution of sensilla on the outer surface

also on the teeth of the gonapophyses VIII of the former three species (Fig. 10e). These sensilla can be morphologically further categorized into eight types: (1) Campaniform sensilla type I (SCa I) ($\sim 5.0 \mu\text{m}$ in diameter), with the center raising to become a protuberance from the slightly depressed surrounding area, are densely distributed on the dorsal surface of the gonapophyses VIII and scattered dorsally on the gonapophyses IX of *C. atrata*, *Pl. kaempferi*, *M. mongolica*, *Hy. maculaticollis*, *Kar. caelatata*, *S. yangi*, *Pl. hilpa*, and *T. japonensis* (Fig. 10b); (2) Campaniform sensilla type II (SCa II) ($\sim 3.5 \mu\text{m}$ in diameter), with smooth surface, are tightly inserted into sockets, and densely located on the dorsal surface of the gonapophyses IX of *C. atrata*, *Pl. kaempferi*, *M. mongolica*, *Hy. maculaticollis*, *Kar. caelatata*, *S. yangi*, and *Hu. sanguinea* (Fig. 10c); (3) Campaniform sensilla type III (SCa III) ($\sim 2.0 \mu\text{m}$ in diameter) are located in a shallow pit, distributed dorsally and densely on the gonapophyses IX of *C. atrata*, *Pl. kaempferi*, *M. mongolica*, *Hy. maculaticollis*, *Kar. caelatata*, *S. yangi*, *Ko. yezoensis*, *M. opalifera*, *Kat. neokanagana*, *Po. linearis*, *Hu. sanguinea*, and also on the gonapophyses VIII of *T. japonensis*; this type of sensilla are distributed dorsally and ventrally on both gonapophyses of *Po. linearis* (Fig. 10d); (4) Campaniform sensilla type IV (SCa IV) ($\sim 4.0 \mu\text{m}$ in diameter) are smooth-walled, inserted in loose sockets, and scattered ventrally on the gonapophyses IX of *Pl. kaempferi*, *Pl. hilpa*, *M. opalifera*, and *M. mongolica*, and on the gonapophyses VIII of *Hy. maculaticollis*, *Po. linearis*, *M. opalifera*, and *T. japonensis*; they are also present on the dorsal surface of the gonapophyses IX of *Kar. caelatata* and *Kat. neokanagana* (Fig. 10f); (5) Campaniform sensilla type V (SCa V) ($\sim 2.5 \mu\text{m}$ in diameter), with a molting pore in the center, are situated dorsally on the gonapophyses IX of *Pl. kaempferi* and gonapophyses VIII of *S. yangi*, and also scattered ventrally on the gonapophyses IX of *Kar. caelatata* (Fig. 10g, h); (6) Campaniform sensilla type VI (SCa VI) ($\sim 3.3 \mu\text{m}$ in diameter), smooth-walled, inserted deeply in tight sockets, are sparsely distributed on the dorsal surface of the gonapophyses IX of *Pl. kaempferi*, *Pl. hilpa*, and *Hu. sanguinea* (Fig. 10i); (7) Campaniform sensilla type VII (SCa VII) ($\sim 3.5 \mu\text{m}$ in diameter) are inserted into sockets, located in large, deep depressions, and distribute in a line on the lateral surface of the gonapophyses VIII of *M. mongolica* and *M. opalifera* (Fig. 10j); and (8) Campaniform sensilla type VIII (SCa VIII) ($\sim 2.5 \mu\text{m}$ in diameter), with several teeth-like pegs at the apical portion within each pit. The SCa VIII are distributed on the ventral surface of the gonapophyses IX of *C. atrata*, *Pl. kaempferi*, *Hy. maculaticollis*, and *Kar. caelatata* (Fig. 11a).

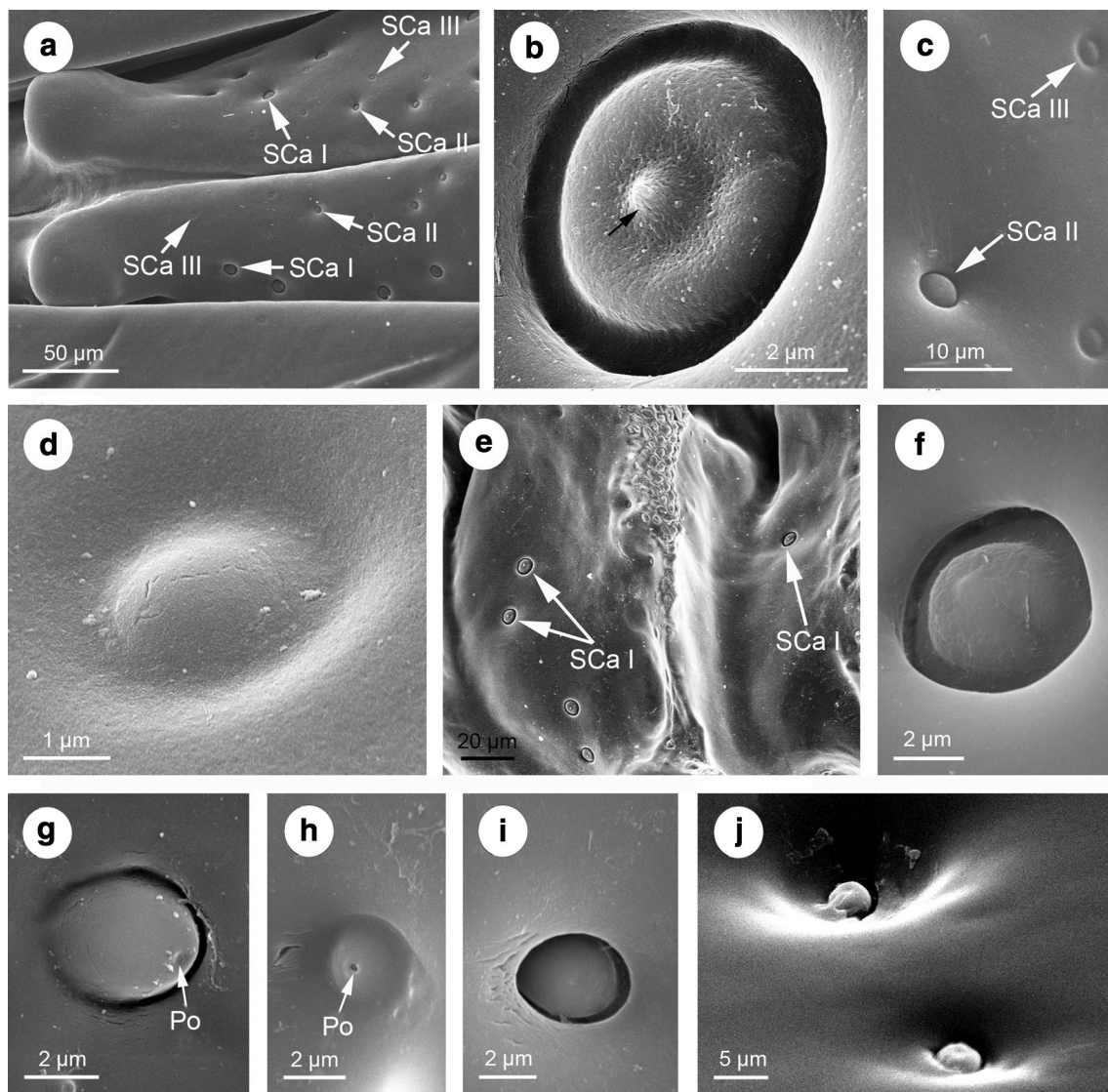


Fig. 10 SEM images of campaniform sensilla. **a–e** and **j** *Meimuna mongolica*, **f** *Hyalessa maculaticollis*, **g** *Karenia caelata*, **h–i** *Platypleura kaempferi*. **a** Dorsal view of the gonapophysis IX, showing campaniform sensilla types I, II, III (SCa I, II, III). **b** High magnification of campaniform sensilla type I (SCa I), showing its center with a protuberance. **c** High magnification of campaniform sensilla types II and III (SCa II, III). **d** High magnification of campaniform sensilla type III (SCa III). **e** Campaniform sensilla type

II located on the teeth of gonapophysis VIII. **f** Campaniform sensilla type IV (SCa IV) with a smooth surface and inserted in a loose socket on the distal part of the gonapophysis VIII. **g** Campaniform sensilla type V (SCa V) with a molting pore (Po) distributed on the gonapophysis IX. **h** Campaniform sensilla subtype V (SCa V) with a molting pore (Po) located on the gonapophysis IX. **i** Campaniform sensilla type VI (SCa VI) on the gonapophysis IX. **j** Campaniform sensilla type VII (SCa VII)

Basiconica sensilla (SBa)

They are morphologically similar to inverted triangles or thick conical pegs, tightly situated in sockets. These sensilla are classified into two types based on the cones that sink into or reach above the cuticle surface. The first type of basiconica sensilla (SBa I) ($\sim 3.9 \mu\text{m}$ in basal diameter) are large sensilla, with their conical pegs inserted in sunken large sockets and the cone tip reaching above the cuticle surface (Fig. 11b). These sensilla are

scatteredly present on the outer surface of the gonapophyses VIII of *M. mongolica* and *M. opalifera*. The second type of basiconica sensilla (SBa II) is approximately $3.0 \mu\text{m}$ in basal diameter, with their conical pegs inserted so deeply in sockets that the cone tip hardly emerges above the cuticle surface (Fig. 11c). They are distributed sparsely on the ventral surface of the gonapophyses IX of *S. yangi*, the outer surface of the gonapophyses VIII and the ventral center of each tooth of *Ko. yezoensis* and *Kat. neokanagana*.

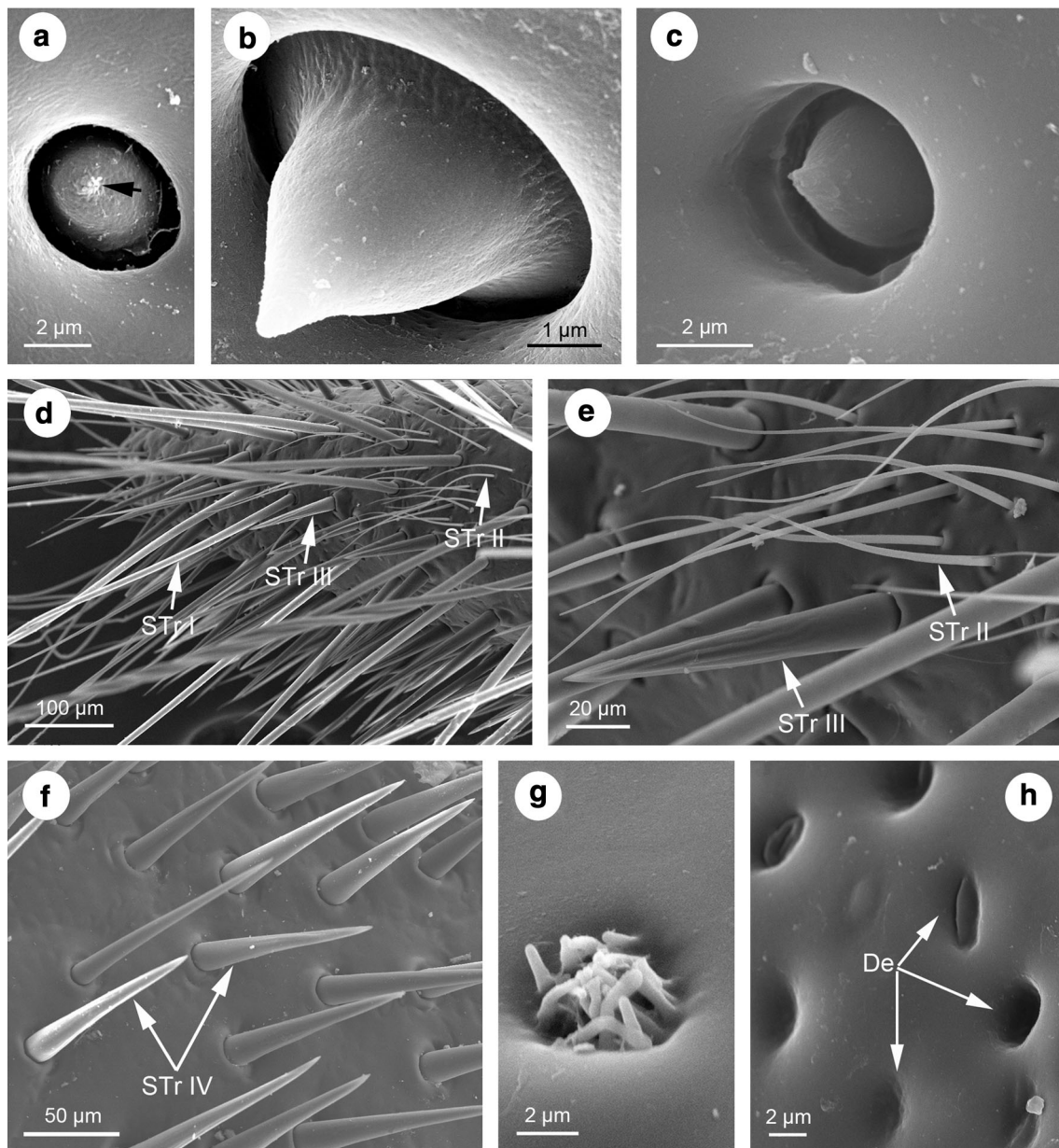


Fig. 11 SEM images of different types of sensilla. **a** *Karenia caelata*, **b** *Meimuna mongolica*, **c**, **f** *Subpsaltria yangi*, **d**, **g** *Cryptotympana atrata*, **e** *Platyleura kaempferi*, **h** *Hyalessa maculaticollis*. **a** A campaniform sensillum (SCa VIII) with apical pegs (black arrow indicates) on the gonapophysis IX. **b** Basiconica sensilla type I (SBa I) on the gonapophysis VIII, showing its cone tip reaching above the surface of the cuticle. **c** Basiconica sensilla type II (SBa II) on the gonapophysis IX, showing its cone tip hardly reaching above the

cuticle surface. **d** Trichoid sensilla types I, II, and III (STr I, II, III) on the gonoplacs. **e** Trichoid sensilla types II, III (STr II, III). **f** Trichoid sensilla type IV (STr IV). **g** Coeloconic sensilla on the gonoplacs. It is devoid of any pit orifice and is situated in a shallow depression. **h** Cuticular depressions (De) of different size and depth on the outer surface of the gonapophysis VIII; some depressions possess longitudinal ridge(s)

Trichoid sensilla (STr)

Hair-like trichoid sensilla are distributed densely on the outer surface of the gonoplacs. They are pointed or blunt, inclined, and arise from flexible sockets. These sensilla are different in size and are morphologically classified into two types: (1) sharply pointed trichoid sensilla, and (2) blunt-

tipped trichoid sensilla. The pointed trichoid sensilla are subdivided into four distinct types based on length and basal diameter. The extremely long, narrow pointed trichoid sensilla (STr I) (~0.70 mm long, ~0.01 mm in basal diameter) are inclined but straightforward toward the apex of the gonoplacs. They are widely distributed on the outer surface of the gonoplacs of *C. atrata*, *Pl. kaempferi*,

M. mongolica, *Hy. maculaticollis*, *Kar. caelatata*, *Pl. hilpa*, *M. opalifera*, *Hu. sanguinea*, *T. japonensis*, and *Po. linearis* (Fig. 11d). The slightly long, narrow pointed trichoid sensilla (Str II) ($\sim 135.0 \mu\text{m}$ in length and $3.0 \mu\text{m}$ in basal diameter) are inclined and slightly curved toward the apex of the gonoplags, uniformly locate on the outer surface of the gonoplags of *C. atrata*, *Pl. kaempferi*, *Kar. caelatata*, *M. mongolica*, *Pl. hilpa*, *M. opalifera*, *Hu. sanguinea*, *T. japonensis*, and *Po. linearis*. The short, wide pointed trichoid sensilla (STr III) ($\sim 110.0 \mu\text{m}$ in length and $17.0 \mu\text{m}$ in basal diameter) are characterized by a longitudinal grooved surface at their distal parts, and project more perpendicularly with the axis of the gonoplags. The STr III is slightly elevated above the cuticle, and are distributed densely on the outer surface of the gonoplags of *Pl. kaempferi*, *Pl. hilpa*, and *M. opalifera* (Fig. 11e). The short, wide pointed trichoid sensilla (STr IV) ($\sim 115.0 \mu\text{m}$ long, $15.0 \mu\text{m}$ in basal diameter) have a smooth surface, which project more perpendicularly with the axis of the gonoplags, also. This type of sensilla distributes densely on the outer surface of the gonoplags of *Pl. kaempferi* and *S. yangi* (Fig. 11f).

Coeloconic sensilla (SCo)

Coeloconic sensilla are pit organs on the cuticle surface of the gonoplags of *C. atrata*. There are approximately 17 teeth-like pegs ($\sim 0.5 \mu\text{m}$ in diameter) within each shallow pits ($\sim 5.0 \mu\text{m}$ in diameter) of a coeloconic sensillum. This type of sensilla are devoid of pit orifices in the center, and only observed on the cuticle surface of the gonoplags of *C. atrata* (Fig. 11g).

Cuticular depressions

These depressions are oval-shaped, and contain a single central orifice. They are scattered on the outer surface of the gonapophyses VIII of *Hy. maculaticollis*, *Pl. kaempferi*, and *T. japonensis*, and on the gonapophyses IX of *M. mongolica*, *Hu. sanguinea*, and *M. opalifera*. These depressions are varying in depth, and some of them contain ridges of different shapes and sizes (Fig. 11h).

Discussion

Morphology of gonapophyses and its taxonomic and phylogenetic significance in Cicadoidea

The present study compared the morphology and ultrastructure of gonapophyses of 13 cicada species representing the three subfamilies of Cicadidae for the first time. The configurations of the gonapophyses in all species

examined in our study are similar to those investigated by Hilsman (1921), who simply made an attempt to find characteristics from the gonapophyses of a few species of Cicadidae for taxonomic purposes. In our present study, a careful morphological investigation of gonapophyses was made, and some remarkable differences may be promising characters for taxonomic and phylogenetic analysis, i.e., the number and shape of teeth, shape of the tongue-shaped slice, curvature of the mesal margin, form of the polygonal cells and/or raised protuberances on the ventral outer margin of the teeth, and the separation of slices apices of the gonapophyses VIII, and the form of protuberances and ridges distributed on the gonapophyses IX, etc.

Among the eight species belonging to the subfamily Cicadinae, the gonapophyses display great similarities in morphology, i.e., many polygonal cells are observed near the ventral outer margin of the teeth on the gonapophyses VIII, excepting for *Platypleura hilpa* and *Pomponia linearis*; and the gonapophyses IX are devoid of regularly arranged ridges, but bear one or two pairs of protuberances. Hilsman (1921) noted that the meeting point of mesal margins (i.e., mesal margins meeting for cephalad or far cephalad of the first tooth) of the gonapophyses VIII and the chitinized degree of the gonapophyses are different among species. However, such structures may be unavailable or not informative for taxonomy, because the mesal margins of the gonapophyses VIII actually do not meet at any point as revealed in our current study, and the chitinized degree of this gonapophyses is difficult to detect simply by optical observation.

The polygonal cells observed near the ventral outer margin of the teeth on the gonapophyses VIII, if present, are significantly different among species: the polygonal cells are devoid of protuberances in *Cryptotympana atrata* and *Tanna japonensis* (Figs. 2c, d, 6d); polygonal cells contain raised protuberances in *Platypleura kaempferi*, *Meimuna mongolica*, *M. opalifera*, and *Hyalessa maculaticollis* (Figs. 3a, b, 4b, c); only a very few polygonal cells and some raised protuberances exist in *Karenia caelatata* (Fig. 9g); only some slightly raised protuberances occur in *Subpsaltria yangi* (Fig. 7b); neither polygonal cells nor protuberances exist in *Pl. hilpa*, *Po. linearis*, *Kosemia yezoensis*, *Katoa neokanagana*, and *Huechys sanguinea*. We speculated that the polygonal cells and protuberances, contacting with tissue of host plants in oviposition, are possible to play a special role which may be related to the selection of oviposition sites in different species.

Our morphology revealed that the gonapophyses in *Ko. yezoensis*, *Kat. neokanagana* and *Hu. sanguinea*, belonging to the subfamily Cicadettinae, appear to be more complicated. They all possess ridges on the outer margin of teeth of the gonapophyses VIII and on the dorsal surface of the

gonapophyses IX. The longitudinal ridges with serrated distal end on the gonapophyses VIII and pectinate ridges on the gonapophyses IX of *Ko. yezoensis* are more developed than those of *Kat. neokanagana* and *Hu. sanguinea*. In comparison, these three species are more similar to *S. yangi*, the solely known species of the subfamily Tettigadinae from China, in having ridges on the gonapophyses. However, *S. yangi* can be easily distinguished from species of Cicadinae by having: (1) exposed, less developed protuberances near the ventral outer margin of the teeth of the gonapophyses VIII (Fig. 7a, b); (2) irregular but developed ridges between the outer and inner margins of the teeth of the gonapophyses VIII (Fig. 7c, d); (3) two pairs of morphologically distinct (spherical and flake-like) protuberances near the apex of the gonapophyses IX (Fig. 7e); (4) regularly arranged ridges exhibiting a herringbone pattern along the midline of the gonapophyses IX (Fig. 7f); and (5) farther distance between the apex of the tongue-shaped slice and the outer margin of the tooth-bearing part of the gonapophyses VIII (Fig. 7a). The genus *Karenia* is currently placed in the Cicadettinae (=Tibicininae auct.), but its systematic placement remains controversial (Boulard 1973, 1976a, b; Chou et al. 1997; Moulds 2005). The gonapophyses of *Kar. caelatata* resemble those of species in the subfamily Cicadinae in morphology. This indicates that it will be more reasonable to place this genus in the Cicadinae, instead of Cicadettinae (=Tibicininae auct.), as previously suggested by Li et al. (2015). The results indicate that the morphological similarities and differences of gonapophyses in Cicadidae, in addition to some other morphological characters, can supply implications for the relationship of groups in this family.

In summary, our results show that the morphological similarities and differences of ovipositor among species of Cicadidae, particularly the gonapophyses, can provide implications for the relationships of groups within this family.

Function of sensilla on the gonapophyses and gonoplacs

Sensilla on gonapophyses supply vital information about oviposition behavior of insects, and some related studies have been conducted for different groups, e.g., the caddisfly *Lype phaeopa* (Stephens, 1836), ectoparasitoid *Habrobracon hebetor* (Say, 1836), stem borer *Chilo partellus* (Swinhoe, 1885), cotton leafworm *Spodoptera littoralis* (Boisduval, 1833), spruce budworm *Choristoneura fumiferana* (Clemens, 1865), mosquito *Dasineura brassicae* Winnertz, 1853, anisopteran *Aeshna cyanea* (Müller, 1764), and the zygopteran *Ischnura elegans* (van der Linden, 1823) (Chadha and Roome 1980; Hallberg and Ahman 1987; Banga et al. 2003; Spänhoff et al. 2003;

Dweck et al. 2008; Rebora et al. 2013). In our investigation, some morphologically different types of sensilla and cuticular depressions were recognized on the gonapophyses and gonoplacs of various cicada species. The sensilla combined with teeth on the ovipositor play an important role in the assessment of potential oviposition sites (Chadha and Roome 1980; Hallberg and Ahman 1987). We speculated that the teeth on the front claw of cicada ovipositor initially contact and cut the twig surface and are followed by the sensilla when ovipositing. The mechanism for cicadas to sense chemical properties of the host plant need to be studied further; and it would be of value to further investigate the sensilla using TEM examination and electrophysiological study.

Trichoid sensilla located on the outer surface of the gonoplacs observed in our present study are morphologically similar to those of the sharpshooter *Homalodisca coagulata* (Hummel et al. 2006). Faucheux (1991) suggested that trichoid sensilla on the ovipositor of the moth *Homoeosoma electellum* serve as mechanoreceptors. Hummel et al. (2006) inferred that this type of sensilla may coordinate the oviposition activities to help females find suitable sites. Electrophysiological and behavioral studies are needed to verify the role of trichoid sensilla on the gonoplacs of cicadas.

Campaniform sensilla densely distributed on the gonapophyses of the examined species are morphologically similar to those on the gonapophyses of the caddisfly *Lype phaeopa* (Spänhoff et al. 2003), sharpshooter *H. coagulata* (Hummel et al. 2006), workers of *Apis mellifera* (King and Fordy 1970), and the parasitic wasp *Orgilus lepidus* (Hawke et al. 1973). These sensilla are considered as thermo- and hygroreceptors (Yokohari et al. 1982), or mechanoreceptors (Liang and Fletcher 2002; Mciver 1985; Schneider 1964; Zacharuk 1985), and presumably serve as receptors for tactile stimuli (Dweck et al. 2008; Spänhoff et al. 2003). The campaniform sensilla observed on the gonapophyses of cicadas may serve as mechanoreceptors to sense stress and pressure coming from the tissue of host plants, but this need to be investigated further.

Coeloconic sensilla are only found on the gonoplacs of *C. atrata* in our present study. This type of sensilla have been frequently reported in insects, e.g., the sharpshooter *H. coagulata* (Hummel et al. 2006), treehopper *Enchenopa* sp. (Dietrich 1989), and the moth *Manduca sexta* (Shields and Hildebrand 1999, 2001). They serve as thermoreceptors in Culicidae (Davis and Sokolove 1975), and as chemo-, thermo-, and/or hygroreceptors in Cicadellidae and Acrididae (Altner et al. 1981; Hummel et al. 2006). The function of this type of sensilla in *C. atrata* needs further investigation based on transmission electron microscopy and electrophysiological studies.

Basiconica sensilla have been previously observed on the antennae of many insects, e.g., the ground beetle *Bembidion properans* (Merivee et al. 2002), mosquitoes *Aedes aegypti*, *Culex pipiens*, *A. atropalpus*, and *A. epac-tius* (Bowen 1995) and the myrmecophilous beetle *Paussus favieri* (Di Giulio et al. 2012). They were also observed on the labium of the cotton stainer *Dysdercus fasciatus* (Peregrine 1972). This type of sensilla are known as chemoreceptors (Gracco and Catalá 2000), but recently hypothesized as mechanoreceptors (Di Giulio et al. 2012). The basiconica sensilla distributed on the outer surface of the gonapophyses VIII of *M. mongolica* and the ventral surface of the gonapophyses IX of *S. yangi* may serve as mechanoreceptors.

The various sensilla and their distribution on the gonapophyses of cicadas in the present study supply information not only for future study of taxonomy and phylogeny of the Cicadoidea, but also for researches of oviposition behavior and oviposition-site selection of cicadas. Future exploration of phylogeny based on morphology and ultrastructure of cicada gonapophyses together with other informative data is desirable.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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