

A comparative study of the proventricular structure in twenty Chinese Tettigoniidae (Orthoptera) species

Yinliang Wang, Yuetian Su, Xue Zhang, Na Li & Bingzhong Ren*

Wang, Y., Su, Y., Zhang, X., Li, N. & Ren, B. 2012: A comparative study of the proventricular structure in twenty Chinese Tettigoniidae (Orthoptera) species. — Entomol. Fennica 23: 140–148.

This study focuses on the proventriculus and the alimentary canal of twenty Tettigoniidae species among three subfamilies, Tettigoniinae, Phaneropterinae and Conocephalinae. Each part of the alimentary canal and the inner structure of proventriculus were examined under optic microscope and scanning electron microscopy. As a result, the length of each part of the alimentary canal and the inner structure of proventriculus were highly associated with feeding habits. Carnivorous species always had a short foregut and long cilia on the base of the sclerotized appendix in proventriculus, whereas herbivorous species always had a longer foregut and a highly sclerotized proventriculus. These results increase understanding of the alimentary canal in Tettigoniidae and will be useful in future studies of their feeding habits.

*Y. Wang, X. Zhang & B. Z. Ren, Jilin Key Laboratory of Animal Resource Conservation and Utilization, School of Life Sciences, Northeast Normal University, Renmin St. 5268, Changchun, China CO130024; *Corresponding author's e-mail: bzren@163.com*

Y. Su, Neurosurgery, the Second Norman Bethune Hospital of Jilin University, Yatai St. 991, Changchun China CO130024

N. Li, Traditional Chinese Institution, Shenyang Pharmaceutical University, Shenyang, 110016, China

Received 26 December 2011, accepted 19 March 2012

1. Introduction

The proventriculus is an organ situated between the foregut and midgut, its lumen lined by cuticle with teeth-like projections. These, together with associated well-developed muscles, aid food trituration process (Isely & Alexander 1949).

The proventriculus is comprised of several sclerotized plates (Chapman 1998), whose structure has been adaptively associated with insect diet (Caetano 1988, Bland & Rentz 1991). Moreover, many studies have shown that the morphol-

ogy of proventriculus can provide useful taxonomic characters for Orthoptera (Judd 1948, Bland & Rentz 1991, Fontanetti & Zefa 2000, Fontanetti *et al.* 2002), Coleoptera (Judd 1947, Yahiro 1990), Isoptera (Lebrun 1985), Trichoptera (Gibbs 1967) and Hymenoptera (Roche & Wheeler 1997, Serrão 2001, 2005, 2007). According to a new updated file of orthopteran species, there are more than 6,000 described Tettigonioidae species in the world (Eades *et al.* 2012), and there are more than 300 species of Tettigoniidae in China (Li & Ren 2007), How-

Table 1. List of twenty Chinese Tettigoniidae species examined with their collection locations and feeding habits.

Species	Location	Feeding habit
Tettigoniinae		
<i>Gampsocleis sedakovii</i> (Walker 1869)	Jilin, Tianbei	Omnivorous
<i>G. ussuriensis</i> (Adelung 1910)	Jilin, Songhua Lake	Omnivorous
<i>G. gratiosa</i> (Brunner von Wattenwyl 1888)	Inner Mongolia	Omnivorous
<i>Uvarovites inflatus</i> (Uvarov 1924)	Jilin, Zuoja	Herbivorous
<i>Tettigonia caudata</i> (Charpentier 1845)	Jilin, Tianbei	Omnivorous
<i>T. cantans</i> (Fuessly 1775)	Jilin, Songhua Lake	Omnivorous
<i>T. ussuriana</i> (Uvarov 1939)	Jilin, Tianbei	Omnivorous
<i>Metrioptera ussuriana</i> (Uvarov 1926)	Jilin, Baishan	Herbivorous
<i>M. bonneti</i> (Bolivar 1890)	Jilin, Songhua Lake	Herbivorous
<i>M. bicolor</i> (Philippi 1830)	Heilongjiang, Dailing	Omnivorous
<i>M. engelhardti</i> (Uvarov 1926)	Jilin, Yaoling	Herbivorous
<i>Atlantiscus sinensis</i> (Uvarov 1923)	Jilin, Yaoling	Carnivorous
Phaneropterinae		
<i>Phaneroptera falcata</i> (Poda 1761)	Jilin, Tianbei	Omnivorous
<i>Ph. nigroantennata</i> (Brunner von Wattenwyl 1878)	Jilin, Tianbei	Omnivorous
<i>Ducetia japonica</i> (Thunberg 1815)	Jilin, Zuoja	Omnivorous
<i>Elimaea fallax</i> (Bey-Bienko 1951)	Jilin, Tianbei	Omnivorous
Conocephalinae		
<i>Conocephalus fuscus</i> (Fabricius)	Inner Mongolia	Herbivorous
<i>C. chinensis</i> (Redtenbacher 1891)	Heilongjiang, Dailing	Herbivorous
<i>Ruspolia nitidula</i> (Scopoli)	Inner Mongolia	Herbivorous
<i>R. jezoensis</i> (Matsumura Shiraki 1908)	Jilin, Qingfeng	Herbivorous

ever, the inner structures of the proventriculus and the alimentary canal as a whole are still poorly known in Tettigoniodea.

In the present paper, the whole alimentary canal and the inner structures of the proventriculus in twelve species of Tettigoniinae, four species of Phaneropterinae and four species of Conocephalinae were investigated using stereomicroscope and scanning electron microscopy. The objective was to reveal the ultramorphological differences of the alimentary canal between representatives of these three largest subfamilies of Tettigoniidae.

2. Material and methods

2.1. Studied material

We used adult males as our study material. The species and collection localities are listed in Table 1. We classified the species by their morphology. The number of individuals studied for each species was 5–10.

2.2. Scanning electron microscopy

The male samples were immediately pre-treated with 8% formaldehyde solution. After 12–24 hours water flushing, the alimentary canal was carefully isolated from the specimen by ophthalmic scissors. The length of each part of the alimentary canal was measured under stereomicroscope (SMZ-168, Motic). Then the proventriculus was isolated from dissected guts and dissected with scissors through a single longitudinal cut. Subsequently, the samples were transferred to 15% cold KOH for 12 hours to facilitate the removal of muscle and connective tissue. The remaining flap of sclerotised tissue was washed, fixed 1–2 h in 2.5% glutaraldehyde, rinsed in 0.05 M phosphate buffer (pH 7.2), dehydrated in ascending alcohol series (50–100%), and critical-point dried. After dried, the material was placed on aluminum supports attached with double-faced tape and sputter coated with gold. The proventriculus was examined with scanning electron microscope (S-570, Hitachi) under an acceleration voltage of 20 kV. The lengths of each part of the alimentary canal and inner structure of

proventriculus were measured under SEM and Digital calipers, and characters were observed under optic microscopy.

The following abbreviations are used for the studied structures

- *sa*: sclerotized appendix
- *sp*: sclerotized partition
- *mt*: medium tooth
- *lt*: lateral teeth
- *sl*: sclerotized lobes

3. Results

3.1. Gross morphology of the alimentary canal

The lengths of the whole alimentary canal (Fig. 1), foregut, midgut, hindgut, proventriculus and gastric caeca were measured under stereomicroscope among three subfamilies Tettigoniinae, Phaneropterinae and Conocephalinae. The results are shown in Table 2. The foregut in Tettigoniinae was obviously longer than the midgut and hindgut, which accounts for about 40% of the whole alimentary canal. In the subfamily Phaneropterinae, the midgut accounted for over half of the whole alimentary canal, the hindgut for 20%, and the foregut for 33%. For the subfamily Conocephalinae, the proportions of different parts of the gut were very similar to the subfamily Tettigoniinae, i.e. a long foregut accounting for about 45%, the midgut and hindgut accounting for 25% each.

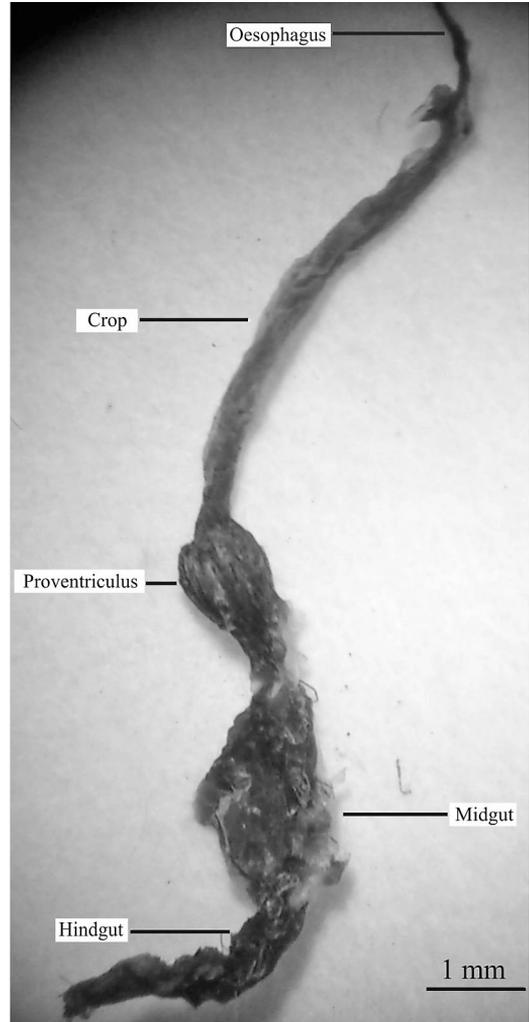


Fig. 1. Alimentary canal of Tettigoniidae species.

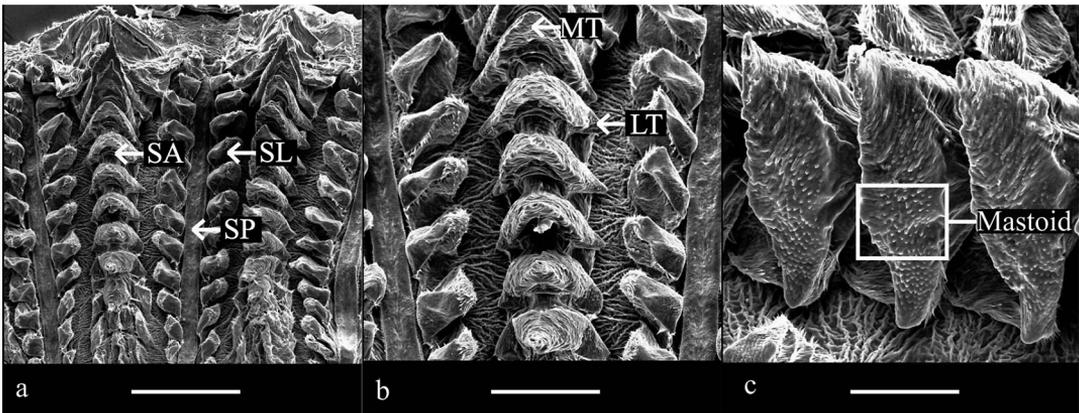


Fig. 2. Internal view of the proventriculus of *Gampsocleis sedakovii* (Walker 1869). – a. Distant view of longitudinal folds, scale 600 μ m. – b. One longitudinal fold, scale 300 μ m. – c. Lateral view of sclerotized appendices, scale 150 μ m.

Table 2. Length (mm) of alimentary canal of twenty Tettigoniidae species. Length of proventriculus was included in foregut and total length.

Species	Total length	Fore-gut	Mid-gut	Hind-gut	Proven-triculus	Gastric caeca	Body length
<i>G. sedakovii</i>	55.60	22.10	16.80	16.70	4.20	5.96	40.10
<i>G. ussuriensis</i>	54.79	22.43	15.72	16.64	4.78	4.78	36.00
<i>G. gratiosa</i>	49.90	22.00	13.00	14.90	5.00	5.76	39.20
<i>U. inflatus</i>	51.88	20.42	15.20	16.26	3.12	4.22	18.20
<i>T. caudata</i>	47.90	24.80	10.60	12.50	3.68	5.46	32.20
<i>T. cantans</i>	45.50	24.10	10.40	11.00	3.90	5.78	31.00
<i>T. ussuriiana</i>	46.30	24.50	10.60	11.20	3.16	4.10	32.25
<i>M. ussuriiana</i>	37.26	14.34	10.20	12.72	2.82	3.14	18.75
<i>M. bonneti</i>	41.30	15.46	11.50	14.34	2.58	6.32	19.00
<i>M. bicolor</i>	35.68	13.70	9.58	12.40	1.94	3.00	21.75
<i>M. engelhardti</i>	34.20	13.00	9.00	12.20	2.20	3.00	17.50
<i>A. sinensis</i>	43.10	16.20	12.30	14.60	3.64	3.76	30.75
<i>P. nigroantennata</i>	44.44	16.30	22.56	5.58	1.24	2.40	15.00
<i>P. falcata</i>	49.52	17.66	25.10	6.76	2.10	4.16	14.50
<i>D. japonica</i>	38.90	20.50	12.20	6.20	2.40	3.12	18.50
<i>E. fallax</i>	47.06	20.94	15.96	10.16	2.52	3.52	17.50
<i>C. fuscus</i>	22.54	11.00	6.00	5.54	1.80	3.10	16.25
<i>C. chinensis</i>	21.68	9.28	7.10	5.30	1.64	1.94	16.75
<i>R. jezoensis</i>	34.94	14.84	10.22	9.88	2.20	4.72	20.75
<i>R. nitidula</i>	37.40	15.16	11.40	10.94	2.50	3.80	27.00

Table 3. Characters of proventriculus of twenty Tettigoniidae species. Lengths and widths in μm . Abbreviations: *sa*, sclerotized appendix; *sp*, sclerotized partition; *mt*, medium tooth.

Species	No. of <i>sa</i>	Length of <i>mt</i>	Width of <i>mt</i>	Width of <i>sp</i>	Length of <i>sa</i>
<i>G. sedakovii</i>	15	354.55	107.14	136.36	1,800.00
<i>G. ussuriensis</i>	15	357.12	94.29	139.29	1,850.00
<i>G. gratiosa</i>	15	340.91	136.36	147.27	2,359.09
<i>U. inflatus</i>	12	261.70	69.11	99.82	1,190.18
<i>T. caudata</i>	16	364.29	85.71	96.43	1,865.45
<i>T. cantans</i>	16	415.18	120.54	93.75	2,181.82
<i>T. ussuriiana</i>	14	200.89	93.75	120.54	1,647.32
<i>M. ussuriiana</i>	12	352.36	96.73	72.18	1,174.55
<i>M. bonneti</i>	12	349.09	123.64	87.27	1,258.18
<i>M. bicolor</i>	12	374.91	101.22	82.91	1,084.36
<i>M. engelhardti</i>	12	381.45	78.18	93.82	1,266.55
<i>A. sinensis</i>	19	477.27	109.09	163.64	2,290.91
<i>P. nigroantennata</i>	10	234.55	98.18	30.36	1,263.23
<i>P. falcata</i>	10	226.36	89.45	44.28	1,266.83
<i>D. japonica</i>	13	211.82	78.55	34.36	929.64
<i>E. fallax</i>	15	220.18	96.73	46.91	1,077.82
<i>C. fuscus</i>	10	180.60	64.00	54.60	313.20
<i>C. chinensis</i>	10	163.64	54.55	54.66	224.14
<i>R. jezoensis</i>	13	347.09	103.64	96.73	1,043.27
<i>R. nitidula</i>	13	354.55	109.09	90.91	1,327.27

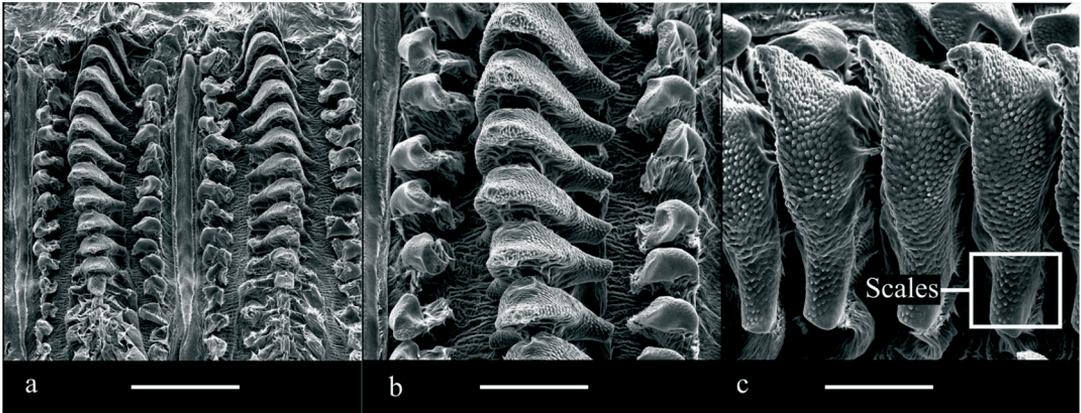


Fig. 3. Internal view of the proventriculus of *Tettigonia caudata* (Charpentier 1845). – a. Distant view of longitudinal folds, scale 600 μm . – b. One longitudinal fold, scale 250 μm . – c. Lateral view of sclerotized appendices, scale 150 μm .

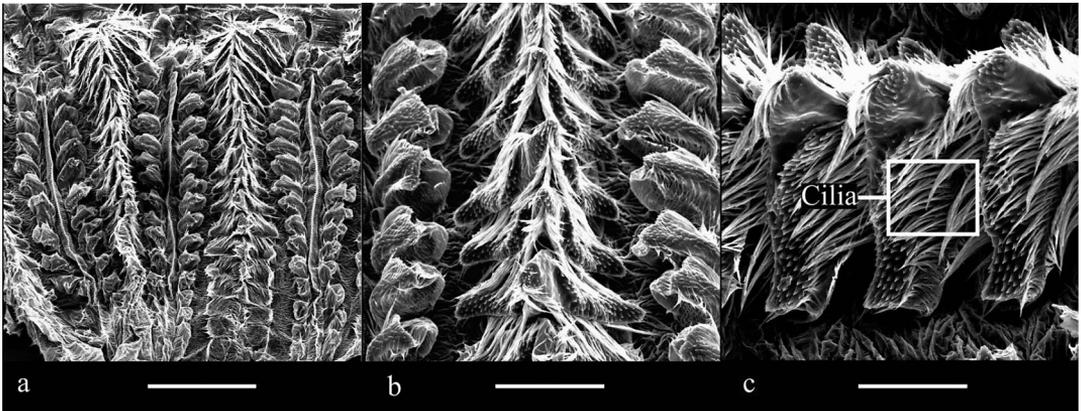


Fig. 4. Internal view of the proventriculus of *Phanerotera nigroantennata* (Brunner von Wattenwyl 1878). – a. Distant view of longitudinal folds, scale 430 μm . – b. One longitudinal fold, scale 150 μm . – c. Lateral view of sclerotized appendices, scale 100 μm .

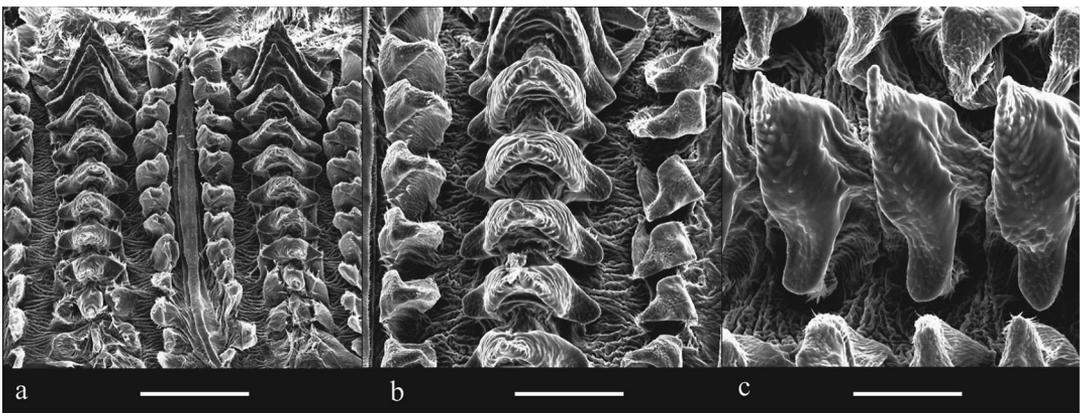


Fig. 5. Internal view of the proventriculus of *Uvarovites inflatus* (Uvarov 1924). – a. Distant view of longitudinal folds, scale 380 μm . – b. One longitudinal fold, scale 200 μm . – c. Lateral view of sclerotized appendices, scale 120 μm .

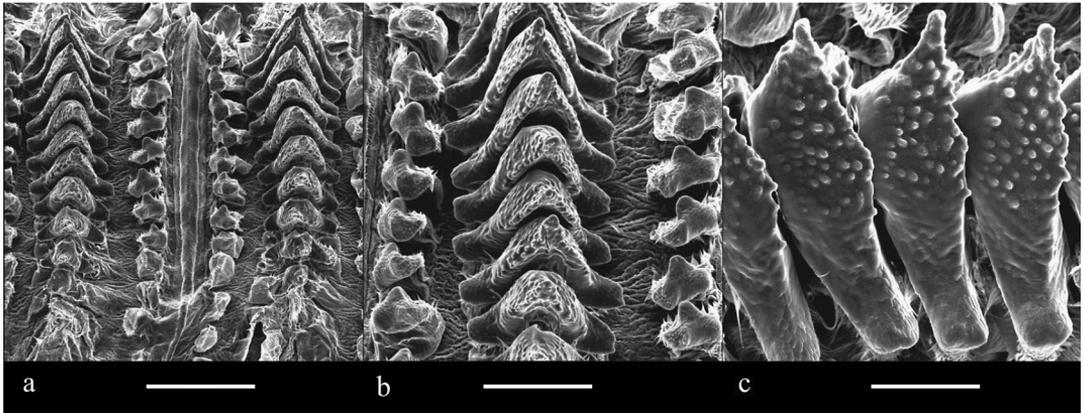


Fig. 6. Internal view of the proventriculus of *Metrioptera ussuriana* (Uvarov 1926). – a. Distant view of longitudinal folds, scale 380 μm . – b. One longitudinal fold, scale 200 μm . – c. Lateral view of sclerotized appendices, scale 100 μm .

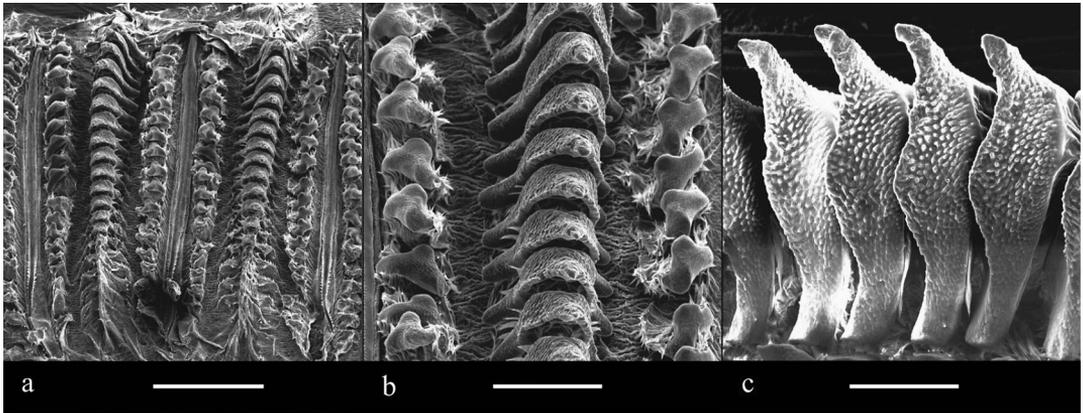


Fig. 7. Internal view of the proventriculus of *Atlanticus sinensis* (Uvarov 1923). – a. Distant view of longitudinal folds, scale 750 μm . – b. One longitudinal fold, scale 270 μm . – c. Lateral view of sclerotized appendices, scale 176 μm .

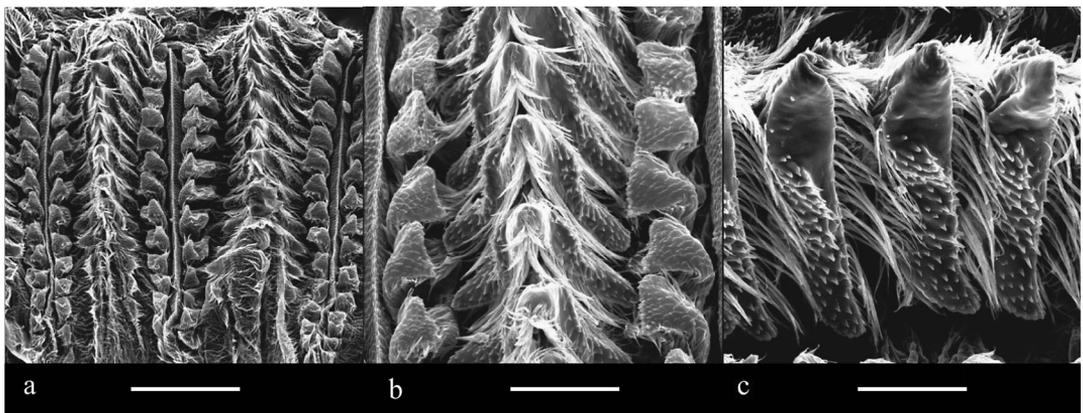


Fig. 8. Internal view of the proventriculus of *Ducetia japonica* (Thunberg 1815). – a. Distant view of longitudinal folds, scale 270 μm . – b. One longitudinal fold, scale 100 μm . – c. Lateral view of sclerotized appendices, scale 75 μm .

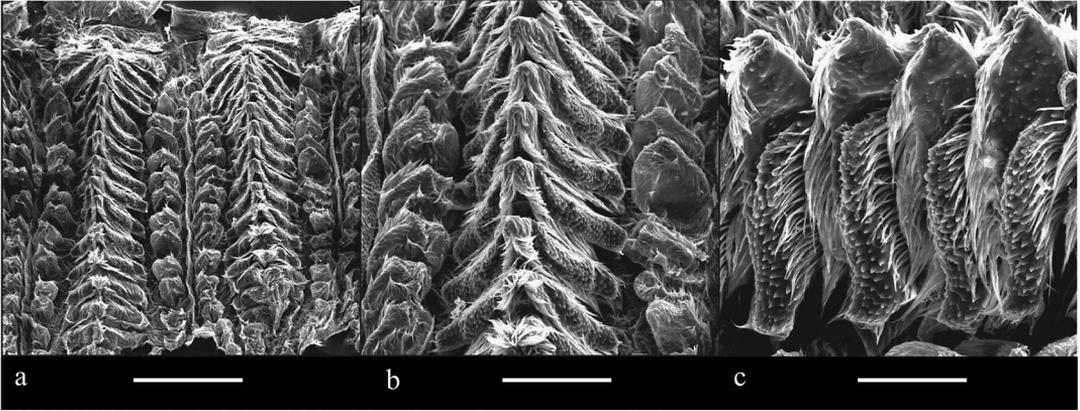


Fig. 9. Internal view of the proventriculus of *Elimaea fallax* (Bey-Bienko 1951). – a. Distant view of longitudinal folds, scale 380 μm . – b. One longitudinal fold, scale 150 μm . – c. Lateral view of sclerotized appendices, scale 86 μm .

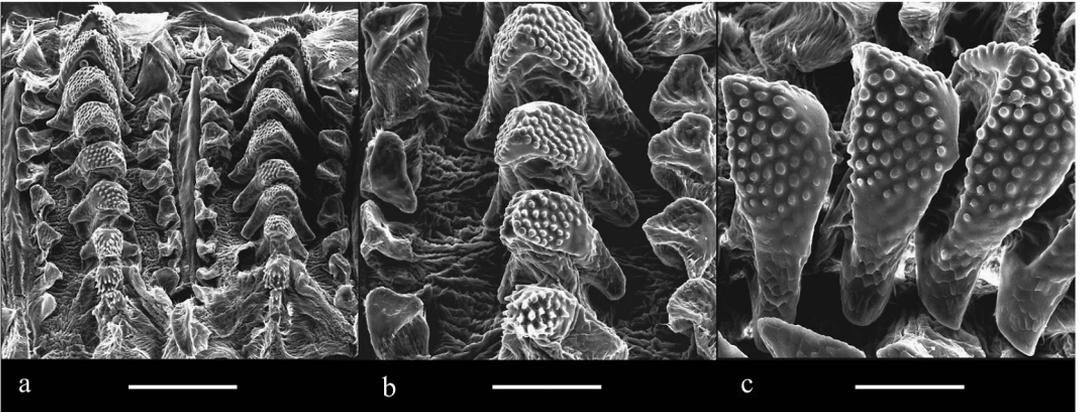


Fig. 10. Internal view of the proventriculus of *Conocephalus fuscus* (Fabricius). – a. Distant view of longitudinal folds, scale 231 μm . – b. One longitudinal fold, scale 100 μm . – c. Lateral view of sclerotized appendices, scale 75 μm .

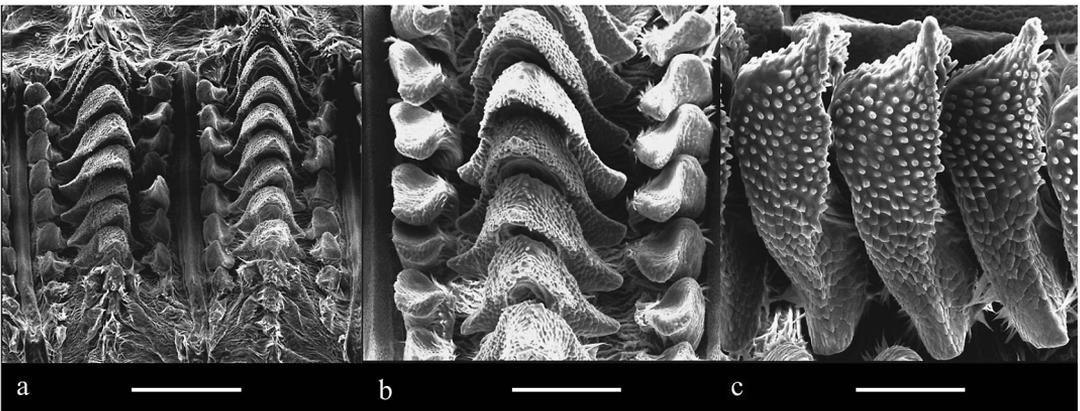


Fig. 11. Internal view of the proventriculus of *Ruspolia jezoensis* (Matsumura & Shiraki 1908). – a. Distant view of longitudinal folds, scale 380 μm . – b. One longitudinal fold, scale 176 μm . – c. Lateral view of sclerotized appendices, scale 100 μm .

3.2. Structure of proventriculus

Five characters of proventriculus were analyzed among all the species, which were the length of proventriculus, the number of *sa* on each longitudinal fold, the length and width of *sa*, and the width of *sp* between adjacent rows (Table 3).

The proventriculus of all the species examined showed the basic morphological pattern for katydids, i.e. it was formed by six longitudinal folds with complex rows of heavily sclerotized teeth. The sclerotized appendix (*sa*) was formed of a central projection, the medium tooth (*mt*) and two lateral expansions, the lateral teeth (*lt*). *Sa* was covered with some mastoids (Fig. 2c), scales (Fig. 3c) and cilia (Fig. 4c). These varied with species. On either side of the rows of *sa*, small expansions could be observed, called sclerotized lobes (*sl*) (Figs. 2, 3, 5). Bundles and rows of bristles lined the bases of the teeth. There was a sclerotized partition (*sp*) between adjacent rows (Fig. 2).

3.2.1. *Tettigoniinae*

The studied species of *Tettigoniinae* had *sa* covered with some scales and cilia on the base of it. They also had an obvious *mt*. The structures of the proventriculus also varied among the different genera. Species of *Gampsocleis* had a relatively smooth *sa* covered with irregular mastoids, with cilia on the base. This genus had the longest proventriculus of all the species we studied, because these species also had a larger body than the others (Table 2). In species of *Uvarovites*, *mt* was not very obvious compared with the other species, and the top of *mt* bended toward the body, with thinning cilia on the base (Fig. 5). Species of *Tettigonia* had a similar proventriculus to that of the genus *Gampsocleis*, but they were smaller and the mastoids distributed on *sa* were more regular than in *Gampsocleis* (Fig. 3). In species of *Metrioptera*, the mastoids were mainly distributed on the top of *mt*, and there were thick cilia on the base of *lt* (Fig. 6). In species of *Atlanticus*, a slender and sharp *mt* could be observed on *sa*, and there were also thick cilia on the base of *lt* (Fig. 7).

3.2.2. *Phaneropterinae*

The proventriculus of this subfamily was generally smaller than that of the subfamily *Tettigoniinae*. They had a smooth *mt* and always extremely thick and long cilia distributed all over *sa*. There were no considerable differences in the specific structure of the proventriculus among the three genera *Phanerotera*, *Ducetia* and *Elimaeta* (Figs. 4, 8–9).

3.2.3. *Conocephalinae*

The species of *Conocephalinae* had no cilia on the *sa*, and *mt* was not obvious. The top of *sa* was covered with mastoids and scales distributed on the base. There was no considerable difference between the two genera, *Conocephalus* and *Ruspolia* (Figs. 10, 11).

4. Discussion

Despite their same basic anatomical features, the inner structures of proventriculus showed diversity among three subfamilies of *Tettigoniidae*. The features of proventriculus and the length of each part of the alimentary canal were highly associated with the feeding habits. As early as in 1966, Uvarov discussed the relationship between the shape of the digestive system and feeding habits of Orthoptera species (Uvarov 1966). But feeding habits of most katydids were still unknown. Berneys and Chapman (1970) identified the feeding habits of Orthoptera species by the undigested food in the guts.

Our results showed that the species of the subfamily *Phaneropterinae* have a short foregut and a longer midgut and hindgut, may be because these species are carnivorous or omnivorous insects, and their food items are easier to be grinded than those of herbivorous species. The foregut is the main place for grinding food, so herbivorous species need a larger one. Species of the subfamily *Conocephalinae*, mostly herbivorous insects, have a longer foregut but a short midgut and hindgut.

The inner structures of proventriculus were also highly associated with the feeding habits. In the subfamily *Phaneropterinae*, the mastoids on

the *mt* were not obvious and there always were long cilia on the base of *sa*. The function of these long cilia might be to help them to transport the semi-liquid food. In the subfamily Conocephalinae, the tooth of proventriculus was highly sclerotized, and had many mastoids covered with *mt*. We assume that the function of these mastoids is to grind the vegetable food, such as plant fibers and pieces of leaves. In the subfamily Tettigoniinae, the species of which are mainly omnivorous, the characters of the proventriculus are also intermediate between the subfamilies Phaneropterinae and Conocephalinae.

There are more than 6,000 described Tettigoniidea species which belong to 1,199 genera around the world (Eades *et al.* 2012). Our results cover only a little fraction of the whole family. However, our study includes most of the common species which occur in China, and gives a better understanding of the alimentary canal of katydids. Furthermore, we examined the anatomical aspects of the alimentary canal and proventriculus of Tettigoniidae for the first time and discussed their relationship to their diet. Further examination of more Tettigoniidae species are needed for a robust assessment of Tettigoniidae phylogeny and feeding habits.

Acknowledgements. This work was supported by the Natural Science Foundation of China (No. 31172133) and the Fundamental Research Funds for the Central Universities (No. 11SSXT153). We are extremely grateful to Carlos Pena and an anonymous referee for the valuable comments on the manuscript. Thanks also to the members of our lab for collecting material. This study was supported by the Central Lab, School of Life Sciences, Northeast Normal University, Changchun, China.

References

- Bland, R. G. & Rentz, D. C. F. 1991: Studies in Australian gryllacrididae: The proventriculus as a taxonomic character. — *Invertebrate Taxonomy* 5: 443–455.
- Caetano, F. H. 1988: Anatomia, histologia e histoquímico sistema digestivo e excretor de operárias deformigas (Hymenoptera, Formicidae). — *Naturalia* 13:129–174. [In Portuguese]
- Chapman, R. F. 1998: *The Insects Structure and Function*. — Cambridge University Press, Cambridge, pp. 38–40.
- Eades, D. C., Otte, D., Cigliano M. M. & Braun H. 2012: Orthoptera Species File Online. Version 2.0/4.0. <http://Orthoptera.SpeciesFile.org> (Site visited on 10 March, 2012).
- Fontanetti, C. S. & Zefa, E. 2000: Morphological characterization of the proventriculus of *Gryllus assimilis* Fabricius (Orthoptera, Gryllidae). — *Revista Brasileira de Zoologia* 17: 193–198.
- Fontanetti, C. S., Zefa, E., Passetti, F., Mesa, A. 2002: Morphological characterization and comparative analysis of the proventriculus from three species of *Endecous* Saussure, 1878 (Orthoptera: Gryllidae: Phalangopsinae). — *Entomotropica* 17: 15–23.
- Gibbs, D. G. 1967: The proventriculus of some trichopterous larvae. — *Journal of Zoology* 152: 245–256.
- Isely, F. B. & Alexander, G. 1949: Analysis of insect food habits by crop examination. — *Science* 109(2823): 115–116.
- Judd, W. W. 1947: The proventriculus of *Macrobasis unicolor* Kirby (Coleoptera: Meloidae). — *Annals Entomological Society of America* 40: 518–521.
- Judd, W. W. 1948: A comparative study of the proventriculus of Orthopteroid insects with reference to its use in taxonomy. — *Canadian Journal of Research* 26: 93–161.
- Lebrun, D. 1985: Structure digestives et regimes alimentaires des termites. — *Actes Collection Insectes Sociaux* 2: 43–44.
- Li, N. & Ren, B. Z. 2007: Karyotypes of Tettigoniidae (Orthoptera: Tettigoniodea) in Northeast China. — *Zootaxa* 1457: 61–68.
- Roche, R. K. & Wheeler, D. E. 1997: Morphological specializations of the digestive tract of *Zacryptocerus rohweri* (Hymenoptera: Formicidae). — *Journal of Morphology* 234: 253–262.
- Serrão, J. E. 2001: A comparative study of the proventricular structure in corbiculate apinae (Hymenoptera: Apidae). — *Micron* 32: 379–385.
- Serrão, J. E. 2005: Proventricular structure in solitary bees (Hymenoptera: Apoidea). — *Organisms, Diversity & Evolution* 5: 125–133.
- Serrão, J. E. 2007: Proventricular structure in the bee tribe Augochlorini (Hymenoptera: Halictidae). — *Organisms, Diversity & Evolution* 7: 175–180.
- Uvarov, B. P. 1966: *Grasshoppers and locusts: a handbook of general acridology*. — Cambridge University Press, Cambridge, Vol. I.
- Yahiro, K. 1990: A comparative morphology of the alimentary canal in the adults of ground-beetles (Coleoptera). — *Esakia* 1: 35–44.