

# Chorion of a South African Heel-walker, *Karoophasma biedouwensis* Klass *et al.*: SEM Observations (Insecta: Mantophasmatodea)

Toshiki UCHIFUNE<sup>1)</sup>, Ryuichiro MACHIDA<sup>1)</sup>, Tadaaki TSUTSUMI<sup>2)</sup>  
and Koji TOJO<sup>3)</sup>

<sup>1)</sup> Sugadaira Montane Research Center, University of Tsukuba, Sugadaira Kogen, Ueda, Nagano 386–2204, Japan

<sup>2)</sup> Biological Laboratory, Faculty of Education, Fukushima University, Fukushima, Fukushima 960–1296, Japan

<sup>3)</sup> Department of Biology, Faculty of Science, Shinshu University, Matsumoto, Nagano 390–8621, Japan

E-mail: uchito@sugadaira.tsukuba.ac.jp (TU)

## Abstract

The chorion of *Karoophasma biedouwensis* was examined in detail using scanning electron microscopy (SEM). The egg is divided into a main body region and an anterior cap region. The chorion of the body region is a three-layered structure, *i. e.*, a thin and fibroid outermost surface layer, an exochorion with a solid basement layer and numerous long outward projections, and a homogeneous endochorion: the basement layer of the exochorion and the endochorion are tightly connected to each other by numerous small rods. The cap region is subdivided into zones 1 to 3. The chorionic structure in the anteriormost zone of the cap region or zone 3 is basically the same as that in the body region. In zone 2, the outermost surface layer is practically lacking, and the basement layer of the exochorion is ill-developed. In the posteriormost zone of the cap region or zone 1, the outermost surface layer and exochorion are practically absent, and the chorion is represented by the endochorion, which strongly upheaves outwards.

Fifteen to 19 micropyles are located in zone 1. The micropyles of Mantophasmatodea are characterized by: 1) their position near the anterior egg pole, 2) their plural number, 3) their circular arrangement centered at the anterior egg pole, and 4) the outward extension over their external opening. Major micropylar features of Mantophasmatodea are in good agreement with those of Grylloblattodea, reinforcing the proposed affinity of these orders.

## Introduction

Mantophasmatodea, polyneopteran members, have been studied in various disciplines since their discovery (Klass *et al.*, 2002). These studies have suggested this order to have a closer affinity with Grylloblattodea (morphology: Klass *et al.*, 2002; molecular phylogeny: Jarvis and Whiting, 2003; Terry and Whiting, 2005; spermatology: Dallai *et al.*, 2003, 2005; embryology: Machida *et al.*, 2004; Tsutsumi *et al.*, 2004; Uchifune and Machida, 2005a, b, c).

Egg membranes of Mantophasmatodea have been examined light- and electron microscopically (Klass *et al.*, 2002; Zompro *et al.*, 2002; Machida and Tojo, 2003; Machida *et al.*, 2004; Tsutsumi *et al.*, 2004). The egg membranes of Mantophasmatodea are characterized by: 1) a honeycomb pattern on the surface, 2) a 'cap structure' in the anterior region of the chorion, 3) a three-layered organization of the chorion (a thinnest outermost surface layer, an exochorion with numerous vertical projections and an endochorion with numerous vertical aeropyles), and 4) a thin and fragile vitelline membrane. No micropyles, however, have been reported in Mantophasmatodea (*cf.* Klass *et al.*, 2002; Zompro *et al.*, 2002; Machida *et al.*, 2004), and mantophasmatodean egg membranes need to be studied in further detail. In the present study, we examine the egg structures of *Karoophasma biedouwensis* by SEM, utilizing their eggs just before oviposition.

## Materials and Methods

Adult females of *Karoophasma biedouwensis* Klass *et al.* were collected in August, 2005, in Biedouw, Western Cape

Province, South Africa. The matured eggs were isolated from the oviduct of females and fixed with Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde) buffered with 0.1 M HCl-sodium cacodylate (pH 7.5). The fixed eggs and chorions were dehydrated in a graded ethyl alcohol series and transferred to *t*-butyl alcohol. They were dried in a *t*-butyl freeze drier (VACUUM DEVISE VFD-21S), coated with gold, and observed under an SEM (TOPCON SM-300).

## Results

The egg of *Karoophasma biedouwensis* was ellipsoidal with 2.0–2.5 mm long and 1.0–1.2 mm short diameters, and divided into two regions: a main body region and an anterior cap region (Fig. 1A). The former uniformly shows a honeycomb pattern on its surface and a three-layered organization in section: the outermost surface layer, an exochorion, and an endochorion (Fig. 1B, C). The outermost surface layer is thin and fibroid. The exochorion is composed of a homogeneous basement layer and numerous long outward projections. The endochorion is homogeneous. The exochorion and endochorion are spaced, and remain in contact with each other *via* numerous small rods (Figs. 1C, 2D).

The cap region ['cap structure' in Tsutsumi *et al.* (2004) and Machida *et al.* (2004)] occupies *ca.* 15% of the anterior part of the egg (Fig. 1A). This region is subdivided into three zones (Fig. 2A, B). Zone 1, with a width of *ca.* 25  $\mu\text{m}$ , is a narrow ridge defining posteriorly the cap region, with little of the honeycomb pattern developed (Fig. 2B). Sections of zone 1 reveal that both the outermost surface layer and exochorion thin down to be indistinct, but the endochorion is much thickened and upheaves outwards (Fig. 2C, E). Zone 2, *ca.* 200  $\mu\text{m}$  in width, shows little of a honeycomb pattern either (Fig. 2B), because the outermost surface layer is ill-developed and the long outward projections are exposed. Sections of zone 2 reveal that the exochorion there is represented only by long outward projections, without its basement layer developed, and that the endochorion shows the same thickness as in zone 3 (Fig. 2C–E). The long outward projections are thickened at their bases, which keep in contact with the endochorion *via* numerous small rods (Fig. 2C–E). Zone 3, *ca.* 250  $\mu\text{m}$  wide, is the same as the body region in superficial and sectional structures (Fig. 2B–D), although zone 3 is more abundant than the body region in the number of small rods between the exochorion and endochorion (Fig. 1C *vs.* 2D).

We have revealed the micropyles for mantophasmatodean eggs, in the present study, in *Karoophasma biedouwensis*. They number 15 to 19, being irregularly arranged at the border between zones 1 and 2 (Fig. 3A). Externally, each micropyle is a 20–30  $\mu\text{m}$  wide, anteriorly directed opening with a flat funnel-like hood: the hood is an

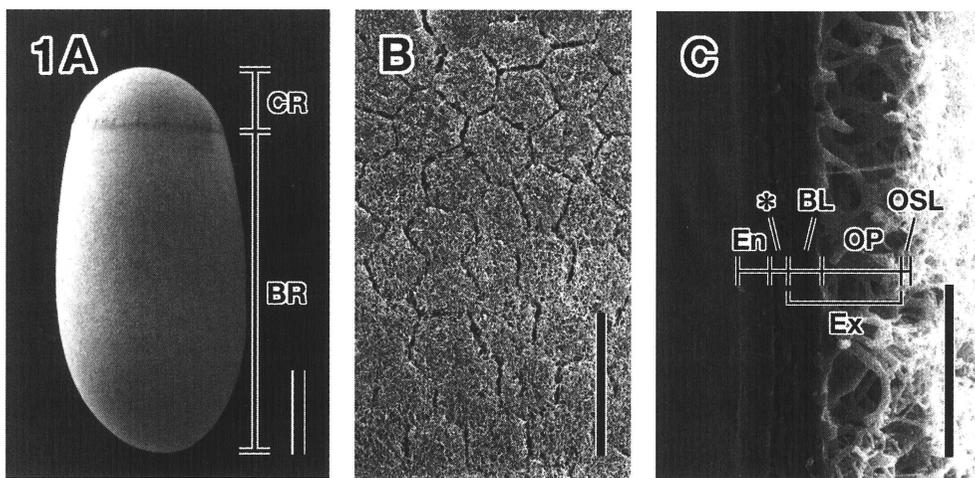


Fig. 1 Egg and chorionic structure in the body region of *Karoophasma biedouwensis*, SEMs. Anterior to the top. A. An egg. B, C. Superficial (B) and sectional (C) structures of the chorion in the body region. An asterisk shows the region with rods intervening between the exochorion and endochorion. BL: basement layer of the exochorion, BR: body region, CR: cap region, En: endochorion, Ex: exochorion, OP: long outward projections in the exochorion, OSL: outermost surface layer. Scales = A: 500  $\mu\text{m}$ ; B: 50  $\mu\text{m}$ ; C: 10  $\mu\text{m}$ .

anterior extension of the zone 1 (Fig. 3A, B). Internally, each micropyle is observed to be situated along a faint line just on the back side of zone 1 with a *ca.* 5- $\mu$ m wide orifice, which is equipped with a flap-like structure overhung from the anterior (Fig. 4A, B).

### Discussion

The present observations for *Karoophasma biedouwensis* have confirmed our previous findings regarding mantophasmatodean egg membranes (Klass *et al.*, 2002; Zompro *et al.*, 2002; Machida and Tojo, 2003; Machida *et al.*, 2004; Tsutsumi *et al.*, 2004) as well as provided some novel information.

### Chorion

The eggs of Mantophasmatodea are divided into two regions or the body and cap regions and have a honeycomb pattern on their surface.

#### 1. Surface structure

The present observations newly revealed the honeycomb pattern on the egg surface to be faint or practically lacking in zones 1 and 2 of the cap region. This may be related to the fact that the outermost surface layer is ill-developed in zones 1 and 2. Therefore, the honeycomb pattern may be due to the outermost surface layer.

#### 2. Body region

The chorion of the body region is a three-layered structure, *i. e.*, a thin and fibroid outermost surface layer, an exochorion with a solid basement layer and numerous long outward projections, and a homogeneous endochorion: the basement layer of the exochorion and the endochorion are tightly connected to each other by numerous small rods (see Fig. 1C). The 'exochorion' of Tsutsumi *et al.* (2004) and Machida *et al.* (2004) corresponds to our basement layer of the exochorion. They regarded the rods connecting the exochorion and endochorion as derivatives of the exochorion, but this affiliation should be discussed further.

#### 3. Cap region

The present observations revealed that three regions (zones 1 to 3) are distinguished in the cap region of *Karoophasma biedouwensis*. The anteriormost zone 3 is basically the same as the body region in superficial and sectional structures (Fig. 1C *vs.* Fig. 2D): the rods between the exochorion and endochorion are more abundant in zone 3 than in the body region.

Zone 2, posterior to zone 3, practically loses the outermost surface layer and the basement layer of the exochorion, seemingly represented by the long outward projections of the exochorion and the endochorion. There, the long outward projections are thickened at their bases. This may be explained by the absence of the basement layer as a defined structure, which is well conceived to be integrated with the bases of the long outward projections, because the rods and the long outward projections with thickened bases are in direct contact.

The posteriormost zone 1 is characterized by a thick upheaved endochorion and practically no outermost surface layer or exochorion. Tsutsumi *et al.* (2004) and Machida *et al.* (2004) simply called zone 1 the 'circular ridge.'

Tsutsumi *et al.* (2004) and Machida *et al.* (2004) failed to distinguish zones 2 and 3 in their observations of the *Karoophasma biedouwensis* chorion, and misleadingly concluded that the exochorion and endochorion are fused to each other in the 'exo-endochorion' in the cap region.

### Micropyles

In the present study, micropyles were first found in mantophasmatodean eggs. This enables us to incorporate Mantophasmatodea into comparisons of Polyneoptera in terms of micropylar features. The micropyles of Mantophasmatodea are characterized by: 1) their position near the anterior egg pole, 2) their multiple number (15–19), 3) their circular arrangement centered at the anterior egg pole, and 4) the outward extension over their external opening.

#### 1. Comparison with Grylloblattodea

Grylloblattodea were suggested to share some features of egg membranes with Mantophasmatodea (Tsutsumi *et*

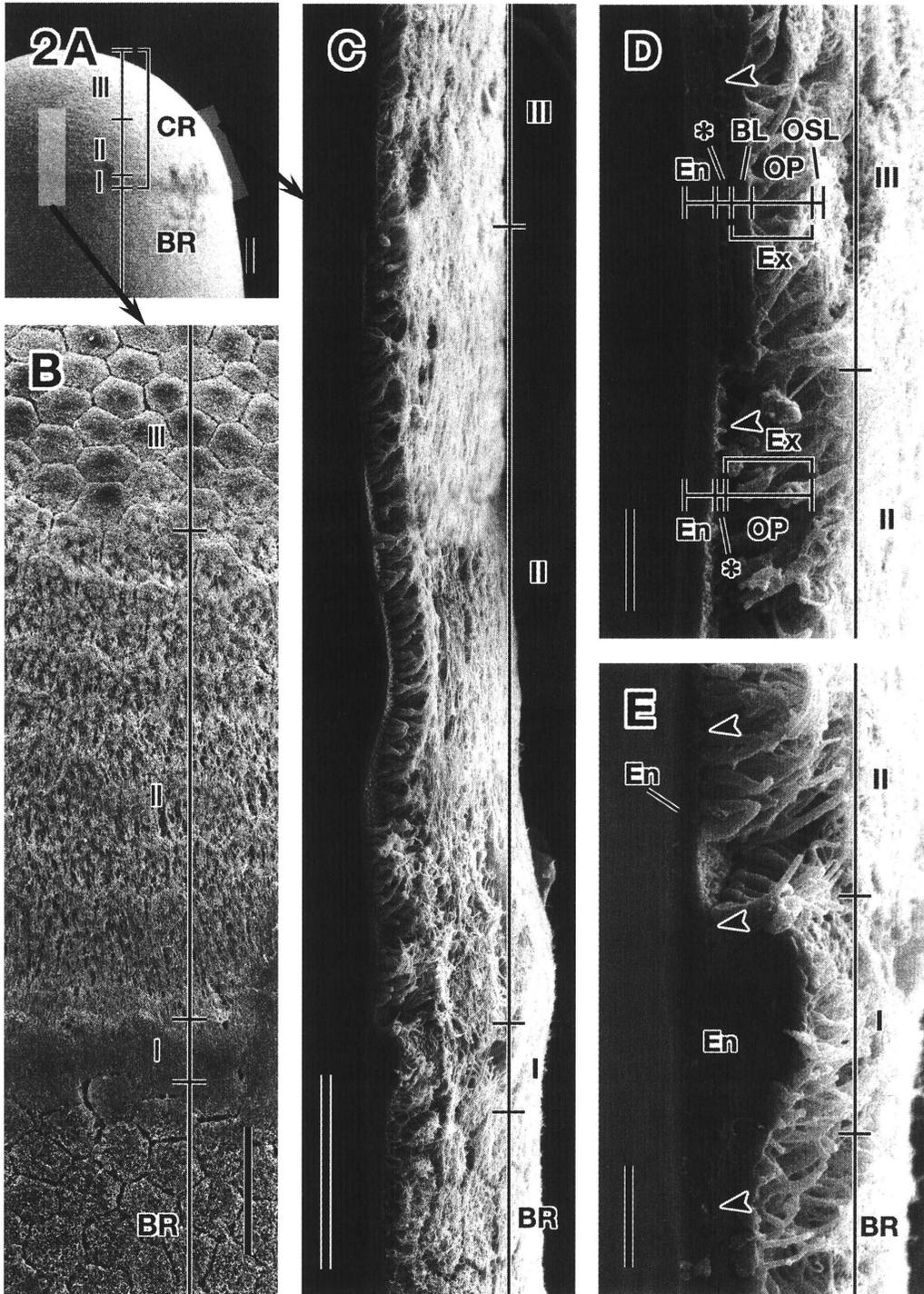
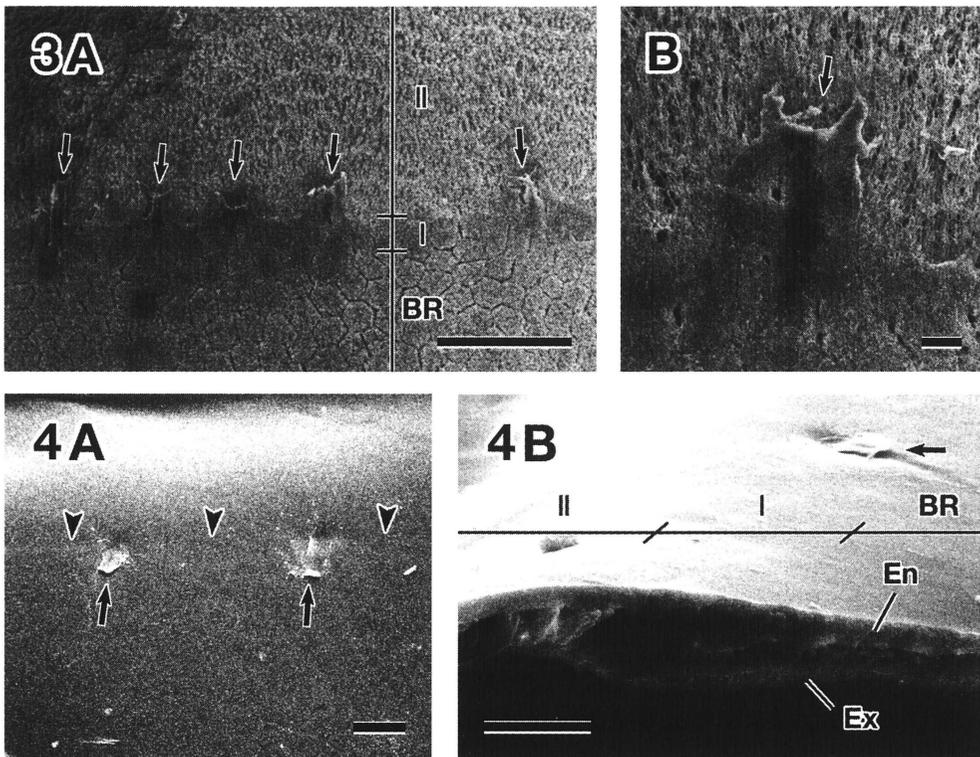


Figure 2



Figs. 3, 4 Micropyles of *Karoophasma biedouwensis* eggs, SEMs.

Fig. 3 A surface view around the border of the cap and body regions. Micropyles are situated in zone 1, which extend slightly anterior to the area of zone 2. Anterior to the top. B. Enlargement. Arrows show the micropyles with a funnel-like hood. Scales = A: 100  $\mu$ m; B: 10  $\mu$ m.

Fig. 4 Interior view around the region with micropyles. A. Micropyles (arrows) are observed to be situated along a faint line (arrowheads) just to the back of zone 1. Anterior to the top. B. Internal view of the cap region, accompanied by a fractured facet, in order to show the location of the micropyle on the interior surface. Anterior to the left. A micropyle (arrow), which is equipped with a flap-like structure overhung from the anterior, is visible. Scales = 10  $\mu$ m.

BR: body region, En: endochorion, Ex: exochorion, I, II: zones 1 and 2 in the cap region.

*al.*, 2004, Uchifune and Machida, 2005a, b, c; see Introduction). The present study revealed that the Mantophasmatodea have micropyles with some important features (Nos. 1–3 above-mentioned) common to those in Grylloblattodea, which are multiple openings (2–10) in the chorion circular arranged near the anterior egg pole (Uchifune and Machida, 2005b, c). Mantophasmatodean and grylloblattodean micropyles do, however, differ in their external features: the micropyles of Mantophasmatodea have a long outward extension, whereas those of Grylloblattodea are a simple opening without an extension. This difference between the two orders may not be essential, but the extensions over the mantophasmatodean micropyles may be a specialized modification, brought about by the development of long outward

Fig. 2 Chorionic structure in the cap region of *Karoophasma biedouwensis*, SEMs. Anterior to the top. A. Anterior region of an egg. B. A surface view of the chorion in the region surrounded by a square in A. C. Sectioned view in the region shown in another square in A. D, E. Enlargements of C, showing zones 2 and 3 (D) and zone 1, zone 2 and the body region (E). Arrowheads show the rod structures, which intervene between the basement layer of the exochorion and endochorion in zone 3 and the body region, and on which the long outward projections of the exochorion stand in zone 2. Asterisks show the region with rods intervening between the exochorion and endochorion. BL: basement layer of the exochorion, BR: body region, CR: cap region, En: endochorion, Ex: exochorion, OP: long outward projections in the exochorion, OSL: outermost surface layer, I–III: zones 1 to 3 in the cap region. Scales = A: 100  $\mu$ m; B, C: 50  $\mu$ m; D, E: 10  $\mu$ m.

projections of the exochorion, which may be regarded as some adaptation to the arid environment in which Mantophasmatodea live.

Thus, we recognize a close resemblance in micropylar structure between Mantophasmatodea and Grylloblattodea, and propose a closer affinity between them, as has been suggested for other aspects of egg membranes.

## 2. Comparison with Phasmatodea and Embioptera

Mantophasmatodean eggs seemingly resemble those of Phasmatodea and Embioptera, in possessing a cap region (Mantophasmatodea) and an operculum (Phasmatodea and Embioptera), as suggested by Zompro *et al.* (2002), Machida *et al.* (2004) and Tsutsumi *et al.* (2004). However, the mantophasmatodean and the phasmatodean and embiopteran eggs show great contrast concerning micropylar structures. While mantophasmatodean micropyles are characterized as aforementioned, Phasmatodea and Embioptera share the following peculiar features: 1) a single micropyle, 2) a micropyle that is positioned on the ventral side of the egg, and 3) a micropyle that is accompanied by a micropylar plate (Phasmatodea: Sellick, 1997, 1998; Zompro, 2004; Embioptera: Melander, 1903; Zompro, 2004; Jintsu and Machida, in preparation), so that Jintsu and Machida (in preparation) suggested a closer affinity of these orders, regarding these features as synapomorphies.

## 3. Comparisons with other polyneopteran members

Micropyles have been studied in all polyneopteran orders except for Zoraptera (see Tsutsumi *et al.*, 2004). With the exception of Phasmatodea and Embioptera, in which there is only a single micropyle, micropyles are multiple in number in polyneopteran orders, and can be categorized according to their position and arrangement: for example, a circular arrangement in the anterior region of the egg with one additional micropyle at its center in Mantodea (Iwaikawa and Ogi, 1982), a semicircular or localized arrangement in the posterior region of the egg in Isoptera (*e. g.*, Knower, 1900), and a circular arrangement in regions other than the anterior region of the egg in Caelifera (*e. g.*, Roonwal, 1954). However, our knowledge of the micropyles in Polyneoptera remains too scanty to generalize the micropylar characteristics for each order and to develop phylogenetic comparisons (cf. Hinton, 1981).

Only Grylloblattodea completely share a set of micropylar features found in Mantophasmatodea.

*Acknowledgments:* We are indebted to our colleagues at the Sugadaira Montane Research Center, University of Tsukuba for their help in keeping materials. This work was partially supported by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science to R.M. (17370030). Contribution No. 207 from the Sugadaira Montane Research Center, University of Tsukuba.

## References

- Dallai, R., F. Frati, P. Lupetti and J. Adis (2003) Sperm ultrastructure of *Mantophasma zephyra* (Insecta, Mantophasmatodea). *Zoomorphology*, **122**, 67–76.
- Dallai, R., R. Machida, T. Uchifune, P. Lupetti and F. Frati (2005) The sperm structure of *Galloisiana yuasai* (Insecta, Grylloblattodea) and implications for the phylogenetic position of Grylloblattodea. *Zoomorphology*, **124**, 205–212.
- Hinton, H.E. (1981) *Biology of Insect Eggs*. Pergamon Press, Oxford.
- Iwaikawa, Y. and K. Ogi (1982) Chorionic structures of the egg shells of mantis, *Tenodera aridifolia* (Dictyoptera: Mantidae). *Res. Bull. (Nat. Sci. Psychol.) College Gen. Educ., Nagoya Univ.*, (26), 69–83. (in Japanese with English summary).
- Jarvis, K.J. and M.F. Whiting (2003) New insights in grylloblattodean phylogeny. *Entomol. Abh.*, **61**, 146–147.
- Klass, K.-D., O. Zompro, N.P. Kristensen and J. Adis (2002) Mantophasmatodea: A new insect order with extant members in Afrotropics. *Science*, **296**, 1456–1459.
- Knower, H.M. (1900) The embryology of a termite (*Eutermes rippertii?*). *J. Morphol.*, **16**, 505–568.
- Machida, R. and K. Tojo (2003) Heel walker, a new insect order Mantophasmatodea. *Kontyû to Shizen*, **38** (6), 26–31. (in Japanese).
- Machida, R., K. Tojo, T. Tsutsumi, T. Uchifune, K.-D. Klass, M.D. Picker and L. Pretorius (2004) Embryonic development of heel-walkers: Reference to some prerevolutional stages (Insecta: Mantophasmatodea). *Proc. Arthropod. Embryol. Soc. Jpn.*, **39**, 31–39.
- Melander, A.L. (1903) Notes on the structure and development of *Embia texana*. *Biol. Bull.*, **4**, 99–118.
- Roonwal, M.L. (1954) The egg-wall of the african migratory locust, *Locusta migratoria migratorioides* Reiche and Frm. (Orthoptera, Acrididae). *Proc. Natl. Inst. Sci. India*, **20**, 361–370.
- Sellick, J.T.C. (1997) Descriptive terminology of the phasmid egg capsule, with an extended key to the phasmid genera based on egg structure. *Syst. Entomol.*, **22**, 97–122.
- Sellick, J.T.C. (1998) The micropylar plate of the eggs of Phasmida, with a survey of the range of plate form within the order. *Syst.*

- Entomol.*, **23**, 203–228.
- Terry, M.D. and M.F. Whiting (2005) Mantophasmatodea and phylogeny of the lower neopteropterous insects. *Cladistics*, **21**, 240–257.
- Tsutsumi, T., R. Machida, K. Tojo, T. Uchifune, K.-D. Klass and M.D. Picker (2004) Transmission electron microscopic observations of the egg membranes of a South African heel-walker, *Karooophasma biedouwensis* (Insecta: Mantophasmatodea). *Proc. Arthropod. Embryol. Soc. Jpn.*, **39**, 23–29.
- Uchifune, T. and R. Machida (2005a) Grylloblattodea: A proposed affinity between Grylloblattodea and Mantophasmatodea. *Biol. Sci.*, **57** (1), 35–39. (in Japanese).
- Uchifune, T. and R. Machida (2005b) Embryonic development of *Galloisiana yuasai* Asahina, with special reference to external morphology (Insecta: Grylloblattodea). *J. Morphol.*, **266**, 182–207.
- Uchifune, T. and R. Machida (2005c) Egg membranes of *Galloisiana yuasai* Asahina (Insecta: Grylloblattodea). *Proc. Arthropod. Embryol. Soc. Jpn.*, **40**, 9–14.
- Zompro, O. (2004) *Revision of the genera of the Areolatae, including the status of Timema and Agathemera* (Insecta, Phasmatodea). Goecke & Evers, Keltern-Weiler.
- Zompro, O., J. Adis and W. Weitschat (2002) A review of the order Mantophasmatodea (Insecta). *Zool. Anz.*, **241**, 269–279.