

# Mating and genital coupling in the primitive earwig species *Echinosoma denticulatum* (Pygidicranidae): implications for genital evolution in dermapteran phylogeny

YOSHITAKA KAMIMURA <sup>\*,1,2</sup> & CHOW-YANG LEE <sup>1</sup>

<sup>1</sup>Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia — <sup>2</sup>Department of Biology, Keio University, 4-1-1 Hiyoshi, Yokohama 223-8521, Japan; Yoshitaka Kamimura \* [kamimura@fbc.keio.ac.jp] — \* Corresponding author

Accepted 11.iii.2014.

Published online at [www.senckenberg.de/arthropod-systematics](http://www.senckenberg.de/arthropod-systematics) on 8.iv.2014.

## Abstract

Dermoptera (earwigs) shows much diversity in the genital structures, the presence of either one or two male intromittent organs (penes) being one striking aspect. The members of several groups (Karschiellidae, Eudermoptera, Arixeniina, and Hemimerina) possess a single functional penis, while others have a pair of penes. The latter condition is considered to be plesiomorphic in Dermoptera. Despite its importance for inferring the phylogeny of Dermoptera, it is presently unclear how the ancestor of earwigs acquired paired penes. To estimate the mode of mating and sperm transfer in the common ancestor of extant earwigs, this study examines the mating behavior and genital coupling of the primitive earwig species *Echinosoma denticulatum* Hincks, 1959 as a representative of Pygidicranidae, one of the basal-most assemblages of earwigs. Staged mating experiments, including surgical manipulation of male penes revealed the following characteristics for this species: (1) males use only one of the paired penes for a single genital coupling; (2) both penes are likely functional; (3) there are no consistent biases in usage of the penes; (4) laterality of the penis-use pattern is not related to the direction of rotation of the male abdomen to establish genital coupling; (5) sperm are transferred directly into the spermatheca, a female sperm storage organ; (6) the spined area of the penis inflicts wounds on the vagina around the spermathecal opening, which bears many setae, during copulation. Characteristics (1)–(4) are considered to be plesiomorphic and may represent the condition of the common ancestor. Traumatic penetration during copulation is reported for earwigs for the first time. Together with intermittent acceptance of courting males by females only after a certain interval, these results suggest sexual conflict over mating in this species.

## Key words

Evolution of laterally paired structures, dermapteran phylogeny, Pygidicranidae, genital evolution, mating behavior, insemination process, traumatic mating.

## 1. Introduction

The insect order Dermoptera (earwigs) shows much diversity in the genital structures, including size, number and laterality of both male and female genital components, and the degree of differentiation of female com-

ponents, making them valuable study models of genital evolution (e.g., POPHAM 1965; STEINMANN 1986, 1989, 1990, 1993; KAMIMURA 2000, 2004a,b, 2006, 2007b, 2013, 2014; KAMIMURA & MATSUO 2001; KLASS 2001,

2003; KAMIMURA & IWASE 2010; SCHNEIDER & KLASS 2013). The presence of either a single or a pair of male intromittent organs (penes) is an especially striking feature of earwigs.

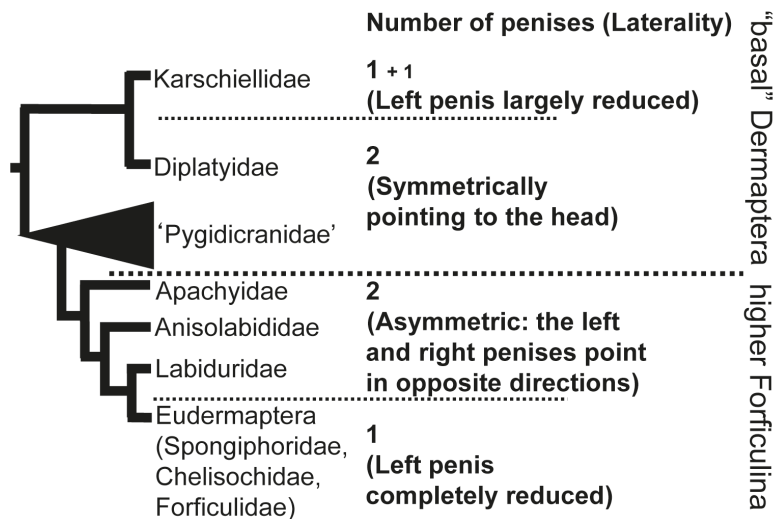
Dermaptera is generally subdivided into three taxa: Hemimerina, Arixeniina and Forficulina. Members of the former two taxa live on mammals (hamster rats [*Crictomys* spp.] in Africa and bats in Asia, respectively) and show many adaptations to their phoretic-epizoic lifestyle (NAKATA & MAA 1974). According to the classification of SAKAI (1982), Forficulina comprises eight families of typical free-living earwigs: Pygidicranidae, Diplatyidae, Anisolabididae, Apachyidae, Labiduridae, Spongiphoridae, Chelisochidae, and Forficulidae. Karschiellinae of the Pygidicranidae in Sakai's system is sometimes treated as the ninth family Karschiellidae (e.g., POPHAM 2000; HAAS & KLASS 2003; JARVIS et al. 2005); here we follow this view. Figure 1 shows the currently most elaborate phylogenetic hypothesis for Forficulina. Several studies have suggested that both Hemimerina and Arixeniina are in-group members of Forficulina (KLASS 2001; HAAS & KLASS 2003; JARVIS et al. 2005; TWORZYDLO et al. 2012; KOCAREK et al. 2013), but their phylogenetic placements in Forficulina have not been settled.

Males of all Dermaptera have a genital (phallic) organ placed at the midline of the body in the reflected membrane above the coxosternum of abdominal segment IX (subgenital plate). In six families the genitalic organ includes a pair of penes, with few exceptions (Fig. 1; for genital terminology, see Fig. 2). Among the "basal" dermapteran families (Karschiellidae, Pygidicranidae, and Diplatyidae), the right and left penes are symmetrical in Pygidicranidae and Diplatyidae, both pointing to the head when not in copulation, while in Karschiellidae the left penis is strongly reduced (e.g., STEINMANN 1986). In Apachyidae, Anisolabididae, and Labiduridae of the "higher Forficulina", penes are asymmetric when in repose: the left and right penes point in opposite directions (e.g., POPHAM 1965; STEINMANN 1989; KAMIMURA & MATSUSO 2001; KAMIMURA 2006; Fig. 1). The members of the other three families of the higher Forficulina, which are collectively termed Eudermaptera, have only one penis without exception (Fig. 1; STEINMANN 1990, 1993), as do the phoretic earwigs (Hemimerina and Arixeniina; NAKATA & MAA 1974). Importantly, however, in these taxa (Eudermaptera, Hemimerina and Arixeniina) either the ejaculatory ducts and gonopores are still paired, or there is at least some reminiscence of a formerly paired condition by the presence of a vestigial ejaculatory duct on the non-functional side (e.g., SNODGRASS 1959: fig. 4H,I; POPHAM 1965: fig. 4; KAMIMURA 2006: fig. 3C, table 4). Although the detailed relationships among the majority of the families of earwigs are still unsettled, all previous studies have assumed or estimated that paired penes represent the ancestral state (POPHAM 1985; SAKAI 1987; HAAS 1995; HAAS & KUKALOVÁ-PECK 2001; COLGAN et al. 2003; HAAS & KLASS 2003; KAMIMURA 2004b; JARVIS et al. 2005). In addition, to our knowledge, no examples of reversals from the status 'asymmetrically paired' to the

status 'symmetrically paired' or from 'single penis' to the status of 'asymmetrically paired' have been reported. Thus, the number and direction of penes are considered stable characters and are used to define higher taxa in Dermaptera (Fig. 1; BURR 1915a,b, 1916; POPHAM 1965; STEINMANN 1986, 1989, 1990, 1993).

Irrespective of the importance for inferring the phylogeny of Dermaptera and its placement in the polyneopteran insect orders, the evolutionary reason how the ancestor of earwigs acquired paired penes has remained unexplained. In anisolabidids such as *Anisolabis maritima* (Bonelli, 1832) and *Euborellia plebeja* (Dohrn, 1863), males have two functionally competent penes although they use only one of them during a single genital coupling (KAMIMURA 2000; KAMIMURA & MATSUSO 2001). Each penis bears an elongated virga, which is a heavily sclerotized process which contains the terminal part of the ejaculatory duct and has the gonopore on its tip. Since the virgae are fragile, they sometimes break off during mating; therefore, the remaining counterpart in paired penes functions as a spare (KAMIMURA & MATSUSO 2001). This phenomenon can explain the modern function of paired penes in this specialized group. However, because not all earwigs, especially those in basal groups, have thin and elongated virgae, this spare function is not likely sufficient to explain the evolution of paired penes and virgae in the ancestor of earwigs. The usage pattern of paired penes has been investigated for a few representative species of Diplatyidae, Anisolabididae and Labiduridae. In *Diplatys flavicollis* Shiraki 1907 (Diplatyidae), *Anisolabis littorea* (White, 1846) (Anisolabididae), *A. maritima* and *E. plebeja*, males use or are ready to use the right or left penes without great bias (GILES 1961; KAMIMURA & MATSUSO 2001; KAMIMURA 2003b, 2004a). In contrast, males of *Labidura riparia* (Pallas, 1773) (Labiduridae) predominantly use the right penis for insemination, although both penes are functional without any detectable morphological differentiations between them (KAMIMURA 2006). Previous phylogenetic studies based on morphology and/or molecular data have suggested a sister-group relationship between the Labiduridae and Eudermaptera (Spongiphoridae + Chelisochidae + Forficulidae), which is characterized by a single penis (SAKAI 1987; HAAS 1995; WIRTH et al. 1999; HAAS & KUKALOVÁ-PECK 2001; HAAS & KLASS 2003; COLGAN et al. 2003; KAMIMURA 2004b; JARVIS et al. 2005; Fig. 1). Therefore, extant labidurids may have retained morphological and/or behavioral characters that led eventually to the loss of the less frequently used left penis in the common ancestor of the Eudermaptera (KAMIMURA, 2006, 2007b).

Despite its importance for estimating the evolutionary history of earwig genitalia, the detailed functional morphology of male and female genitalia has been studied for only one species of the basal Dermaptera, *D. flavicollis* (Diplatyidae; KAMIMURA 2004a). The members of Diplatyidae have paired penes, each of which bears a bifurcated virga (e.g., STEINMANN 1986). In several diplatyids, each of bifurcated virgae bears two independ-



**Fig. 1.** The most elaborate phylogenetic hypothesis for the Forficulina (modified from HAAS & KLASS 2003) with the characteristics of the male genitalia of each group. Although HAAS & KUKALOVÁ-PECK (2001) originally proposed that Karschiellidae is the first offshoot of the present Dermaptera, while paraphyletic Diplatyidae is basal-most to the other extant Forficulina, subsequent detailed examination of female genitalia by KLASS (2003) supported the monophyly of two taxa, Diplatyidae (*Haplodiplatys* and *Diplatys*) and Karschiellidae + Diplatyidae (HAAS & KLASS 2003).

ent gonopores, yielding four exits of ejaculates per male (POPHAM 1965; STEINMANN 1986; KAMIMURA 2004a). Female diplatyids also have multiple spermathecae (POPHAM 1965; KLASS 2003; KAMIMURA 2004a). Fixation and examination of copulating pairs of *D. flavicollis* revealed that males inserted only one of the paired virgae into the females during a single genital coupling. To determine whether this method of penis use actually represents a plesiomorphy in the extant earwigs, it is especially important to examine the functional aspects of the genital morphologies in the members of Pygidicranidae, whose subgroups ("subfamilies" and "genera") represent the most basal clades of earwigs together with Diplatyidae (+ Karschiellidae) in the estimated earwig phylogenies (HAAS & KLASS 2003; Fig. 1).

In this study, we examined the mating behavior, genital morphology and genital coupling in the earwig *Echinosoma denticulatum* Hincks, 1959 (Pygidicranidae: Echinosomatinae). For this species, taxonomists described the presence of a pair of symmetrical penes, each bearing a single slender virga, and a well-developed spined area on each penis (HINCKS 1959). The internal genitalia of females has not been described, but female genitalia including cuticulated parts of the gonoducts are known for the congener *E. yorkense* Dohrn, 1869 (KLASS 2003). Courtship and mating behaviors of pygidicranids have been reported for only one species, *Tagalina papua* (Bormans, 1903) by MATZKE & KLASS (2005), in which both males and females show no apparent precopulatory courtship and copulation is long (14–20 hours). During the course of the present study, we found that the stout spines of the penis inflict wounds on female genitalia during copulation. This type of mating, termed traumatic mating by LANGE et al. (2013), has been reported in several insect taxa but not previously from earwigs (LANGE et al. 2013). We here report the occurrence of traumatic mating in earwigs for the first time and discuss the significance of this phenomenon.

## 2. Materials and methods

### 2.1. Insects and rearing

Two males and about fifty nymphs of *Echinosoma denticulatum* were collected from forested areas of Bukit Jambul and Bukit Kukul, Penang Island (05°30'N 100°28'E), Malaysia, during May–November 2012. Field-collected adults were individually placed in a separate plastic vessel (60 mm diameter, 40 mm high) with plaster of Paris at its base and kept at  $26 \pm 1^\circ\text{C}$  (12 h photoperiod). All animals were provided with water, unlimited amounts of commercial cat food and a small piece of peeled tree bark for harborage (and an oviposition site for mated females; see below). Nymphs were kept in a group (up to 40 individuals) in vessels of a similar setting, and checked for imaginal eclosion every three days. Laboratory-raised virgins of both sexes (6–44 days old; including F1 and F2 generations) were used for various types of observation and experiments described below. All of the following observations and experiments were conducted under the same laboratory conditions.

### 2.2. Frequency and duration of copulation

To observe courtship and mating behaviors, a virgin male (but in two cases, field-collected males of unknown age) and a virgin female were introduced into a mating arena (identical to the rearing vessel but without harborage) with a transparent plastic lid. To avoid possible effects of inbreeding and kin-recognition, pairing of a male and a female from the same full-sib family (i.e., offspring of

the same male-female pair) was avoided. Their behavior was recorded for 24–72 hours by a video camera (GZ-MG980S; Victor, Kanagawa, Japan) using a time-lapse recording function (one frame per two seconds). Observations in dark periods (1900–0700 hour) were conducted under a dim red light. The number of pairs observed simultaneously ranged from one to three, based on the availability of adults. Because of the limitation in the number of video recorders, the starting time, ending time and duration of video recording varied according to the schedule of other experiments. After video recording, the females were individually reared for two months to produce offspring. After two months, the spermatheca was dissected out from the females in insect Ringer's solution (0.9 g NaCl, 0.02 g CaCl<sub>2</sub>, 0.02 g KCl and 0.02 g NaHCO<sub>3</sub> in 100 ml water) under a stereo microscope (EZ vision, Saxon, Guangzhou, China) and then examined under light microscopes (BX53 and CX21, Olympus, Tokyo; 40–400×). A total of 21 pairs were observed. The subsequent dissection revealed that one female had malformed genitalia and one female that died before two months after the recording could not be dissected because of the bad condition of the body. These two females were removed from the subsequent analysis. Genitalia from fifteen laboratory reared virgin females were also examined using the same method.

### 2.3. Penis-use patterns and surgical treatment

Two mating experiments were conducted to examine the use pattern and the insemination potential of the paired penes of *E. denticulatum*. In the first experiment, adult pairs were released into a mating arena (see 2.2.). Ten minutes after the initiation of copulation, the mating pairs were instantaneously fixed by pouring liquid nitrogen into the vessel. These samples were later dissected to observe the genital coupling and to determine which penis had been used for insemination.

For the second experiment, either the right or left penis, randomly determined, was excised from males that were lightly anaesthetized with carbon dioxide using fine

forceps ( $n = 23$  in total; Fig. 2D). After at least six days following surgery, the males were individually paired with a virgin female. All of the pairs copulated at least 10 min. When copulation lasted over 60 min, the pairs were artificially dislodged. At the initiation of copulation, the male mating posture (direction of abdominal rotation; Fig. 2A) was also recorded. As in KAMIMURA (2006), the direction of this abdominal rotation was defined as clockwise (CW) or counterclockwise (CCW), as viewed from the head of the male (see Fig. 2A for an example of CCW rotation). The insects were then stored in a freezer (–20°C) for later dissection, in which the success of the surgical treatment and insemination were examined for the male and female samples, respectively. All statistical analyses of the data were conducted using the R 3.0.1 software (R CORE TEAM 2013).

## 3. Results

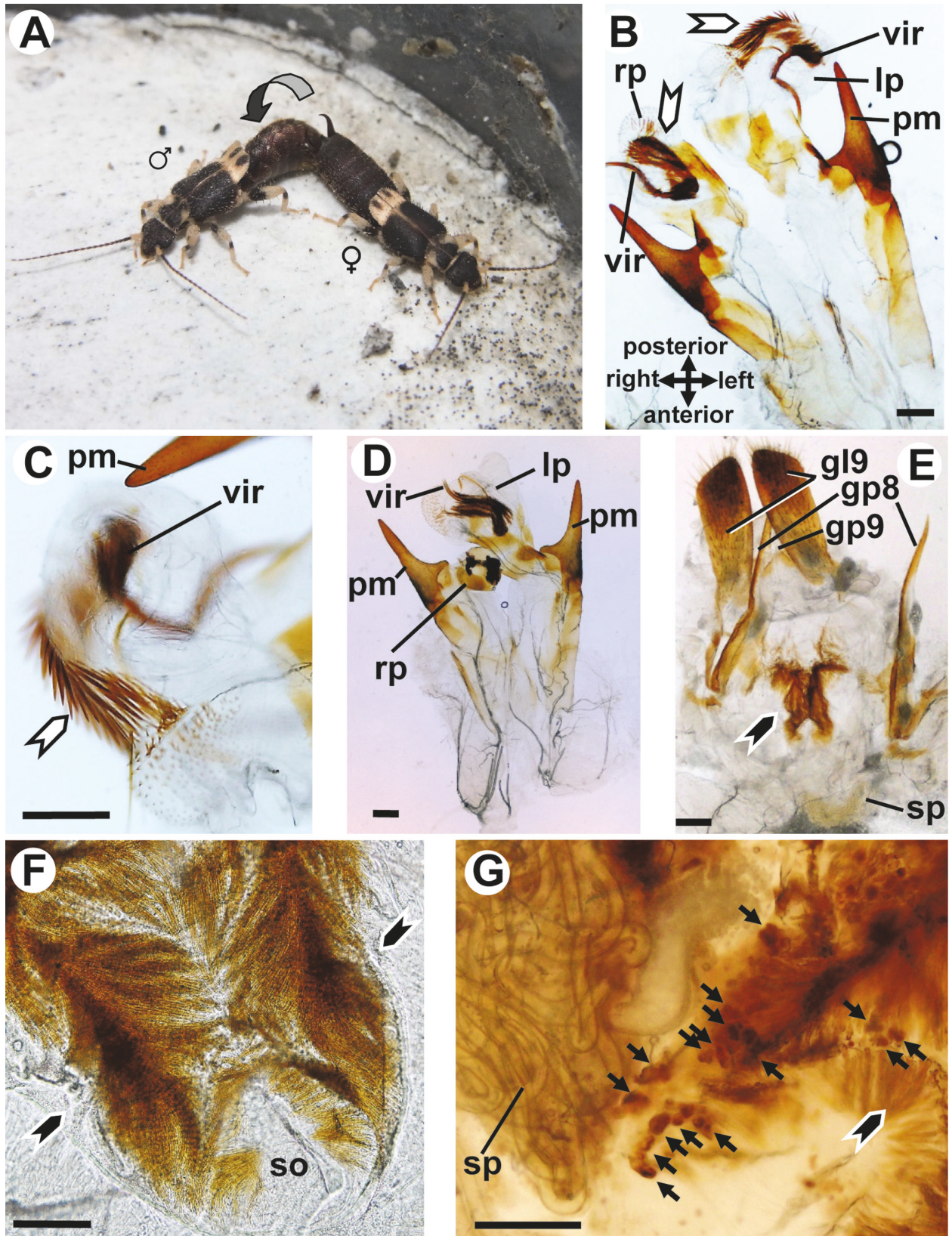
### 3.1. Courtship and mating behavior

When a virgin female and virgin male pair was placed in a mating arena ( $n = 19$ ), the male actively courted the female by directing his abdomen and forceps toward the female abdomen, simultaneously rotating the abdomen 180° around the anterior-posterior axis and retreating to establish genital contact. The first copulation usually took place within a few minutes (up to 38 minutes), irrespective of the start time of the observation (Fig. 3). In 12 of the 19 cases, repeated matings were observed in the same pairs. Later rearing and dissection revealed that two females did not lay eggs and had no detectable sperm in the spermatheca. These two uninseminated females (the two samples at bottom of Fig. 3) mated two and six times, respectively, while the 17 inseminated females mated up to four times (Fig. 3). Copulation lasted  $153 \pm 94$  min (mean  $\pm$  SD,  $n = 41$  including all copulations of all pairs). The average duration of the first copulation was longer than those of the second and third copu-

→ **Fig. 2.** Mating posture (A), male genitalia (B–D) and female genitalia (E–G) of *Echinosoma denticulatum*. In this paper, the terminologies of K<sub>L</sub>ASS (2003) and KAMIMURA (2014) are followed for female and male genital structures, respectively. The spined areas of the male penis and the setaceous parts of the female genitalia are indicated by the open (in B and C) and filled arrowheads (in E–G), respectively. **A:** Mating posture. The male (left) has the abdomen twisted in a counterclockwise (CCW) direction (indicated by the gray curved arrow) to establish genital coupling with the female (right). **B:** Male genitalia, entire view from dorsally, penes extended and endophallic sac of left penis (lp) evaginated. **C:** The spined area of the penis. **D:** Male genitalia with the right penis excised. **E:** Female genitalia. **F:** The setaceous area in the vagina of a virgin female. **G:** The setaceous area of a mated female with many melanized patches indicated by the arrows. Similar patches were not detected in the same area of virgin females, indicating that these are repaired wounds inflicted by the male penial spines. All photographs except for (A) are shown in the direction indicated in (B). – Abbreviations: gl9, gonoplac IX; gp8, gonapophysis VIII; gp9, gonapophysis IX; lp, left penis; pm, paramere; rp, right penis; so, spermathecal opening; sp, spermatheca; vir, virga. – Scale bars: 200  $\mu$ m in B–E, 100  $\mu$ m in F, G.

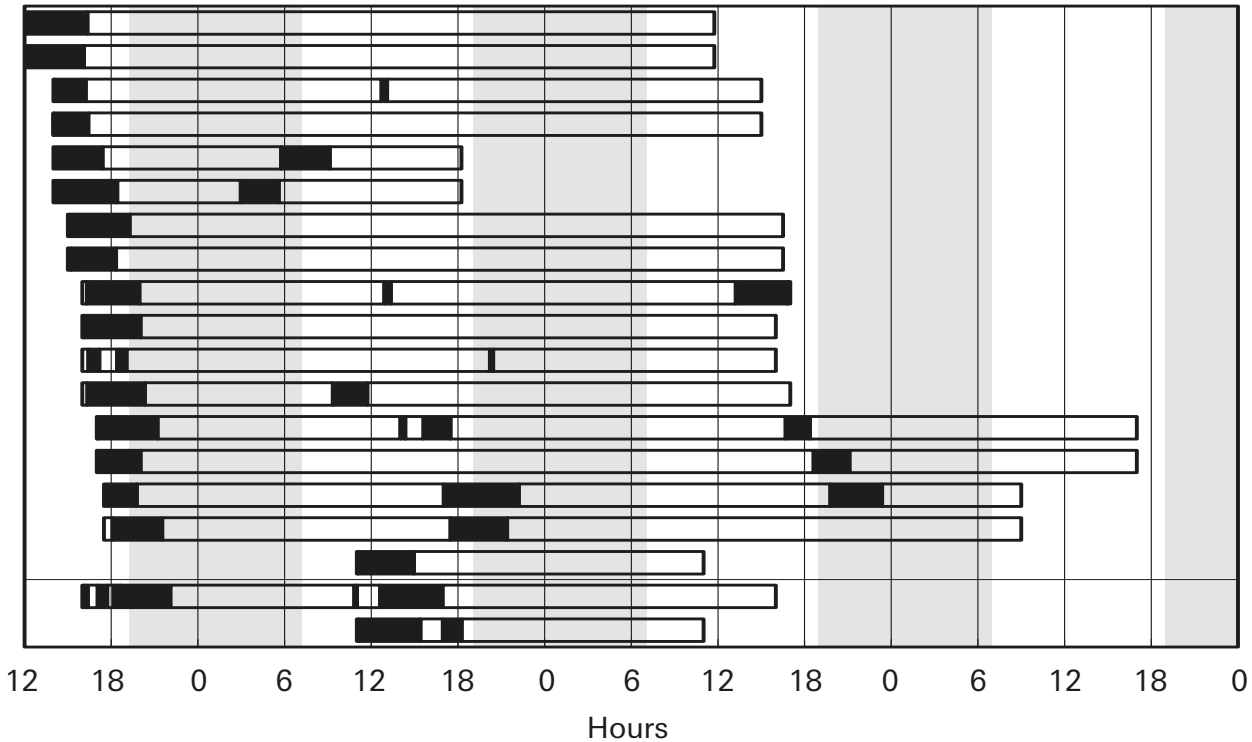
lations (Table 1). However, there were no significant differences among them when we analyzed only the cases in which the male successfully inseminated the female (Table 1). Although the males continued to court the female frequently after their first mating, the females usually escaped from the courting male and accepted them

only after a certain interval (Fig. 3). In the cases in which the female was inseminated, copulations were repeated at 8–24 hour intervals with a few exceptions (Fig. 4). In contrast, the two uninseminated females repeatedly mated with the same male at intervals of less than four hours with one exception (Fig. 4).

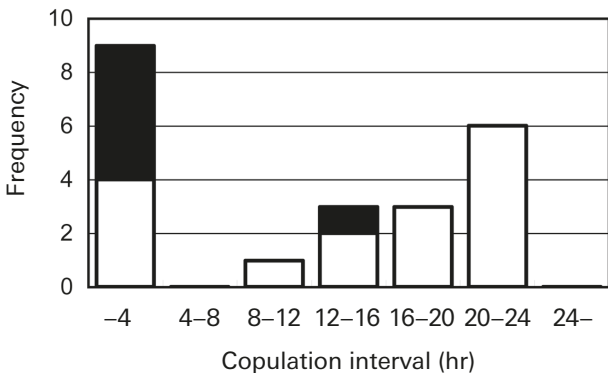


**Table 1.** Copulation durations (in min; mean  $\pm$  SD [*n*]) in *Echinosoma denticulatum*. There were significant differences among the durations of the first, second and third copulations when all samples observed were included (Kruskal-Wallis test;  $\chi^2_2 = 6.80$ ,  $P = 0.033$ ), but the differences were insignificant when only pairs in which the females were inseminated were analyzed ( $\chi^2_2 = 5.03$ ,  $P = 0.080$ ).

	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>
All cases	194 $\pm$ 72 [19]	117 $\pm$ 99 [12]	115 $\pm$ 99 [5]	107 $\pm$ 98 [3]	12 [1]	259 [1]
Females inseminated	201 $\pm$ 59 [17]	128 $\pm$ 105 [10]	139 $\pm$ 97 [4]	57 $\pm$ 62 [2]	—	—



**Fig. 3.** Copulation duration and frequencies in 19 pairs of *Echinosoma denticulatum*. Each rectangle represents the time window of video recording for each pair, in which the filled parts indicate copulation. Later dissection revealed that the two bottom-most females were not inseminated, while the others were inseminated. Dark periods are indicated by gray shading.



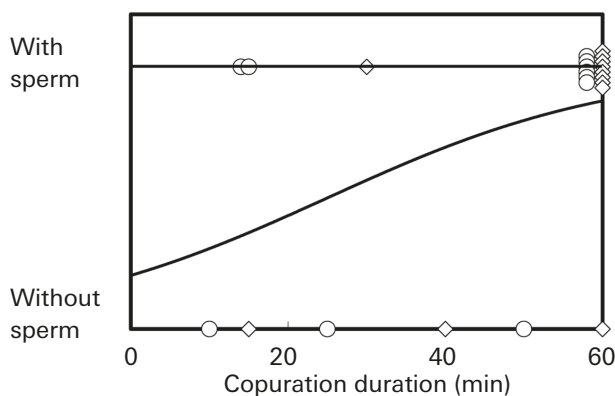
**Fig. 4.** Intervals between successive matings in repeatedly mating pairs of *Echinosoma denticulatum*. The data for pairs where females were inseminated (10 pairs, 16 intervals) and for pairs where females remained uninseminated (2 pairs, 6 intervals) are shown by open and filled bars, respectively.

### 3.2. Genitalia, genital coupling and copulatory wounds

As described by HINCKS (1959), each of the paired penes of *E. denticulatum* bears an area of many strong spines, each about 100  $\mu\text{m}$  long, and a slender virga in the endophallus, which is evaginated during copulation (Fig. 2B,C). The female genital structure is essentially similar to what KLASS (2003) described for *Echinosoma yorkense*: anteriorly to two pairs of the major ovipositor components (a pair of gonapophyses VIII and a pair of gonoplacs IX [= coxal lobes IX]), a single, long, tubular spermatheca without internal branching opens into the dorsal side of the vagina (Fig. 2E). The vagina of *E. denticulatum* is membranous, concave to the dorsal side, and covered with many long (ca. 150  $\mu\text{m}$ ) setae (Fig. 2E,F).

**Table 2.** The relationship between the direction of abdomen rotation during copulation (CW or CCW) and the penis used in *Echinosoma denticulatum*.  $P = 0.41$  (Fisher's exact probability test).

	Clockwise (CW)	Counter-clockwise (CCW)
Right penis used (Left penis excised)	5	6
Left penis used (Right penis excised)	8	4



**Fig. 5.** The relationship between insemination success and copulation duration revealed by surgical experiment in *Echinosoma denticulatum*. The matings were artificially terminated at 60 min after initiation. The open circles and open diamonds represent the data for the right- and left-penis-excised males, respectively. The curved line shows predictions of probability of insemination from logistic regression ( $\chi^2_1 = 4.64$ ,  $P = 0.031$ ).

The length and orientation of the setae varied with the region in the vagina, but most setae are directed posteriorly (from inner parts towards the entrance of vagina).

In the freeze-fixation experiment of mating pairs ( $n = 12$ ), five males used the right penis. In these males, the right penis was directed posteriorly for genital coupling, while the left penis pointed toward the head as when in repose. For the other seven males, the opposite condition was observed. There was no significant bias in the laterality of penis use (5 right:7 left; binomial test,  $P = 0.77$ ). The penes used were dilated (endophallic sac evaginated, possibly caused by increased hemolymph pressure) and the virga and spined area were exposed outside (as shown in Fig. 2B). When the right (left) penis was used, it was inserted into the female genital chamber through the membranous region on the right (left) side of the right (left) gonoplac IX (note that because of the end-to-end mating posture and  $180^\circ$  rotation of the male abdomen around the anterior-posterior axis, the right side of the male contacts the right side of the female). Although the male penis was easily separated after melting of the samples, in the frozen samples, the spined area of the penis in use and the setaceous area of the vagina were in contact, and the virga was inserted directly into the spermatheca.

All 19 females used for the observation of normal mating behavior, including the two uninseminated ones, showed a number of melanized patches around the setaceous area posterior to the spermathecal orifice on which the spines of the male penis pressed during genital coupling (Fig. 2G). None of the virgin females examined ( $n = 15$ ) had detectable melanized patches in the area, indicating that these were repaired wounds inflicted by the male penial spines during copulation.

### 3.3. Surgical experiment

As described above, the freeze-fixation experiment revealed that there was no consistent bias in the laterality of penis use (five and seven males used the right and left penes, respectively). However, this fact does not necessarily indicate that a single male of *E. denticulatum* can use both the right and left penes. A surgical experiment was conducted to examine whether only one of the two penes is functional or not. The males from which one of the paired penes had been artificially excised (12 and 11 cases for the right and left, respectively), courted and copulated easily with a virgin female, just as normal males. Fifteen pairs copulated for 60 min and were artificially dislodged. All but one females of these pairs had detectable sperm in the spermatheca (Fig. 5). The other eight pairs spontaneously terminated copulation before 60 min after initiation (Fig. 5); among these, the probability of insemination increased with the duration of copulation, indicating a variation in the intervals between establishment of genital coupling and the initiation of sperm transfer (Fig. 5). If only one of the paired penes is functional, only 50% of the penis-excised males are expected to successfully inseminate the female. However, the observed insemination success (17 of 23 cases) is higher than expected from this null hypothesis (binomial test,  $P = 0.035$ ). Considering that several pairs copulated only once for a short time in this experiment, and that in a few cases females remained uninseminated even after prolonged mating with a normal male (Fig. 3), it is highly likely that both right and left penes of *E. denticulatum* are functional. The direction of abdominal rotation was biased toward neither the CW nor CCW direction (13:10; binomial test,  $P = 0.68$ ), with no significant relationship to the laterality of penis use (Table 2).

## 4. Discussion

This study showed the following characteristics of mating in *E. denticulatum*: (1) males use only one of the paired penes for a single copulation; (2) both the right and left

penes are likely functional; (3) there is no consistent bias in the usage of the right or left penes; (4) the laterality of penis-use pattern (right or left) is not related to the direction of abdominal rotation (CW or CCW); (5) one virga is inserted directly into the spermatheca to transfer sperm; (6) the spined area of the penis (endophallus) inflicts wounds on the vaginal wall around the spermathecal opening, on which females bear many setae, during copulation. Among these characteristics, (1) is shared by all other earwigs examined to date (KAMIMURA 2004a, 2006) and is likely to be a common feature of earwigs. Apparently, the vagina of *E. denticulatum* can only accommodate one penis at a time. Characteristic (2), the insemination competency of both paired penes, was also confirmed for *D. flavicollis* (Diplatyidae; KAMIMURA 2004a), *E. plebeja* (Anisolabididae; KAMIMURA & MATSUO 2001) and *L. riparia* (Labiduridae; KAMIMURA 2006) and is also likely to be common among earwigs with paired penes. In contrast, the lack of consistent lateral bias in penis use [characteristic (3)] observed in *E. denticulatum* is likely shared by diplatyids and anisolabidids. This supports the view that the conspicuous right-handedness in *L. riparia* is an apomorphy, which may have resulted in the reduction of the left side penis in the sister clade Eudermaptera (KAMIMURA 2006; see Introduction for details).

How did the ancestor acquire a paired condition of penes and gonopores, and how has the paired state been maintained in many extant earwigs? In several earwig species with paired penes, damaged penes or virgae are sporadically observed and considered to have occurred accidentally during copulation (KAMIMURA & MATSUO 2001; KAMIMURA 2003a, 2006). Although further scrutiny is needed for *Echinosoma*, we failed to detect any naturally caused genital damage in the males used for the mating experiments. In many insect taxa, the evolution of asymmetric genitalia is associated with asymmetric copulatory positions (LUDWIG 1970; HUBER et al. 2007). Thus, another possibility is that the direction of abdominal rotation is related to the laterality of penis use, that is, one penis for CW and another for CCW rotation of the male abdomen (KAMIMURA 2003b). To our knowledge, the present study is the first attempt to address laterality in the mating posture of basal Dermaptera. However, as in the two species of the higher Forficulina studied to date (*E. plebeja*, KAMIMURA 2003b; *L. riparia*, KAMIMURA 2006), no consistent correlation was observed between the two asymmetric traits in *E. denticulatum* (Table 2). Several recent studies have supported a sister relationship between Dermaptera and Plecoptera (stoneflies) among the polyneopteran insect orders (KJER 2004; YOSHIZAWA & JOHNSON 2005; MISOF et al. 2007; ISHIWATA et al. 2011; YOSHIZAWA 2011; WAN et al. 2012). Moreover, several stonefly species also have paired orifices of ejaculatory ducts that apparently function as sperm exits (BRINK 1956a,b). Thus, the origin of paired earwig penes may date back to the common ancestor of these two orders.

This study revealed the occurrence of a closed sperm transfer; i.e., direct insertion of the virga into the spermatheca for sperm transfer [characteristic (5)], in a mem-

ber of the basal Dermaptera for the first time. This feature differs from the open system of the diplatyid *D. flavicollis*, in which two male gonopores of a bifurcated virga are closely pressed against a row of two or three spermathecal openings, but are not directly inserted into the spermathecae (KAMIMURA 2004a). According to the phylogenetic relationships proposed by HAAS & KLASS (2003), it is presently equivocal whether the common ancestors of the extant earwigs possessed a closed or an open system of transfer (Fig. 1). The virgae of many members of two subfamilies of Pygidicranidae, Pyagrinae and Esphalmeninae, are bifurcated, suggesting the presence of four exits for sperm per male as in some diplatyids (e.g., BRINDLE 1984; SAKAI 1985; STEINMANN 1986). The genus *Pyagra* Audinet-Serville, 1831 (Pyagrinae) is estimated to be the basal-most clade in the paraphyletic Pygidicranidae, while the placement of Esphalmeninae has not been settled (HAAS & KLASS 2003). Thus, possibly in association with the evolution of non-bifurcated virgae, the closed system of sperm transfer may have evolved in a clade of the paraphyletic Pygidicranidae and be presently shared by “the Pygidicranidae (not necessarily all members, but including *Echinosoma*) + higher Forficulina.” Genital couplings in other representatives of the basal Dermaptera should be examined to confirm this hypothesis.

Functional aspects of associated sclerites or spines of the earwig penis have been examined in only two earwig species to date. The male penis of *Paralabellula dorsalis* Burmeister, 1838 (Spongiphoridae; referred to as *Paralabella dorsalis* in BRICEÑO 1997; cf. KEVAN & VICKERY 1997) possesses several areas with hardened spines (referred to as “toothed plates” in BRICEÑO 1997). During copulation, the membranous penis is inflated and these spines contact areas around the female gonopore and the lateral wall of the vagina (BRICEÑO 1997). Males of *D. flavicollis* possess three differently shaped accessory sclerites, specifically U-, rod- and saber-shaped sclerites, in each penis (KAMIMURA 2004a). In an established genital coupling, two lateral pouches in the female genital chamber receive the U- and rod-shaped sclerites, while the saber-shaped sclerite contacts the female subgenital plate (KAMIMURA 2004a). Although these structures are supposed to function as anchorage devices during copulation, occurrence of copulatory trauma has not been reported for these two species (re-examination by Y. Kamimura of the female samples used in KAMIMURA 2004a failed to reveal any traces of wounds).

Traumatic mating has been reported for members of seven insect orders (Orthoptera, Hemiptera, Coleoptera, Strepsiptera, Diptera, Lepidoptera and Hymenoptera; LANGE et al. 2013) and the present study adds Dermaptera to the list as the eighth order. LANGE et al. (2013) divided the examples of traumatic mating into three categories: traumatic insemination, traumatic secretion transfer and traumatic penetration. In cases of the former two categories, wounds function as the entrance of semen and seminal secretions (without sperm) into the female body, respectively (see HOTZY et al. 2012; LANGE et al. 2013;



TATARNIC et al. 2014 for examples of these categories). In *E. denticulatum*, because the wounds are distributed outside of the spermatheca, while after dissection semen is found inside the sclerotized spermatheca, the copulatory wounds are unlikely to function as an entrance of either sperm or seminal fluid into the female hemocoel. The case of *E. denticulatum* thus represents an example of traumatic penetration. There is continuing debate about the function and significance of this type of traumatic mating (LANGE et al. 2013). Although many experimental or observational studies support the view that wounding is a side effect of mate-anchoring during copulation (e.g., TEUSCHL et al. 2007; KAMIMURA & MITSUMOTO 2011; OKUZAKI et al. 2012), the wound itself or its associated damage may increase the paternity gain of the focal male via stimulatory functions (EBERHARD 1996, 2010). Damaging the female itself can produce similar effects via induction of immediate reproductive output (termed terminal reproductive output) or reduction of the probability of remating (JOHNSTONE & KELLER 2000; MORROW et al. 2003). Although there is no confirmative empirical support for these hypotheses, the observed repeated copulation by female *E. denticulatum* that took place only after a certain interval is a possible candidate phenomenon that can be explained by the latter hypothesis. Future studies are warranted to substantiate this possibility as well as the circadian rhythms of both sexes. Moreover, two females remained uninseminated after prolonged repeated matings by apparently normal males (Fig. 3) and frequent premature dislodgement of matings with the surgically treated males (Fig. 5), both of which have not been reported for other earwig species, also indicate conflict between the sexes with respect to control over the outcome of mating.

Female insects can develop specialized structures, such as pouches, sclerotized plates and thickened walls, in their genitalia where male genitalia inflict wounds; these are considered counteradaptations for traumatic mating (e.g., RÖNN et al. 2007; KAMIMURA 2007a, 2012). Detailed female genital structures were reported for only two other species of the genus *Echinosoma*: *E. afrum* (Palisot de Beauvois, 1805) (GILES 1963) and *E. yorkense* (KLASS 2003). In these species, males lack conspicuous penial spines (GILES 1963; SAKAI 1985; STEINMANN 1986). In *E. yorkense* much of the vaginal walls show a dense cover of minute hair-like elements, which are much shorter than the setae of *E. denticulatum* (KLASS 2003; K.-D. KLASS, pers. comm.), while detailed description of *E. afrum* by (GILES 1963) lacks any mentions of setae or bristles on the vaginal wall. Thus, the long setae on the vaginal wall likely represents a counteradaptation of female *E. denticulatum* to the male genital spines. Interestingly, female *Paralabellula dorsalis* also bear sclerotized spines around the gonopore (the orifice of the common oviduct) and some of the teeth of the male toothed plate mesh with them during copulation (BRICEÑO 1997). However, such spiny structures are totally absent from both male and female genital structures of the congeners *Paralabellula curvicauda* (Motschulsky, 1863) and

*Paralabellula rotundifrons* (Hincks, 1954) (STEINMANN 1990; SAKAI 1993; Y. Kamimura, unpublished data). Thus, such a possible counteradaptation is likely to have evolved independently multiple times in distantly related earwig lineages (Pygidicranidae in the basal Dermaptera and Spongiphoridae in the higher Forficulina). To date, no comprehensive study has been conducted on sexual conflict over mating in any species of earwig (KAMIMURA 2014). These groups should be excellent study models for this topic of evolutionary biology.

## 5. Acknowledgements

This study was conducted with the approval of the Economic Planning Unit, Malaysia (Reference No. UPE: 40/200/19/2844). We thank H.-S. Tee for his assistance in insect rearing, M. Nishikawa for identification of species and providing important references, G. Veera Singham for his help in the freeze-fixation experiment and the staff of the Electron Microscope Unit, School of Biological Science, Universiti Sains Malaysia for their help in microscopic observations. We are also grateful to K.-D. Klass and F. Haas for their helpful comments on an earlier version of the manuscript. This study was partly supported by a grant for overseas research from Keio University and a Grant-in-Aid for Scientific Research (Kakenhi, No. 22770058) from the Japan Society for the Promotion of Science to Y.K.

## 6. References

- BRICEÑO R.D. 1997. Genitalic structure and copulation in *Paralabellula dorsalis* (Dermaptera: Labididae). – *Revista de Biología Tropical* **45**: 1107–1116.
- BRINDLE A. 1984. The Esphalmeninae (Dermaptera: Pygidicranidae): a group of Andean and South African earwigs. – *Systematic Entomology* **9**: 281–292.
- BRINK P. 1956a. Reproductive system and mating in Plecoptera I. – *Opsucula Entomologica* **21**: 57–96.
- BRINK P. 1956b. Reproductive system and mating in Plecoptera II. – *Opsucula Entomologica* **21**: 97–128.
- BURR M. 1915a. On the male genital armature of the Dermaptera Part 1: Protodermaptera (except Psalidae). – *Journal of the Royal Microscopical Society* **1915**: 413–447.
- BURR M. 1915b. On the male genital armature of the Dermaptera Part 2: Psalidae. – *Journal of the Royal Microscopical Society* **1915**: 521–546.
- BURR M. 1916. On the male genital armature of the Dermaptera Part 3: Eudermaptera. – *Journal of the Royal Microscopical Society* **1916**: 1–18.
- COLGAN D.J., CASSIS G., BEACHAM E. 2003. Setting the molecular phylogenetic framework for the Dermaptera. – *Insect Systematics and Evolution* **34**: 65–79.

- EBERHARD W.G. 1996. Female Control: Sexual Selection by Cryptic Female Choice. – Princeton University Press, Princeton, New Jersey. 501 pp.
- EBERHARD W.G. 2010. Rapid divergent evolution of genitalia: theory and data updated. Pp. 40–78 in: LEONARD J.L., CÓRDOBA-AGUILAR A. (eds), The Evolution of Primary Sexual Characters in Animals. – Oxford University Press, Oxford, UK.
- GILES E.T. 1961. The male reproductive organs and genitalia of *Anisolabis littorea* (White) (Dermaptera: Labiduridae). – Transactions of the Royal Society of New Zealand (Zoology) **1**: 203–213.
- GILES E.T. 1963. The comparative external morphology and affinities of the Dermaptera. – Transactions of the Royal Entomological Society, London **115**: 95–164.
- HAAS F. 1995. The phylogeny of the Forficulina, a suborder of the Dermaptera. – Systematic Entomology **20**: 85–98.
- HAAS F., KLASS K.-D. 2003. The basal phylogenetic relationships in the Dermaptera. In: KLASS K.-D. (ed.), Proceedings of the 1st Dresden meeting on insect phylogeny: “Phylogenetic Relationships within the Insect Orders” (Dresden, September 19–21, 2003). – Entomologische Abhandlungen **61**: 138–142.
- HAAS F., KUKALOVÁ-PECK J. 2001. Dermaptera hindwing structure and folding: New evidence for familial, ordinal and superordinal relationships within Neoptera (Insecta). – European Journal of Entomology **98**: 445–509.
- HINCKS W.D. 1959. A Systematic Monograph of the Dermaptera of the World. Part II. Pygidicranidae excluding Diplatyinae. – British Museum of Natural History, London. 218 pp.
- HOTZY C., POLAK M., RÖNN J.L., ARNQVIST G. 2012. Phenotypic engineering unveils the function of genital morphology. – Current Biology **22**: 2258–2261.
- HUBER B.A., SINCLAIR B.J., SCHMITT M. 2007. The evolution of asymmetric genitalia in spiders and insects. – Biological Reviews **82**: 647–698.
- ISHIWATA K., SASAKI G., OGAWA J., MIYATA T., SU Z.-H. 2011. Phylogenetic relationships among insect orders based on three nuclear protein-coding gene sequences. – Molecular Phylogenetics and Evolution **58**: 169–180.
- JARVIS K.J., HAAS F., WHITING M.F. 2005. Phylogeny of earwigs (Insecta: Dermaptera) based on molecular and morphological evidence: reconsidering the classification of Dermaptera. – Systematic Entomology **30**: 442–453.
- JOHNSTONE R.A., KELLER L. 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. – American Naturalist **156**: 368–377.
- KAMIMURA Y. 2000. Possible removal of rival sperm by the elongated genitalia of the earwig, *Euborellia plebeja*. – Zoological Science **17**: 667–672.
- KAMIMURA Y. 2003a. Effects of broken male intromittent organs on the sperm storage capacity of female earwigs, *Euborellia plebeja*. – Journal of Ethology **21**: 29–35.
- KAMIMURA Y. 2003b. Genital structure and asymmetry in mating behavior of earwigs. – Nature & Insects **38**: 42–45. [in Japanese]
- KAMIMURA Y. 2004a. Mating behaviour and insemination in *Diplatys flavicollis*, an earwig with double-barrelled penises and a variable number of female sperm-storage organs. – Journal of Zoology **262**: 37–46.
- KAMIMURA Y. 2004b. In search of the origin of twin penises: molecular phylogeny of earwigs (Dermaptera: Forficulina) based on mitochondrial and nuclear ribosomal RNA genes. – Annals of the Entomological Society of America **97**: 903–912.
- KAMIMURA Y. 2006. Right-handed penises of the earwig *Labidura riparia* (Insecta, Dermaptera, Labiduridae): Evolutionary relationships between structural and behavioral asymmetries. – Journal of Morphology **267**: 1381–1389.
- KAMIMURA Y. 2007a. Twin intromittent organs of *Drosophila* for traumatic insemination. – Biology Letters **3**: 401–404.
- KAMIMURA Y. 2007b. Possible atavisms of genitalia in two species of earwig (Dermaptera), *Proreus simulans* (Chelisochidae) and *Euborellia plebeja* (Anisolabididae). – Arthropod Structure & Development **36**: 361–368.
- KAMIMURA Y. 2012. Correlated evolutionary changes in *Drosophila* female genitalia reduce the possible infection risk caused by male copulatory wounding. – Behavioral Ecology and Sociobiology **66**: 1107–1114.
- KAMIMURA Y. 2013. Promiscuity and elongated sperm storage organs work cooperatively as a cryptic female choice mechanism in an earwig. – Animal Behaviour **85**: 377–383.
- KAMIMURA Y. 2014. Pre- and postcopulatory sexual selection and the evolution of sexually dimorphic traits in earwigs (Dermaptera). – Entomological Science. doi:10.1111/ens.12058
- KAMIMURA Y., IWASE R. 2010. Evolutionary genetics of genital size and lateral asymmetry in the earwig *Euborellia plebeja* (Dermaptera: Anisolabididae). – Biological Journal of the Linnean Society **101**: 103–112.
- KAMIMURA Y., MATSUO Y. 2001. A “spare” compensates for the risk of destruction of the elongated penis of earwigs (Insecta: Dermaptera). – Naturwissenschaften **88**: 468–471.
- KAMIMURA Y., MITSUMOTO H. 2011. Comparative copulation anatomy of the *Drosophila melanogaster* species complex (Diptera: Drosophilidae). – Entomological Science **14**: 399–410.
- KEVAN D.K.McE., VICKERY V.R. 1977. An annotated provisional list of non-saltatorial orthopteroid insects of Micronesia, compiled mainly from the literature. – Micronesica **30**: 269–353.
- KJER K.M. 2004. Aligned 18S and insect phylogeny. – Systematic Biology **53**: 506–514.
- KLASS K.-D. 2001. The female abdomen of the viviparous earwig *Hemimerus vosseleri* (Insecta: Dermaptera: Hemimeridae), with a discussion of the postgenital abdomen of Insecta. – Zoological Journal of the Linnean Society **131**: 251–307.
- KLASS K.-D. 2003. The female genitalic region in basal earwigs (Insecta: Dermaptera: Pygidicranidae s.l.). – Entomologische Abhandlungen **61**: 173–225.
- KOCAREK P., JOHN V., HULVA P. 2013. When the body hides the ancestry: Phylogeny of morphologically modified epizoic earwigs on molecular evidence. – PLoS ONE **8**: e66900.
- LANGE R., REINHARDT K., MICHELS N.K., ANTHES N. 2013. Functions, diversity, and evolution of traumatic mating. – Biological Reviews **88**: 585–601.
- LUDWIG W. 1970. Das Rechts-Links-Problem im Tierreich und beim Menschen. Reprint of 1932 edition. – Springer-Verlag, Berlin. 496 pp.
- MATZKE D., KLASS K.-D. 2005. Reproductive biology and nymphal development in the basal earwig *Tagalina papua* (Insecta: Dermaptera: Pygidicranidae), with a comparison of brood care in

- Dermoptera and Embioptera. – Entomologische Abhandlungen **62**: 99–116.
- MISOFF B., NIEHUIS O., BISCHOFF I., RICKERT A., ERPENBECK D., STANICZEK A. 2007. Towards an 18S phylogeny of hexapods: accounting for group-specific character covariance in optimized mixed nucleotide/doublet models. – *Zoology* **110**: 409–429.
- MORROW E.H., ARNQVIST G., PITNICK S. 2003. Adaptation versus pleiotropy: why do males harm their mates. – *Behavioral Ecology* **14**: 802–806.
- NAKATA S., MAA T.C. 1974. A review of the parasitic earwigs. – *Pacific Insects* **16**: 307–374.
- OKUZAKI Y., TAKAMI Y., TSUCHIYA Y., SOTA T. 2012. Mating behavior and the function of the male genital spine in the ground beetle *Carabus clathratus*. – *Zoological Science* **29**: 428–432.
- POPHAM E.J. 1965. The functional morphology of the reproductive organs of the common earwig (*Forficula auricularia*) and other Dermaptera with reference to the natural classification of the order. – *Journal of Zoology* **146**: 1–43.
- POPHAM E.J. 1985. The mutual affinities of the major earwig taxa (Insecta, Dermaptera). – *Zeitschrift für Zoologische Systematik und Evolutionforschung* **23**: 199–214.
- POPHAM E.J. 2000. The geographical distribution of the Dermaptera (Insecta) with reference to continental drift. – *Journal of Natural History* **34**: 2007–2027.
- R CORE TEAM 2013. R: A Language and Environment for Statistical Computing. – R Foundation for Statistical Computing, Vienna, Austria.
- RÖNN J., KATVALA M., ARNQVIST G. 2007. Coevolution between harmful male genitalia and female resistance in seed beetles. – *Proceedings of the National Academy of Sciences USA* **95**: 6217–6221.
- SAKAI S. 1982. A new proposed classification of the Dermaptera with special reference to the check list of the Dermaptera of the world. – *Bulletin of Daito Bunka University* **20**: 1–108.
- SAKAI S. 1985. *Dermapterorum Catalogus. XVI–XVIII: Iconographia I–III. Pygidicranidae and Diplatyidae*. – Daito Bunka University, Tokyo. Pp. 698–1080.
- SAKAI S. 1987. Phylogenetic and evolutionary information on Dermaptera from the point of view of insect integrated taxonomy. Pp. 496–513 in: BACETTI B.M. (ed.), *Evolutionary Biology of Orthopteroid Insects*. – Ellis Horwood, Chichester.
- SAKAI S. 1993. *Dermapterorum Catalogus. XXV. Iconographia IX. Spongiphoridae*. – Daito Bunka University, Tokyo. Pp. 4691–5285.
- SCHNEIDER K., KLASS K.-D. 2013. The female genitalic region in Eudermaptera (Insecta: Dermaptera). – *Zoologischer Anzeiger* **252**: 183–203.
- SNODGRASS R.E. 1959. A revised interpretation of the external reproductive organs of male insects. – *Smithsonian Miscellaneous Collections* **135**(6): 1–60.
- STEINMANN H. 1986. Dermaptera. Catadermaptera I. – *Das Tierreich* **102**: 1–343.
- STEINMANN H. 1989. Dermaptera. Catadermaptera II. – *Das Tierreich* **105**: 1–504.
- STEINMANN H. 1990. Dermaptera. Eudermaptera I. – *Das Tierreich* **106**: 1–558.
- STEINMANN H. 1993. Dermaptera. Eudermaptera II. – *Das Tierreich* **108**: 1–711.
- TATARNIC N.J., CASSIS G., SIVA-JOTHY M.T. 2014. Traumatic insemination in terrestrial arthropods. – *Annual Review of Entomology* **59**: 245–261.
- TEUSCHL Y., HOSKEN D.J., BLANCKENHORN W.U. 2007. Is reduced female survival after mating a by-product of male-male competition in the dung fly *Sepsis cynipsea*? – *BMC Evolutionary Biology* **7**: 94.
- TWORZYDLO W., LECHOWSKA-LISZKA A., KOCAREK P., BILINSKI S.M. 2013. Morphology of the ovarioles and the mode of oogenesis of *Arixenia esau* support the inclusion of Arixeniina to the Eudermaptera. – *Zoologischer Anzeiger* **252**: 410–416.
- WAN X., KIM M.I., KIM M.J., KIM I. 2012. Complete mitochondrial genome of the free-living earwig, *Challia fletcheri* (Dermaptera: Pygidicranidae) and phylogeny of polyneoptera. – *PLoS ONE* **7**: e42056.
- WIRTH T.R., GUELLEC L., VEUILLE M. 1999. Directional substitution and evolution of nucleotide content in the cytochrome oxidase II gene in earwigs (dermapteran insects). – *Molecular Biology and Evolution* **16**: 1645–1653.
- YOSHIZAWA K. 2011. Monophyletic Polyneoptera recovered by wing base structure. – *Systematic Entomology* **36**: 377–394.
- YOSHIZAWA K., JOHNSON K.P. 2005. Aligned 18S for Zoraptera (Insecta): phylogenetic position and molecular evolution. – *Molecular Phylogenetics and Evolution* **37**: 572–580.