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

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**Abstract**

Dermaptera (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 components, 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; Kläss 2001,

The insect order Dermaptera (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 components, 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; Kläss 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 [Cricetomys 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; Tworzylko 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 & Matsuo 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 remiscence of a formerly paired condition by the presence of a vestigial ejaculatory duct on the non-functional side (e.g., Snodgrass 1959: fig. 4H; 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 & Kukalova-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 anisolabids 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 & Matsuo 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 & Matsuo 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 & Matsuo 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 & Kukalova-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. flavidollis (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-
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 Pyginicranidae, 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 (Pygicerianidae: 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 cuticuized parts of the gonoducts are known for the congener E. yorkense Dohrn, 1869 (KlaSS 2003). Courtship and mating behaviors of pygicerianids 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 Kukus, 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 ± 1°C (12 h photoperiod). All animals were provided with water, unlimited amounts of commercial cat food and a small piece of peeled tree bark (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$_2$, 0.02 g KCl and 0.02 g NaHCO$_3$ 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 $180^\circ$ 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 un inseminated 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-

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Fig. 2. Mating posture (A), male genitalia (B–D) and female genitalia (E–G) of *Echinosa denticulatum*. In this paper, the terminologies of KAESS (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: gp9, 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 µm in B–E, 100 µm 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).
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 µm 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 µm) setae (Fig. 2E,F).
All 19 females used for the observation of normal mating behavior, including the two un inseminated 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 (binominal 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 un inseminated 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; binominal 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...
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* (Diplaytidae; 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) (Kjer 2004; Yoshizawa & Johnson 2005; Misof et al. 2007; Ishiiwa 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 member of the basal Dermaptera for the first time. This feature differs from the open system of the diplaytid *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, Pyragrinae and Espanhlenineae, are bifurcated, suggesting the presence of four exits for sperm per male as in some diplaytids (e.g., Brindle 1984; Sakai 1985; Steinmann 1986). The genus *Pyagra* Audinet-Serville, 1831 (Pyragrinae) is estimated to be the basal-most clade in the paraphyletic Pygidicranidae, while the placement of Espanhlenineae has not been settled (Haas & Klass 2003). Thus, possibly in association with the evolution of non-bifurcated virga, 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 *Para­labellula 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 hemocoeil. 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 re-mating (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 uniseminated 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* (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 congener *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.

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6. References


KAMIMURA & LEE: Reproductive biology of Echinosoma


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...


Popham E.J. 1965. The functional morphology of the reproductive organs of the common earwig (Forficula auricularia) and other Dermoptera with reference to the natural classification of the order. – Journal of Zoology 146: 1–43.


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