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Authors: Wang, Li-Ping, Ji, Bao-Zhong, Zhao, Zheng-Ping, and Cao, Dan-Dan

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ANATOMICAL STUDY ON THE MORPHOLOGY OF THE REPRODUCTIVE SYSTEMS AND THE SCALE BRUSHES OF *DIORYCTRIA RUBELLA* HAMPSON (PYRALIDAE, PHYCITINAE)

LI-PING WANG[1], BAO-ZHONG JI[1*], SHU-WEN LIU[2], ZHENG-PING ZHAO[1], DAN-DAN CAO[1], & ZHONG-XIANG XU[3]

[1]Southern Modern Forestry Collaborative Innovation Center, College of Forestry,
Nanjing Forestry University, Nanjing 210037, P.R. China

[2] The Administration Bureau of Dr. Sun Yat-Sen's Mausoleum, Nanjing 210014, P.R. China

[3] Nanjing Entry-Exit Inspection and Quarantine Bureau, Nanjing 211106, P.R. China

*Corresponding Author, E-mail: jbz9885@njfu.edu.cn

ABSTRACT. The morphology of the reproductive systems and the coupling mechanism between the male and the female genitalia during copulation in *Dioryctria rubella* Hampson are described. Differing from those in other genera of Phycitinae moths, the corpus bursae possesses a well-developed signum, and the seminal duct does not exhibit a bulla seminalis, instead arising from the ventral surface of the corpus bursae. The ultrastructural observation shows that the spines on the inner surface of the ductus bursae and the cervix bursa are complementary to that of cornuti on the outer surface of vesica in position and direction, which elucidates the matching mechanism between the ductus bursae and the vesica. The male possesses well-developed scale brushes, which consist of six pairs of overlapped structures. Finally, the morphological differences of scale brushes between *D. rubella* and other *Dioryctria* species and related genera, and their function during copulation are discussed.

Additional key words: *Dioryctria rubella*, reproductive system, scale brushes, ultrastructure

The pine shoot moth, *Dioryctria rubella* Hampson (Pyralidae), ranges over more than 20 provinces in China, and also occurs in Philippines, Japan, Russia and many European countries (Liang et al. 2011). The larva bores into the terminals and cones and thereby causes severe losses to the cones and seeds of *Pinus massoniana*, *P. thunbergii*, and *P.taeda*. It is quite difficult to control this insect pest because of its concealed foraging habit and overlapping generations, and methods for managing this insect, such as chemical, physical and biological control have been improved for over 30 years (Wu et al. 1986; Zhao et al. 1992; Liang et al. 2011). Knowledge of the morphology of the reproductive organs is prerequisite to studies of reproductive biology as well as to investigations of sex pheromone. Up to now, only a few studies on the morphology of reproductive systems have been reported in Pyralidae. Fatzinger (1970) detailed the internal reproductive systems of *Dioryctria abietella*. This is the only species of genus *Dioryctria* in which reproductive organs have been researched. Only eight other species of Pyralidae have had their reproductive morphology described (Srivastava & Srivastava 1959; Ye & Lu 1964; Song et al. 1965; Davis 1968; Beals & Berberet 1976; Miskmen et al. 1983; Jones et al. 1984; Liao 1988; Song et al. 2012). Although Wang & Song (1985) described briefly the genitalia morphology of this moth, the morphology of the reproductive systems of *D. rubella* has not been reported in detail before.

The scale brushes play an important role in releasing of the male sex pheromone in Lepidoptera (Birch et al. 1990). Simonsen & Roe (2009) described morphology of the scale brushes in male Phycitinae moths and discussed their phylogenetic value. In this paper, we describe the anatomical structures of male and female reproductive systems and the scale brushes of *D. rubella* in detail so as to supply some basic information for further studies on reproductive biology and the sex pheromone. Anatomical terminology follows Klots (1970) and Kristensen (2003).

MATERIALS AND METHODS

Insect materials. The larvae and pupae were field–collected from April to October in 2013, from Sanjie, Anhui province, China. They were kept in the 15–20 mm long larval feeding tunnels, which were natural or man-made. The terminals were plugged into the matrix which can absorb water, and covered with glass tubes to avoid evaporation. Afterwards, the terminals were maintained at 25–28 degrees centigrade and about 62% RH on a 14:10 (L:D) cycle. The adults were collected and used as anatomical materials, they were either dissected instantly or fed with 0.5% honey water prior to study. All samples (30 females, 25 males) used for dissection were alive and unmated.

Anatomy. Moths were dissected in Ringer solution (6.5g NaCl, 0.14g KCl, 0.12g $CaCl_2$, 0.2g $NaHCO_3$, 0.01g NaH_2PO_4, and diluted with water to 1000ml) under the light microscope JN-JSZ6S. Meanwhile, the structures of reproductive systems were observed, measured and described. The photographs were captured using a computer and video camera mounted on a stereoscopic microscope, afterwards, figures were sketched according to the printed photographs. The dissecting structures were kept in stationary liquid (40% formaldehyde, 1.25ml glacial acetic acid, 10g chloral hydrate, and diluted with water to 100ml) for further observation.

Scanning electron microscopy. In order to investigate the relationship between the ductus bursae and the vesica, the ultrastructure in the inner surface of the ductus bursae were observed under the scanning electron microscopy (SEM). The ductus bursae was fixed in stationary liquid mentioned above for three weeks. Then, it was washed with 0.1 M phosphate buffer, pH 7.2, for 20 mins, operated twice. Afterwards, the sample was dehydrated in a graded acetone, 30%, 50%, 70%, 90% for 15 mins respectively, 100% for 20 mins and three times, then submitted to the critical point drying method, using superdry CO_2. Finally, the sample was placed on metallic support, coated with a thin layer of gold and examined under the SEM FEI Quanta 200.

RESULTS

Internal female reproductive organs. The internal female reproductive organs (Fig. 1) of *D. rubella* are composed of paired ovaries, a pair of lateral oviducts, one common oviduct, a genital chamber, the spermatheca along with its gland, and accessory glands.

The ovaries are secured within the abdomen by fine tracheae and some fat bodies, and normally loop back and forth 2 or 3 times within the abdomen. Each ovary is composed of 4 ovarioles, with an average length of 10.05 mm, which connects to the calyx of each lateral oviduct. The ovarioles fuse at their apex, contact closely throughout their length, and each of them is composed of a thin membranous tunica propria containing oocytes from 7 to 10, with newly emerged female containing the greater number.

The common oviduct branches anteriorly into two lateral oviducts, with an average length of 0.90 mm, and slightly constricts before joining the genital chamber. The lateral oviduct expands into the base of ovarioles. The seminal duct and the spermathecal duct open into the anterior end of the genital chamber, while the common duct of the accessory glands opens into the dorsum of the posterior region of the genital chamber, slightly anterior of the opening to the rectum. The genital chamber terminates into a fleshy, telescopic ovipositor equipped with numerous sensory hairs and an ovipore.

The spermathecal duct, 1.25 mm long, convolutes into 10 small spiral loops. The convolute directions of the loops are reversed at the center of the spermathecal duct. The spermathecal chamber, which opens through

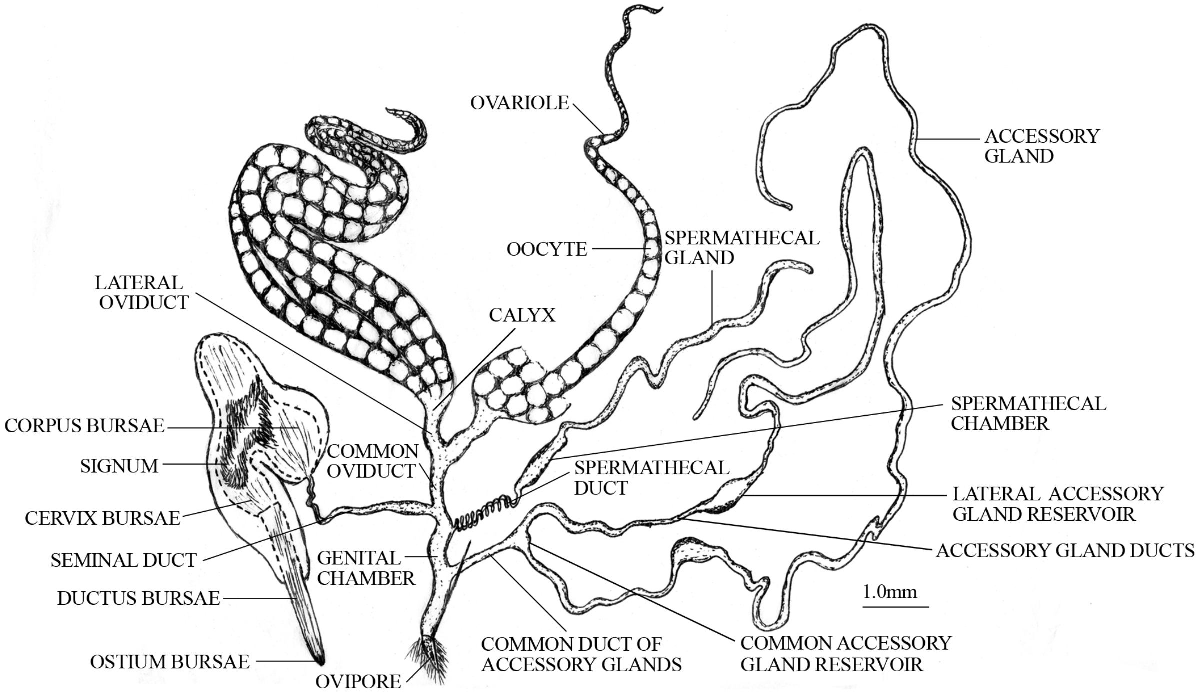


FIG. 1. Female reproductive system of *D. rubella*

the spermathecal duct, is an elongated sac. The spermathecal gland appears as a diverticulum of the spermathecal chamber and it terminates into 2 branches or not, depending on the individuals.

The common duct of the accessory gland attaches to the genital chamber and distally expands into an indistinctive common accessory gland reservoir. The separate accessory gland ducts attach to the common reservoir respectively. Each accessory gland also possesses a kidney-shaped reservoir near its base, the lateral accessory gland reservoir, with an average length of 0.96 mm. The anterior half of the accessory gland duct is finer than the posterior one. These reservoirs dilate or contract depending on the contents of the accessory glands. Each accessory gland arises from the anterior end of the lateral reservoir and is folded several times in the abdomen.

External female reproductive organs. The bursa copulatrix is a large, membranous organ (Fig. 1). The sclerotized ductus bursae, 2.68 mm in length, about eight times as long as width, is noticeably narrowed and musculated, anteriorly joins the cervix bursae and opens externally in the intersegmental cuticula of the 7th and 8th abdominal sternites through the ostium bursae. The membranous, U-shaped corpus bursae arise as a pouch from the cervix bursae. The internal surface armed with numerous spines, more than 500, about 0.18 mm long, which form a ring-like signum. The seminal duct arises from the ventral surface of the corpus bursae, coils three times at its anterior half near the corpus bursae, and slightly enlarges toward the posterior half near its junction with the genital chamber. There was not a bulla seminalis in the enlarged region, nor eggs found inside

The posterior opening of the genital chamber, the ovipore, is located below the anus. The papillae anales are derived from the 10th segment; they are weakly sclerotized and terminate in a nipple–shaped terminus. The papillae anales are covered with numerous long sensory hairs, and the hairs are 0.3 mm long in average. The margin of the papillae anales bears the posterior apophyses. There are a pair of lateral sclerites in the 8th abdominal segment, which bear a pair of anterior apophyses. Both of posterior and anterior apophyses are slightly sclerotized (Fig. 2).

Internal male reproductive organs. The internal male reproductive organs (Fig. 3) of *D. rubella* include two testes, a pair of vasa deferentia, the ductus ejaculatorius duplex, a ductus ejaculatorius simplex, and a pair of accessory glands.

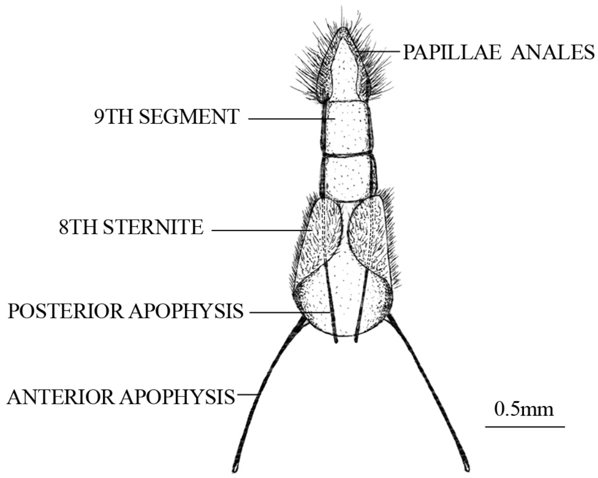


FIG. 2. Terminal female abdominal segments of *D. rubella*.

The two testes are encased by a scrotum which appears as a thin, transparent membrane and form a complex testis. The complex testis is 1.04 mm in diameter, supported by tracheae and other viscera in the dorsal abdominal cavity between the 3rd and 4th segments, and easily distinguished by the brilliant claret-red of the scrotum. The paired vasa deferentia arise from the ventral surface of the testis and join to the ductus ejaculatorius duplex respectively. Each vas deferens possesses two seminal vesicles, which appear as two significant dilations, the anterior enlargement and posterior enlargement, each of them is 0.82 mm long. The anterior enlargements cross at their middle parts.

The ductus ejaculatorius duplex consists of 2 tubular organs, fusing at their posterior terminals to form the ductus ejaculatorius simplex and giving rise to the accessory glands at their anterior terminals. Each branch of the ductus ejaculatorius duplex receives one vas deferens, and the junctions are near the accessory glands and locate at the one third of ductus ejaculatorius duplex. The accessory glands are uniform,

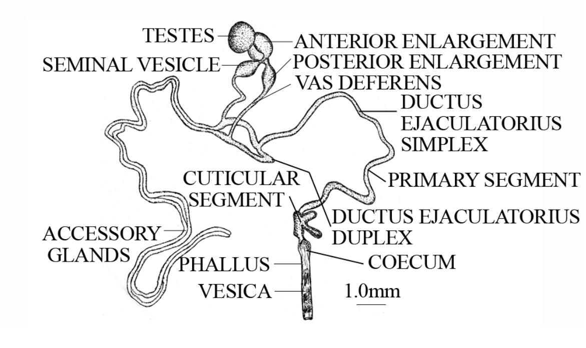


FIG. 3. Male reproductive system of *D. rubella*.

remain in close contact throughout their length and are intertwined among other organs within the abdominal cavity. Both of accessory glands are slightly club shaped at their terminals.

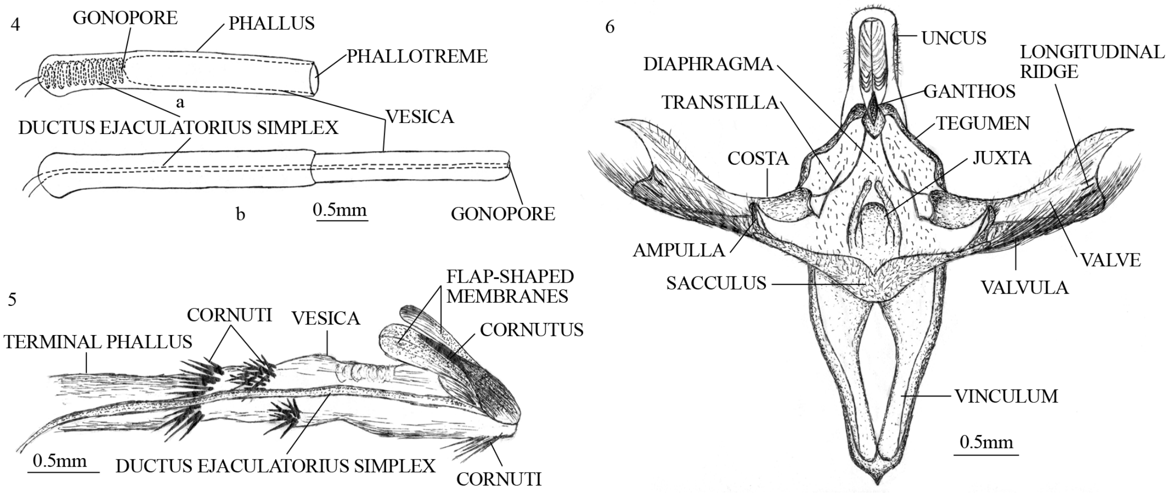
The anterior end of the tubular ductus ejaculatorius simplex connects to the ductus ejaculatorius duplex and posteriorly extends to the vesica. The ductus ejaculatorius simplex consists of 2 morphological areas, the primary segment and the cuticular segment. The primary segment appears as a thin, membranous tube, the cuticular segment is muscular area, differentiated by a lateral diverticulum and terminates into the phallus.

External male reproductive organs. The phallus is dorsolaterally supported by the transtilla with strip-typed muscles attaching to the margin of the 9th tergum, ventrally supported by the juxta. The phallus, which bears a caecum at the phallobase, is slender, strongly sclerotized tubular-shaped, and about seven times as long as wide. The ductus ejaculatorius simplex within the phallus combines with well–developed muscles fibers, convolutes into many spiral loops and joins to the base of vesica. These structures make the vesica possible to evert or invaginate freely during copulation. The terminal phallus is a tubular structure and the vesica is located in the lumen. Owing to its eversible and retractable abilities, the vesica, together with the phallus, constitute a telescopic structure.

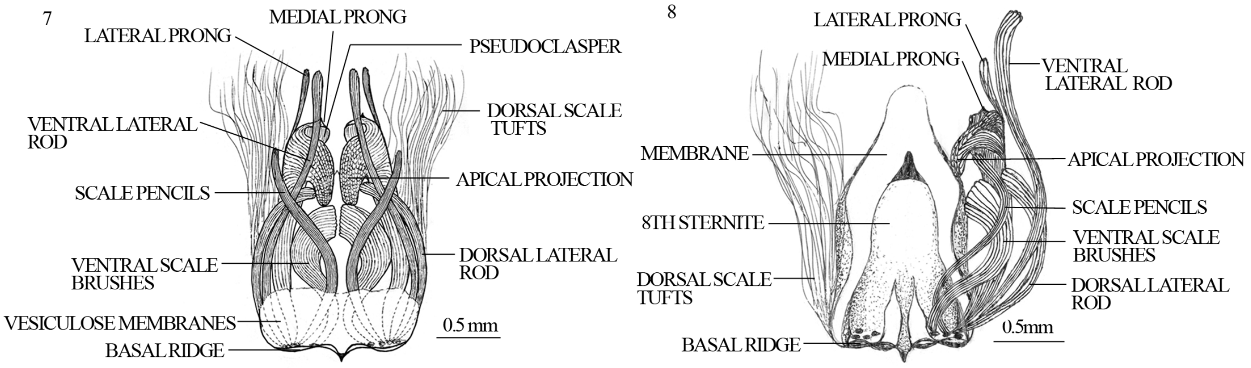
The membranous vesica, which is invaginated in the lumen of the phallus and forms a true gonopore (Fig. 4b), the primary gonopore or aperture of the ductus ejaculatorius simplex, opens externally at the apex membranous vesica. The opening at the terminal phallus, which results from the invagination of vesica, is actually a false gonopore, the secondary gonopore or the phallotreme (Fig. 4a). The phallotreme disappears along with the evaginable vesica, and the surface of vesica exposes one large spine-like cornutus, with an average length of 0.78 mm (cornutus in Fig. 5), five to ten slender cornuti on the opposite side to the cornutus (cornuti in Fig. 5), and one to three sets of smaller straight cornuti (cornuti in Fig. 5) (with an average of 72), 0.28 mm long. The direction and position of cornuti vary with the position of vesica. When the vesica everts, the cornuti point to the phallobase (Fig. 5). After the vesica invaginating, the cornuti point to the terminal phallus. The cornutus is surrounded by two flap-shaped muscular membranes (flap-shaped membranes in Fig. 5), and the membranes is surrounded by a layer of longitudinal muscle fibers.

When the vesica is in invagination (Fig. 4a), the phallus is about 2.71 mm long, thereinto the vesica is 1.86 mm long. The relative position of the eversible vesica and the phallus is shown in Fig. 4b, and the eversible vesica and the phallus are totally 4.57 mm in length. This is correlated with the telescopic structure of the phallus and the vesica.

The genital capsule of *D. rubella* is composed of the highly modified 9th and 10th abdominal segments (Fig. 6). The anal tube passes beneath the 9th tergum. Uncus with rounded apex, which formed from the 10th segment, is attached to the mid-dorsal surface of the



Figs.. 4–6. Male genital. **4.** The schematic diagram of the phallus and the vesica in male *D. rubella*. **a.** The telescopic structure. **b.** The phallus with eversible vesica. **5.** Eversion of vesica in male *D. rubella*. **6.** Male genitalia of *D. rubella* (the phallus has not been sketched).



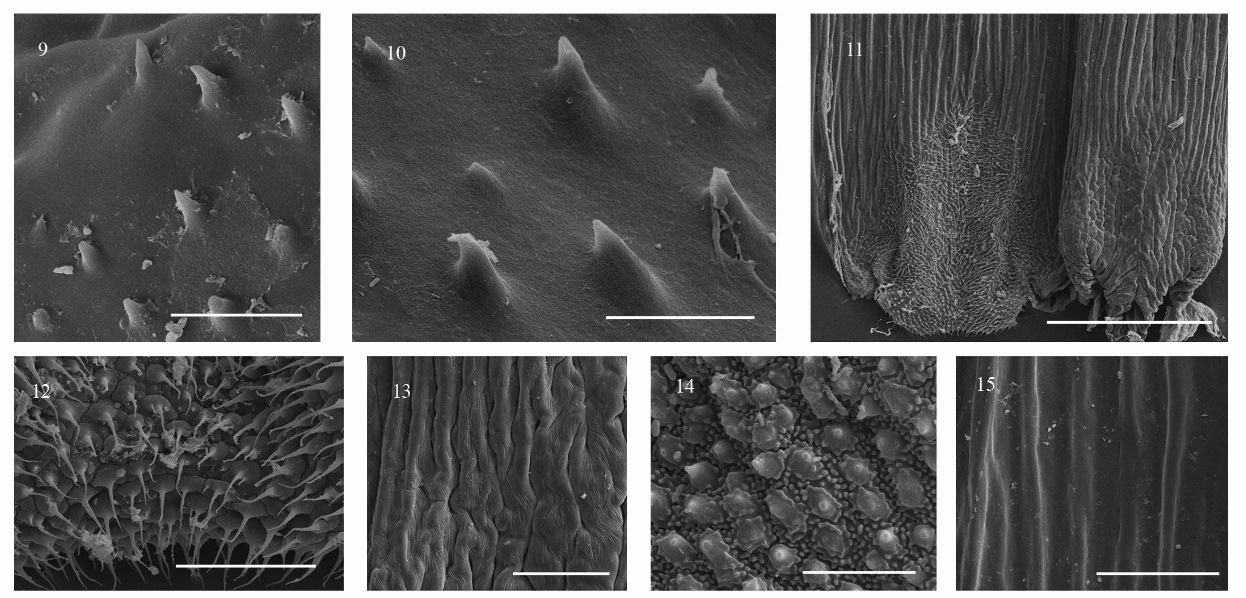
FIGS. 7–8. The modified scale brushes from the 8th abdominal sternite of male *D. rubella*, ventral visual. **7.** Overlapped sight. **8.** Unfolded sight, with partly removed.

9th tergum. Both sides of the uncus are parallel, with the sunken margin. The sclerotized gnathos is small pear-like with protruding cuspides, and connects to the sclerotized shield-shaped tegumen by the hardened, arcuate, and wide arms of both sides. The paired gnathos giving ventrolateral support to the anal tube. The diaphragma, a membranous ventral body wall behind the valve bases and ahead of the 10th segment sclerotizations, is the place where the anal tube and the phallus pass through. Most of the diaphragma derives from intersegmental membrane between the 9th and 10th segment (Klot 1970). The juxta, which is the most consistently developed diaphragma sclerotization, closely associated with the vinculum, round-shaped with two finger-type arms terminally equipped with fine hairs. Another kind of diaphragma sclerotization is transverse bridge, the transtillas, which is between the upper basal valve corners, extending above the phallus. The paired valves derive from the 9th segment (Ferro & Akre 1975), articulate with the vinculum along the posterior margin, and are armed with numerous setae, which are primarily serving to clasp the female abdominal apex during copulation (Kristensen 2003). The dorsal costa widens terminally and tapers to a long, sclerotized, falcate process, with the following three longitudinal ridges. The ventral valvula connected to the sacculus is membranous and bears many long setae. The sclerotized sacculus is located in the base-ventral of the valve and covered with shorter setae. The mediodorsal, hooked, sclerotized ampulla, ventrally connected to the terminal sacculus and dorsally associated with costa, also armed with numerous short setae. The vinculum is fused with the lateral margin of the 9th tergum. The vinculum joins the tegumen with its two arms, and ventral part sinks as a “V”.

Scale brushes in male adult. The scale brushes (Fig. 7) originate from the basal ridge of the strongly sclerotized and U-shaped the 8th sternite (Fig. 8), are composed of six pairs of overlapped structures. A bigger U-shaped, slightly sclerotized membrane is ventrally attached to the 8th sternite, and dorsolaterally attached to the male genitalia. The most dorsal components are a pair of dorsal scale tufts lateral to the 8th sternite, which are comprised by multiple very long piliform scales. Two pairs of long rods originate laterally from the base of the 8th sternite. The dorsal lateral rods are short and thick, while the ventral lateral rods are very long. The pseudoclaspers originate from the latero-basal portion of the 8th sternite, medially and posteriorly to the two pairs of lateral rods. These structures are composed of a thick shaft and head with a curved inner margin, a large downward pointed ventral hook, apical projection, medial prongs and lateral prongs. Ventral to the pseudoclaspers are a pair of large ventral scale brushes, which are laterally curved, originate from the centro-dorsal of the 8th sternite. The scale pencils are a pair of long and narrow structures, loosely composed of a few scales, originating ventro-laterally to the ventral scale brushes.

The six pairs of scale brushes are equipped with many parallel and scaly ridges on surface, and they are flexible at the base, so that they can unfold. The scale brushes are covered with vesiculose membranes (Fig. 7).

Scanning electron microscopy of the inner wall of the ductus bursae. The inner wall of the cervix bursa and the ductus bursae are armed with some spines (Figs. 9–10), which are evenly distributed at one side of the base of the ductus bursae, with an average length of 10.0 μm (Fig. 10). The setae are unevenly distributed on the internal surface of ostium bursae



Figs. 9–15. SEM micrographs of the inner surface of the ductus bursae. **9.** Anterior end of the ductus bursae, showing spiculate surface (Scales 20 µm). **10.** The inner wall of the cervix bursae (Scales 20 µm). **11.** The ostium bursae (Scales 300 µm). **12.** Dense, long setae of the ostium bursae (Scales 40 µm). **13.** Plicated muscles of the ostium bursae (Scales 50 µm). **14.** Membranes joining to the ostium bursae (Scales 10 µm). **15.** Middle part of the ductus bursae (Scales 50 µm).

(Fig. 11), one side is equiped with dense, long setae (Fig. 12), and the remainder is plicated muscles (Fig. 13). However, the spiculate surface and the setaceous surface are on opposite sides of the ductus bursae. The membranes, which attached to the ostium bursae are sags and crests, with some big humps surrounded by numerous small ones (Fig. 14). The remainder part of the ductus bursae is smooth, with longitudinal rumples (Fig. 15).

Discussion

In general, the morphological characters of reproductive organs in *D.rubella* are similar to that of *D. abietella* and other Pyralidae species (Fatzinger 1970; Srivastava & Srivastava 1959; Ye & Lu 1964; Song et al. 1965; Davis 1968; Beals & Berberet 1976; Miskmen et al. 1983; Jones et al. 1984; Liao 1988; Song et al. 2012). However, there are some obvious differences, for example, the seminal duct of some species in the genus *Passadenoides*, *Etielloides* and *Endotricha* arises from corpus bursae near the junction of ductus bursae and corpus bursae (Ferris 2004; Ren & Li 2006; Sun & Li 2009). As for *D. rubella* and some other *Dioryctria* species, it arises from the ventral surface of the corpus bursae (Fatzinger 1970; Wang & Song 1985). Furthermore, no bulla seminalis such as described in *D. abietella* has been found in *D. rubella*, which seems to be acting as a pumping organ to assist movement of sperm through the seminal duct (Fatzinger 1970). Callahan & Cascio (1963) suggested that peristaltic movements of the seminal duct were also beneficial to sperm transmission in species which lack a bulla seminalis, and the seminal duct in *D. rubella* may function in this way.

D. rubella is of the U-shaped corpus bursae and the well-developed signum equipped with widespread, numerous spines. These are similar to some *Dioryctria* moths (Wang & Song 1985), and different from other species of the genus *Endotricha*, which the corpus bursae are round and the signum is indistinctive (Sun & Li 2009), the signum even absent in some *Etielloides* and *Lipographis* moths (Ren & Li 2006; Ferris 2012). The signum aids in retaining the spermatophore in the corpus bursae as the phallus is removed and the ultimate shape of the spermatophore is determined by the corpus bursae (Ferro & Akera 1975). The developed signum and the U-shaped corpus bursae may imply a strong retaining capacity and the U-shape of the spermatophore in *D. rubella*.

In *D. rubella*, the exact mating process has still not been reported. Some speculations may be inferred according to the morphology of external reproductive organs. There is a fine matching between the outer surface of vesica and the inner surface of the ductus bursae as well as the cervix bursae. The spines on the inner surface of the ductus bursae and the cervix bursae are complementary to the cornuti on the outer surface of vesica, moreover, the directions of spines and the cornuti are opposite to each other, which give the male a firm hold on the female during copulation and spermatophore formation (Ferro & Akera 1975; Callahan 1958). In ditrysian Lepidoptera, the phallus and the vesica insert into the ductus bursae and the cervix bursa during copulation, and *D. rubella* may copulate in the same way.

Male scent structures from abdomen of Pyralidae have many descriptive terms, such as coremata (Bradley 1968; Bradley 1969), corema (Wang & Song 1985), scale tufts (Mutuura & Munroe 1974; Ren & Li 2006), hair pencils (Sasaerila et al. 2003), culcita (Ren et al. 2011; Liu & Li 2012), scale brushes (Simonsen & Roe 2009). In this paper, we adopt the Simonsen's term and describe the scale brushes structure of *D. rubella* in detail. Simonsen & Roe (2009) examined structural morphology of scale brushes within the genus *Dioryctria* and two closely related genera by the scanning electron microscopy. Though variable in size and shape, the scale brushes of these species are clearly comprised by overlapped scales except for *Pyla criddlella*, which only has a pair latero-ventral scale tufts on the 8th sternite. The ultrastructures of the scale brushes are diversiform in species of genus *Dioryctria* and *Sciota*. There existed numerous pores on the cuticula of the scale brushes in *Sciota termitalis*, *D. pseudotsugella* and *D. fordi*, and some secretions were found around the pores (Simonsen & Roe 2009). They suggested that the scale brushes of these species might have excretory functions. The pores are neither found in *D. rubella*, nor in *D. abietivorella*, *D. auranticella*, and *D. yiai*, which may imply that the scale brushes only function to emit sex pheromone in these species. Whether the vesiculose membranes associated with the scale brushes in *D. rubella* have the excretory function of sex pheromone still need to be investigated.

In some Pyralidae species, sex pheromone is emitted from the scale brushes when the male is close to the female (Phelan & Baker 1990). During the study on the reproductive behavior of *D. rubella*, we found that some females ran after the males. Whether male sex pheromone exists in *D. rubella* still need to be further studied. Phelan & Baker (1990) suggested that male species equipped with well-developed scale brushes exhibited a more complex, interactive courtship sequence. It may imply a higher sequence of courtship behavior in *D. rubella*.

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