

### *Symposium 3*

## **Eversion of Drone Endophallus and the Probable Mating Process in *Apis dorsata***

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### **SUMMARY**

The knowledge of the process of eversion of the drone endophallus enables one to understand the natural mating process, and helps to collect the semen from the everted endophallus. The thorax of a drone was squeezed and then pressure on the abdomen was gradually increased. Seven relatively distinct stages of endophallus eversion were described. At first the vestibulum everts. Next the ventral and the medial cornua evert, followed by eversion of the lateral ones. Then, the cervix everts. A small drop of 0.2mm<sup>3</sup> of semen was observed at its end in 50 drones. In stage 6, the fimbriate lobe and the bulbus appear. In the last stage 7, the fimbria appear on the fimbriate lobe.

Probable positions of the endophallus during mating is described. The ventral cornua touch the last dorsal sclerite of the queen from outside. The medial cornua evert next inside queen's sting chamber. The lateral cornua embrace the queen's abdomen from the ventral side. Next, the cervix everts. Its tip is positioned against the orifice to queen's reproductive organs. Further eversion results in the injection of semen into queen's oviducts. The following eversion of the bulbus and the fimbriate lobe pushes the endophallus out of queen's sting chamber.

**Keywords:** *Apis dorsata* / endophallus eversion / reproductive organ

### **INTRODUCTION**

The eversion process of the drone's endophallus of *Apis mellifera* was described by Woyke (1955, 1958) and Woyke and Ruttner (1958), and of *Apis cerana* by Ruttner *et al.* (1973). Everted endophalli of *Apis dorsata* drones were described by Simpson (1970), and Koeniger *et al.* (1990) and of *Apis laboriosa* by McEvoy and Underwood (1988). However, the eversion process of the endophallus of *A. dorsata* drones remains undescribed. Therefore investigations were conducted to examine this phenomenon. The figures presented enable us to speculate about the possible position of the endophallus during natural mating.

Different authors have given different names to the cornua of the endophallus of *A. dorsata*. Sometimes the authors gave the same names to two different pairs of cornua. The longest cornua which

reach the most upper position during eversion are called the ventral ones. Therefore, I have chosen the most appropriate name for each pair of cornua given by previous authors (table 1).

## MATERIALS AND METHODS

The investigations were started at the Central Bee Research Institute in Poona, India in 1973/74, continued in the Bee Biology Research Unit, Chulalongkorn University in Bangkok, Thailand in 1992, followed in the Bee Division of the Agricultural University, Warsaw, Poland and finished in Dabur Apicultural Center in Jugedi, Nepal in 1999.

*A. dorsata* drones of various ages were examined. Presumably sex mature drones were caught during drone flights at the dusk. Eversion of hundreds of drones was investigated. Special attention was paid to develop a method, which would result in repeatable positions of different parts during the eversion of the endophallus. At first, the thorax of a drone was squeezed. Sometimes this resulted in partial eversion of the endophallus. Next the pressure on the abdomen was gradually increased. This resulted in different stages of eversion of the endophallus. When the internal pressure was decreased, the endophallus collapsed, and the cornua changed their position. Repeatable positions of different parts were obtained only in endophalli under full internal pressure. Therefore only such were investigated and are here described.

Sometimes the sticky yellow mass on the cornua glued together different parts of the endophallus. Therefore the eversion was conducted in two ways: either in the air or submerged under water in a small container. While still under the pressure, (in air or under water) the endophallus was examined under a stereo microscope, and was drawn with the aid of a Nikon drawing apparatus. The drawings were scanned into a computer and were elaborated with CorelDRAW 6.0 software. Female reproductive organs of three *A. dorsata* queens were examined.

## RESULTS

### Eversion stages of drone endophallus

Gradual increase of the pressure inside the abdomen of the *A. dorsata* drone results in seven relatively distinct stages of endophallus eversion. At first the vestibulum everts partially. Rudiments of all cornua also appear (Figure 1, 1). In stage 2, the vestibulum everts completely. The hairy ventral vestibular field is now visible. The ventral cornua evert about halfway, to the part where the broadened tubes become narrower. The medial cornua evert also about halfway, until a small additional cornu projects. The lateral cornua are everted very little. The tips of the partly everted cornua observed directly without magnification or in photos look rounded. However, under a microscope a concavity to the uneverted part of the cornu is visible. A narrower part starts to evert from the hump of the dorsal cornua (Figure 1, 2). In step 3, the medial cornua evert completely (Figure 1, 3). They embrace between themselves the ventral cornua (Figure 2, 3a). The additional small cornua of the medial ones also evert completely. The dorsal cornua evert completely in dorso-lateral direction. In stage 4, the ventral cornua evert completely, or almost completely, and the lateral ones to about half their length (Figure 1, 4). In step 5, the tips of ventral cornua curve in ventral and oral direction and the lateral cornua evert completely (Figure 1, 5). The lateral cornua are much longer (about 11 mm) than the ventral ones (about 7 mm). The cervix also everts completely. Hairy patches are visible on 9 - 11 small humps on its ventral side. A small hairy patch, covered with sparse hairs (setae), is visible on the dorsal side at the end of the everted cervix (Figure 1, 5).

Increase of the pressure caused the appearance of small drop of semen at the end of the cervix. A drop of semen was observed in 50 drones. The drop appeared at the ventral part of the end of cervix. The semen was creamy-light brown coloured, similarly like in *A. mellifera*. Its volume was about 0.2 mm<sup>3</sup>. This stage of eversion is the right one to collect the semen into the tip of a strig. Further increase of the pressure resulted in a flow of mucus. The mucous was white coloured and was distinctly differentiated from the semen. Then rapidly the next stage 6 of the eversion occurs. In this step all cornua are completely everted, as well as the cervix (Figure 1, 6). Two new structures appear at the end of the cervix. A quadripartite fimbriate lobe with 2 distal and 2 dorsal lobes appears. The distal lobes are larger than the dorsal ones. No fimbria (fringes) are visible at this stage of eversion. The partly everted bulbus protrudes in ventral - posterior direction. Step 7 presents the completely everted endophallus (Figures 1, 7 and 2, 7a). Quite high

pressure is required to reach this final stage of eversion. The lobes of the fimbriate lobe enlarge. Fimbria appear on the two dorsal and the two distal lobes (Figure 2, Fl). The ventrally directed bulbus everts completely. The orifice (connection) to the ductus ejaculatorius is now situated at the end of the bulbus in ventral - anterior direction.

The different stages of eversion occur as a result of some obstacle to continuous eversion. These are: decrease of the diameter of the tubes of cornua, appearance of hairy patches and fields in the cervix, and the necessity of eversion of new structures such as the small additional cornua in the medial ones, or eversion of the fimbriate lobe and bulbus at the end of everted cervix. Some additional pressure is required to evert the fimbria of the fimbriate lobe. Of course, all intermediate stages of eversion exist. However, those presented above are most frequently found among different stages induced by manual increase of internal pressure in drone abdomen. The stages should not be considered as stops during natural mating. They present different positions through which the endophallus must pass to reach the final one of complete eversion.

### Probable Mating Process

The endophallus has no muscles. Thus to pull it out, the internal pressure inside drone's abdomen must be increased, similarly as it was done in this investigation. Increase of internal pressure results in eversion of the endophallus. The knowledge of the sequence and the position of different parts during eversion of the endophallus under internal pressure enables one to predict the probable events occurring during natural mating. No doubt, during natural mating, the endophallus comes in touch with the tip of the queen's abdomen during stage 1 of the eversion. Ventral cornua are the first to evert halfway (Figure 1, 2).

They cannot penetrate inside the sting chamber of the queen, because their further eversion would push the endophallus out of the abdomen. Thus the ventral cornua must touch the last dorsal sclerite from outside (Figure 3a). The cornua serve as guidance to position the endophallus in the right place. Their sticky substance attaches the endophallus to the abdomen. If the tip of the queen's abdomen is opened to some extent, the medial cornua evert next inside the queen's sting chamber. The cornua embrace the sting from both sides, similarly to the cornua in *A. mellifera* and *A. cerana*. Their tips press from inside the last abdominal sclerite. Thus this sclerite is grasped like in pliers between the medial cornua from inside and the ventral ones from outside (Figure 3a). This fixes the endophallus to the tip of queen's abdomen.

The lateral cornua which evert next probably do not penetrate inside the sting chamber either, because their tips direct laterally (Figure 2, 3a) and upwards (Figure 1, 4). Later on, the cornua approach each other (Figure 2, 7a). Thus, they embrace the queen's abdomen from the opposite side (ventral) than the ventral cornua (Figure 3b). It is not certain whether or to what extent the tips of the cornua are detached from the queen's abdomen. In the meantime the cervix everts. Its tip must be positioned against the orifice to the queen's reproductive organs (Figure 3b). Inside the cervix is the bulb containing semen and mucus. With further eversion, the cervix releases the opening to the bulbus containing semen and mucus. Consequently, the semen is injected into the queen's oviducts. At this stage of mating the tip of drone's everted cervix must stick very tightly to the orifice conducting to queen's vagina. This is assured by the grasping action of all three pairs of cornua pointing backwards. Further eversion of the bulbus and the eversion of the fimbriate lobe push the endophallus out of the queen's sting chamber. The queen is now ready to mate with the next drone.

### DISCUSSION

The method of examination of fresh endophalli under internal pressure enabled repeatable investigations conducted in different times and places. The figures show, that the positions of cornua are not accidental, but the successive stages present a continuation of the eversion of a previous stage. The present investigation determined the right eversion stage to collect the semen from everted endophallus of *A. dorsata* drones.

The positions of different parts of the everted endophallus described until now differ very much from those presented in this paper. Previous authors examined endophalli conserved in alcohol that failed to preserve the natural positions of fresh endophalli under internal pressure. The small everted bulbus as well as the fimbriate lobe without fimbria indicate that none of the hitherto published papers described the completely everted endophallus of *A. dorsata*. Rather the last but one stage No 6 of this paper was described.

Finding *A. dorsata* in copula would help to verify the probable position described here. A photo of *A. dorsata* drone in copula with *A. mellifera* queen carcass is published (Koeniger *et al.*, 1994). However, the mating was an unsuccessful one, and the drone endophallus got stuck in queen's abdomen. This shows that the natural mating process in *A. dorsata* must occur in a different manner.

In *A. mellifera* and *A. cerana* the tips of everted cornua do not reach the tip of everted cervix containing the semen on its end. Thus the cornua can enter queen sting chamber. The function of the large cornua in *A. mellifera* and *A. cerana* is replaced by the median cornua in *A. dorsata*. The everted ventral and lateral cornua of *A. dorsata* are much longer than the everted cervix containing a drop of semen on its end. Much higher pressure is required to evert the cervix completely and to eject the semen from its tip, than to evert both cornua completely. If both cornua entered queen sting chamber, they would push the endophallus out of queen abdomen before the everted cervix with semen reached the orifice to the queen's vagina. Thus it seems credible that those cornua embrace the queen's abdomen from outside. The large fimbriate lobe pointing backwards eases the detachment of the pair.

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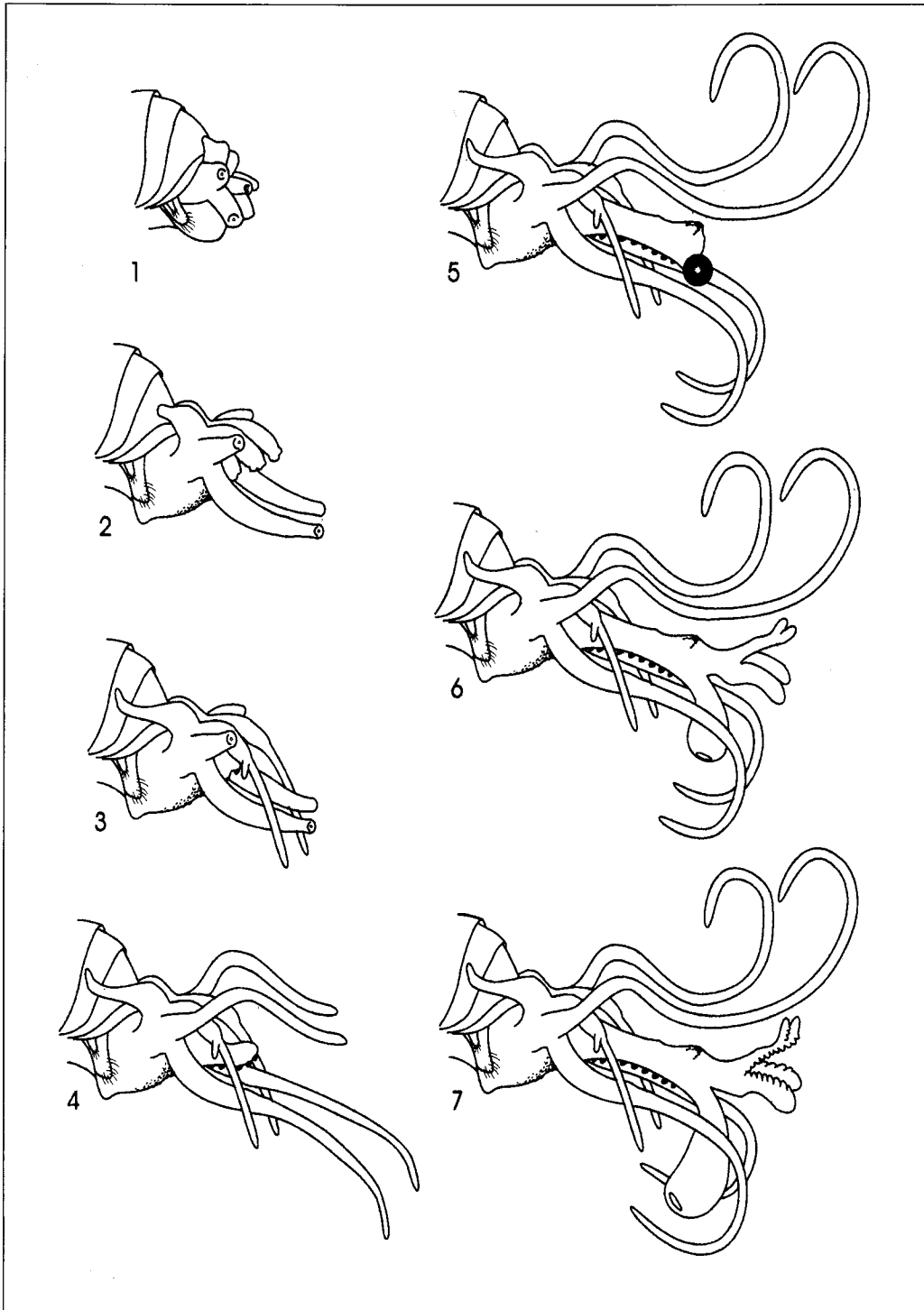
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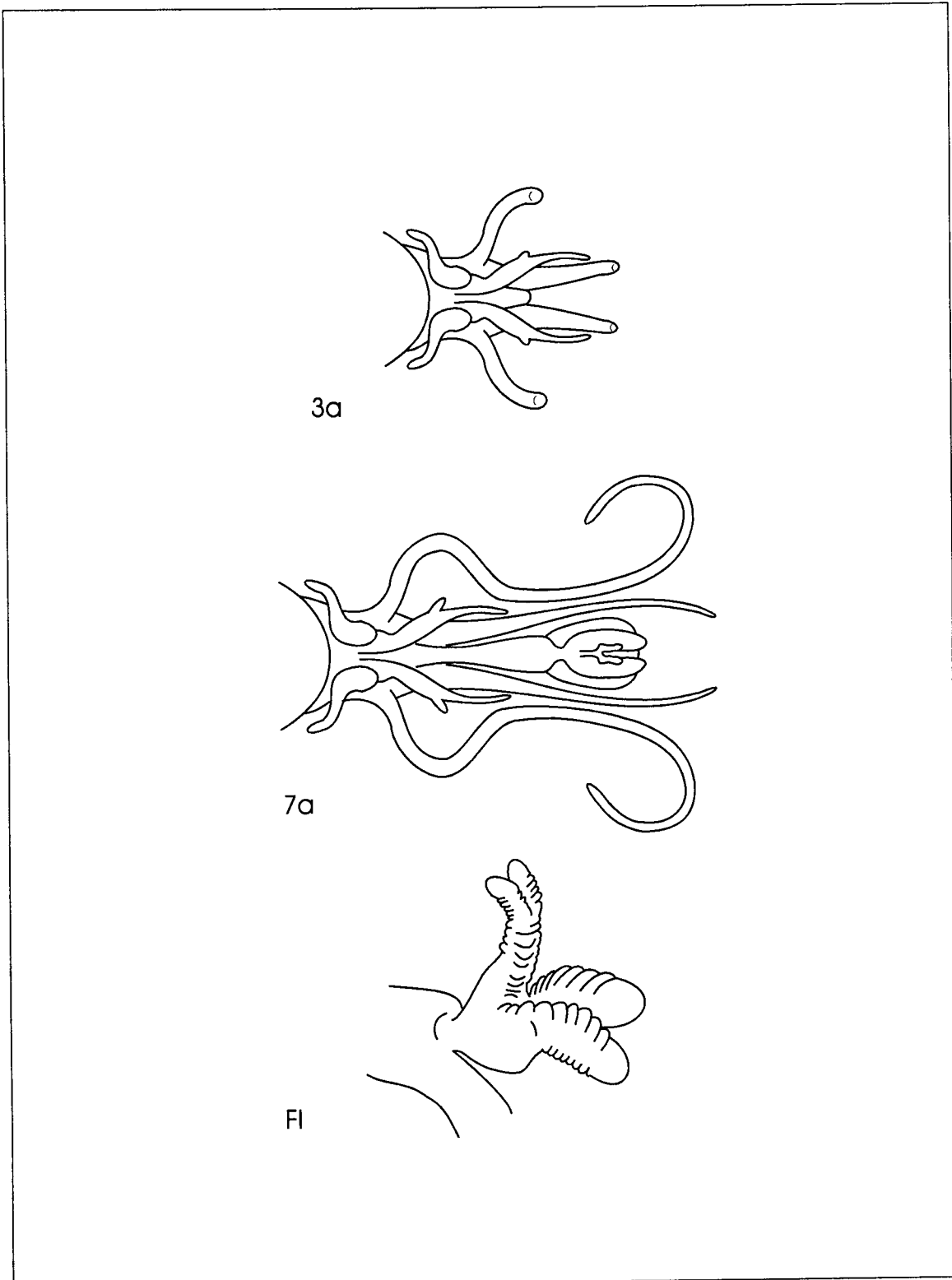
**Table 1** Names given by different authors to cornua of *Apis dorsata* endophallus

| Simpson<br>1970              | McEvoy &<br>Underwood 1988 | Koeniger, Mardan &<br>Ruttner 1990 | Woyke<br>2000 |
|------------------------------|----------------------------|------------------------------------|---------------|
| Upper anteriorly projecting  | Medial                     | Dorsal (1)                         | Dorsal        |
| Upper posteriorly projecting | Medial                     | Dorsal (2)                         | Medial        |
| Lateral                      | Lateral                    | Ventral (3)                        | Lateral       |
| Basal                        | Lateral                    | Ventral (4)                        | Ventral       |

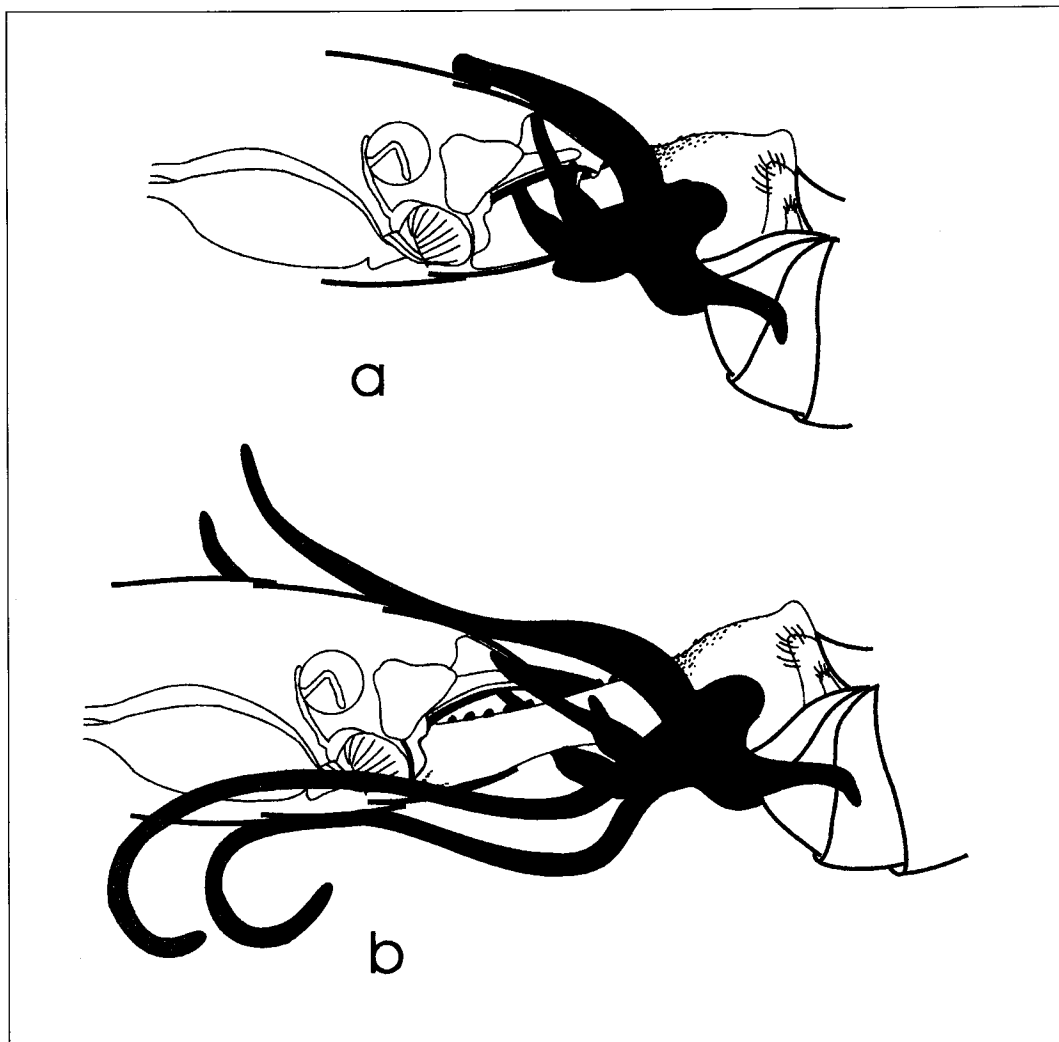
Figure 1 Lateral view of seven stages (1 – 7) of eversion of *Apis dorsata* drone endophallus.



**Figure 2** Dorsal view of two stages of eversion of *Apis dorsata* endophallus, 3a - stage tree, 7a - stage seven, and laeral view of fimbrate lobe of completely everted endophallus.



**Figure 3** Probable positions of the drone endophallus during the mating process in *A. dorsata*. a – initial position, b – position during injection of semen into queen's reproductive organs.





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