Zoomorphology (2018) 137:535–544
https://doi.org/10.1007/s00435-018-0411-1

ORIGINAL PAPER

Functional anatomy of the vesica in *Eugnosta magnificana* (Insecta: Lepidoptera: Tortricidae)

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Received: 16 March 2018 / Revised: 23 May 2018 / Accepted: 4 June 2018 / Published online: 8 June 2018

Abstract
This study is the first attempt to elucidate the detailed function of the internal musculature of the phallus in Lepidoptera with respect to complementary female structures. Based on the examination of *Eugnosta magnificana*, a leaf-roller species with a large elaborate vesica (endophallus), the attachment points of the muscle bundles are described and their putative function is discussed. Some parts of the vesica (two large diverticula with cornuti) are not everted in living specimens, though their artificial eversion is possible after maceration with KOH. Direct observation of everted vesica of living moths showed that its large diverticula are capable of at least two movements: forth-and-back and flexion–extension. Hypothetical position of the internal male and female genitalia during copulation is proposed. It appears that the corpus bursae consists of two compartments with different functions: anterior for spermatophore digestion and posterior for interaction with the vesica. Some evidence of a stimulating function by non-deciduous cornuti was found.

Keywords Phallic musculature · Endophallus · Copulation · Movements of vesica · Non-deciduous cornuti

Introduction

Insect genitalia have long attracted the attention of entomologists, mainly for taxonomic purposes. Among Lepidoptera detailed descriptions of the cuticular skeletons of the external and internal (female) genitalia exist for the vast majority of known taxa. However, the situation is quite different in regards to the musculature and functional morphology of these structures. Fundamental works on the functional morphology of the male genitalia include Kuznetzov and Stekolnikov (2001) and references therein; papers dedicated to the internal musculature of Tortricidae include those by Kuznetzov and Stekolnikov (1973, 1977), who examined these structures in a phylogenetic context. The external genitalia are studied in great detail, but the intrinsic musculature of the phallus remains practically untouched: a retractor muscle of the vesica is reported without further details on its functioning. The only work revealing protracting function of a muscle of the endophallus is that of Eaton (1988).

During copulation, the internal genitalia are largely in close contact, and complementary male/female structures can be noticed, leading researchers to formulate hypotheses explaining the correspondence, e.g., lock-and-key mechanism and cryptic female choice (Eberhard 1985; Cordero and Baixeras 2015).

The purpose of this study is to examine the intrinsic musculature of the phallus using *E. magnificana*, a representative of the tribe Cochylini, a group characterised by complicated internal genitalia. This species was selected during juxtaposing of male and female genitalia of various Cochylini species. The disparity in sizes of the internal genitalia in *E. magnificana* is remarkable: the length of the everted vesica considerably exceeded the length of the inflated bursa (Fig. 1) and an explanation was needed. The simplest one was that the vesica is only partially everted during copulation, but to test this, living individuals in copulation were needed. *E. magnificana* is a common species in southern Bulgaria but never abundant, therefore experiments with ceasing copulation at consecutive stages and serial morphology method (Callahan 1958) were inapplicable. A partial solution to the problem was treatment of living males with dichlorvos, an insecticide that causes eversion of the vesica (Dang 1993). The experiments with this compound conducted by me confirmed my preliminary assumption that

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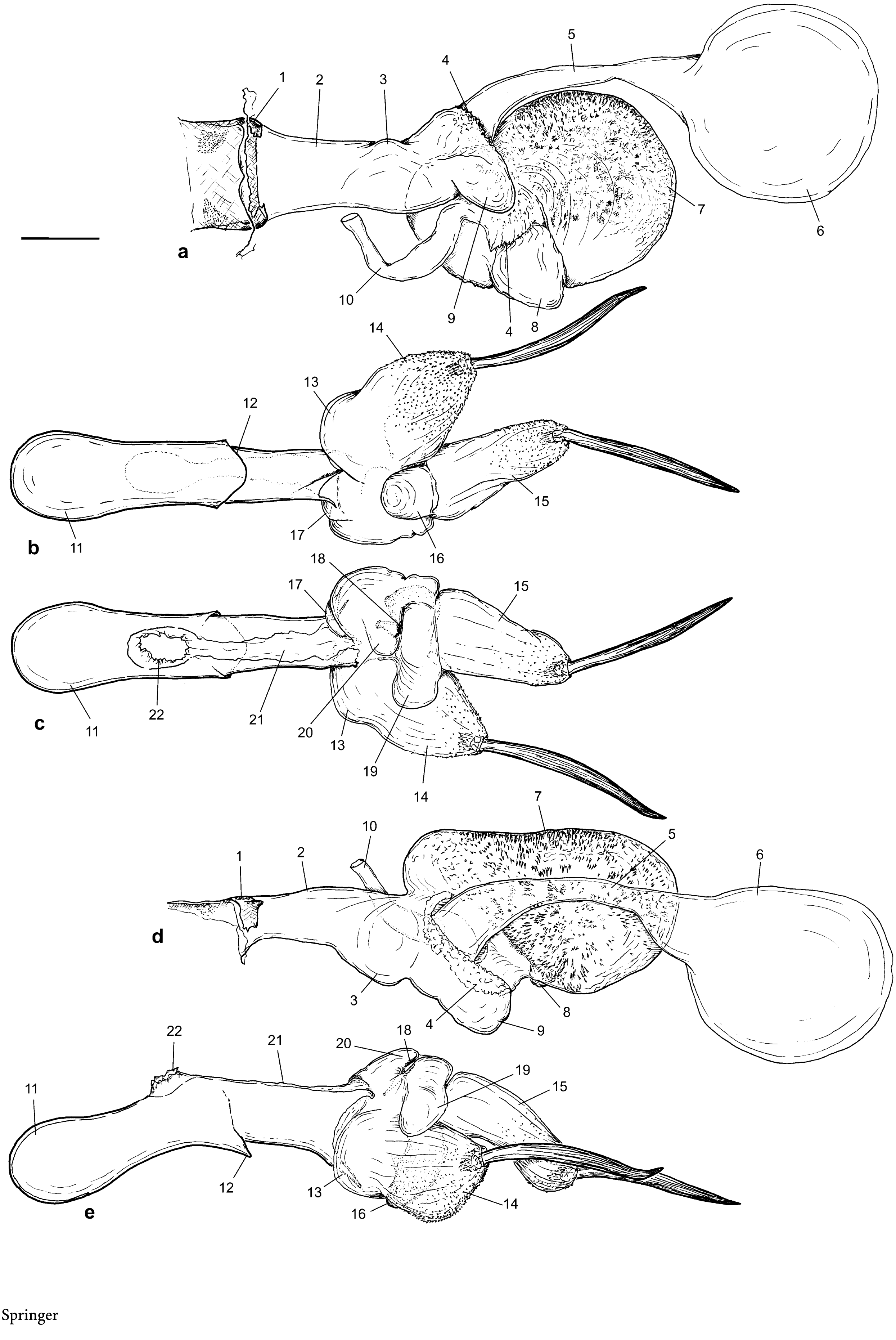
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◂**Fig. 1** Internal genitalia (inflated KOH preparations). **a** Female, ventral view, Bulgaria, Pirin Mts., Ilindentsi, 500 m a.s.l., 7.v.2010. **b** Male, ventral view, Bulgaria, Black Sea Coast, Bolata, 40 m a.s.l., 3.vi.2017. **c** Male, dorsal view, ibid. **d** Female, right view, the same from **a**. **e** Male, left view, the same from **b**. Note the spatial correspondence between **a, b** and **c, d**, likely the copulation is performed in this position of the internal genitalia. 1 sclerotisation of ostium, 2 ductus bursae, 3 protuberance of ductus bursae, 4 tendinous stripe, 5 ductus of accessory bursa, 6 accessory bursa, 7 corpus bursae, 8 anterior protrusion of corpus bursae, 9 posterior protrusion of corpus bursae, 10 ductus seminalis, 11 coecum, 12 caulis, 13 basal protrusion of the sinistro-ventral diverticulum, 14 sinistro-ventral diverticulum with cornutus, 15 dextro-dorsal diverticulum with cornutus, 16 ventral diverticulum, 17 crescent-shaped diverticulum, 18 gonopore sclerotisation, 19 posterior dorsal diverticulum, 20 anterior dorsal diverticulum, 21 membranous dorsal side of the phallus, 22 membranous extension of the phallus around the ductus ejaculatorius simplex. Scale bar 250 µm, all to scale

most of the vesica is eversible but its large diverticula are not everted completely. In this way, the bursa can accommodate the vesica, and moreover, there appear to be special protrusions of the bursa corresponding to the tips of cornuti.

Materials and methods

For purposes of the present work, the phallus and the vesica (= endophallus) are referred to as internal male genitalia, and the ductus bursae, bursa copulatrix and its cuticular extensions are referred to as internal female genitalia. For anatomical observations of phalli freshly collected specimens were used. Living moths were collected in the field with light traps and transferred to the laboratory from the following localities: 2 males, Bulgaria, Black sea Coast, Kaliakra Cape: Bolata, N 43°23′10″, E 28°28′21″, 40 m a.s.l., 3.vi.2017; 2 males, Bulgaria, Struma River, Kozhuh Hill, N41°27′43″, E 23°15′27″, 210 m a.s.l., 4.viii.2017. They were treated with 2,2-dichlorovinyl dimethyl phosphate (dichlorvos) following the method of Dang (1993). This fumigant insecticide is a neurotoxin causing eversion of the vesica at low concentration. My previous experience showed that the vapour concentration of the insecticide is the most critical step and overdosage is a frequent side effect. To avoid failure, a small piece of cardboard 1 × 1 cm was soaked with a drop of liquid dichlorvos and put in a polypropylene container of 100 ml with a cap. The following day, the cardboard was removed and the container was left open for 5 min. A living moth was released into the container, then the lid was sealed. The insecticide accumulated in the walls of the container released slowly achieving low vapour concentration. A container prepared this way can be used many times for at least 3 days. When the moth shows signs of disorientation, it must be transferred immediately into a clean container to avoid overdosage. After a few minutes it almost stops moving. At this stage the moths were immobilised with minuten needles driven through the wings to a piece of Plastazote© and observed under a Stemi 2000 (Zeiss) stereomicroscope. When the movements of the vesica ceased, the moths were fixed in cold (− 20 °C) 70% ethanol. The low temperature prevented muscle contractions and retraction of the vesica. For anatomical investigation, the fixed specimens were dissected in 5% ethanol. The abdomen was cut longitudinally with a microknife and the skin was removed. The phallus was extracted with fine forceps and a knife, together with a part of ductus ejaculatorius simplex; the muscles on the external surface of the coecum were removed. The phallus was observed and photographed under an Amplival (Carl Zeiss Jena) compound microscope with a polarising device with 1/4 λ plate, a trino tube with MF Messprojectiv 4:1 and a digital camera Canon EOS 700D. Because the muscle fibres are birefringent, they become distinguishable under polarised light without special staining. Subsequently, most of the phallic tube was removed, leaving only the coecum, and the muscle fibres were traced with a 0.15-mm minuten needle under magnification of a stereomicroscope. Additionally, the vesica was cut into pieces without cutting the muscle fibres, and each piece was studied using a polarised compound microscope. Three male specimens were studied in this way. After initial observation, the phallus of a fourth male was macerated in hot 10% KOH solution, and the vesica was processed following the procedure described by Zlatkov (2011). The prepared phallus was immobilised with a curved minuten needle inserted through the "opening" when submerged in Euparal essence on a cavity slide. It was drawn with a microscope camera lucida from three aspects: dorsal, ventral and left. To achieve better three-dimensional perception, the compound microscope was modified for stereoscopic observation following Hammond (1996). After drawing, one of the diverticula was severed, cut longitudinally to be opened, and mounted on a slide with Euparal. The slide was photographed with an AxioStar Plus (Zeiss) compound microscope equipped for phase contrast with digital camera ProgRes CT3 (Jenoptik). The remaining phallus was also permanently mounted on a slide and observed under phase contrast. Macerated phalli of seven specimens preserved in the collection of the National Museum of Natural History, Sofia and prepared in accordance with these techniques were used for comparison.

The structure of the female genitalia was studied on a dry female specimen whose abdomen was processed following standard procedure with KOH maceration and staining with chlorazol black (e.g., Robinson 1976). The only deviation from the method of Robinson is that before clearing in Euparal essence, the bursa was inflated by injecting absolute ethanol through the ostium with a glass capillary, in a manner similar to that used on the phallus. Female genitalia were drawn in the manner as the male genitalia and eventually mounted in Euparal.

To test the function of diverticula, a plastic model was made from a piece of a wooden pencil, a polyethylene glove, and a polypropylene test tube with its bottom cut off (Fig. 6). The dimensions of the pencil and glove finger were selected to be approximately proportional to the dextro-dorsal diverticulum of the vesica and its cornutus. The rubber end of the pencil was tied with copper thread to the tip of the inverted middle finger of the glove. Two thin copper threads were attached to the periphery of the rubber end of the pencil. The other fingers and most of the glove were tightened with rubber bands to the test tube, and the model was filled with water. The movements were tested by pulling the copper threads and changing the configuration of the glove in relation to the tube.

Results

Structure of macerated phallus (Fig. 1b, c, e). The phallus is tubulous with coalescent caulis, large coecum and membranous dorsal region merged with the vesica. A small triangular ventral process is present at the distal end of the sclerotised tube as well as a narrow lateral sclerotised extension on each side. The vesica comprises several more or less differentiated compartments. The most remarkable of them are two large diverticula, with dextro-dorsal and sinistro-ventral positions, each bearing a cornutus on its apex. The dextro-dorsal diverticulum is longer and with a smaller diameter than the other, subcylindrical, and has a slightly sinuate cornutus. This cornutus is attached not at the apex but slightly ventrally. The sinistro-ventral cornutus is large, conical, with distinct basal protrusion and a more sinuate cornutus. The shape and diameter of the distal part of this diverticulum may vary in specimens from the same population (Fig. 2). Either diverticulum has acanthae on the distal half, more numerous on the ventral side (Fig. 4c). The cornuti are large (ca. 0.6 × the length of the sclerotised phallus), aciculate, capitate (terminology by Anzaldo et al. 2014), and of nearly equal length. Their proximal third is smooth, the remaining part has large longitudinal ribs separated by deep grooves shallowing toward the tip. In ethanol fixed specimens, the large lumen of the cornuti appears filled with a transparent jelly-like material. The sockets have thread-like cuticular tendons attached to their periphery so the opening of the socket is not obliterated (Fig. 4b). The tendons at the ventral side of cornuti are longer and more numerous. The two cornuti-bearing diverticula are attached to the voluminous basal part of the vesica which also bears a large smooth conical diverticulum on the ventral side (ventral diverticulum), emerging at the base of the dextro-dorsal diverticulum, two less differentiated protrusions dorsally at the base of the diverticula (anterior and posterior dorsal diverticulum) and a small crescent-shaped diverticulum at the very end of the phallus located dextro-dorsally. The gonopore is slit-like, with a weak sclerotisation, and located dorsally between the posterior and anterior dorsal diverticulum.

Eversion in vivo (Fig. 3). One of the moths treated with dichlorvos at first everted a small part of the dorsal portion of the vesica and ejected drops of transparent colourless ejaculate, solidifying in seconds, then everted the entire basal portion together with the widening at the base of the sinistro-ventral diverticulum (Fig. 3a). Subsequently, the basal half of the sinistro-ventral diverticulum everted gradually as a maximum of 2/3 of the corresponding cornutus protruded outside; meanwhile, the vesica everted and inverted at intervals of 5–10 s (Fig. 3b). Following that, a whitish ejaculate was ejected. The dextro-dorsal diverticulum started everting, and when 1/2 of its cornutus

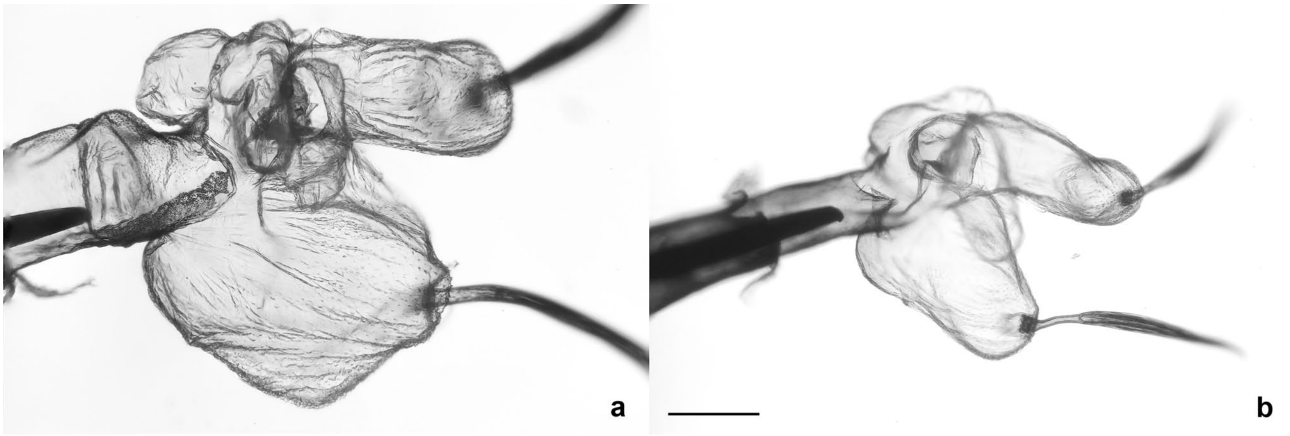


Fig. 2 Variability of vesica, dorsal view (inflated KOH preparations). **a** Bulgaria, Pirin Mountains, Kalimantsi, 340 m a.s.l., 22.vii.2010. **b** Bulgaria, Pirin Mountains, Ilindentsi, 500 m a.s.l., 9.x.2008. Note the considerable difference in the general size of vesica and the shape of the sinistro-ventral diverticulum. Scale bar 250 µm, both to scale

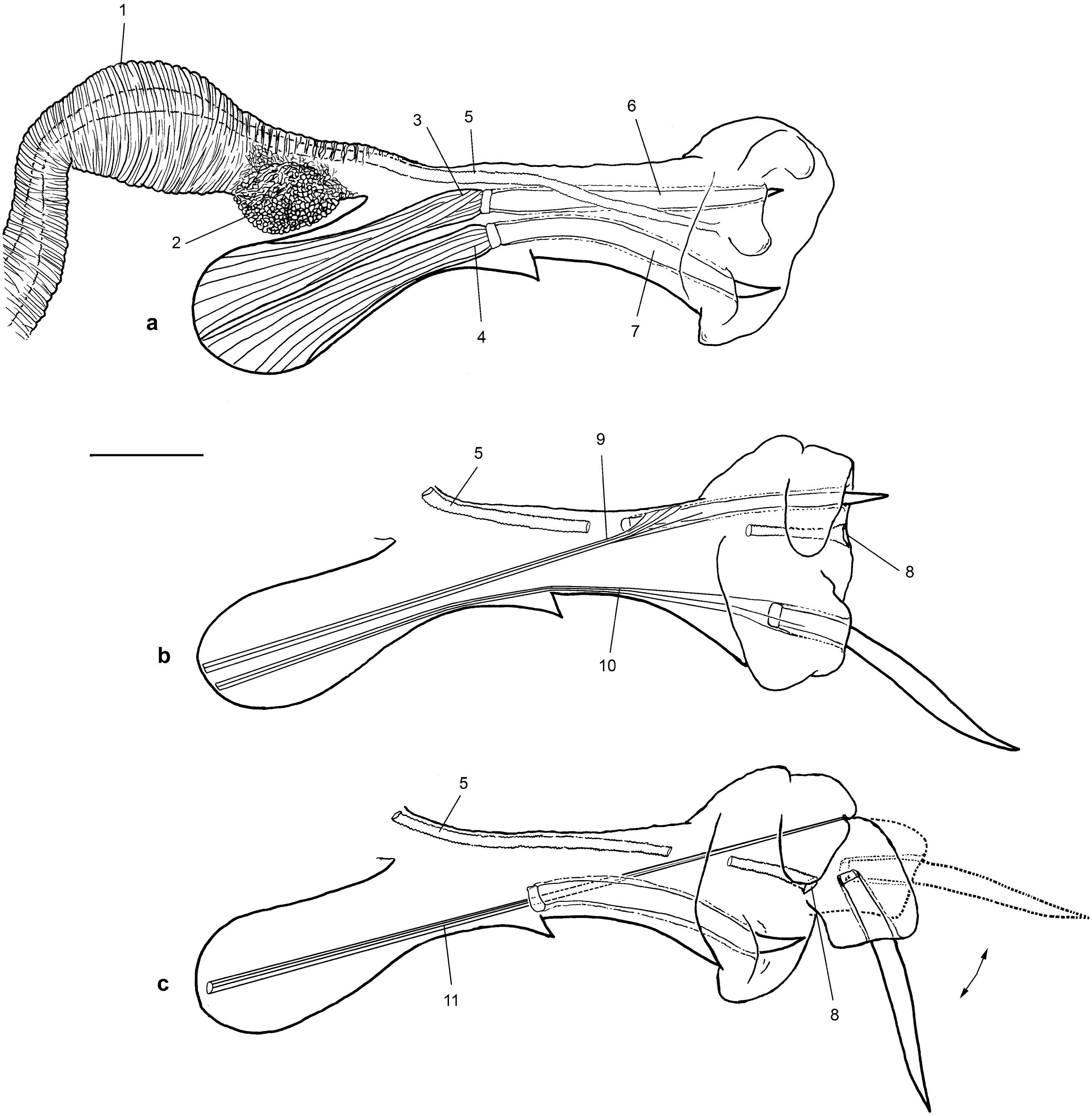
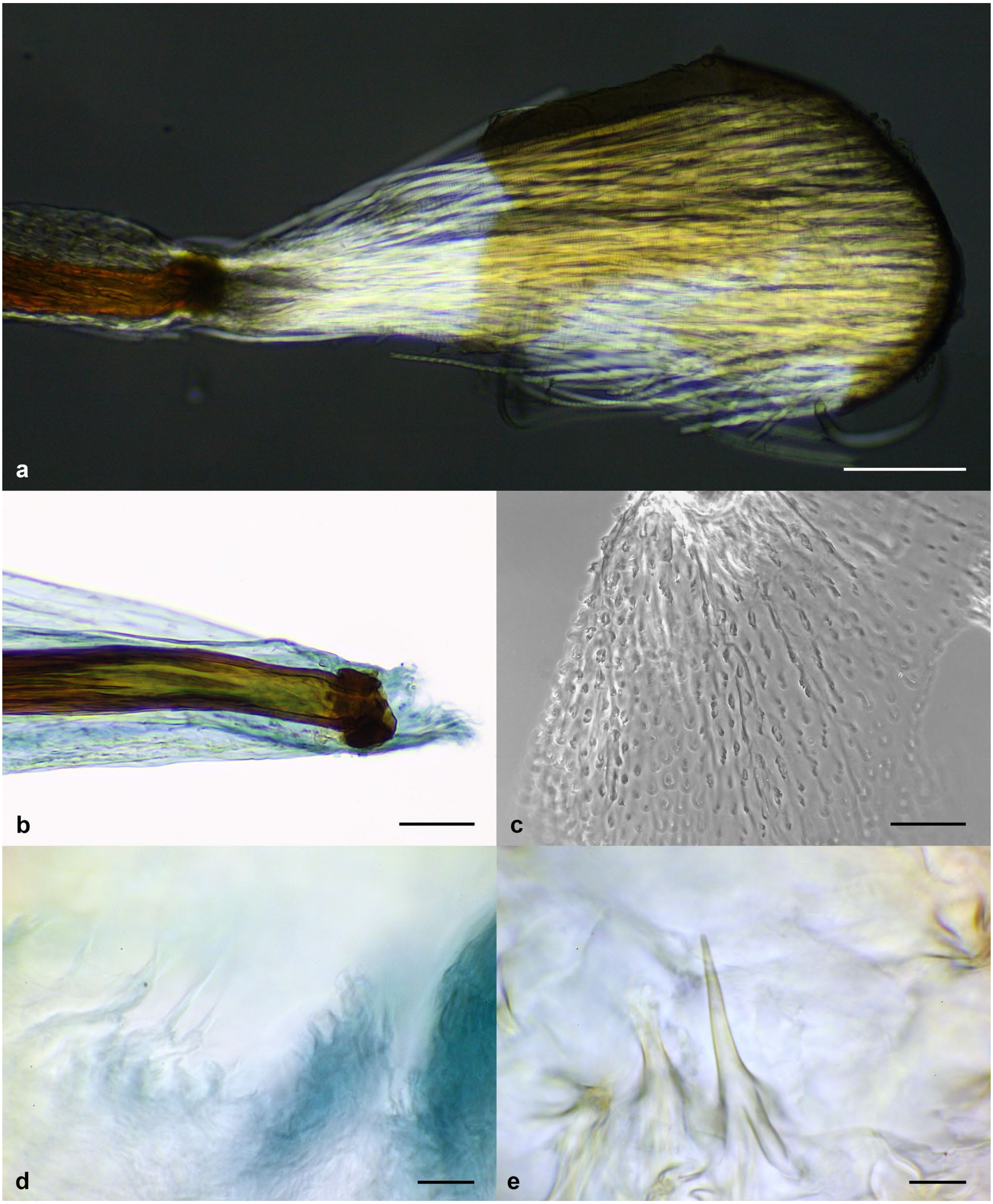


Fig. 3 Intrinsic phallic musculature and eversion of the vesica in vivo, left view. The three parts of the figure illustrate different muscle bundles. **a** Initial stage of eversion. **b** Eversion of the sinistro-ventral diverticulum. **c** Eversion of the dextro-dorsal diverticulum and its flexion/extension movements. 1 circular muscle fibres of ductus ejaculatorius simplex; 2 bulbus ejaculatorius with fibres of the muscle mph. it-v; 3 dorsal branch of the muscle mph. il-t with chiasma; 4 ventral branch of the muscle mph. il-t; 5 distal area of ductus ejaculatorius simplex (severed in **b, c**); 6 dextro-dorsal diverticulum and its cornutus; 7 sinistro-ventral diverticulum and its cornutus; 8 gonopore, 9 muscle fibres of the dextro-dorsal diverticulum; 10 muscle fibres of the sinistro-ventral diverticulum; 11 muscle fibres inserted at the base of the dextro-dorsal diverticulum. Scale bar 250 µm, all to scale

was protruded, the ventral cornutus and its diverticulum retracted. The dextro-dorsal diverticulum together with its cornutus protruded at ca. 2/3 of its length and retracted several times, then flexed and extended in a ventral direction again several times (Fig. 3c). Later on, the diverticula alternated with each other in protrusion–retraction movements as the dextro-dorsal one continued its periodical ventral flexion. Finally, the dextro-dorsal diverticulum



retracted instantly and the rest of vesica pulsed extensively (2–5 times/s), meanwhile slowly retracting to some degree. No further changes were observed in the next 45 min, therefore I fixed the specimen in ethanol. The other specimens treated with dichlorvos did not demonstrate the full set of described events. One of them everted its vesica in a similar way but ventral flexion of the dextro-dorsal cornutus was not observed. The other moths died shortly

◀**Fig. 4** Details of phallus and bursa copulatrix. **a** Dorsal branch of the muscle mph. il-t originating from the coecum and inserting in the base of the dextro-dorsal cornutus. Most of the phallic tube and vesica were removed; view from the frontal plane. Polarised light microscopy after clearing in Euparal essence. **b** Basal part of the sinistro-ventral cornutus with diverticulum inverted. Note that the numerous tendons attached to the periphery of the socket are longer and denser at the ventral side. KOH maceration and staining with chlorazol black. **c** The same diverticulum from **b**, everted, cut longitudinally and flattened. The numerous acanthae on its distal part are arranged in longitudinal rows. Phase contrast microscopy. **d** Tendinous area at the base of the anterior protrusion of corpus bursae. KOH maceration and staining with chlorazol black. **e** Acanthae on the internal surface of corpus bursae. KOH maceration. Scale bars **a** 100 µm, **b** and **c** 50 µm, **d** and **e** 20 µm

after treatment with dichlorvos, without everting the entire vesica, perhaps due to overdosage of the insecticide.

Intrinsic phallic musculature (Fig. 3). The protuberant distal portion of ductus ejaculatorius simplex is known as the bulbus ejaculatorius. In *E. magnificana* it is occupied by muscular structure consisting of fibres with various orientations and presenting the muscle mph. it-v, designated also as m_{28}, musculus phallicus internus transversus: Kuznetzov and Stekolnikov (2001) and *ph-tr*: Kristensen (2003). This muscle formation is most likely responsible for increasing the hydrostatic pressure during eversion of the vesica (Kristensen 2003). The area of the ductus ejaculatorius between the bulbus ejaculatorius and phallic tube is equipped with semicircular muscle fibres on the dorsal side. The ductus ejaculatorius inside the phallus passes along its dorsal side and connects the gonopore. The phallic tube is largely occupied by muscle fibres of a large muscle, designated as mph. il-t; referred to as m_{21}, musculus phallicus internus longitudinalis, by Kuznetzov and Stekolnikov (2001) and *ph-int* by Kristensen (2003). It originates from a large area of the coecum and inserts in different parts of the vesica. Two main branches of the muscle are easily recognised: dorsal and ventral, attached via tendons to the periphery of the sockets of dextro-dorsal and sinistro-ventral cornutus, respectively (Fig. 4a). Part of the fibres of the dorsal branch originates from the left side of the coecum and inserts at the right side of the dextro-dorsal socket, which is manifested as a muscular chiasma (Fig. 3a). In a similar manner, a few fibres are inserted on the right side of the distal part of the dextro-dorsal diverticulum (Fig. 3b). A small bundle of ca. 30 fibres originates from the left side of the coecum and inserts into a small, weakly sclerotised plate on the dorsal side at the base of the dextro-dorsal diverticulum (Fig. 3c). Additionally, a couple of fibres are attached to the gonopore. The ventral branch of the muscle is as large as the dorsal one, no chiasma was observed. A few fibres are attached to the distal part of the sinistro-ventral diverticulum.

Macerated female genitalia (Fig. 1a, d). A detailed description is provided for the internal female genitalia as they are of particular interest for this study. The ostium is weakly sclerotised dorsally, with ventral and dorsal walls tightly pressed to each other at rest. The ductus bursae is completely membranous, tubular, with a dextro-ventral protuberance anteriorly. It grades into the corpus bursae forming a large ventral protrusion pointed antero-laterally. The ductus of the accessory bursa (Razowski 1983; = pseudobursa; Kristensen 2003) emerges from its left side, and the ductus seminalis on the right side. A second protrusion of similar size and shape as the first one is located on the left ventral side of the corpus bursae, more anteriorly than the other. Both protrusions consist of thick, resilient transparent cuticle with wrinkles and tendons. The tendons are particularly longer and denser in the base of the protrusions, forming distinctive stripes (Fig. 4d). Most of the dorsal and a limited area of the ventral internal walls of the corpus bursae are covered with numerous sclerotised sharp-tipped acanthae (sometimes called signa, Fig. 4e), most probably serving in breaking the spermatophore envelope; there is evidence for such a function in other Lepidoptera (Galicia et al. 2008).

Discussion

Movements of the vesica and possible interaction with the female genitalia. The eversion of the vesica is probably achieved with increased haemolymph pressure inside the phallus (but see Naumann 1987 for alternative mechanism), which is easily viewed under a stereomicroscope. The inversion (retraction) is achieved by contraction of the muscle mph. il-t, which is essentially a retractor. Presumably, all movements of the vesica are accomplished by a balance of the two opposing forces: increased hydrostatic pressure possibly resulting from contractions of the mph. it-v and antagonistic muscle contraction of the mph. il-t. No protracting muscle fibres were observed, which is consistent with that reported by most previous authors who identify only retracting intrinsic muscle, although Eaton (1988) illustrated muscles protracting the vesica (“aedeagus extensor”) in *Manduca sexta* (Linnaeus, 1763) (Sphingidae).

The cornuti-bearing diverticula are never completely everted in a living moth; this is consistent with my preliminary assumption that there is no physical space for complete vesica eversion into the bursa copulatrix. When the everted vesica is juxtaposed to the inflated bursa copulatrix (Fig. 1; I accept that the phallic tube is inserted to the point where the juxta encounters the lamella antevaginalis), the spatial correspondence between the cornuti-bearing diverticula and the two cuticular protrusions on the ventral side of bursa become obvious. It can be assumed that during copulation the cornuti are inserted into these protrusions. The smooth ventral diverticulum is located at the insertion of the ductus seminalis. It should be emphasised that this diverticulum was not observed to evert, but it still may serve as a stopper during ejaculation, perhaps preventing the ductus seminalis from penetrating spermatophore material (Fig. 5). The large diverticula have numerous acanthae on their distal halves, arranged in longitudinal rows. As the acanthae are devoid of receptors and are immovable, their only possible function is mechanical (Richards and Richards 1979; Gorb 1997). Their arrangement conforms to the longitudinal grooves of the cornuti. When a diverticulum is not completely everted, the increased hydrostatic pressure presses its walls against the basal half of the cornutus (the distal one is protruding outside) and the acanthae are inserted into the longitudinal grooves of the cornutus. In this way the diverticulum and its cornutus form a mechanical entity that inhibits axial rotation. Perhaps this is important during ventral flexion of diverticula. The ventral flexion can be explained with unilateral contractions of the muscle fibres attached to the ventral tendons of the socket. The tendons are better developed at the ventral sides of the sockets, which probably corresponds to a greater number of muscle fibres. The asymmetrical attachment of the dextro-dorsal cornutus to its diverticulum may also contribute to unilateral flexion, which was supported by the plastic model used to test the functioning of diverticula (Fig. 6). When the stick of the model is attached slightly asymmetrically to the tip of the plastic bag, the lateral flexion is considerably enhanced. The ventro-dorsal movement (extension) of the diverticulum may due to contraction of the contralateral muscle fibres or relaxation of all fibres with hydrostatic pressure as an antagonist. The muscular chiasma at the socket of the cornutus is probably also related to its ventral flexion–extension, though one can assume that this muscle arrangement is related to axial movements. Such movements were not observed; however, they are unlikely given the position of acanthae on the diverticulum. The plastic model also demonstrated that the configuration of the cuticle at the base of diverticulum is important for the direction of movement. In this way differences in the three-dimensional configuration of the vesica surrounding diverticula lead to different movements and different stimulation of the female, which can be interpreted as a prerequisite for divergence and speciation through cryptic female choice. Though the anatomy of the vesica appears relatively constant, some of its structures may vary considerably. For example the protrusion at the base of the sinistro-ventral diverticulum is relatively constant in shape probably because it must be accommodated in the protuberance of ductus bursae. On the other hand, size variation in the distal part of the sinistro-ventral diverticulum is remarkable (Fig. 2). Given that it is non-eversible during copulation, presumably its shape is not crucial for proper functioning. The reason for the variation is unclear; it may be caused by environmental factors, seasonal polymorphism, or cryptic taxonomic diversity. This variation was not juxtaposed with the female genitalia.

The absence of ventral flexion in the sinistro-ventral cornutus may be related to the absence of muscular chiasma at its socket, but it may be also an artefact of insecticide poisoning, as flexion–extension movements are possible without chiasma, which was demonstrated by the plastic model. It should be noted that the arrangement of the tendons of the socket is asymmetrical in the same way as in the dextro-dorsal cornutus. Outside the main branches of the mph. il-t, attached to the sockets of cornuti, there are also fibres with other insertion points. The fibres attached to the distal parts

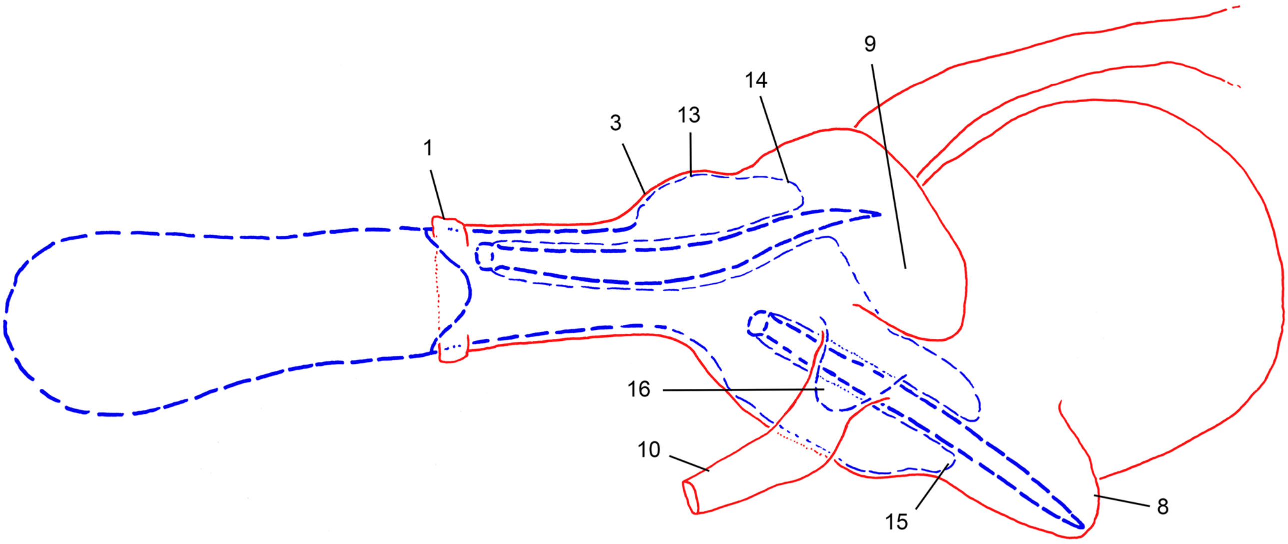


Fig. 5 Hypothetical position of the internal genitalia during copulation when the dextro-dorsal diverticulum is everted. Broken blue line, male structures; continuous red line, female structures. See the legend of Fig. 1

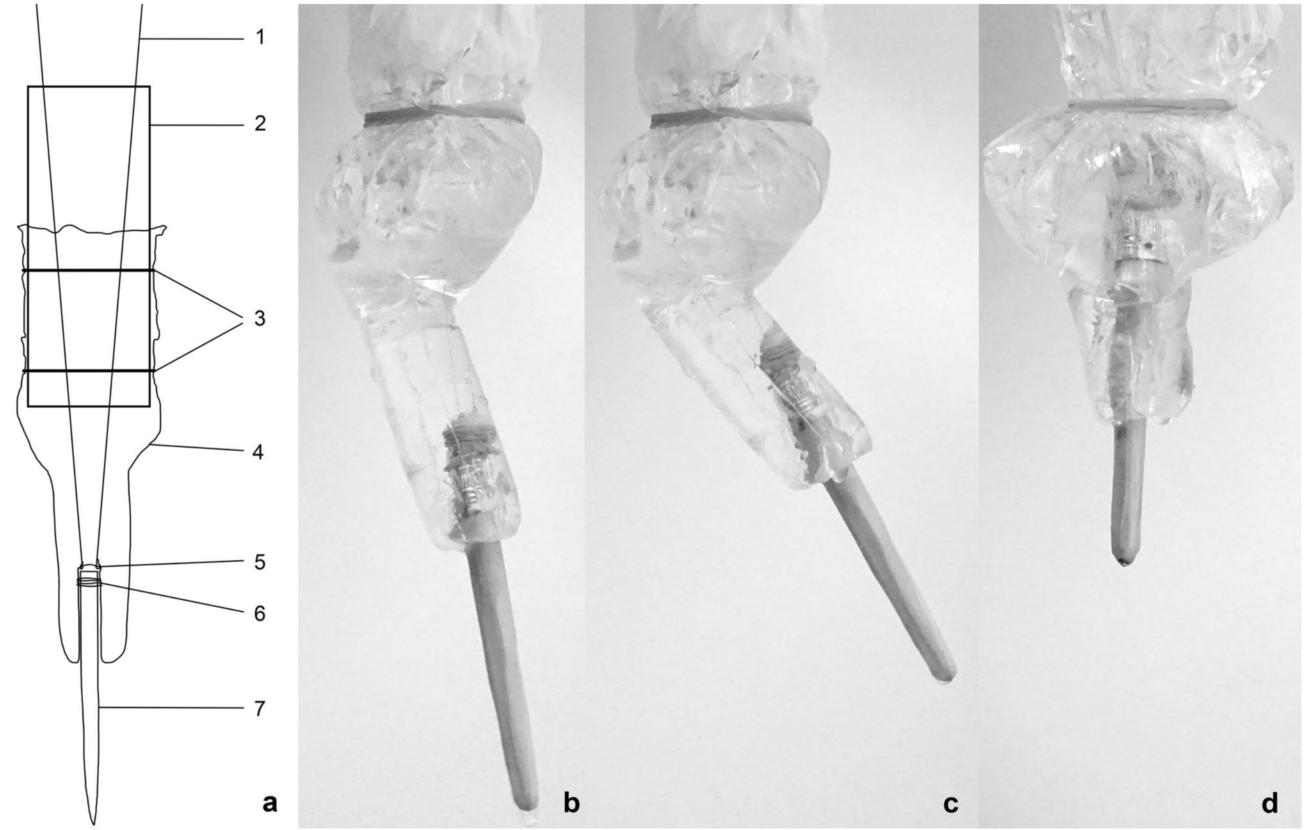


Fig. 6 Plastic model used for testing the movements of diverticula and cornuti. **a** Scheme of the model. **b** Extended position. **c** Flexion after pulling the treads. **d** Retraction after further pulling the treads. 1 copper tread, 2 polypropylene test tube with bottom cut off, 3 rubber band, 4 middle finger of polyethylene glove, 5 attachment of the copper treads, 6 copper tread winding, 7 piece of wooden pencil

of diverticula (distal referring to their location in completely everted diverticulum; in natural position it is proximal) may assist the general retraction of the vesica. It is possible that the small bundle attached to the base of the dextro-dorsal diverticulum has the same function. Seemingly, this bundle is not powerful enough to contribute considerably to the ventro-dorsal movement of the diverticulum, though at a glance its position hints at such a function.

The possible events during copulation may be the following (Fig. 5). After complete insertion of the phallus (the juxta and the lamella antevaginalis are in tight contact), the dorsal part of vesica starts eversion and fills the protuberance of ductus bursae and the posterior area of corpus bursae. The ventral diverticulum is everted filling the origin of ductus seminalis and ejaculate is ejected into the corpus bursae. The sinistro-ventral cornutus via eversion of its diverticulum is inserted into the more posterior protuberance of the corpus bursae, then it is retracted and the dextro-dorsal cornutus is inserted into the more anterior protuberance of corpus bursae. When inserted into protuberances, the cornuti are moved forth and back stimulating the female. Eventually the entire vesica is retracted. The consequence of all these events is hypothetical and based on eversion observed in one specimen (though other two specimens performed partially the same movements) poisoned with dichlorvos, which may differs from the real copulation.

What is the role of the insertion of cornuti in specialised female structures? Stimulation of the female is a feasible explanation. The walls of corpus bursae in different Lepidoptera have stretch-receptors (Sugawara 1979), which may be present also in the wall of the ventral protuberances of *E. magnificana*. The pressure from protruding cornuti may stimulate the receptors. This assumption supports one of the hypothetical functions of the non-deciduous cornuti in Lepidoptera (Cordero 2010). As the vesicae of many Cochylini are adorned with the same type of cornuti, one can speculate that they have the same or similar stimulating function. Though not observed, the muscularis of the protuberances is probably well developed regarding the numerous tendons on their surface. Contractions of their fibres can cause deformations related to interaction with the cornuti. Other options for reception are hardly feasible as microscopical examination of the cuticle failed to find any traces of cuticular receptors (e.g., setae). Additional or even completely different functions of cornuti cannot be dismissed without direct observation of their motility during copulation however. One of them is manipulating the spermatophore, as it was observed in Noctuidae (Callahan and Chapin 1960).

Presumably, the corpus bursae in *E. magnificana* is divided into two compartments with different function: (1) anterior part for spermatophore storage and digestion and (2) posterior part for interaction with the vesica and stimulation. This condition resembles that found in some Noctuidae (Mikkola 1992) and is typical for many Cochylini representatives (unpublished data), but is remarkably different from those in other Tortricidae lineages, e.g., Eucosmini (Zlatkov 2016) which usually have reduced vesica and short tubular phallus. In the tribe Eucosmini the interaction between the internal genital structures is limited to the distal part of ductus bursae, the only male structures reaching corpus bursae are the deciduous cornuti (if present). The role of the accessory bursa in Cochylini is not understood, a resorptive or secretive functions are possible since the main compartments of the corpus are occupied by additional activities. The two different compartments of the corpus bursae in *E. magnificana* (and probably other Cochylini species) may have evolved driven by different selection pressures: cryptic female choice for the posterior compartment and sexual conflict via spermatophore for the anterior compartment.

Acknowledgements I am indebted to Joaquin Baixeras (Valencia, Spain) for providing literature and commenting on an earlier draft of the paper. John W. Brown (Washington, USA) improved the paper linguistically and also provided important suggestions for which I am grateful.

Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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