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A short review of the skeletal anatomy of male genitals of genus *Eupithecia* CURTIS, 1825 (Lepidoptera, Geometridae).

Vladimír KUBÍN & Josef PROCHÁZKA

Abstract

The study deals with the composition of the skeleton of the male genitalia of genus *Eupithecia* CURTIS, 1825. Separated parts of the genitalia, including some organel not described so far (e.g. Suspensorium recti), were depicted through the light and scanning electron mikroskopy. Suspensorium recti lies ventrally from the tegumen fixed to the cranial base of uncus. The examination of the cross sections of aedeagus brought some findings concerning the morphological character of cornuti. The study brings only anatomical significant information, without any useability for determination purposes.

Key words: Lepidoptera, Geometridae, *Eupithecia*, Male genitalia, Skeletal anatomy.

Zusammenfassung

Bei dieser Arbeit handelt es sich um anatomische Beschreibung der männlichen Genitalien der Gattung *Eupithecia* CURTIS, 1825. Mit dem Rasterelektronenmikroskop wurden einzelne Teile der Genitalien photographiert. Manche Organe wurden bislang nicht beschrieben. Insbesondere handelt es sich dabei um das Suspensorium recti. Dieses Organ liegt ventral vom Tegumen und vermittelt die Befestigung von Canalis rectalis. Bei der Untersuchung der Querschnitte der Aedeagen wurden die morphologischen Differenzen der Cornuti entdeckt. Diese Arbeit dient ausschließlich anatomischen Zwecken; für eine

Determination ist sie nicht geeignet.

Introduction

With many species of genus *Eupithecia* CURTIS, 1825, the anatomical structure of male and female genitals represents the sole reliable criterion for exact determination (MIRONOV 2003, WEIGHT 1987, 1988, 1990, 1991, 1993, PETERSEN 1910 et. al.). For this purpose, preparation of abdomens with dilutions of NaOH under highed temperatures is the most frequent method of investigation (BENNET 1929), soft organs (musculature, digestive tract and all.) being liquidated in this process. For examination, only the sclerotized skeleton remains, formed by firm chitin which, due to NaOH technique, changes into chitosan while acidum aceticum is released. The morphological features of the skeleton, however, do not change (OBENBERGER 1952). In our research, we used this preparation technique, so that our description focused only on the anatomy of skeleton, not on the complet anatomy. Our anatomical survey does not serve any determination purposes.

Material and methods

We have used preserved dry collection material, only in some cases fresh material immediately after catch (Figs. 13, 40, 41, 42). The preparation technique was the same as in our former studies (KUBÍN & PROCHÁZKA 2014b). Clearing solution (15'–30') was used for better visualization of details (OTČENÁŠEK 1990). Some preparations were coloured with 0,1% water solution of amidoblack. For cross sections the material was routinely embedded in parafin and cut with a microtom of fa Reichert. After routine prepatarion (KUBÍN 2003) the objects were photographed using the scanning electron microscope TESLA BS 300 on FOMA 100 ASA film (Figs. 25, 31, 38, 48, 49, 50). For examining some preparations (Figs. 8, 9, 10, 15, 17, 23) the scanning electron microscope HITACHI – SU was used and digital photography applied. All remaininng preparations were photographed with light microscope using the digital camera COSMOS 530 (3MP). All preparations are deposited in the collection of the first author.

Abbreviations: - N. nov. denotes organs newly named by the authors;
- SEM denotes Scanning electron microscope.

Results

Genitals (Figs. 1, 2, 3) were dismantled into anatomical wholes. Firstly, the tegumen complex and vinculum were separated (Fig. 4).

Tegumen and vinculum

Tegumen (PIERCE 1914, Povolný 1956) forms the upper covering of the genitals. Thus the genitals proper are covered dorsally with the tegumen and ventrolaterally with valves (Fig. 3). Tegumen is formed by two sclerotized arms which close the membrane – membrana teguminis posterior N. nov. (Figs. 5, 6) with alveolar structures (Fig. 8). This struc-

ture will be the subject of a separate contribution. The cranial part of tegumen is strengthened by a membrane which does not have any verifiable structure – *membrana teguminis* anterior N. nov. (Fig. 5). Cranially the tegumen ends closed with an anatomically right joint – *articulatio vincularis* N. nov. which connects it with caudal arms of the vinculum (Figs. 14, 15). The joint facilitates considerable movability of the tegumen against vinculum (Fig. 3) (PIERCE 1914). In some kinds the massive arch of the vinculum may be interrupted in the medial line. Vinculum contains the massive saccus (Figs. 3, 16, 17). Tegumen is connected caussally with uncus (PENTOUREAU 1895, KUBÍN & PROCHÁZKA 2012) (Figs. 4, 5, 6, 7, 9). Uncus is a firm and strongly sclerotized formation whose surface does not reveal any visible structures (Figs. 9, 12). The two arms of the tegumen connect on the cranial side of the uncus in an arch varied to kind – *sinus teguminis* N. nov. (Figs. 5, 6). It is connected with the uncus ligamentally and with flexible chitin (SHARPLIN 1963) (Fig. 7).

Suspensorium recti

The suspensory apparatus of the rectum – *suspensorium recti* N. nov. is attached to the cranial base of uncus (Figs. 4, 5, 6, 11, 12.). After their arched course, fully independent on the tegumen, its two arms continue in the cranial direction and conjugate approximately in the half of tegumen (Fig. 5). Here *canalis rectalis posterior* N. nov. (Figs. 4, 5, 11) joins the suspensory apparatus, oriented caudally and converting into *conus recti* (Fig. 9) with anal aperture (Fig. 10). After leaving the *suspensorium recti*, *canalis rectalis posterior* continues as *canalis rectalis anterior* N. nov. to the intestine (Fig. 13). Where they are connected, flexible chitin can be seen (SHARPLIN 1963), which secures some movability. The whole complex of *suspensorium recti* lies ventrally under the tegumen and serves only for the fixation of the rectum (Figs. 4, 5, 6, 11, 12).

Labides

Labides (PIERCE 1914) – (*labida* pl. *labides*) are important component, representing the fundamental supporting system of the genitals (Figs. 18, 19, 24). They are noticeably sclerotized segments connected with a joint – *articulatio lacertalis* N. nov. to *lacerti* (sg. *lacertus*) N. nov. *Lacerti* are simple (Figs. 21, 22), or bifurcated (Fig. 20). *Lacerti* fix the *fulturae aedeagi* N. nov. (sg. *fultura*) (Figs. 19, 20, 21, 22) active as supports of aedeagus during erection (Fig. 19). *Fulturae aedeagi* are richly covert with typical sensitive thorns – *setae* (sg. *saeta*) (Figs. 19, 20, 22, 23) (OBENBERGER 1952, SONDGRASS 1993). The conjunction of *lacerti* with *fulturae* is firm, all movement is produced only by joints – *articulatio lacertalis* (Figs. 19, 20, 21, 22). At the cranial end *papillae* (sg. *papilla*) settled with *setae* are attached to the labides (Fig. 24). Approximately in the half of the labides *iunctura flexibilis* is attached to them, forming a significant part of the costal joint (*articulatio costalis*) (KUBÍN & PROCHÁZKA 2014b).

Transtilla

Transtilla (PIERCE 1914) is an unpaired formation connecting the dorsocranial costas prominences lying on the medial ends of the left and right costal. It is significantly variable as regards different kinds and individual cases (Figs. 26, 27, 28, 29).

Crista valvae

A sclerotized slightly S-curved formation, crista valvae N. nov. lies on the cranial side of valvae. It runs between the regions of costal and lingular joints (Fig. 30). In its course it is significantly variable, and underdeveloped in some kinds. Its purpose and significance have not been clarified yet.

Valvae

The anatomy of valvae was detailed described in our previous study (KUBÍN & PROCHÁZKA 2014b).

Aedeagus

Aedeagus (PIERCE 1914) (Fig. 3) is the most important part of male genitals. It is a chitin wrapping without any distinct structure with exception of praephalus (KUBÍN & PROCHÁZKA 2014a), covering vesica proper (penis, phallus) (NIELSEN 1957). Vesica is a ciliary body, laying in the aedeagus movably, without any mechanical attachment with it (Fig. 31). Vesica contains sclerotized formations – cornuti (sg. cornutus) (Figs. 32, 35, 37). The shape and number of cornuti are important and generally applied criteria for the determination of the kinds of genus *Eupithecia* (KUBÍN & PROCHÁZKA 2014a). In this genus, cornuti are non decidui and do not remain in the female genitalia (bursa copulatrix) after copulation (CORDERO 2010).

In our work we succeeded in distinguishing two forms of cornuti, according to their morphological character. Firstly, cornuti arise through a mere strengthening of heavily sclerotized plicas of the vesica; these appear in the kinds categorized in the 3rd group (Cornuti dentate on the caudal margin) (Figs. 32, 33). Quite different forms of cornuti occur in the kinds belonging to the 2nd group of our classification (KUBÍN & PROCHÁZKA 2014a). They are independently localized solitary bodies of long cylindrical form ending mostly caudal directed tips (Figs. 35, 37, 38). In cross sections their walls have a spherical shape (Figs. 36, 39). The function of cornuti has not been explained adequately so far (CORDERO 2010).

Spermatophors are conveyed into aedeagus through the ductus ejaculatorius (Fig. 40) which before entering aedeagus passes through a semispherical body – bulbus ejaculatorius (PIERCE 1914) (Fig. 41). Bulbus ejaculatorius is strongly oxydized with numerous tracheolas (Fig. 42); this formation lies either close to aedeagus (Fig. 41) or at certain distance from it (Fig. 40).

In the cranial, closed part of aedeagus (caecum of aedeagus), transparent, sharply delimited bodies may be found quite often (Fig. 43), artefacts arising probably in the course of preparation.

In its praephalic part, aedeagus is connected to anellus with sparse thin tissue (Figs. 44, 45). Anellus (PIERCE 1914, POKORNÝ 1956) is a caudally lying (usually semi-) ring formation, separated from juxta with a narrowed neck, heavily infiltrated with flexible chitin (SHARPLIN 1963, GORB 2001, KUBÍN & PROCHÁZKA 2014b), mostly triangular shape (Figs. 45, 47). This formation enables movement between juxta and anellus. The flexible chitin, as an inner structure, is not visible in the scanning electron microscope, depicting only the surface of objects (Fig. 48). Praephalus, the most caudal, open part of aedeagus, has a well – marked structure both on the surface and internally (FÄNGER & NEUMANN 1998)

(Figs. 49, 50). It is the area where the tissue binding aedeagus to anellus is externally attached. On the inner side of the cross section of aedeagus in its praephalic part, a fine, but very dense dentation is apparent, which participates in the fixation of vesica to ostium bursae of the female in the course of copulatio (OBENBERGER 1952) (Fig. 51).

Juxta

Juxta is a sizable formation without any surface structure (Figs. 18, 44, 45, 46, 48); it is an important structure fixing the whole apparatus of aedeagus, especially during erection in the course of copulation. Besides, the valves are also connected with it by means of a very firm tonguelike joint – articulatio lingularis (KUBÍN & PROCHÁZKA 2014b). In the dorsoventral projection, juxta appears as a flat formation, but in actual fact it has a groove-like shape. (Fig. 46).

Discussion

This paper represents a brief information of skeletal anatomy of the male genitals. As a study model genus *Eupithecia* was chosen owing to its morphological homogeneity. In the anatomy of butterflies there exist many parts which are not defined sufficiently. That is why we named them with international Latin terms (EYER 1924, DIAKONOFF 1954).

In the lateral view of male genitals (Fig. 3) tegumen has a noticeably special position, closing genitals proper from the dorsal side. Besides, tegumen serves as a support of the ventrally lying suspensory apparatus of rectum (suspensorium recti). Thus it may be assumed that the whole system (tegumen plus uncus) forms an important fixative component of the last part of the digestive tract.

Uncus is a noticeable part of male genitals. Its function in *Eupithecia* has not been clarified yet. Should it participate actively in copulation, there should be some morphologically demonstrable places on the dorsal side of the female abdomen, where uncus could catch hold in a similar way as cumillae boltei (Boltes pockets) described in MIKKULA (1994) on the 7th sternite of some kinds of Canadian *Eupithecia*. However, we have never found any similar places on the tergites of female abdomens. With regard to the localization of suspensorium recti and even of the conus recti alone, any participation of uncus in copulation seems highly questionable.

The examination of cross sections of aedeagus, for the purpose of extending our knowledge of the morphology of cornuti, confirmed the sorting of the kinds of *Eupithecia* into groups, which facilitates determination (KUBÍN & PROCHÁZKA 2014a). What remains to be elucidated is the anatomical fixation of individual cornuti of group 2 to the corpus of vesica. The assessment of the activity of these cornuti during copulation surpasses the frame of this study.

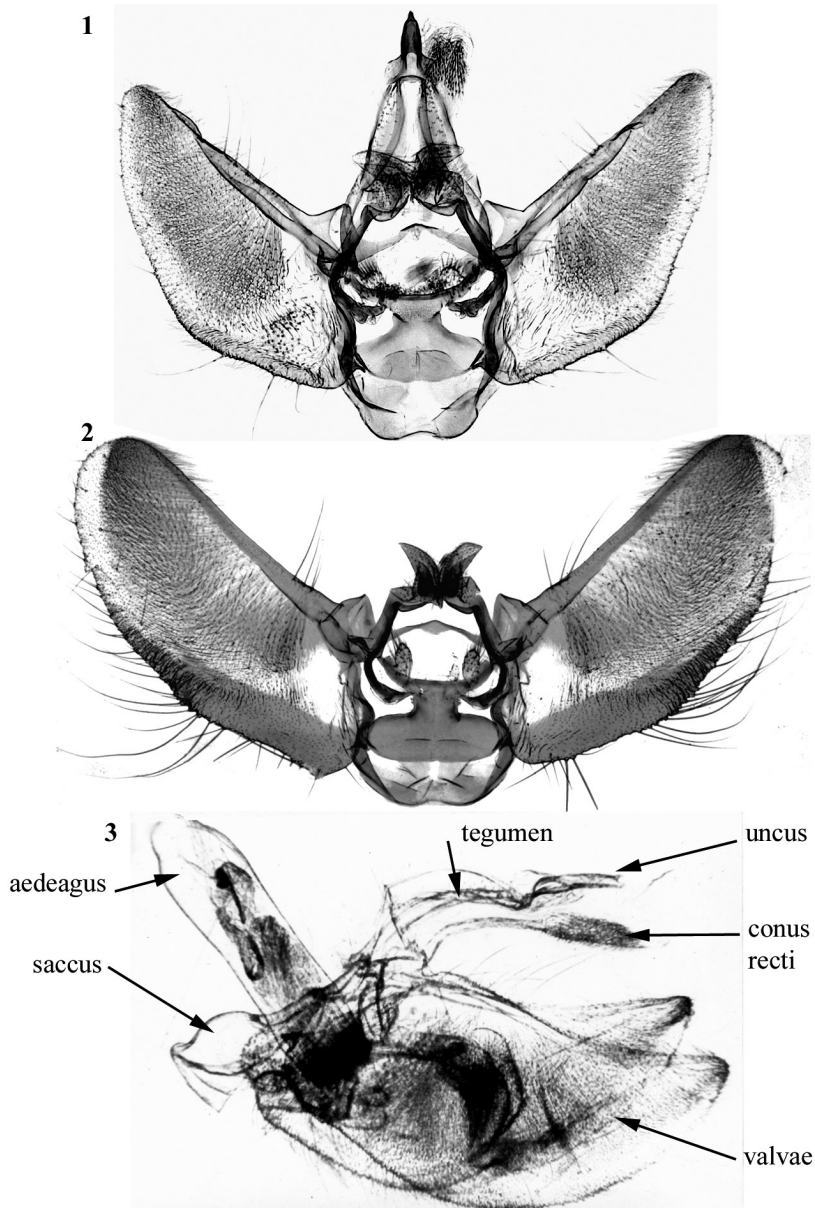
Acknowledgements

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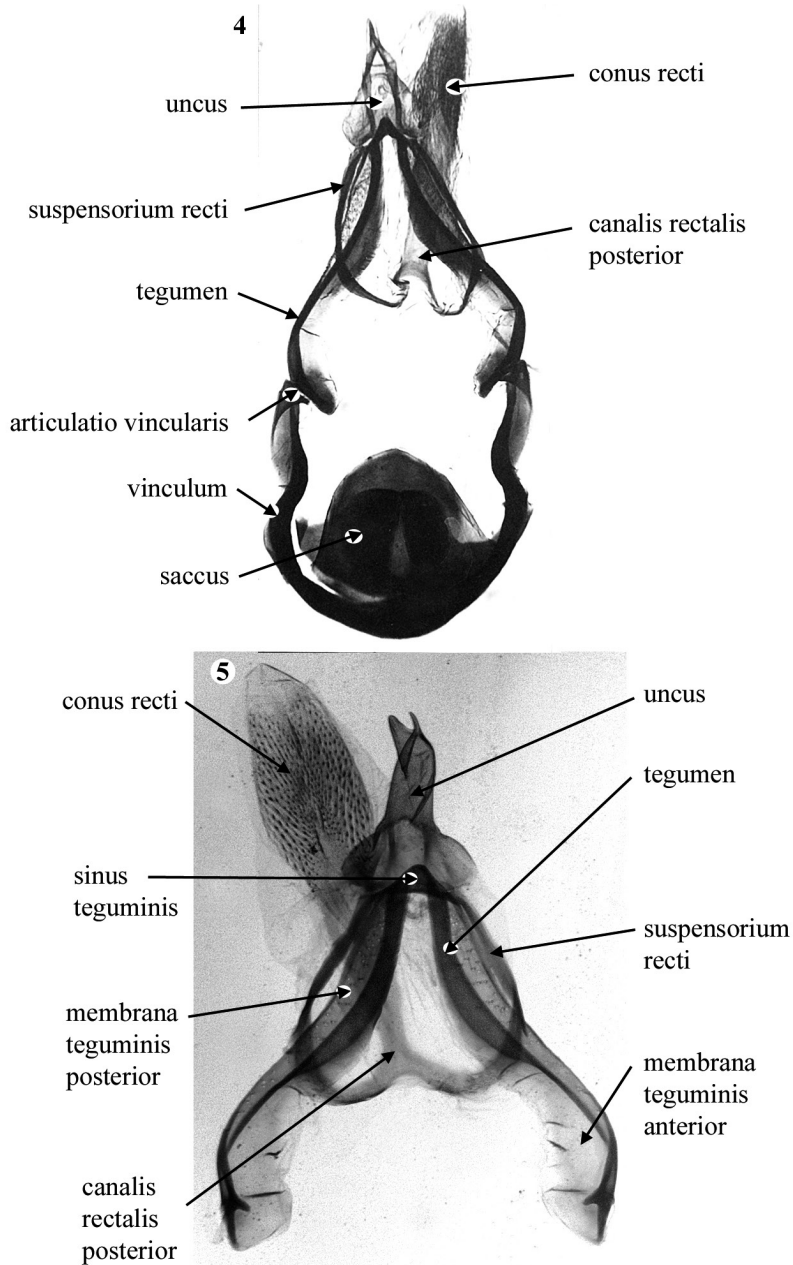
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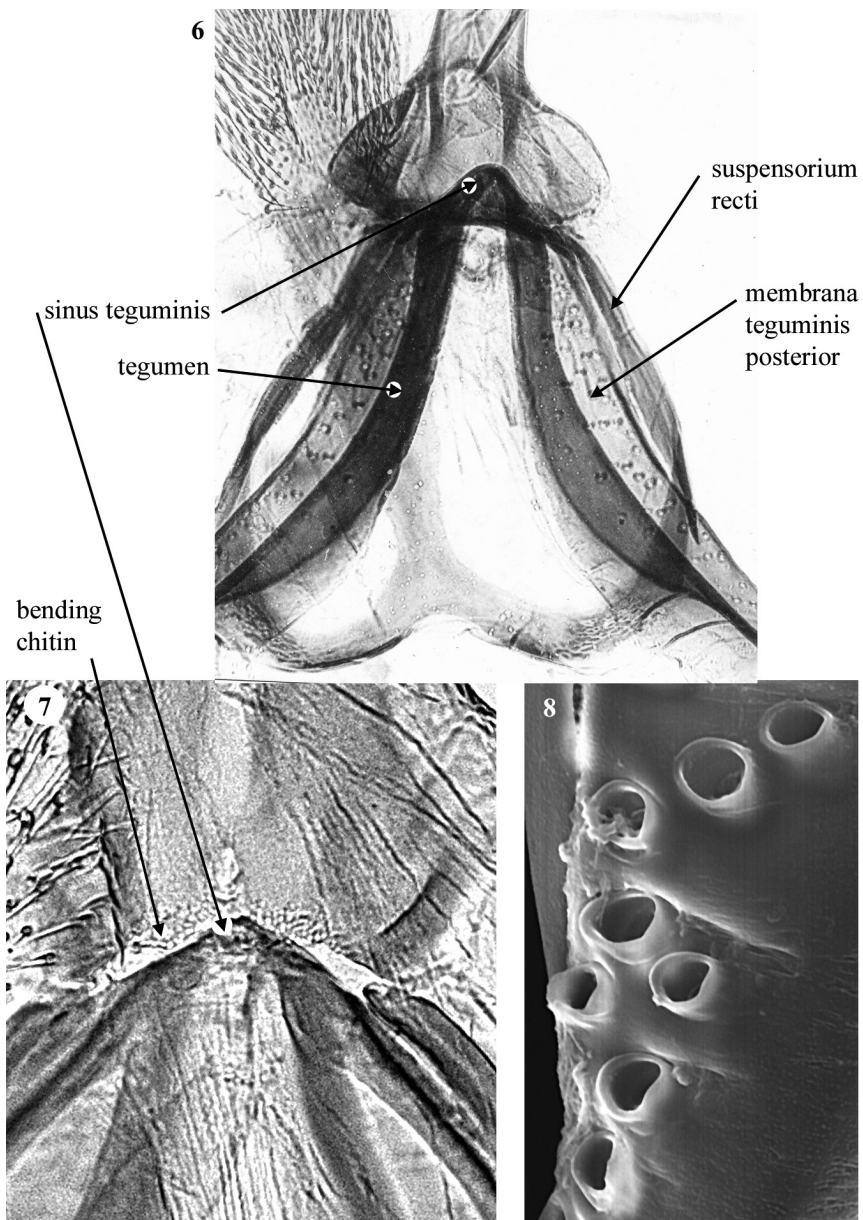
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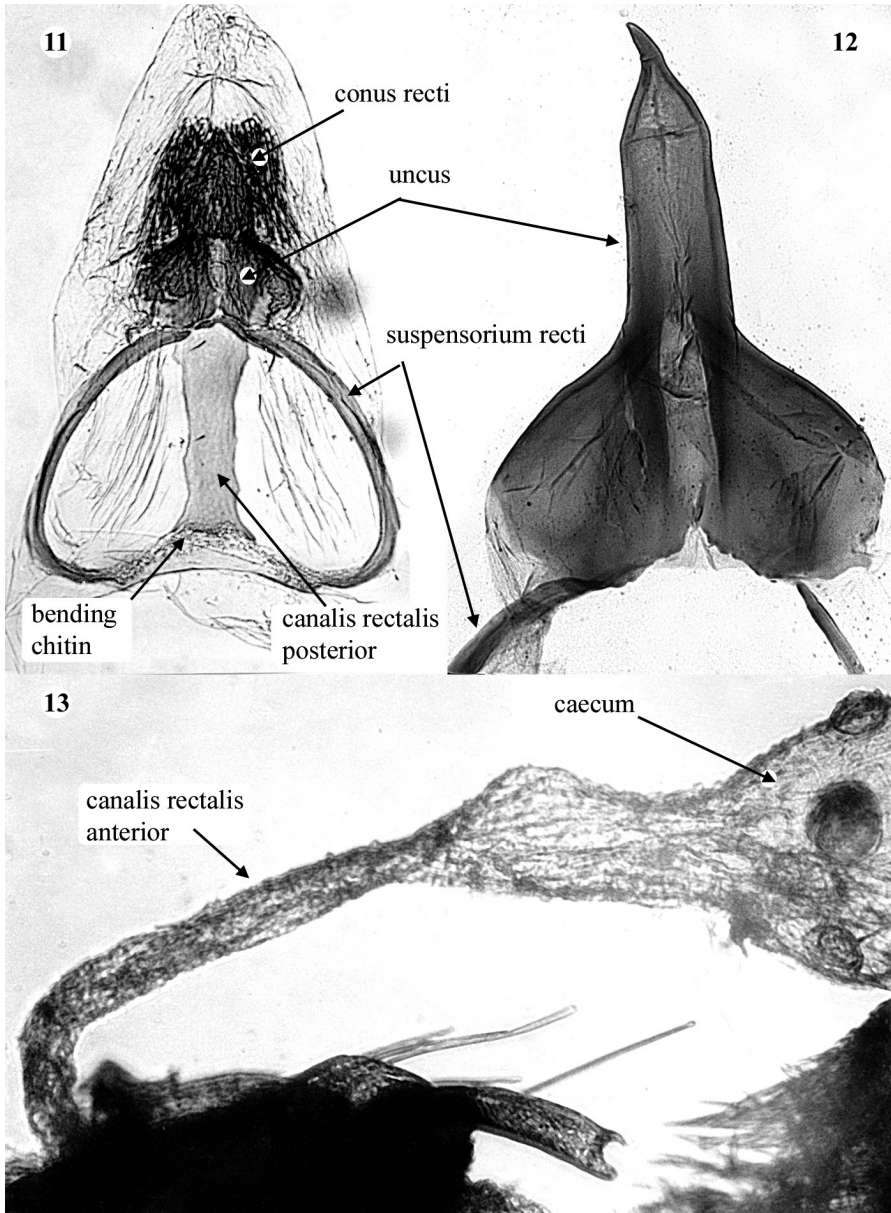
Figs 1 - 3. 1. *E. dodoneata*: the dorsolateral view of the total of genitals after preparation. 2. *E. virgaureata*: after removal of the tegumen. 3. *E. subfuscata*: the lateral view.



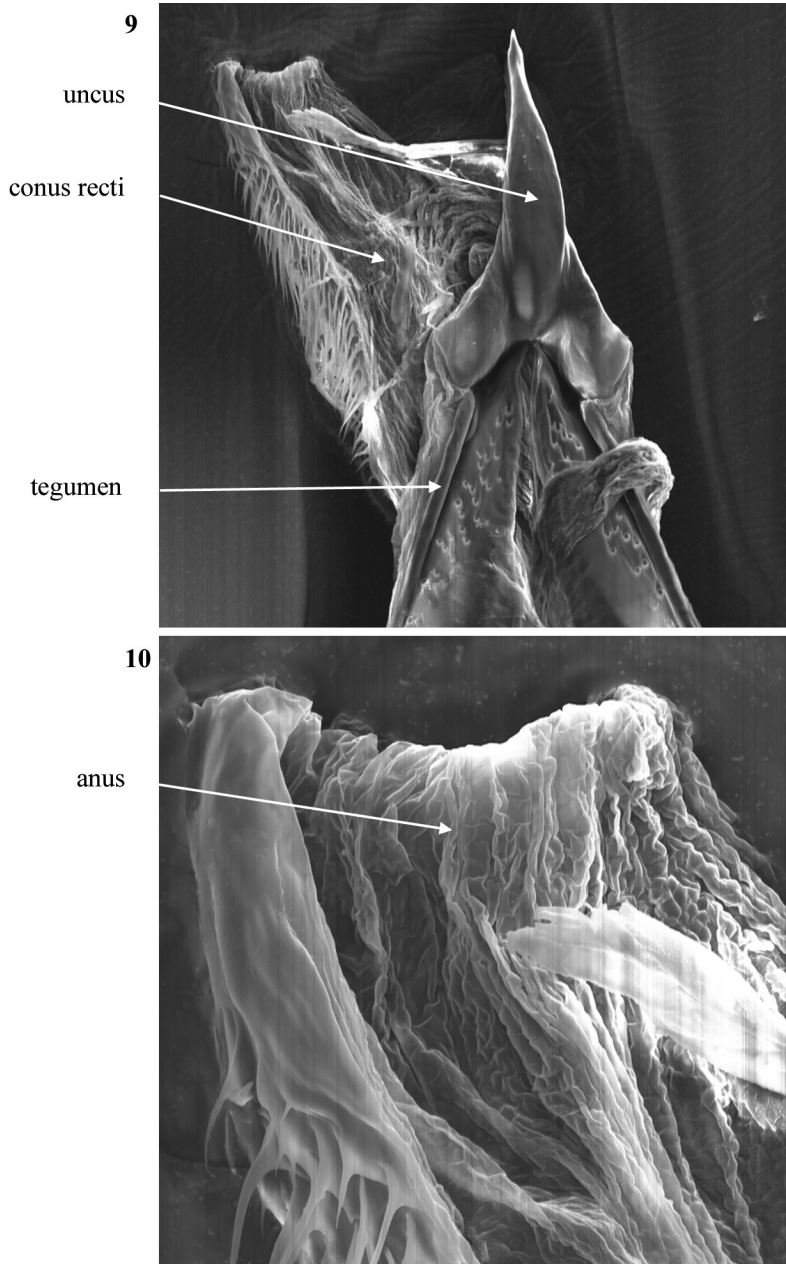
Figs 4 - 5. 4. Isolated tegumen and vinculum. 5. The separated tegumen.



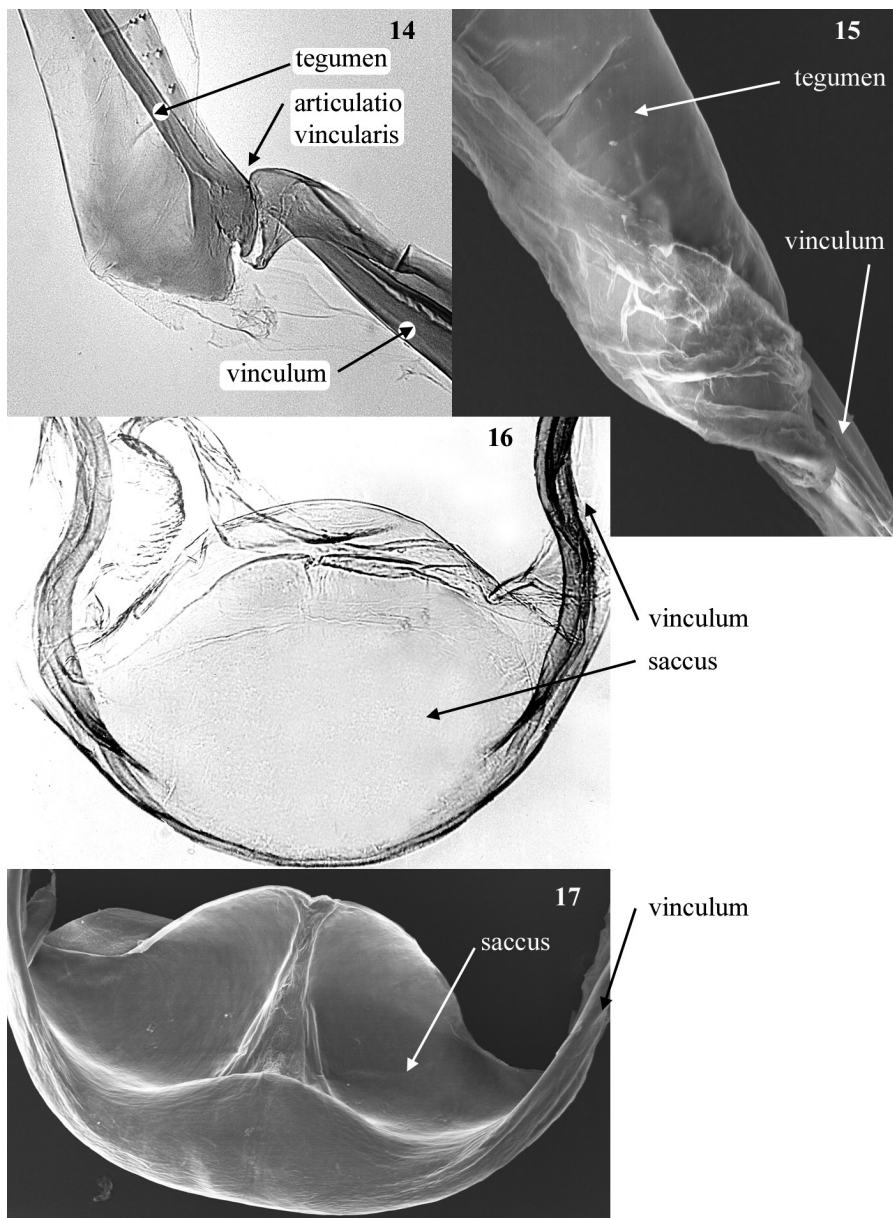
Figs 6 - 8. 6. Sinus teguminis, the connection of both arms of tegumen.
 7. The bending chitin in connection of sinus teguminis in region of uncus.
 8. Alveoli of the membrana teguminis posterior of tegumen SEM Magn.1500x.



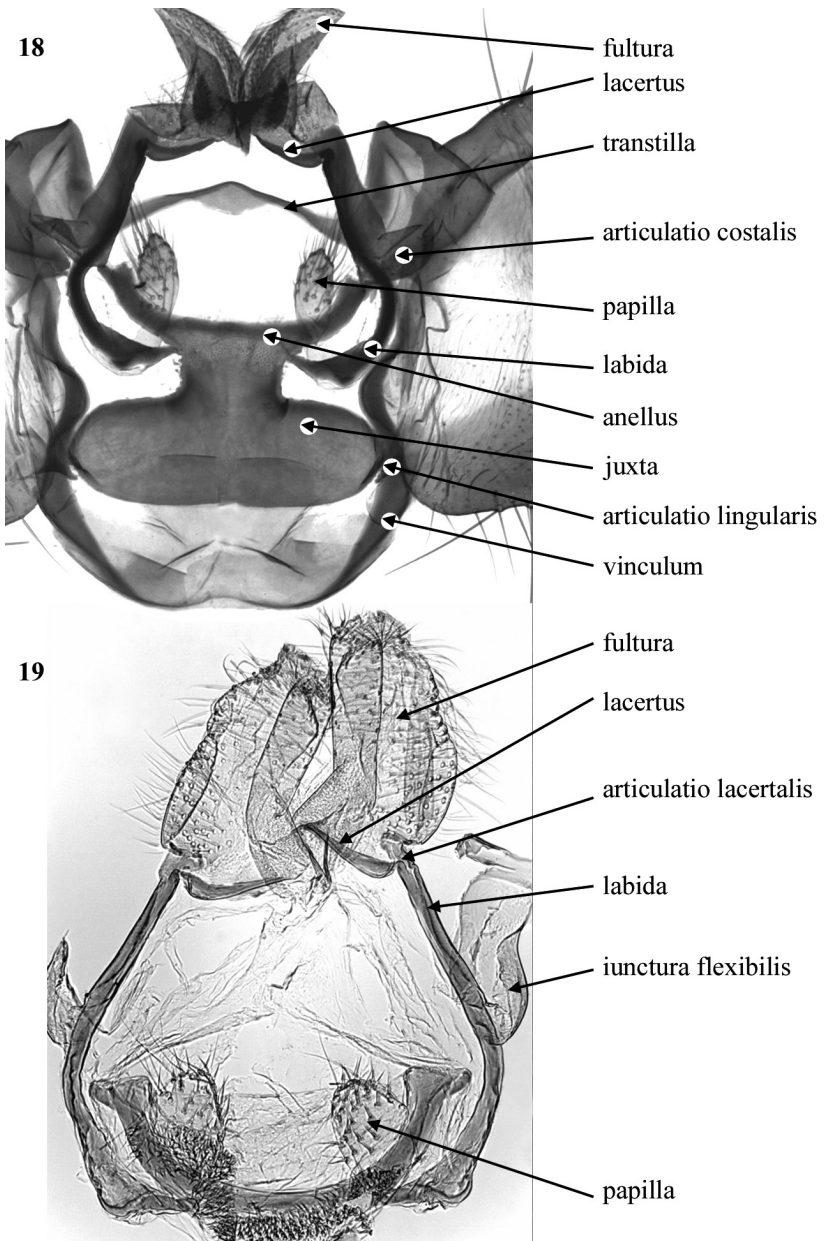
Figs 11 - 13. 11. Rectal complex. Suspensorium recti and canalis rectalis. 12. Attachment of uncus with suspensorium recti. 13. Canalis rectalis anterior, connecting caecum with suspensorium recti (unvisible).



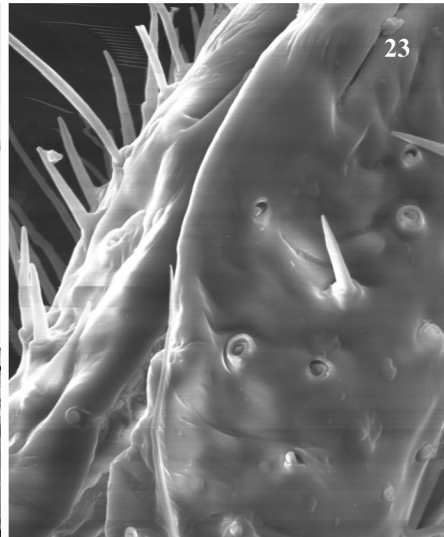
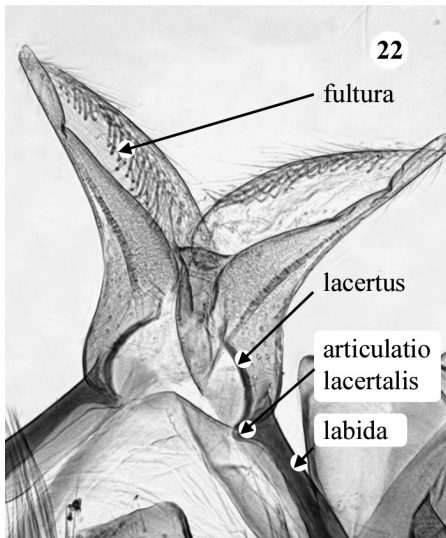
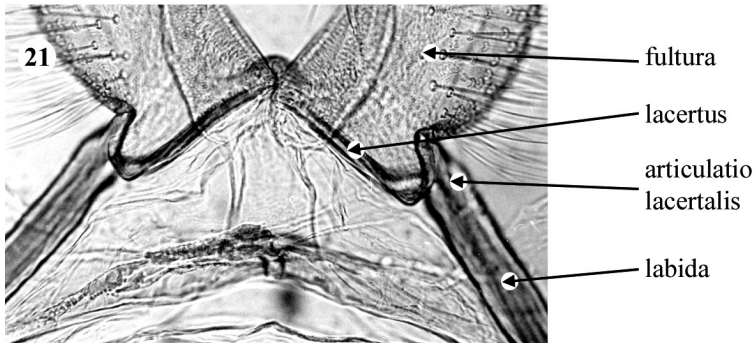
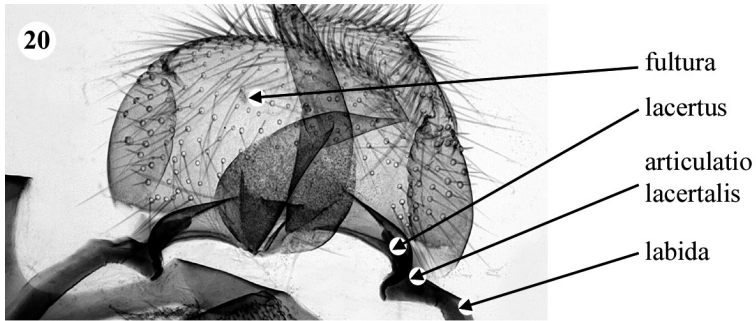
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Figs 14 - 17. 14. Tegumen - vinculum: articulatio vincularis. 15. Articulatio vincularis SEM Magn. 400x. 16. Vinculum and saccus. 17. Vinculum and saccus SEM Magn. 150x.

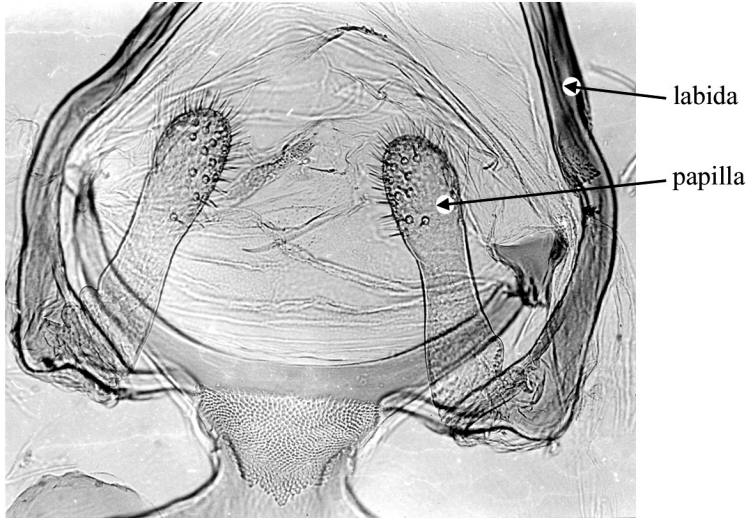


Figs 18 - 19. 18. General view for better orientation.
19. Preparation of the separated labides.

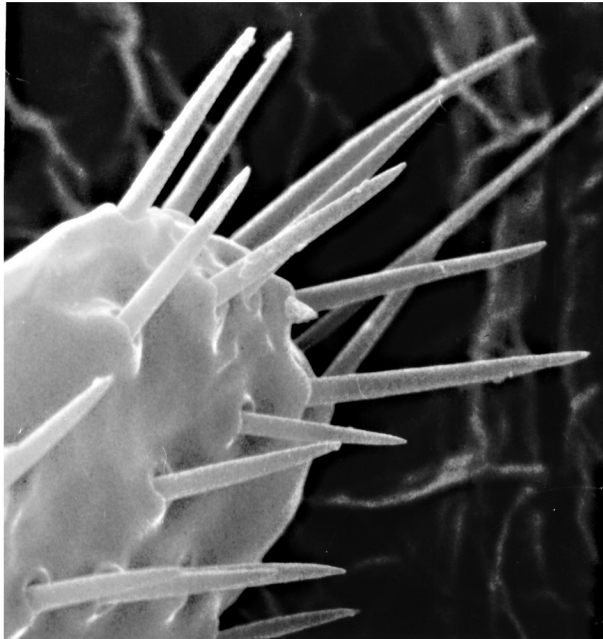


Figs 20 - 23. The caudal end of labides. 20. Fultura aedeagi and bifucated lacerti *E. intricata*. 21. Lacerti *E. dodoneata*. 22. Lacerti *E. virgaureata*. 23. Fultura aedeagi SEM Magn. 1000x.

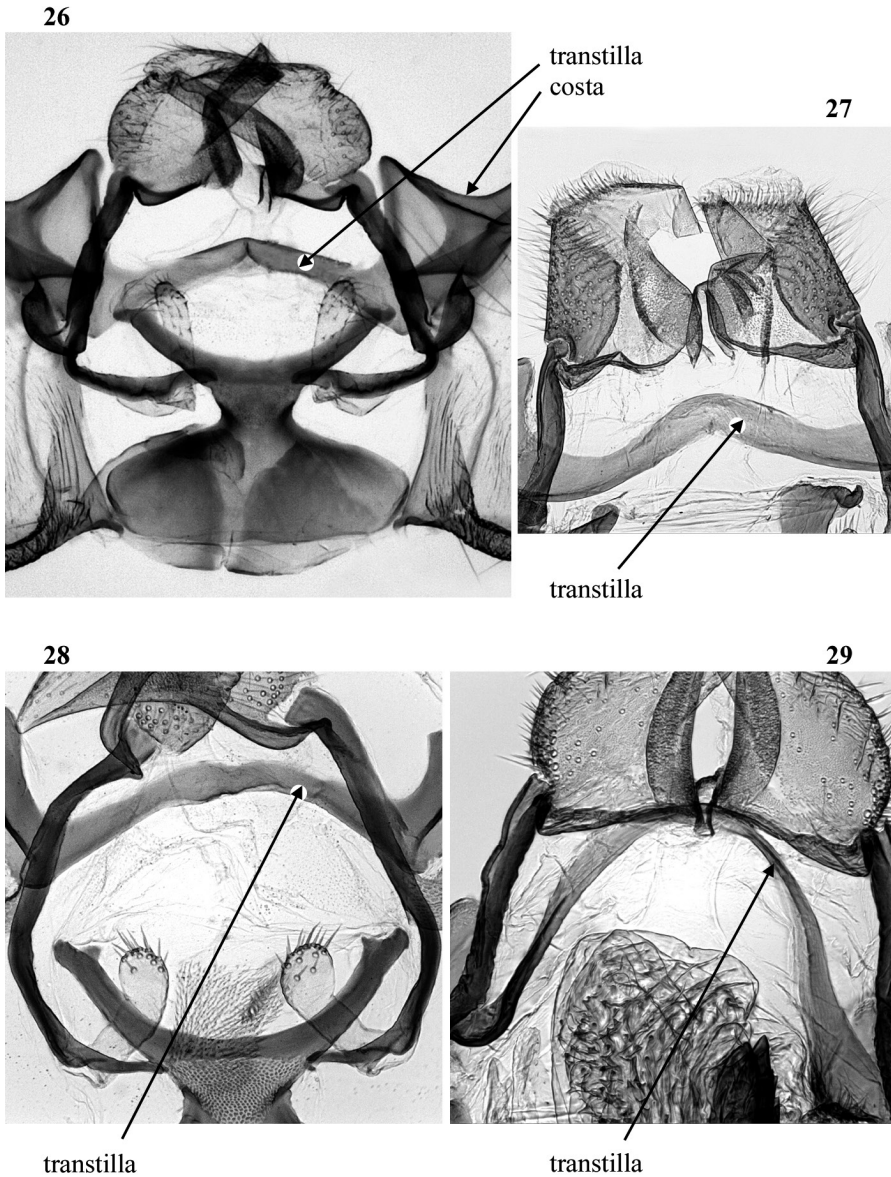
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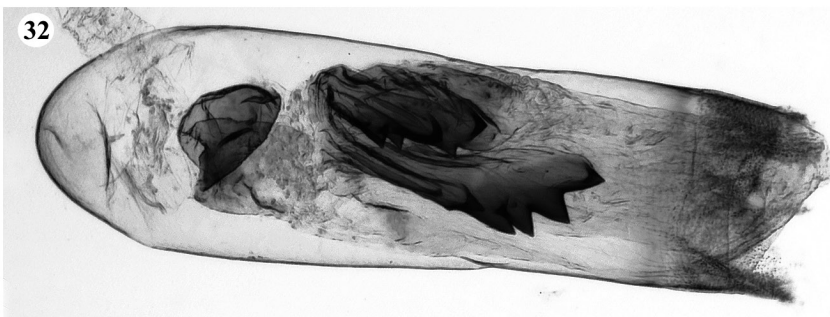
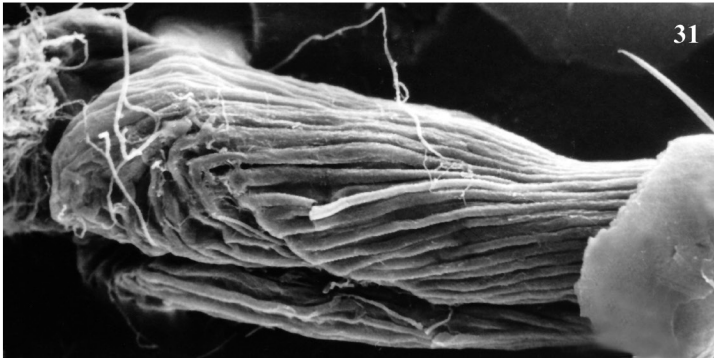
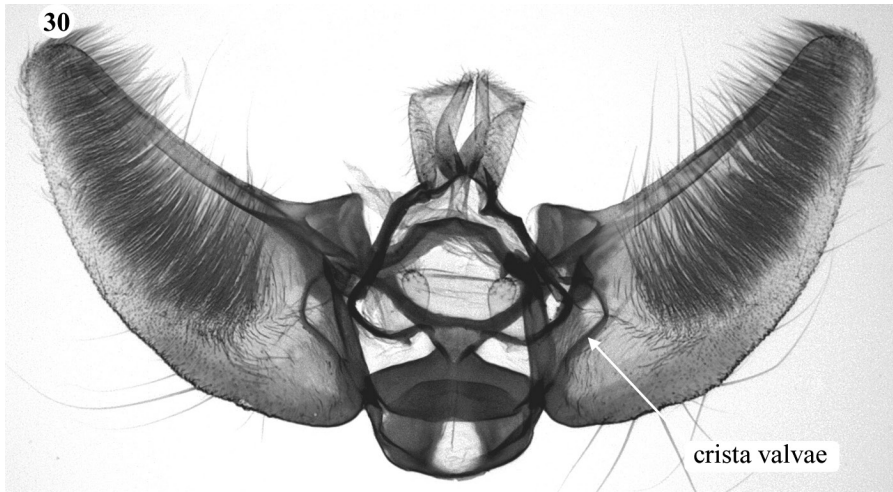
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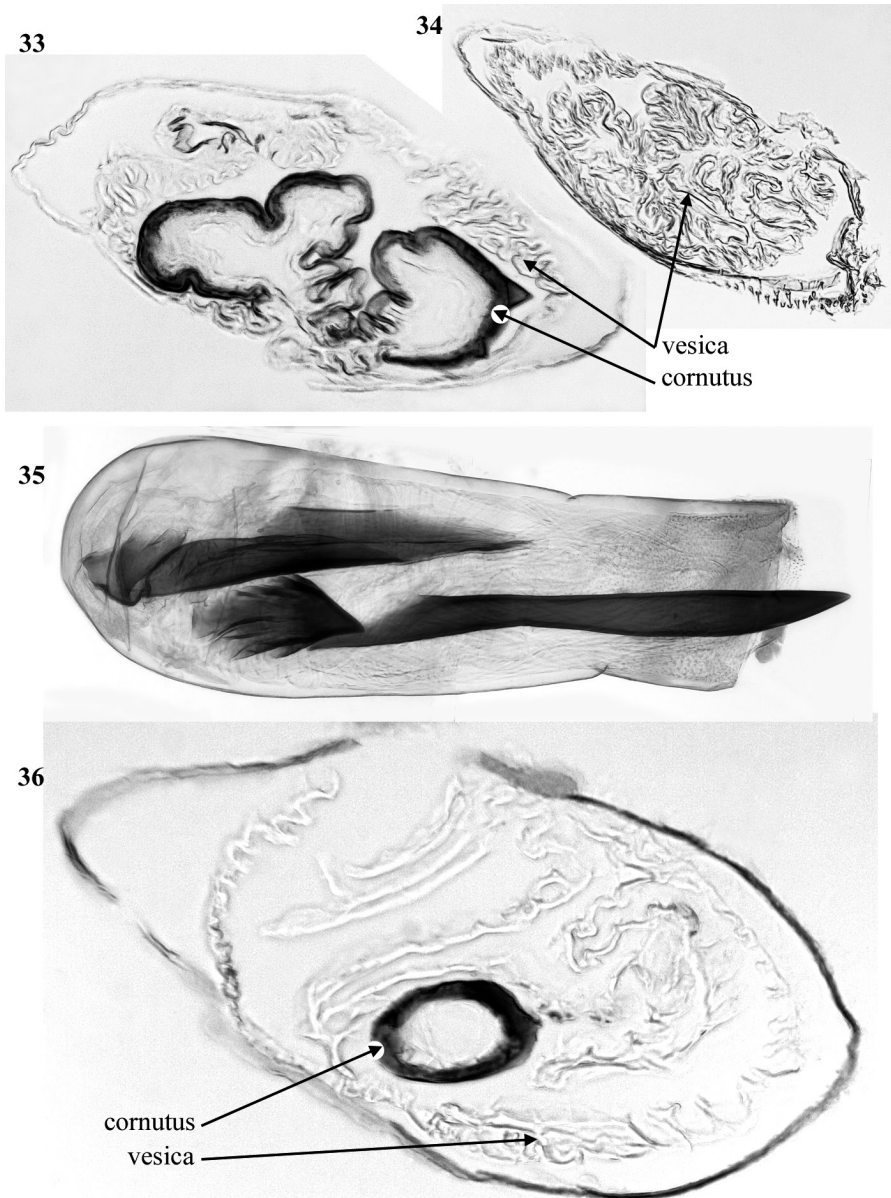
Figs 24 - 25. 24. The cranial part of labides with papillae.
25. Papilla SEM Magn. 700x.



Figs 26 - 29. 26. Connection of right and left valva - transtilla *E. selinata*.
 27. Transtilla *E. lanceata*. 28. Transtilla *E. vulgata*.
 29. Transtilla *E. exigua*.

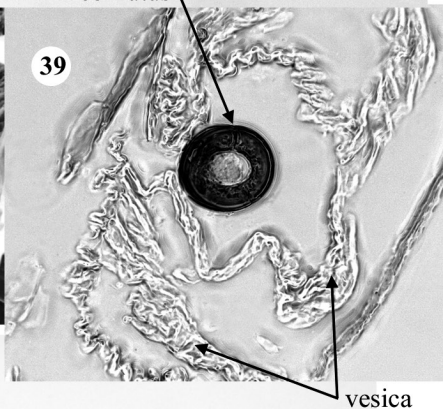
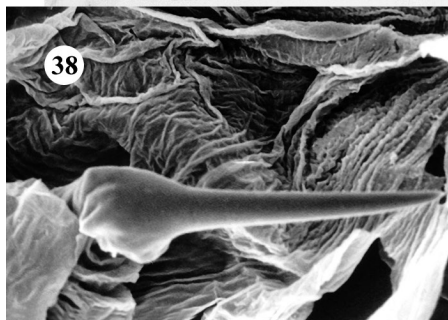
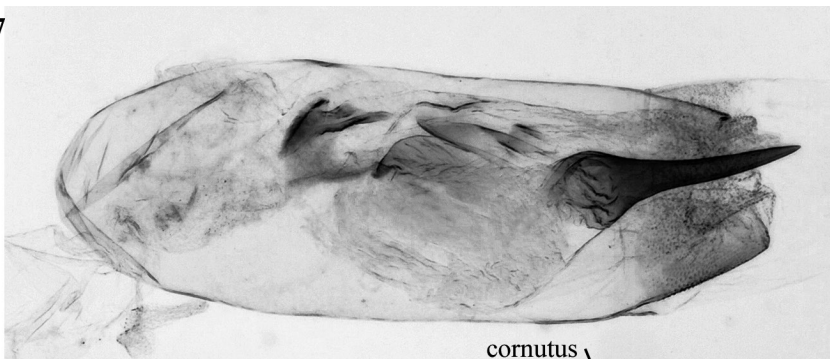


Figs 30 - 32. 30. The crista valvae lying between the region of articulatio costalis and that of articulatio lingularis on the middle area of valvae. 31. Vesica SEM Magn. 310x. 32. *E. subumbrata*: aedeagus after extraction from genital.

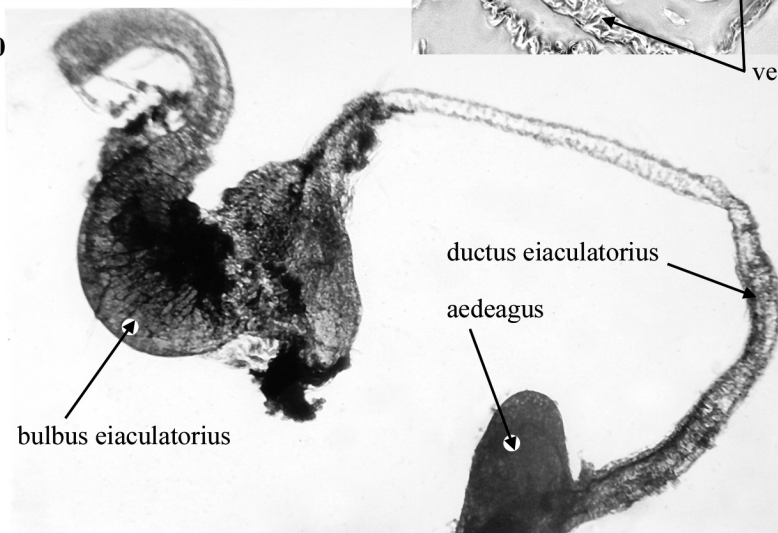


Figs 33 - 36. 33 - 34. *E. subumbrata* . 33. Aedeagus in cross section. 34. The end of aedeagus without cornuti. 35 - 36. *E. abbreviata*. 35. Aedeagus after extraction from genitals. 36. Cross section with solitary cornutus.

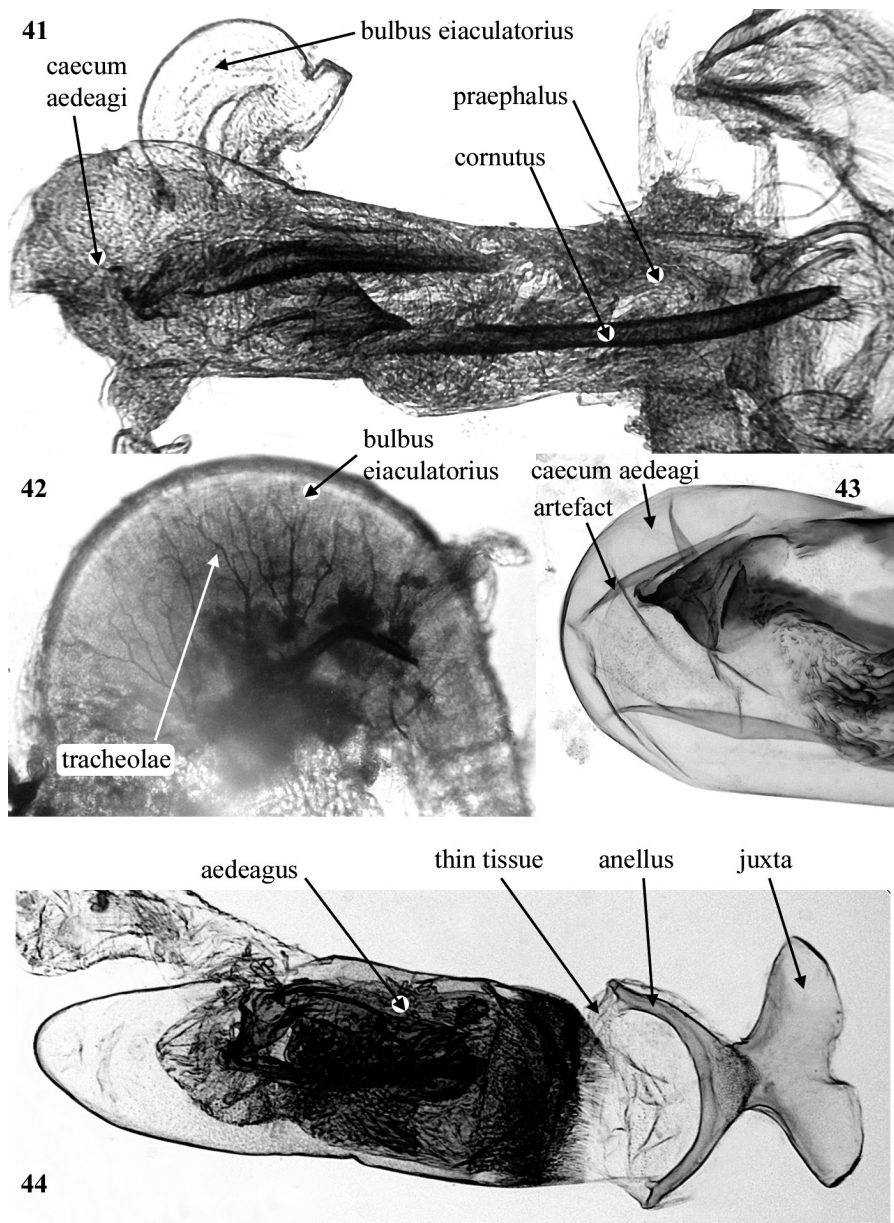
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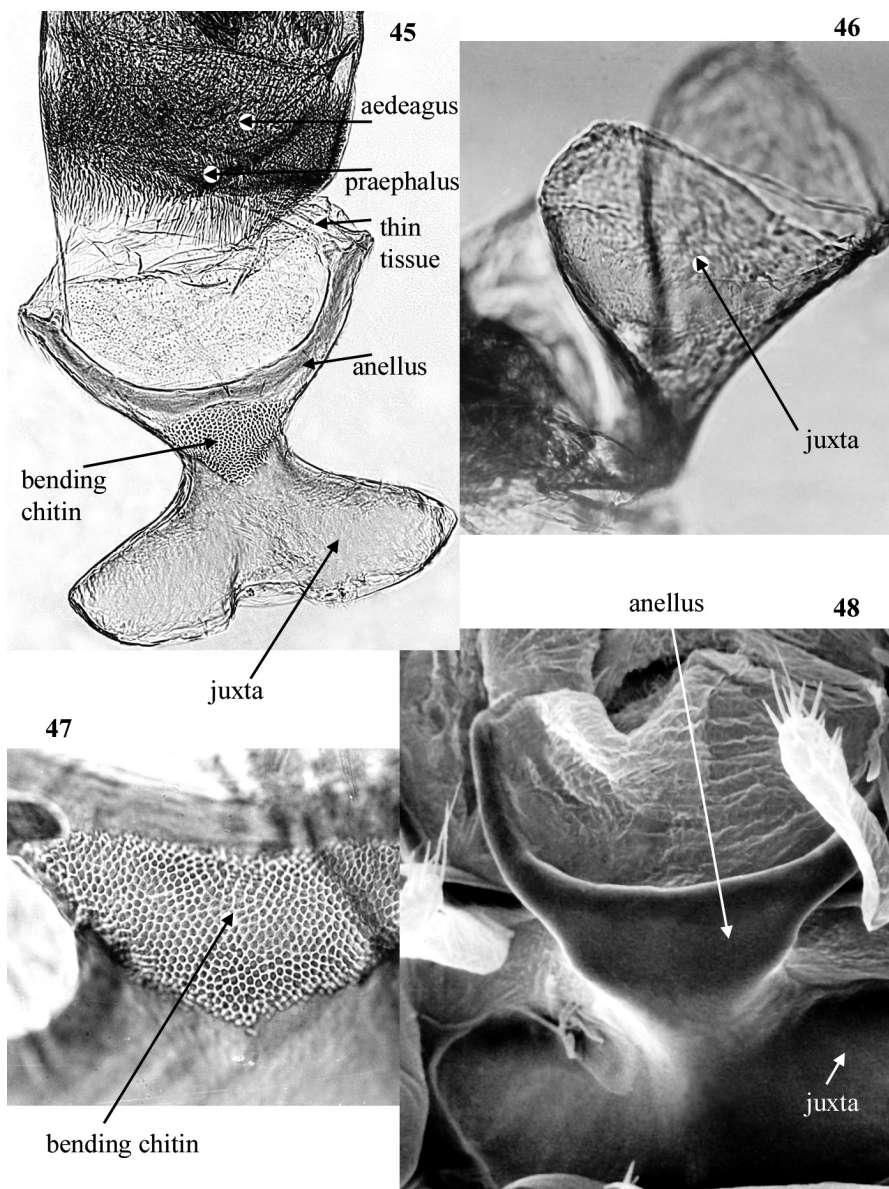
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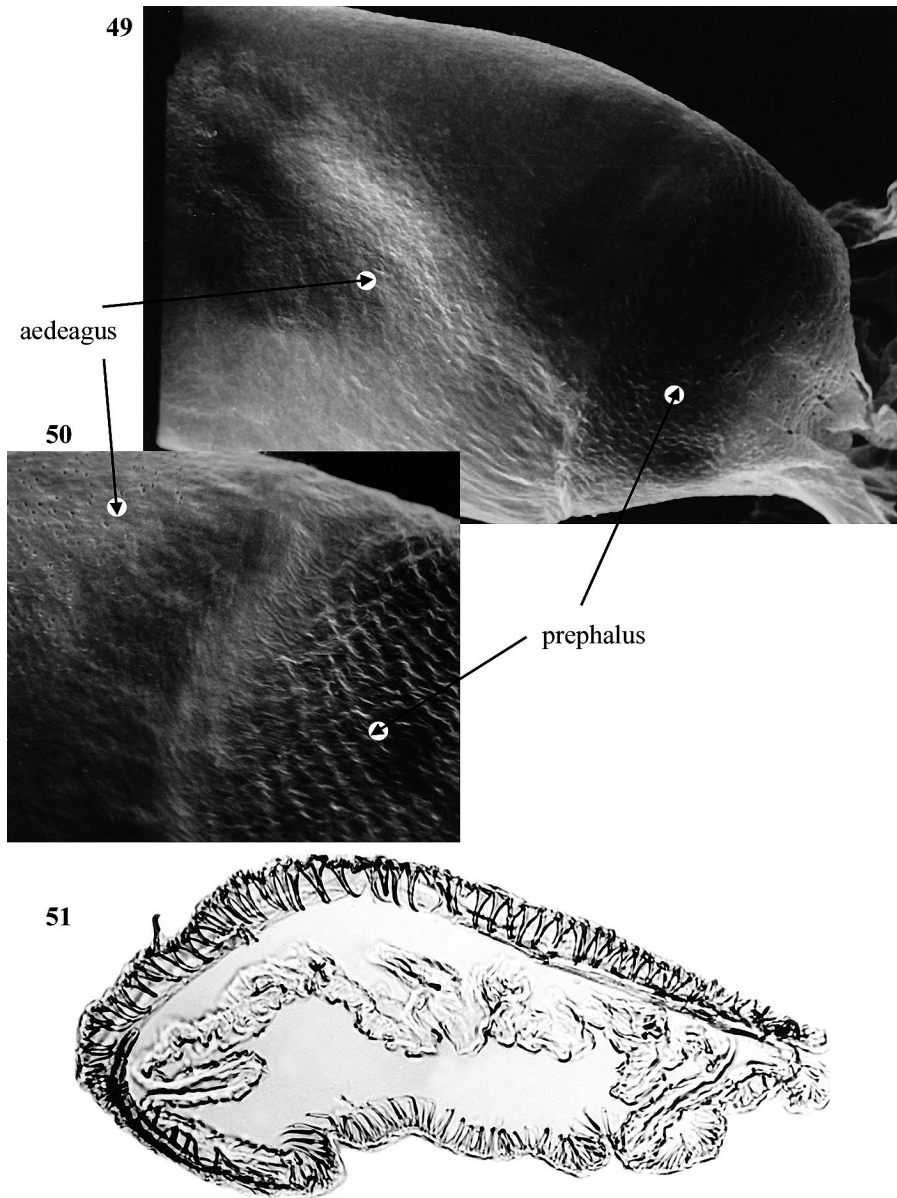
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Figs 45 - 48. 45. Connection of aedeagus to anellus. 46. Juxta the lateral view. 47. Bending chitin in the connection of juxta to anellus. 48. Juxta, anellus SEM Magn. 450x.



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Authors' addresses:

Vladimír KUBÍN
V rovinách 14
Praha 4
140 00
Czech Republic
E-Mail: morfologievk@seznam.cz

Josef PROCHÁZKA
Za Potokem 372
Nižbor
267 05
Czech Republic
E-Mail: prochazka.nizbor@seznam.cz

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A-4052 Anselden, Austria; maximilian.schwarz@liwest.at.

Redaktion: Fritz GUSENLEITNER, Biologiezentrum Linz, f.gusenleitner@landesmuseum.at;
Roland GERSTMEIER, Lehrstuhl f. Zoologie, TU München, gerstmei@wzw.tum.de;
Thomas WITT, Tengstraße 33, D-80796 München, thomas@witt-thomas.com
Berthold CLEWING, Akademischer Verlag München, avm@druckmedien.de;
Harald SULAK, Museum Witt München, h.sulak@atelier-sulak.de;

Mitarbeiter: Karin TRAXLER, Biologiezentrum Linz, bio.redaktion@landesmuseum.at;
Heike REICHERT, Museum Witt München, heike_reichert66@web.de;
Erich DILLER, Zool. Staatssammlung München, Erich.Diller@zsm.mwn.de.

Adresse: Entomofauna, Redaktion und Schriftentausch Thomas WITT, c/o Museum Witt München,
Tengstr. 33, 80796 München, Deutschland, thomas@witt-thomas.com;
Entomofauna, Redaktion c/o Fritz GUSENLEITNER, Lungitzerstr. 51, 4222 St. Georgen/Gusen,
Austria, f.gusenleitner@landesmuseum.at.

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