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A METHOD OF GENITALIA PREPARATION AND DRY PRESERVATION FOR COLEOPTERA

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Abstract.—A technique to evert, inflate, and dry preserve internal sacs of male genitalia is discussed. In addition to pigmented structures, many internal sacs observed have an asymmetrical form and microscopic structures on the white-fleshy parts. This technique is compared to more widely used methods of genitalia preservation and study.

Key Words: Genitalia, SEM, preparation, internal sac

Male genitalia and associated structures (collectively called terminalia) are utilized for taxonomic and systematic studies in most insect taxa. The male internal sacs are often ignored in studies of taxa where the sclerotized parameres are distinct among species. Internal sacs can yield additional information in taxa where the parameres are similar among species (e.g. Chandra 1991).

Most methods of internal sac (IS) (or endophallus) preparation and preservation are variations of two themes: 1) relax and mount on microscope slides, or 2) relax and store with glycerin in microvials. Microscope slides are mostly used for small genitalia that are studied at high magnifications with a compound microscope (transmitted light). With this method there are moderate distortions due to the weight of the coverslip. Some researchers omit the slides altogether and preserve the genitalia on a paper point in a drop of mounting medium (Angus 1969: 2).

Larger genitalia are stored in microvials under glycerin. The glycerin preserves the soft tissues and prevents them from drying out. This method allows study with the dissecting microscope (reflected light) and avoids distortions due to slide mounting. Some researchers combine the two methods by dissecting the IS's, cutting them down one side and mounting them flattened out on a microscope slide. With this variation all three-dimensional information is lost.

Internal sacs can be studied retracted (folded and held within the dark sclerotized genitalic structures) or everted (extended and swollen as during copulation). Occasionally a specimen is killed with the genitalia everted, but this is not a common occurrence. Techniques to evert the IS's are few and are usually delicate procedures.

Retracted IS's are studied with transmitted light in various small-sized taxa where everting the tissue is difficult or impossible (as illustrated in Gordon and Cartwright 1980, 1988). Larger insects IS's are often everted by pushing and pulling the relaxed tissues with hook-tipped pins or forceps (Sharp and Muir 1912, Sharp 1918, Howden 1982, d'Hotman and Schöltz 1990). After all of the work to prepare genitalia, most attention is given only to the pigmented structures on the IS's.

D'Hotman and Schöltz (1990) everted, compared, and illustrated the genitalia of many scarab beetles showing asymmetrical IS's (e.g., Figs. 1, 2). Thompson (1988) de-

veloped a glycerin-inflating technique to study the fleshy IS's of *Leptostethus* weevils. In both of these studies the IS's were observed and stored in glycerin. Thompson's inflation technique worked well, but the inflated IS's could be studied only when attached to the apparatus and under a dissecting scope. Once the genitalia are removed from the apparatus, they presumably deflate.

The intent of this study was to develop a method where IS's could be easily everted, preserved dry without collapse in their three-dimensional form, and studied under a scanning electron microscope (SEM).

Materials and Methods

This technique involved two major steps: 1) eversion and potential inflation of the IS, and 2) drying the genitalia.

Dry museum specimens were relaxed in a weak solution of detergent water (approximately 1 part detergent : 9 parts water), and the genitalia dissected. The IS's were everted manually with hook-tipped pins and jewelers forceps as in Sharp and Muir (1912: 483–484). These specimens were dried for study, but inflating attempts failed and the tissues remained folded and wrinkled. Inflation with a syringe (Hardwick 1950) did not improve the results.

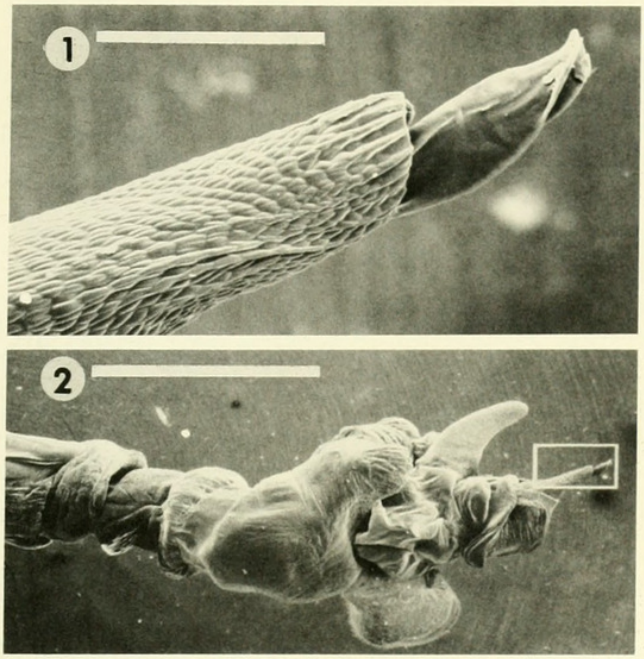
Inflated IS preparations were made from freshly killed specimens, the fresher the better. Rates of tissue hardening varied greatly among specimens depending on size, strength of tissues, and method of killing. Cyanide or ethyl acetate produced the best results. Alcohol submersion worked adequately but appeared to kill the insects in a tense state and rapidly hardened the tissues. Few good inflations were made from old alcohol-killed and preserved specimens.

After the insect died, the genitalia was removed and placed in weak detergent water solution (1 part detergent : 9 parts water). Care was taken to remove genitalia with the associated glands and musculature intact. The genitalia were then covered with detergent water solution, 1 ml in a 4 ml vial was sufficient. The fresh tissues retained much of their membrane integrity and swelled under osmotic pressure. Genitalia were then placed between the thumb and forefinger holding the glands and musculature. With a slow, gentle rolling motion (like squeezing tooth-paste out of a tube from the bottom to top) the tissues were forced up into the median lobe causing the IS to emerge from the apex. A similar technique for everting genitalia has been done with live camel crickets (Orthoptera: Gryllacrididae: *Ceuthophilus*) by T. J. Cohn and the late T. H. Hubbell (unpublished) and is also mentioned by Sharp and Muir (1912: 483). Hook-tipped pins or forceps were often helpful in this process.

Genitalia were again placed in the detergent water solution and the "squeezing" process repeated until the genitalia remained inflated. The time required in the detergent solution varied dramatically among specimens. Larger genitalia often took several days in the solution and several "squeezings." Small genitalia often required only one "squeezing" and a few hours in the detergent.

Delicate IS's could be fixed or hardened before drying. Various chemical fixatives like osmium tetroxide, hexamethyldisalizane (Nation 1983), or formaldehyde may be used. See Glauert (1980) for discussions of various fixation techniques which can be used. I did not employ any of these for this study.

The specimens, once everted and/or inflated, are ready to be dried with the critical point dryer. Other drying techniques (i.e. freeze drying) may be useful, but they were not used here. To be dried in a critical point dryer, the specimens needed to be dehydrated through alcohol baths into 100% ethanol. I raised the alcohol percentage in the vials by slowly adding 70% isopropanol; a few drops at first, then doubling the volume. When near 70%, I poured off the liquid and added straight 70% alcohol. From there a



Figs. 1–2. *Canthon pilularius* (Linnaeus) (Scarabaeidae) genitalia with everted internal sac, dorsal view. 1, Line = 0.18 mm. 2, Line = 1.83 mm.

normal dehydration series was used. I left the specimen in each liquid change from 1 to several hours allowing ample time for the specimen to come to an equilibrium with the solution before changing it.

Once dehydrated and in the third change of 100% ethanol, genitalia were critical point dried with a Tousimis, Samdri®-780A. The genitalia were mounted on a paper point and pinned under the rest of the specimen for study with a dissecting microscope or coated and studied under a scanning electron microscope (SEM). Specimens illustrated here were coated with gold-palladium in a Denton Vacuum DESK II sputter coater and photographed with a Hitachi S-570 SEM. Specimens studied are deposited in the Florida State Collection of Arthropods, Gainesville, Florida.

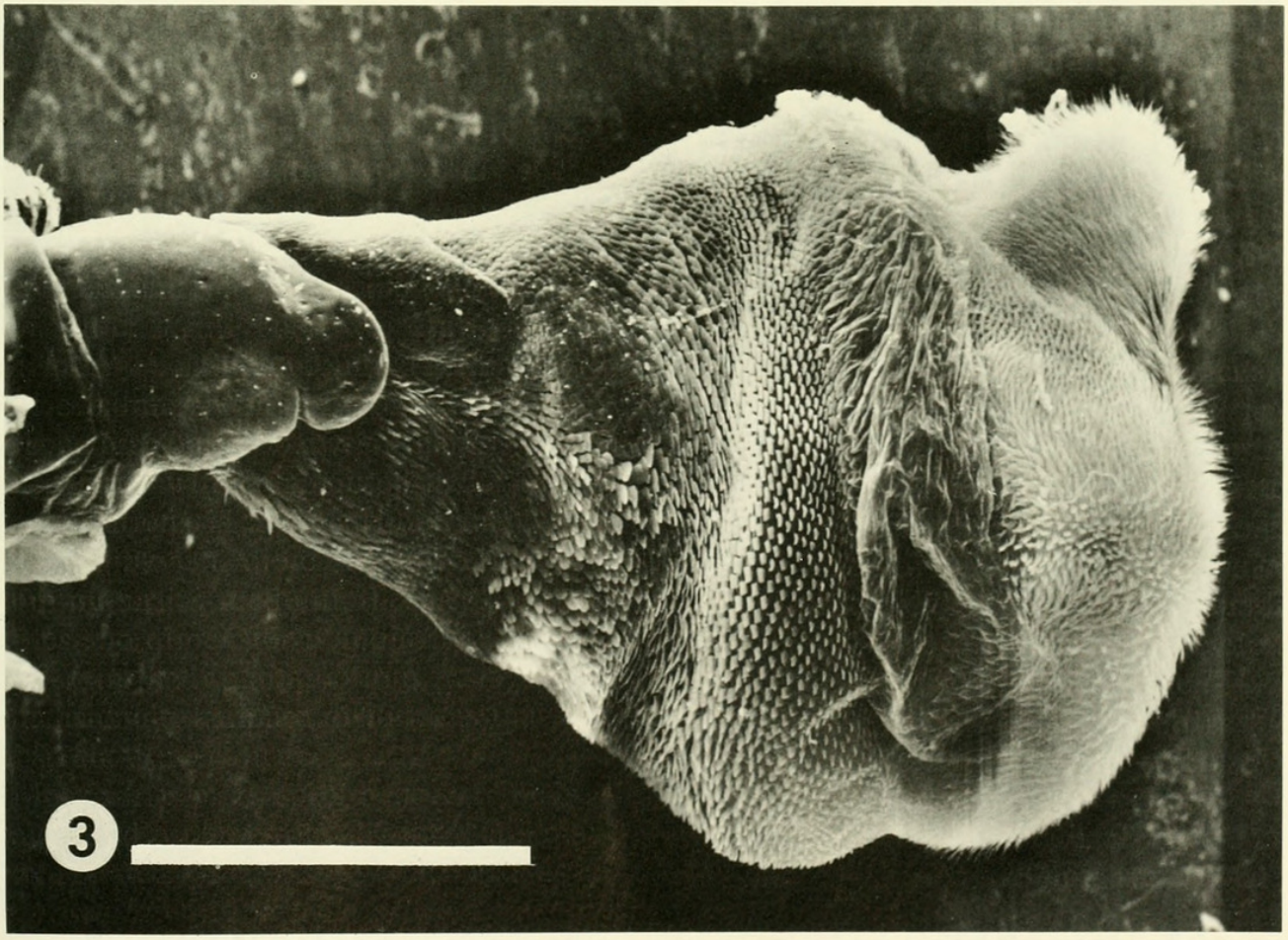
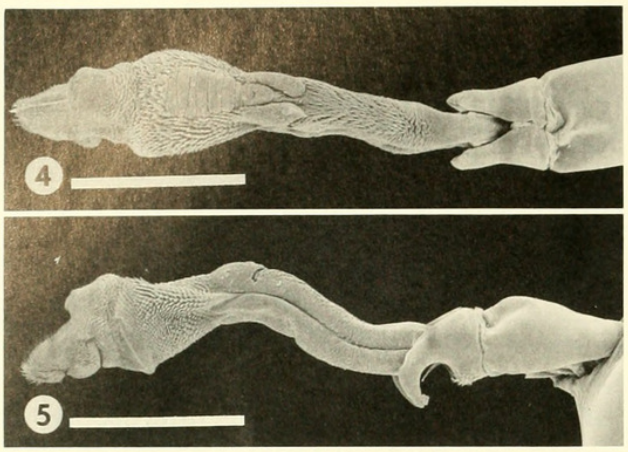


Fig. 3. *Ataenius saramari* Cartwright (Scarabaeidae) genitalia with everted internal sac, lateral view. Line = 0.23 mm.



Figs. 4–5. *Platytomus longulus* (Cartwright) (Scarabaeidae) genitalia with everted internal sac. Line = 0.20 mm. 4, Dorsal view. 5, Lateral view.

Results

The original work was done on *Phyllophaga* (Scarabaeidae) while helping to prepare the SEM genitalia illustrations in Woodruff and Beck (1989). The technique proved useful on various other families and scarab genera, a few of which are illustrated.

In liquid IS's are clear, except for the obvious pigmented structures. When they are critical point dried, soft tissues turn an opaque white and any internal structures are obscured. This white tissue contrasts with pigmented structures and can be studied under a dissecting microscope at lower magnifications (20–100×). Specimens coated for study at higher magnifications (100–1000×) with the SEM lost this contrast. Examination with the SEM revealed varying amounts

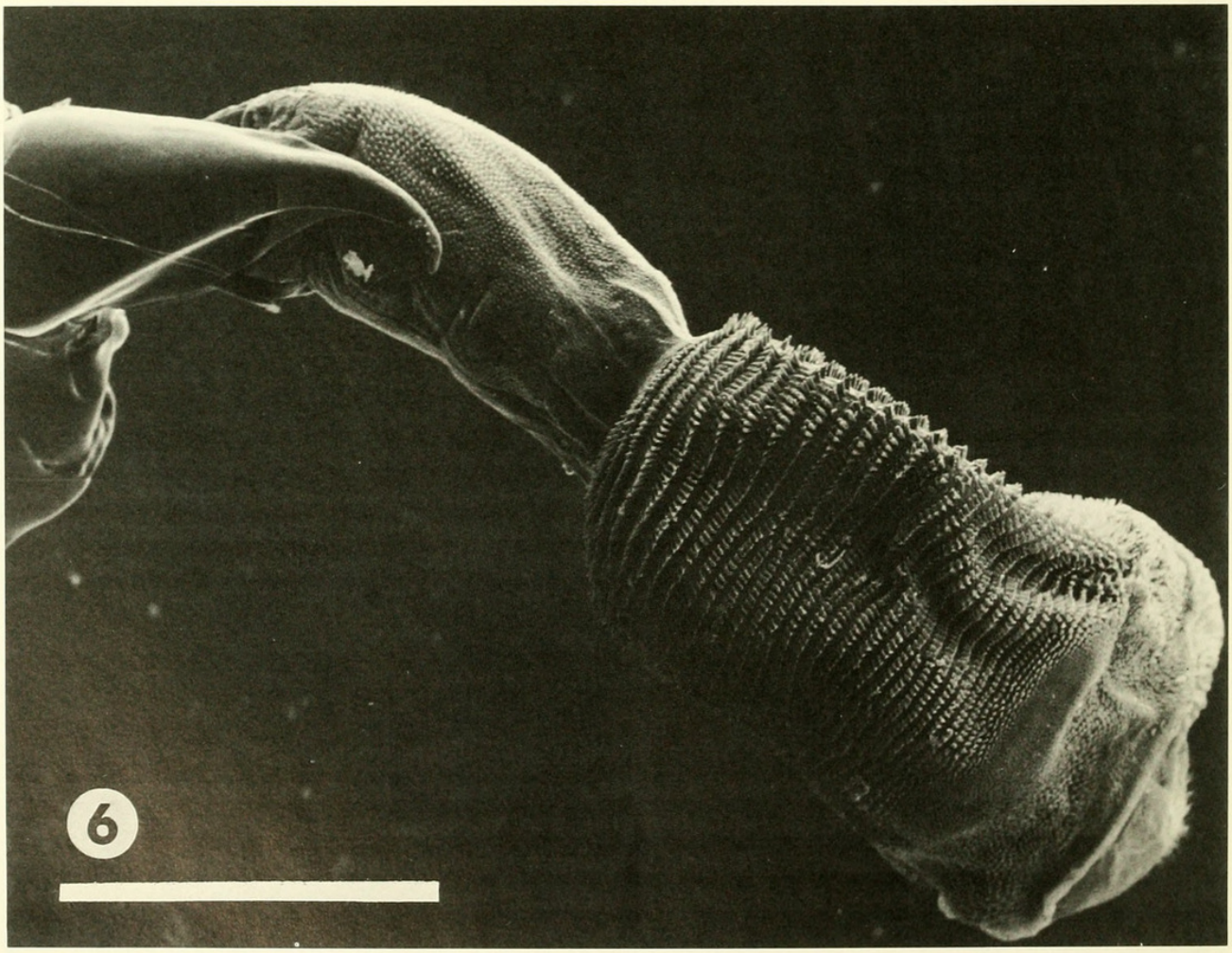
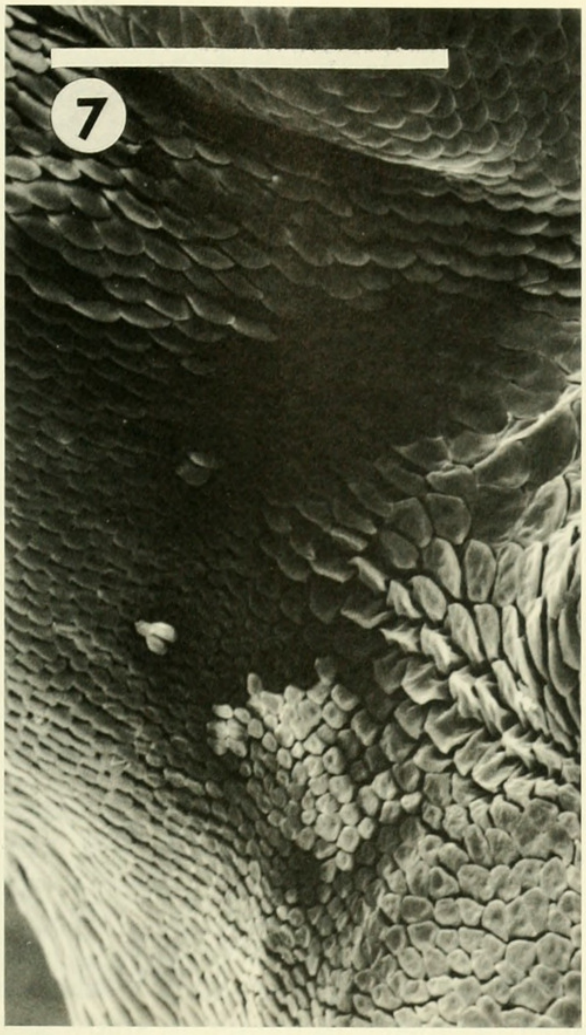
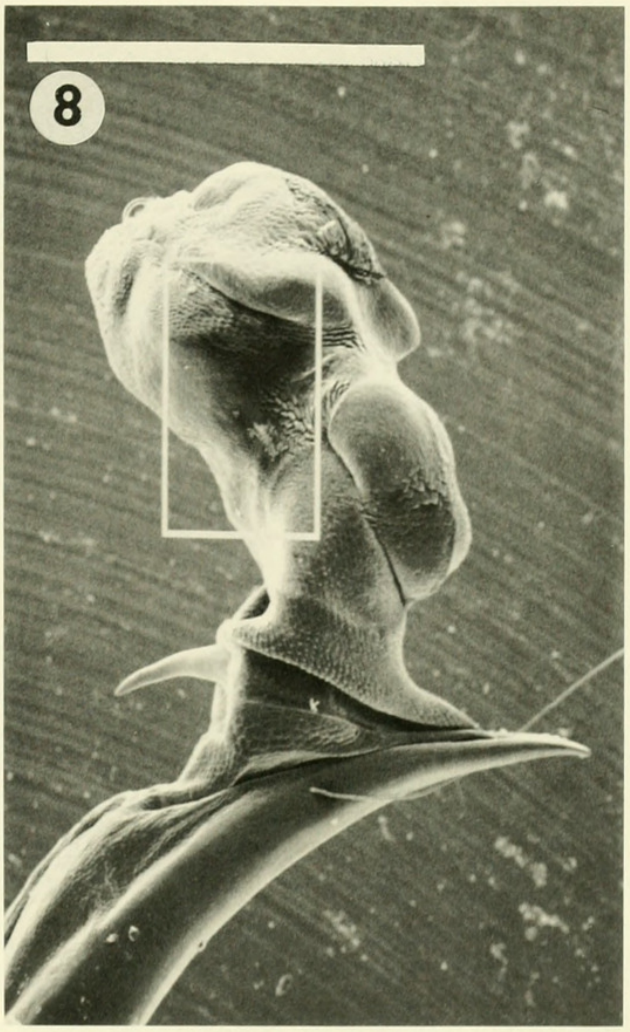


Fig. 6. *Aphodius badipes* Melsheimer (Scarabaeidae) genitalia with everted internal sac, lateral view. Line = 0.60 mm.





Figs. 7–8. *Notiobia terminata* (Say) (Carabidae) genitalia with everted internal sac, lateral view. 7, Line = 0.15 mm. 8, Line = 0.75 mm.

of microstructure on the non-sclerotized tissues not seen with the light microscopes (Figs. 6–13). Small beetles, like *Ataenius saramari* Cartwright (Fig. 3) and *Platytomus longulus* (Cartwright) (Figs. 4, 5), have unpigmented IS's which, under a light microscope, seem to lack structures. The SEM shows this to be in error, as their IS's are covered with patches of scales and spines. The potential usefulness of the SEM in studying IS's for taxonomic and systematic problems is tremendous.

The ease of eversion and inflation was largely dependent upon the amount of tissues associated with the IS. Many scarabs had IS completely enclosed with muscles. These muscles acted like the bulb on an eyedropper, making eversion and inflation easy. Beetles like *Ischyrus* (Erotylidae) simply had strands of muscles and little associated tissue. These genitalia were only everted manually with pins and forceps and inflations were not achieved. In contrast, the complexity of certain dynastine scarab (*Strategus*) IS's made eversion difficult by any method and I have not produced a good preparation.

Discussion of Techniques

All three preparation and storage techniques (microscope slide, microvial, and critical point dried) for IS studies have benefits and limitations. The method used is always limited by the availability of specimens, equipment, and the needs of the researcher.

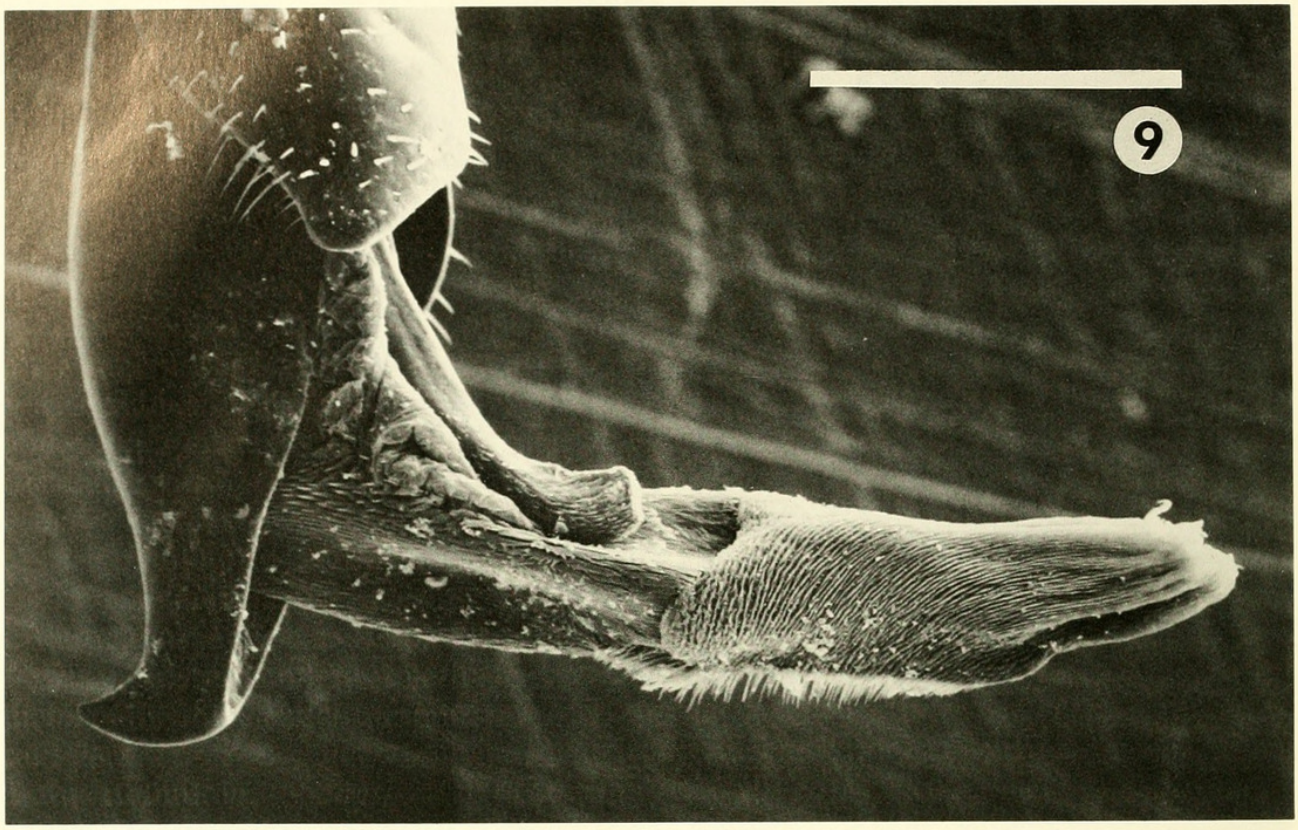
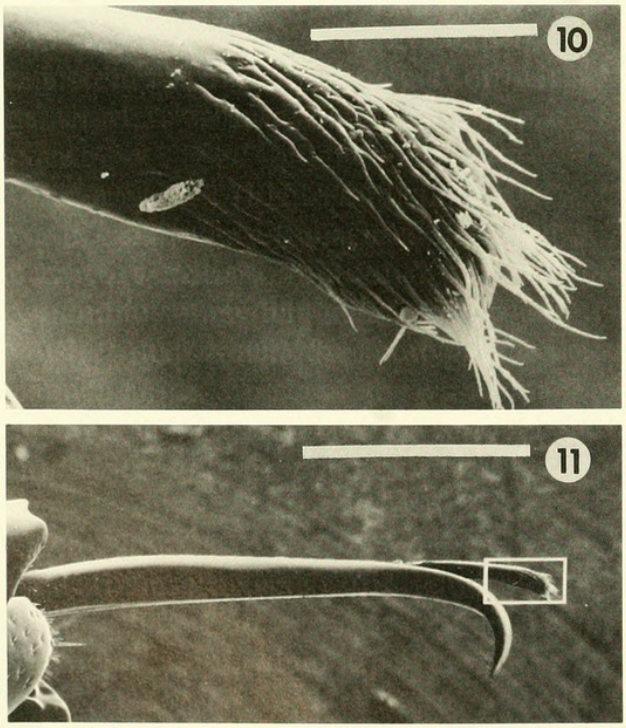


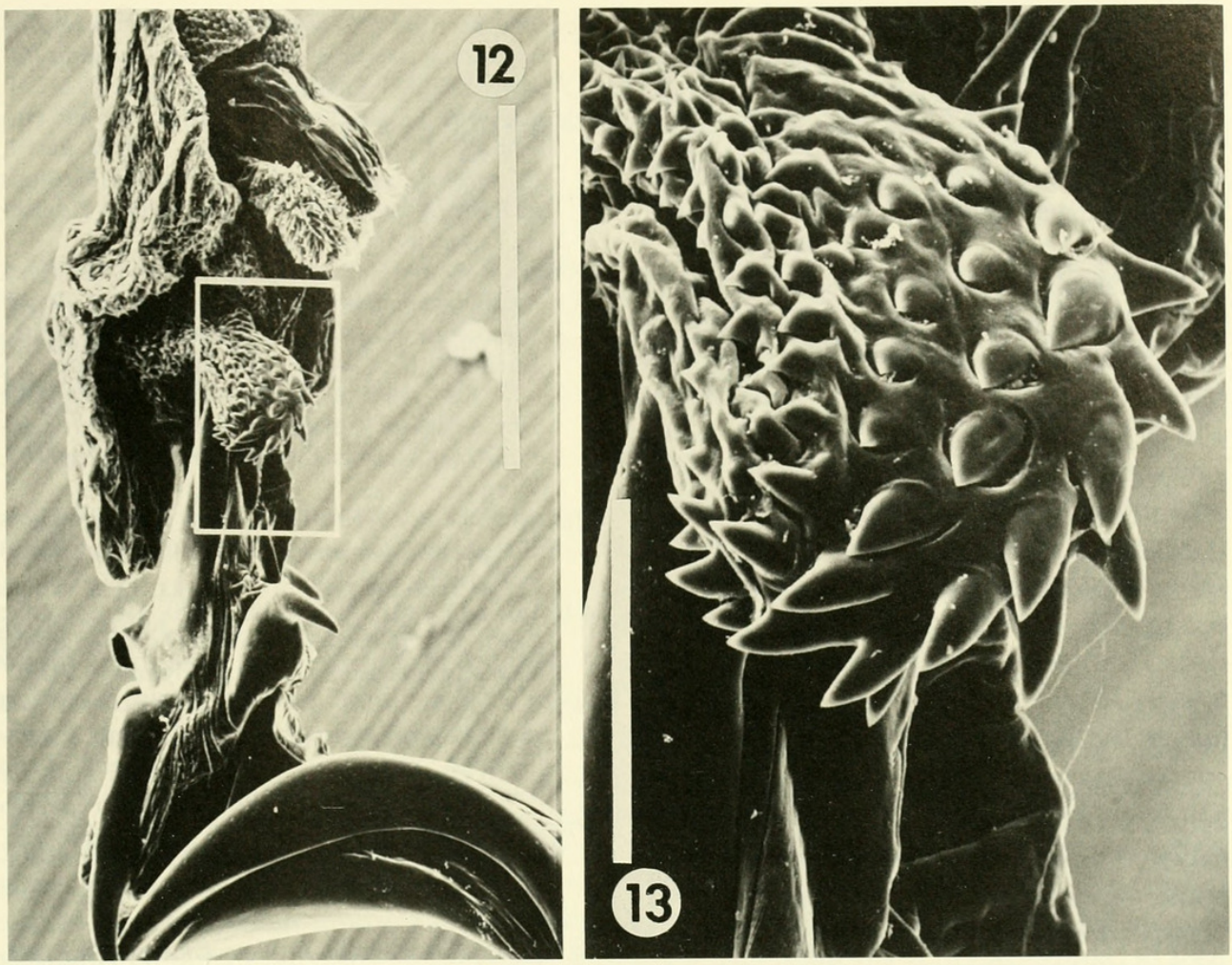
Fig. 9. *Epicauta heterodera* Horn (Meloidae) genitalia with everted internal sac, lateral view. Line = 0.23 mm.



Figs. 10–11. *Saprinus lugens* Erichson (Histeridae) genitalia with everted internal sac, lateral view. 10, Line = 0.06 mm. 11, Line = 0.60 mm.

Microscope slide mounting is the most widely available technique to study specimens at high magnifications. Genitalia can be adequately prepared from dried museum specimens and internal features studied with transmitted light. There are drawbacks to this technique. Slide mounted genitalia are kept separate from the pinned specimen, and the association between them is easily lost. Clarity with transmitted light at high magnifications is adequate but the fleshy microsculpture is difficult to discern. Once set in mounting medium, the specimen becomes two dimensional and can be viewed only from the top and bottom. Being three dimensional, there are distortions that occur as they are flattened.

Microvial-stored specimens can be removed and studied from all views, and may be re-inflated for later study if needed, as in *Leptostethus* preparations described by Thompson (1988). Specimens stored this



Figs. 12–13. *Listronotus echinodori* O'Brien (Curculionidae) genitalia with everted internal sac, lateral view. 12, Line = 0.43 mm. 13, Line = 0.086 mm.

way are generally larger and can be studied with both transmitted and reflected light. Pigmented structures, both internal and external, are obvious and easily studied, whereas unpigmented structures are difficult to discern. Microvials and glycerin are readily available. Problems can arise with the extra weight of the vial when added to the insect pin. The bulk can become a nuisance in usurping storage space and aid in dislodging specimens from the box bottoms. If too much glycerin is used, it can seep through the microvial stopper and soil the label or the specimen. If the specimen is large and takes up most of the insect pin, the microvial is pinned separately next to the specimen. This can lead to a loss of the association between the specimen and its genitalia.

Genitalia dried with a critical point dryer can be studied with a dissecting microscope or a SEM. Being dry, the genitalia can be pinned under the specimen with no chance for loss of the association. The genitalia are light in weight and take up little space, decreasing potential hazards to other specimens. The genitalia contain no liquid which can soil the specimens, although the body oils from specimens may soil the genitalia. The limitations of this technique can be formidable. The equipment needed is expensive and many institutions may not have a critical point dryer available. Dry museum specimens can be used, if a certain amount

of wrinkling is acceptable. The best preparations come from freshly killed material, thus, rare or endangered taxa may be unavailable for study. Critical point drying turns the tissues opaque, eliminating study by transmitted light, but allows study with a SEM.

The use of critical point dryers and SEM's in IS studies have advantages and difficulties depending on the taxa. The exact procedures used for inflating, potential fixation, and drying IS's will vary with each taxon. The method described is not useful for all taxa. Because of the taxonomic information that can be gained, this method should be considered in systematic studies.

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