SCIENTIFIC NOTE

Alternative Techniques to Study Characters of the Genitalia in Lepidoptera

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ABSTRACT - The present note aims to describe two alternative methods for observing genitalia in Lepidoptera. The first one provides means to examine both male and female genitalia without spoiling the scales of the abdomen, preserving it attached to the thorax and aesthetically similar to an unexamined specimen. The second one provides ways of observing certain characters on the male genitalia in a non-destructive way, and does not depend on time-consuming removing and dissection of the abdomen. It is expected that the presented techniques will help on morphological studies and on identifying similar species which consistently differ in genitalic armatures.

KEY WORDS: Morphology, identification, valva, uncus

As noted by Niculescu (1976), many authors recognize the importance of the genitalia as the most informative specific character in the Lepidoptera, especially the valvae of the male genitalia due to their variation (Fox 1953). It is well known that closely related species or species under similar selective pressures tend to show similar patterns, and those patterns are often obscured by intraspecific variations and/or seasonal forms, thus adding an element of confusion in identification. Several of these species are only distinguished by genitalic preparations. The present scientific note aims to make known two alternative techniques to access these structures.

The first technique provides means to remove both male and female genitalia without spoiling the scales of the abdomen, preserving it attached to the thorax and aesthetically similar to an unexamined specimen, while allows morphologic study. The researcher should carry out the following steps: 1) detach the abdomen from the thorax and sink it in distilled water for some 18h to 24h; 2) transfer the abdomen to a petri dish with paraffin wax on the bottom filled with water; 3) fix the abdomen sideways with thin insect pins (size 0 or 1) and cut it antero-posteriorly through the pleura using ophthalmologic scissors (Fig 1a), and cut throughout the inter-segmental membrane between the seventh and eight segments in females or between the eighth and ninth segments in males (Fig 1b); 4) open the abdominal cavity, fixing the tergites and sternites with thin insect pins on the paraffin wax; 5) using a thin forceps, remove the genitalia and with it the abdominal content. After removed, the genitalia may be further prepared as usual. Be careful while removing membranous parts of the genitalia, especially the female's bursa copulatrix which is very easily damaged. To preserve definitively after dissection, put the genitalia in a glycerin-filled small test tube, which should be pinned with the insect afterwards; 6) put the emptied abdomen resting on absorbing paper for a while, and then mount it with insect pins on polystyrene foam. Take it to a low temperature stove (*circa* 30ºC) for a couple of days until it dries completely, then reattach it to the thorax using white glue.

This method has proven to be useful for several taxa of Papilionoidea and Hesperioidea, especially in taxonomic groups where color and pattern of the abdominal scales are an important diagnostic character, as in the Pyrrhopyginae. Even though other methods had been proposed for studying the genitalia without spoiling the abdomen (Chacín 1992), the above-cited method is simpler and suits a wider range of taxa, including small and delicate specimens.

The second technique consists in direct observation of valvae and uncus of the male genitalia without the usual destructive sampling preparations. These structures can be observed by removing the scales of the tip of the abdomen and valvae. The mounted specimen should be fixed sideways in polystyrene foam or other support with the pleural side of the abdomen directed upward. Afterwards, under the stereoscopic microscope and using a thin forceps, the scales should be carefully removed (Fig 2a) and the area cleaned with a soft brush (Fig 2b). In case of asymmetrical genitalia, the procedure should be repeated in both sides.

Although that method is relatively well known among lepidopterists, it's surprisingly unknown among students and many entomologists, including those who curate entomological collections. An example is four closely related species of leafwing butterfly belonging to the genus *Memphis* (Hübner) that have marked resemblance in its facies, but very different genitalic armatures, especially in the posterior tip of the valvae. Their facies show overwhelming variation and overlapping phenotypes, but their genitalia have unique and stable characters. Those characters are readily observed with that technique, eliminating time-consuming and destructive genitalic preparations.

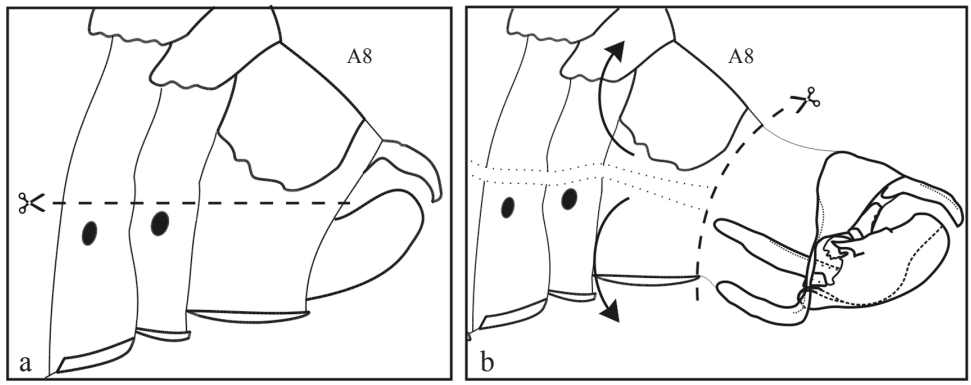

Fig 1 a) Antero-posterior cut through the pleura; b) Cut throughout the inter-segmental membrane between the eighth and ninth segments (male) and genitalia removal.

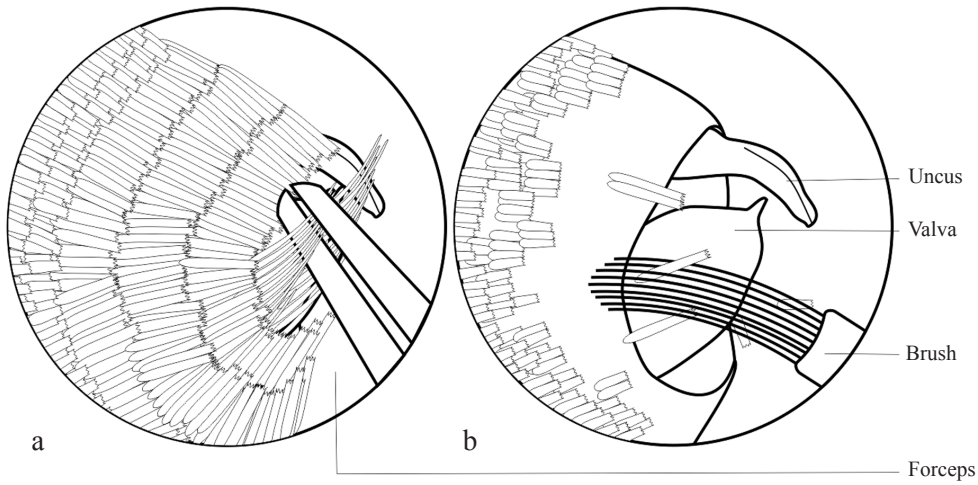

Fig 2 a) Forceps removing the scales from the abdomen and valvae; b) brush cleaning the area after the scales were removed by the forceps.

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