Moths under Magnification

Anthony Thomas (Canada)

Moth species diversity studies and or inventory faunal studies require that all specimens be identified. Most species of the larger moths (the 'macros') can be identified to species simply by a quick examination of the wing pattern. Some specimens may require a closer look and for these a photograph is often as useful as the actual specimen. However there are a few species that are such close 'look-alikes' that the genitalia have to be examined. This requires that these moths be dissected. Very worn individuals of 'macros' and many of the small so-called 'micros' also have to be dissected for species determination. In rare cases wing-scale morphology is useful to separate sibling congeneries.

Methods
There are at least two text descriptions and one on-line description of dissection methods for genitalia (Refs. 1, 2, 3).

Basically, the technique involves removing the abdomen and placing it in 5-10% potassium hydroxide (KOH) overnight. After rinsing in water the genitalia can be teased out from the abdomen using fine forceps. After further rinsing in water, to remove all traces of KOH, the male genitalia can be placed directly in glycerine for examination. Female genitalia are more delicate and it is best to place them in very dilute glycerine, about 5%, and leave until all the water evaporates. Of course, genitalia can be made into permanent mounts using standard techniques.

For examination and photography the standard practice, for males, is to open the valves and examine with the ventral side up. Also, the aedeagus is normally removed but kept with the rest of the genitalia.

Wing-scale morphology is best examined at about 20x or higher magnification.

Use of genitalia to separate sibling species: 1 Crocus Geometers.
In my area there are two medium-size (wingspan about 4 cm) conspicuous yellow moths that show enough intra-specific variation in wing pattern that identification to species requires examination of the genitalia. Figure 1 shows the wing pattern of a Crocus Geometer (*Xanthotype sospeta*) on the left and a False Crocus Geometer (*Xanthotype urticaria*) on the right (note the missing tips of the abdomens – the parts containing the genitalia).

Fig. 1

The False Crocus Geometer is often darker and with more purplish spotting, but variation and wear can change the appearance.

The inter-specific variation for both the male and female genitalia are consistently different but I will detail just the males in this exercise. When first removed from the abdomen, the male genitalia resemble a tiny clam with the two halves opening on the ventral mid-line (Fig. 2, False Crocus Geometer).

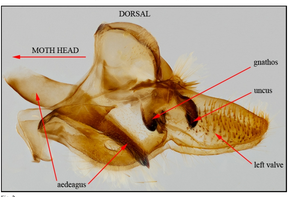

Fig. 2

The major parts useful for species recognition are the two valves, the uncus, gnathos, and the aedeagus. For diagnostic purposes the valves are spread apart and the genitalia flattened as much as possible, without distorting the parts, and mounted such that the valves are pointing upwards and to the sides (Fig. 3).

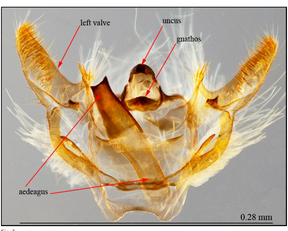

Fig. 3

The aedeagus (phallus, penis) is normally removed but kept with the rest of the genitalia. In these species the difference in the shape of the aedeagi is so clear that is is not necessary to isolate it; its shape is the most obvious feature for separating the males. Note the narrow aedeagus in the False Crocus Geometer (Fig. 3) vs. the bulbous aedeagus in the Crocus Geometer (Fig. 4).

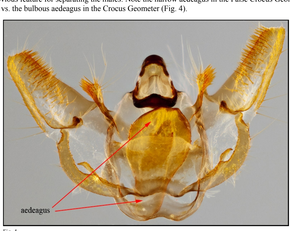

Fig. 4

Apart from the differences in the aedeagi the male genitalia are very similar. In other congeric species the differences are often far greater.

2 Other species
As depicted in the previous images the aedeagi are shown in a 'contracted' state. During mating the internal components of the aedeagus are everted under pressure. For some sibling species it is necessary to inflate the aedeagus to get a specific identification. I have had limited success with the inflation technique but have had a positive result with a Many-lined Wainscot (*Leucania multilinea*) (Fig. 5). The aedeagus has been removed from the genitalia in the left image and has been everted in the far right image. Note the sclerotized terminal spine and the spiral band of spines (termed cornuti). These spines help to provide a grip within the female's reproductive system. Note also the arrow-shaped uncus and the complex valves.

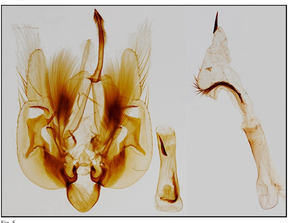

Fig. 5

Use of wing-scales to separate sibling species.
The NE North American Sallow Moths (*Eugnilla* spp.) include 6 species that emerge in the Autumn and overwinter as adults, laying eggs in early Spring. There is one species in the UK, the Satellite (*Eugnilla transversa*), with a similar life-cycle and which superficially resembles the Sidus Sallow of North America (Fig.6).

Fig. 6

In NE North America these Sallows can be separated into two groups based on wing-scale morphology. In the group containing Morrison's (*E. morrisoni*), Straight-toothed (*E. vinulenta*), and Lost (*E. devia*) most of the forewing scales are of the normal form (Fig. 7 left, *vinulenta*). In the other group containing Three-spotted (*E. tristigmata*), Sidus (*E. sidus*) and Franclemont's (*E. cirrpalea*) the outer angles are drawn out into long divergent curving spines which interlock to give the wing, what Forbes called, "*a sort of shredded wheat effect*" (Fig.7 right, *sidus*).

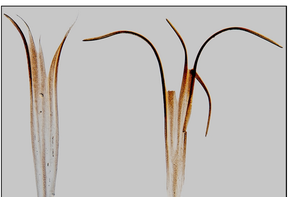

Fig. 7

In the 'normal-scales' group there are a few patches of long-spined scales at the wing base (Fig. 8 left, *vinulenta*) but these are never as long as the spines on the aberrant group (Fig. 8 right, *sidus*). Forbes commented that these super-long-spined scales are not found in any of the other NE North American Noctuidae (Owlet Moths). I am curious to know into which group the European Satellite belongs.

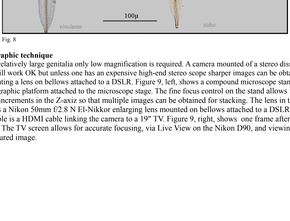

Fig. 8

Photographic technique
For the relatively large genitalia only low magnification is required. A camera mounted of a stereo dissecting scope will work OK but unless one has an expensive high-end stereo scope sharper images can be obtained by mounting a lens on bellows attached to a DSLR. Figure 9, left, shows a compound microscope stand with a photographic platform attached to the microscope stage. The fine focus control on the stand allows for micron increments in the Z-axis so that multiple images can be obtained for stacking. The lens in this image is a Nikon 50mm f/2.8 N El-Nikkor enlarging lens mounted on bellows attached to a DSLR. The grey cable is a HDMI cable linking the camera to a 19" TV. Figure 9, right, shows one frame after capture. The TV screen allows for accurate focusing, via Live View on the Nikon D90, and viewing of the captured image.

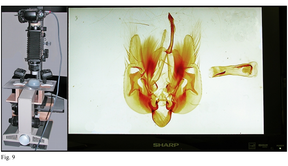

Fig. 9

For the image of the everted aedeagus (Fig. 5, far right) a Nikon 2.5x M Plan objective was used on the bellows with much less extension. For the wing images (Fig. 7) a Nikon 20x M Plan ELWD objective was used. The individual scale images (Fig. 8) were with an Olympus 10x S Plan objective on a compound microscope with a 1.25x intermediate lens + a 2.5x relay lens.

Making genitalia preparations is not for everyone but as Winter stated "*a well prepared slide is unsurpassable for identification, can be a thing of beauty, and makes an intriguing subject for the photographer*".

Relevant sites & References
For UK and European moths "The Lepidoptera Dissection Group" web page has many images and actively solicits images for non-illustrated species:
http://www.dissectiongroup.co.uk/page44.html

For North American species "The Moth Photographers Group" has several images:
http://mothphotographersgroup.msstate.edu/GenitaliaIndex.shtml

1] Holloway, J.D. et al. 1987. CIE Guides to Insects of Importance to Man. 1 Lepidoptera. CAB International Institute of Entomology. London. pp. 14-16, Genitalia Dissection.

2] Winter, W.D. jr. 2000. Basic Techniques for Observing and Studying Moths & Butterflies. The Lepidopterists' Society Memoir Number 5. Los Angeles. Chapter 9 pp. 265-276.

3] http://www.dissectiongroup.co.uk/page37.html

Forbes, W.T. M. 1954. Lepidoptera of New York and Neighboring States. Part III Noctuidae. Cornell University Agicultural Experiment Station, Memoir 329. 433 pp.

Microscope and Photographic Equipment

My basic equipment is an Olympus BH2 with 2x, 4x, 10x, 20x, 40x, 60x, and 100x objectives; Olympus 2.5x NFK relay lens. I also have the components for Phase Contrast, DIC and Polarization. Camera is a Nikon D90 with Nikon PB-6 bellows; Nikon flash in place of Olympus' halogen lamp. For reflected light images I use Nikon CF objectives and El-Nikkor enlarging lenses.
Most images are stacks of several frames processed by Zerene Stacker.

Contact author, email: mothman AT nbnet DOT nb DOT ca

Published in the December 2011 issue of Micscape Magazine, www.micscape.org.