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Preparing wing venation slides of Microlepidoptera

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ABSTRACT. **Preparing wing venation slides of Microlepidoptera.**
Wing venation in Microlepidoptera used to be an important factor governing taxonomic divisions. Today, too, analysis of wing venation frequently supports the taxonomic classification of specimens. The methodology of making wing venation slides has been described in many papers, albeit rather superficially. For staining, various substances are used but these can be costly and difficult to obtain. The present paper describes a simple, inexpensive method of preparing microscope slides of the wing venation of Microlepidoptera. Such slides can be used for detailed microscopic analysis and for preparing drawings of the venation. The method has also proved its worth in the case of the wings of the smaller species of Macrolepidoptera.

KEY WORDS: Lepidoptera, Microlepidoptera, wing venation, microscope slides.

SLIDE PREPARATION

TOOLS AND REAGENTS. Before preparing slides, the following tools and reagents need to be to hand: precision tweezers; a few brushes of size 000, preferably made from natural hair; small glass vessels for staining and rinsing; microscope slides and slide covers; cavity slides with "wells"; an 0.5% aqueous solution of eosin; 20%, 70% and 99% ethanol; distilled water; Euparal; Euparal essence; strips of filter paper; a few blunt entomological pins; ordinary paper; pencil; self-adhesive paper; stereoscopic microscope.

SEPARATING THE WINGS FROM THE SPECIMEN. The right-hand pair of wings to be mounted are removed from the specimen. If this is dry, the wings can be broken off at the base using tweezers or pins; if fresh, the wings are cut off with a scalpel or small scissors. A paper label with the consecutive number of the slide is pinned below the specimen (Fig. 1). After preparation it should be replaced with a proper label, compliant with adopted standards.

Fig. 1. A specimen with the wings removed and a label showing the slide number (photo G. Banasiak).
Ryc. 1. Okaz z oddzielonymi skrzydłami i dołączoną etykietką z numerem preparatu (fot. G. Banasiak).

STAINING. Two self-adhesive labels are prepared with the same slide number of the specimen, written in pencil. One is attached to the staining vessel (Fig. 2), the other to the microscope slide. Pencil marks are resistant to alcohol and its vapour; whilst ball-point pens or fine-liners are not suitable. The wings to be mounted are dipped in 99% ethanol for 2-3 seconds, then steeped in an 0.5% aqueous solution of eosin for about 24 hours. Leaving the wings in the staining solution for a further 24 hours does not significantly improve the staining effect, is unnecessary, and only prolongs the mounting process. If after 24 hours the wings are not uniformly stained, or the staining is patchy, staining can be continued for another 15-20 hours after the wing has been descaled. It is important that the wings are completely submerged in the staining solution. To avoid problems, it is recommended to stain one pair of wings from the same specimen in one vessel.

Fig. 2. A vessel containing eosin with a numbered adhesive label (photo G. Banasiak).
Ryc. 2. Naczynie z eozyną i naklejonym numerem preparatu (fot. G. Banasiak).

DESCALING THE WINGS. The stained wing is rinsed in distilled water and then placed in a slide well containing a few drops of 20% ethanol. Under the stereomicroscope the wings are descaled using two fine brushes: one is used to delicately hold the wing in place, while the other is drawn across the wing from the base to the apex (Fig. 3). If too many scales appear in the liquid, the excess can be drawn off with a scrap of filter paper held in tweezers. Fresh 20% ethanol is then added and the cleaning can continue. Both sides of the wing should be cleaned, delicately and slowly. If the brush movements are too fast or too rough, the wing membrane is liable to break. Particular care is required when preparing slides from old specimens, as these are easily damaged and the scales do not come off so easily. Once all the scales have been removed, the wing is rinsed in 20% ethanol and progress is checked under the microscope. Descaling can be resumed if necessary.

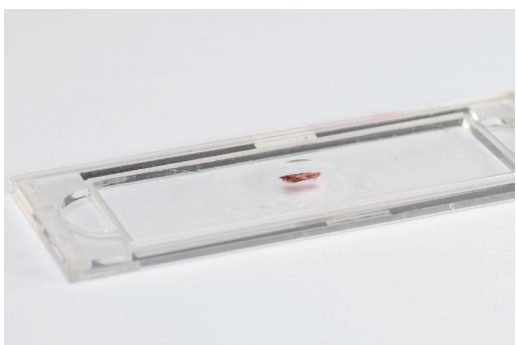

Fig. 3. A wing in a cavity slide (photo G. Banasiak).
Ryc. 3. Skrzydło na szkiełku z łezką (fot. G. Banasiak).

DEHYDRATION. The wings are now briefly dipped in ethanol – in 70% for 10-15 seconds and in 99% for a few seconds. Longer immersion in ethanol causes the wings to become very brittle and easily broken. They are then placed in a few drops of Euparal essence for about a minute. Care should be taken to ensure that the wings are totally submerged in the essence. It is also important to remember not to use the 99% dehydrating ethanol for more than 2-3 slides, as after a few immersions it becomes diluted and is less effective.

PREPARING THE SLIDE. The previously written numbered label is stuck on to the slide. This is now placed in a frame, beneath which is a template of the same size as the cover slip (Fig. 4). Using a frame and template is not absolutely necessary, but it does ensure that the slides are uniformly laid out and that the cover slips lie evenly. A drop of Euparal is now placed on the slide and spread with the entomological pin to the area roughly the same as that of the cover slip (the template is very helpful here – Fig. 5). One drop is an optimal quantity for a 16x16mm cover slip. If larger cover slips are used, then the amount of Euparal has to be proportionally greater. The wings are transferred from the Euparal essence to the slide and, under the microscope, with the aid of two blunt pins or fine brushes, they are carefully straightened out and their position adjusted, so that they lie in the middle of the Euparal-covered area. Gentle pressure is applied to ensure that the upper wing surface is covered with Euparal. The cover slip is coated with a thin layer of Euparal essence and placed over the wing. This is done by standing it vertically to the right of the wing so that its bottom edge is in contact with the Euparal and then gently lowering it, all the time ensuring that nothing underneath moves (Fig. 6). This must be done very carefully so that no air bubbles get under the cover slip; these are very difficult to remove. The position of the cover slip is now finely adjusted.

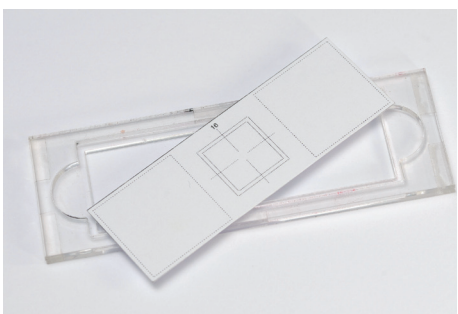

Fig. 4. The slide frame and template (photo G. Banasiak).
Ryc. 4. Ramka i szablon do preparatów (fot. G. Banasiak).

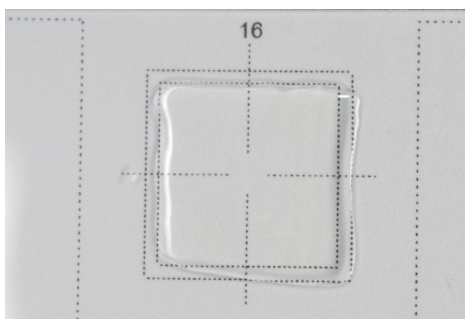

Fig. 5. Euparal spread over the slide (photo G. Banasiak).
Ryc. 5. Euparal rozprowadzony na szkiełku (fot. G. Banasiak).

DRYING. Before being added to the collection, the completed slide has to be dried. At room temperature, it may take several months for the slide to dry out, so during this period it has to be handled very delicately: care must be taken not to apply any pressure to it, not to wipe it and, if possible, to store it in a horizontal position (Fig. 7). Around the edges of the cover slip the Euparal dries out fairly quickly, but underneath it remains liquid for many months. The slightest pressure can then cause the cover slip to move,

irreparably damaging the slide. The slides are stored in purpose-made boxes to protect them from accidental damage and dust. Storing them in numerical order makes it easy to find them quickly.

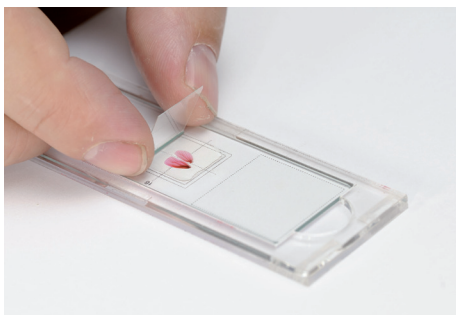

Fig. 6. Applying the cover slip (photo G. Banasiak).
Ryc. 6. Nakładanie szkiełka nakrywkowego (fot. G. Banasiak).

Fig. 7. Completed slides while drying (photo G. Banasiak).
Ryc. 7. Preparaty w czasie suszenia (fot. G. Banasiak).

LABELLING. After drying and removal of the temporary label each slide should be properly labelled. For microscope slides two good-quality ca. 23x23mm self-adhesive labels are needed: one for the left- and the other for the right-hand side of the slide. The left-hand label contains details of the specimen, the right-hand one the slide number, staining agent and medium, preparation date, name of preparer, and the sex and name of the species. The label with the number of the slide is pinned under the completed slide. Each slide must have a unique number in a suitable format, and the numbers must be recorded on paper. Such a register allows one to keep a check on the numbering and to familiarise oneself with the collection of slides without having to take them out of their box. This author uses numbers in the following format – GB-00001: the author's initials followed by a five-digit number. The labels are written out in pencil or laser-printed in black. Both methods are durable and resistant to the vapours of the chemical reagents and ethanol (Fig. 8).

Fig. 8. Slide with labels (photo G. Banasiak).
Ryc. 8. Gotowy i zaetykietowany preparat (fot. G. Banasiak).

STRESZCZENIE

Wykonywanie preparatów użyłkowania skrzydeł Microlepidoptera

W przeszłości użyłkowanie skrzydeł u Microlepidoptera stanowiło istotny czynnik podziałów taksonomicznych. Również współcześnie analiza użyłkowania często pozwala określić przynależność taksonomiczną okazu. Metodyka wykonywania preparatów użyłkowania skrzydeł była opisywana w wielu pracach, zwykle jednak bardzo pobieżnie. Do barwienia stosowane są różne substancje, często trudno dostępne i drogie. W niniejszej pracy opisano prostą, tanią i powszechnie dostępną metodę wykonywania mikroskopowych preparatów użyłkowania skrzydeł Microlepidoptera. Przygotowane preparaty mogą stanowić podstawę do szczegółowych badań mikroskopowych oraz wykonywania rysunków użyłkowania. Metoda ta sprawdza się również w przypadku skrzydeł mniejszych gatunków Macrolepidoptera.

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