Improved solutions of two water-soluble media for mounting beetle genitalia

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Determination of insects, including Coleoptera, often requires the dissection of the last abdominal segments, the separation of the aedeagus and, sometimes, also of its internal structure. The dissected parts are usually embedded, suitably oriented, in a small drop of a mounting medium, either on the same insect card or on an "acetate" transparent card, which is pinned under the specimen on the same pin. Such arrangements allow a simple and easy microscopical examination of the specimen together with the dissected parts, and their conservation, hopefully for many years after dissection.

The properties of a good mounting medium can be summarised as follows:

- To remain permanently clear and transparent (i.e., should not craze, discolor, or crack) so as to allow examination under both transmitted and reflected light.
- To be water soluble to ease handling and also to better preserve the shape and size of the membranous parts (in the writer's experience, it turned out to be very difficult to mount the internal structures of the genus *Dasytes* in anhydrous media).
- To remain soft for a reasonable period of time while preparing the mount.
- To be easily redissolved after hardening in case the parts have to be handled for whatever reason (e.g. examination in a different orientation).
- To be compatible with the mount (either card or transparent "acetate"): a drop of the medium should readily spread on it and permanently adhere after drying.
- To be harmless to the operator.

Advantages and drawbacks of the most widespread mounting media are discussed by Cooter (1991), Halliday (1994) and Stüben (2001). These are summarised below.

Canada Balsam

The most reliable and probably the most frequently used mounting medium is Canada Balsam, a naturally occurring material widely used in microscopy. Although it has been in use for many years it has several disadvantages: it needs xylene as dissolution solvent; it requires dehydration of the parts before mounting (e.g. a passage through 95% ethyl alcohol followed by xylene); on ageing it darkens, reducing the visibility of the embedded parts and often, especially with acetate or vinyl cards, its adherence becomes unsatisfactory.

[*The Coleopterist* 14(1): 29-35, April 2005]

Euparal
A widely used medium has been Euparal. This is easier to use than Canada Balsam because it is water compatible to some extent (i.e. it does not require dehydration). However it contains harmful components, on ageing it sometimes develops cracks or becomes crazed and the parts often require readjustment after 5-15 hours. Euparal is no longer easily obtainable due to its toxicity.

Micro vials
The use of micro vials has been recently recommended by Stüben (2001). In this technique, the dissected parts are placed in a very small glass tube (4mm diameter and 12mm long) partly filled with glycerine, which is pinned through its plastic cork on the same specimen pin. It is reported that the dissected parts are perfectly visible through the glass and the only drawback is related to the cost and the availability of the micro vials (which are manufactured in the USA and are expensive).

DMHF (Dimethyl hydantoin formaldehyde)
DMHF is a perfectly water soluble transparent resin that was first suggested as a mounting medium for microscopy by Steedman (1958). This medium is easily made up, soft parts are readily extended, it is easy to dissolve whenever required and many years of experience on ageing is available. Disadvantages are the rapid formation of a skin, which allows only a short time to prepare the mount, the tendency to retain air bubbles and the positioning of parts that often need readjustment after a few hours. Following the disappearance of Euparal, DMHF has acquired popularity among entomologists (e.g. Angus, 1969; Bameul, 1990). However, there is doubt regarding the future market availability, which seems to be linked to its use in the formulation of hair lacquers.

PVP (polyvinylpyrrolidone homopolymer)
PVP is another transparent and water soluble resin. It appears to be easily available on the chemicals market and like DMHF it is also used as a hair lacquer component. A proprietary PVP based mounting medium has been used by German and Swiss entomologists (Brancucci, 1980: 223; Lompe, 1999: 97) and to a very limited extent by the writer himself, with satisfactory results, although after 20 years ageing several mounts show lack of adherence to the transparent acetate support and the formation of cracks (A. Herrmann, pers. comm.). To the writer's knowledge, the medium was a mixture of PVP, water as a solvent and glycerine as a "plasticizer" to give a smooth appearance to the surface on drying and to reduce the cracking tendency of the hardened droplet.

New solvent for DMHF and PVP
An improved solvent for both DMHF and PVP consists of a mixture of distilled water and propilenglycol mono methyl ether CH_3-CHOH-CH_2-O-CH_3 (1-methoxy-propan-2-ol) (abbreviated PM1). The composition recommended here is 40% water - 60% PM1. This mixture has been found to have a number of advantages,

listed below, the most important being the lower drying speed in comparison to water, which allows easier handling.

PM1 can be easily found on the market, both as a commodity for industrial use (25kg drums) and as a fine chemical for laboratory applications (smaller containers).

PM1 properties are as follows:

Smell	weak, ether like
Freezing point	-100°C
Boiling point	> 117°C - (120°C)
Flash point	> 35°C
Spontaneous combustion temperature	270°C
Density	0.92-0.93g/ml at 20°C

With a boiling point higher than water PM1 is a "slow" solvent, namely the drying time is longer than water. Attention is called however, to the flash point: when heated to over 35°C it becomes easily flammable and, following the EEC (European Economic Community) regulations, its label must include the R10 statement "flammable".

To the writer's knowledge, no adverse effect on health is known for PM1, although the manufacturer recommends that repeated or prolonged contact with the skin should be avoided.

The solvent mixture can be readily manufactured simply by mixing the two solvents in the appropriate ratio: for instance, 25ml (= 25g) water plus 40.8ml (namely 37.5g) PM1 to make up 62.5g of mixture. A high precision level is not required and the quantities shown can be approximated to 25ml water plus 40ml PM1.

Improved DMHF based solution

The improved DMHF solution has the following formulation (by weight):

DMHF	60%
Water-PM1 solvent	40%

Place the desired amount of DMHF, which looks like large crystals, in a wide mouthed glass container (for example 30g) and add the right amount of solvent mixture (20g in our example, which can be approximated to 19-20ml). Cover the container and gently warm it up to 40-50°C for a few hours in a water bath (or, simply, leave it for at least one week at room temperature) until completely dissolved, stirring from time to time with a small glass rod. The solution is ready when no more solid material shows on the container bottom and the viscosity is homogeneous.

This formulation gives a rather low final viscosity: if desired, it can be prepared stronger (DMHF can be raised to 65% and the solvent mixture reduced accordingly to 35%). On usage, however, the viscosity in the bottle goes up and, when required, a few drops of the same solvent mixture can be added (ideally, because water

evaporates quicker than PM1, a correct replenishing solvent should be water 80-85% plus PM 15-20%).

The main advantage of the above-described formulation over the simple water solution is the reduced skin hardening speed, which results in more time to transfer the dissected parts into the drop. Further advantages are less tendency to retain air bubbles, a smoother surface appearance, with no tendency to ripple on drying, and a better wettability of the acetate or card mount. A drawback is that the parts, once introduced, wet into the drop, move around and need fine adjustment after some time: usually not less than 1 hour and not more than 24 hours.

The drying time of the mount depends on the temperature, the ambient humidity and the drop size: usually it requires about 1 hour to become dust-proof and several days to harden. During the hardening process the drop builds up a resilient "skin" which, even if gently punctured with a fine pin, will slowly recover its original shape.

The water solubility of the water-PM1 dissolved DMHF mount is at least equal, even after complete drying, to the usual water dissolved DMHF.

Improved PVP based solution

A PVP (polyvinylpyrrolidone homopolymer) based solution suitable as a mounting medium for microscopic examination of insect parts has been formulated and tested. The PVP is dissolved in a mixture of water and PM1 and a small quantity of polyethylene glycol 400 is added. No experience being available on the long term ageing behaviour of this solution, a simple accelerated ageing cabinet has been assembled for testing purposes.

The PVP based solution has the following formulation (by weight):

PVP K-30 powder*	42%
Water-PM1 solvent	52%
PEG400**	6%

*The K-30 symbol refers to the viscosity of a standard solution, which is linked to the molecular weight of the polymer. PVP K-30 can easily be found in Europe (for example under the trade name Luviskol K 30. It has not been possible to find the lower viscosity grade, namely K 17, which might have been more suitable for this particular application).

**PEG400 is polyethylene glycol of molecular weight 400.

To make the PVP solution, gently heat the solvent mixture up to 50-60°C in a hot water bath (do not use direct flames or electrical heaters: they are both unsuitable and dangerous). While stirring, slowly add the PVP powder until a hazy, viscous solution develops. Then, still stirring, add the PEG400. Leave to stand for about 24 hours until all the small air bubbles have disappeared and the thick solution looks clear and pale amber coloured. If the viscosity is felt to be too high, a small amount of the solvent mixture can simply be added to adjust it.

If the viscosity in the bottle increases with time and usage, it is suggested that a few drops of the same solvent mixture are added (however, such replenishing solvent

should ideally have a composition of about 15-20% PM1 and 80-85% water to take into account the different volatility of the two components).

PEG400 is a commonly available chemical, necessary in the formulation to prevent surface wrinkles and ripples on drying and to reduce the hardness of the dried resin. It replaces glycerine as a "plasticizer", with the advantage of being more stable over time. Apparently its effect is longer lasting and, on accelerated ageing (see below), the hardened medium droplets do not show any brittle behaviour, crazing, cracking or loss of adhesion from the support. To the writer's knowledge, PEG400 should not pose any handling hazard and should be free of adverse effects on the operator's health.

A minor disadvantage of this solution is the rather lower residual volume after the solvent has evaporated (namely less than 50%) resulting in a noticeable volume reduction on drying. Because of this behaviour, it is suggested that the drop placed on the mount is slightly thicker than desired.

Handling of this solution is very much like the previous one. However, PVP seems to be easier to use because it allows a longer handling time (approximately double), dries more quickly so that after 6-8 hours it is already quite hard (depending on temperature, humidity and drop size) and does not show any tendency to retain air bubbles. Once in the medium, the dissected parts show less tendency to move around and readjustments may not always be necessary. However, readjustment, if required, should be made within a few hours from embedding.

Accelerated ageing test

The main concern when using a new mounting medium is its long-term behaviour. As mentioned above, a reasonable level of positive experience is available for DMHF over 20-30 years. The PVP-glycerine system, however, has shown some failures even over a shorter ageing time and some kind of quick testing becomes necessary, at least as a preliminary assessment, for a modified system.

A warm cabinet has been made at home using a metal box (approx. 20x13x7.5cm), a 15-watt lamp and a thermometer to monitor temperature. In this way 1000 hours (6 weeks) cycles have been carried out at the constant temperature of 48-50°C. Comparative tests only have been carried out and no direct relationship between the cabinet results and the ageing behaviour in real conditions can be assumed.

Several PVP based formulations have been tested in comparison with both the PVP-glycerine and the DMHF systems. After 1000 hours (but also after 1500 hours) the results for the above reported PVP-PEG400 formulation, as well as for DMHF, have been very encouraging: no brittleness, cracking or adhesion loss have been seen for them, against a rather poor performance of the PVP-glycerine formulation (and of several other PVP based systems tested).

A PEG 400 concentration of 6% has been felt to be suitable: higher amounts (up to 10%) have been tested with no better performance in the accelerated ageing test but with a significant hardness reduction of the dried medium.

It is also worth noting that the PVP-glycerine formulation, which after 1000 hours in the cabinet clearly becomes brittle with cracks and detachment from support, when exposed again to room conditions of temperature and humidity "recovers" its plastic behaviour to some extent.

Embedding operating suggestions

After dissection, a small drop of solution (either DMHF or PVP) is placed on a transparent plastic card (5x10mm), or on the same insect card, and spread into a round shape with the aid of a pinhead. The parts are then transferred from the dissection water to the medium drop with a micro-pin. The drop on the card remains soft and workable for not less than 30 seconds (DMHF) or 1 minute (PVP), allowing ample time for the transfer.

The card should be left horizontal for some time (2 to 6 hours) and then the orientation of the parts checked. If an adjustment is needed a second small drop of the medium can be placed on top of the first or, alternatively, a small amount of solvent (40% water - 60% PM1) can be applied, to both DMHF and PVP, using a fine brush, over the first drop. The parts can be moved and properly oriented by means of a fine needle and afterwards the card should be left horizontal for several hours (PVP) or 1-2 days (DMHF) to prevent further shifting. However, minor orientation adjustments are still possible after 12 - 24 hours (PVP) or even 2-3 days (DMHF), depending on the room humidity, temperature and drop size.

Comparison

A comparison between DMHF, PVP-PEG400 and Euparal can be summarised as follows:

Property	improved DMHF (water soluble)	PVP-PEG (water soluble)	Euparal (solvent based, proprietary)
Drop appearance	very clear, colourless	clear, colourless to very pale amber	clear, pale amber
Durability in time	good results over > 20 years experience with DMHF in water (the solvent change should not affect ageing)	no experience; 20 years long but limited experience exists on a proprietary PVP formulation	rather good results over many years, but with some problems (loss of clarity and cracking) from time to time
Handling	rather short time allowed for transfer of parts, which are difficult to collect with a pin	easy	easy, although the mount takes a couple of hours to become clear
Drop spreading on an acetate card	acceptable	good	very good

Property	improved DMHF (water soluble)	PVP-PEG (water soluble)	Euparal(solvent based, proprietary)
Drop size reduction on drying	limited	noticeable	noticeable
Parts adjustments after embedding	needed	often needed	needed
Drying	dust-proof after about 1 hour, several days to completion	dust-proof after about 30 minutes, 1-2 days to completion	dust-proof after about 30 minutes, 2-3 days to completion
Toxicity	harmless	harmless	contains toxic components
Solubility of the dry mount	quick and easy in water	easy in water	easy but long in Euparal solvent or ethyl acetate
Market availability	still available in small quantities, but uncertain future	readily available	can still be obtained but is likely to disappear soon

Acknowledgements

Peter Hodge spent time and effort carefully revising the text and his valuable help is gratefully acknowledged. Andreas Herrmann supplied important information on PVP, literature references and copies of several papers. Robert Constantin tested the two formulations and gave useful suggestions. The buyer of Chemetall Italia Srl, Bruno Tappella, has been very helpful when looking for DMHF and PVP on the chemicals market. Lino Bellini, of the Chemetall Italia Srl laboratory, provided samples of PM1 and PEG 400.

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