

Substituting time-consuming pencil drawings in arthropod taxonomy using stacks of digital photographs

CHARLES OLIVER COLEMAN

*Museum für Naturkunde, Invalidenstraße 43, D-10115 Berlin, Germany.
E-mail: oliver.coleman@museum.hu-berlin.de*

Abstract

The most time-consuming part of a taxonomic description is making the illustrations. This contribution shows how to save time by omitting the pencil drawings of arthropod appendages and replacing them by stacks of microphotographs. These are imported into a drawing software package on a computer in order to make a publication-ready line drawing, a technique described in detail in Coleman (2003). The photographic method requires a special treatment of the appendages which is also shown.

Keywords: method, drawing, taxonomy, arthropods, microphotography

Introduction

The high number of undescribed species and the limited time available for research brings the taxonomist on the horn of a dilemma: describe fewer species in great detail or describe more species, but in a not so detailed way. Of course most taxonomists would prefer to deliver high-quality work that will be still useful even in the distant future, as some of our predecessors showed in an exemplary manner (e.g. Stebbing, 1888; Sars, 1889).

The only way out of this dilemma is to speed up the descriptive process. One possibility is the use of DELTA software (Dallwitz *et al.*, 1993; Dallwitz *et al.*, 1998) to quickly generate text descriptions, diagnoses and keys.

However, the most time-consuming aspect of taxonomy is making the illustrations.

Drawing in taxonomy is a two step process, both very time-consuming. First, a pencil drawing is made often using a camera lucida. Then these drawings are traced another time on plates for publication. This inking stage can be sped up by using digital drawing methods (Coleman, 2003).

The idea of this paper is to combine the rapid process of microphotography and the

advantages of a scientific drawing done with a digital drawing board. Moreover, this method is very cost-effective, compared to the purchase of a camera lucida, which for some microscopes is already difficult to buy. This method is especially good for dealing with arthropod appendages. These have to be treated in a special way prior to digitization: muscles must be dissolved, the cuticle decalcified and stained. Following this, the appendages are mounted on microscopic slides.

Material and methods and step by step guide

Preparation and making of permanent slides

If the cuticle of the specimens is thick and calcified the prerequisite for the method is to clear it by maceration, decalcification, staining and mounting the dissected parts before taking the photos.

Maceration

There are two ways of dissolving the muscles inside the appendages.

1) One can use an enzyme solution. I have used two different recipes: a) 3 ml sodium-borate solution in 7 ml aqua dest. with 0.1 g trypsin (modified after Dingerkus & Uhler, 1977); b) enzyrim solution (modified after Niederklopper & Troxler, 2001): NaCl 15 g, Enzyrim 20 ml; Mollecal C 0.5 g in 1 l aqua dest., adjust to ph 8.5–9 with NaCO₃.

2) The appendages are dissected off the body and put in a small vial with a glass lid. Prior to the enzyme treatment the body parts should be washed to remove ethanol or formaldehyde solution. The vial should be put in a warm environment, e.g. a hotplate, at temperatures of 40–45 C (Fig. 1a). Check the vials often and replace any evaporated water. The time required for dissolving the tissue varies from 1–6 hours depending on the size of the object.

3) I prefer the KOH maceration. The appendages are put in a staining block with 70% ethanol and 1–2 pellets of KOH are added. Also add 1 ml of glycerol, which prevents the objects from drying out during further processing. After heating on a hot plate (not more than 50–60C) the KOH dissolves quickly. Add ethanol from time to time to fill up liquid losses. After 2–3 hours the tissue is completely dissolved. Put appendages into fresh glycerol.

Decalcification

When the cuticle is not completely transparent it may be encrusted with calcium carbonate. The calcium carbonate can be eliminated easily by putting the appendages from glycerol into 70% ethanol in which some crystals of citric acid were dissolved. Remaining glycerol prevent gas bubbles from forming inside the appendages.

Staining

After maceration, the body parts are transferred into a fresh staining block with 70% ethanol and some crystals of lignin-pink (Azophloxin, $C_{18}H_{13}N_3Na_2O_8S_2$) are added. Add 1 ml of glycerol and heat on a hotplate (Fig. 1a). Visually control the staining and stop the process when you are content. Then transfer the appendages into fresh glycerol.

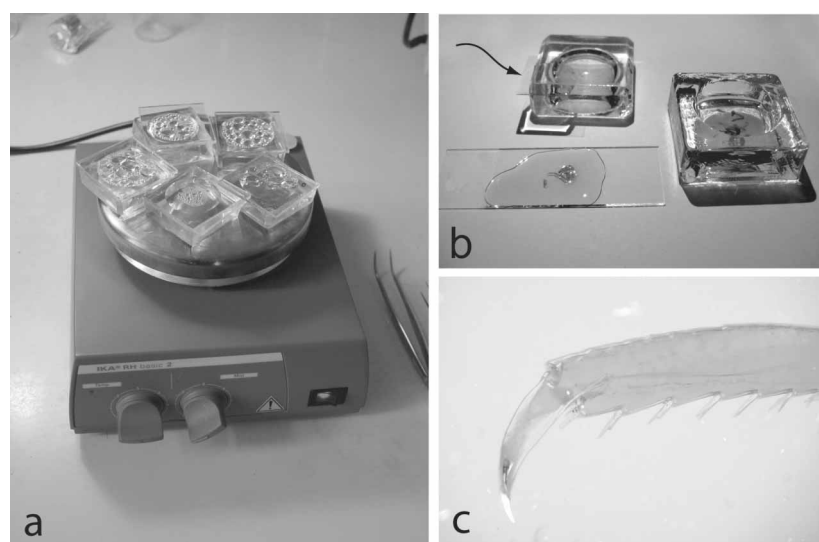


FIGURE 1. Preparation of permanent slides. a, maceration of appendages on a hot plate removing tissue from the appendages; b, creation of permanent slides using Euparal, arrow points to a cover slip with drop of Euparal, resting to allow the mounting medium to becoming somewhat harder; c, lignin-pink stained distal part of a leg of an amphipod crustacean mounted in Euparal.

Making of permanent slides

Two mounting media are recommendable: Canada balsam and Euparal. Both are very long-lasting and there are slides mounted with these media that are more than 100 years old and are still in excellent condition. Canada balsam is rather difficult to handle and some components are very toxic. Thus I prefer Euparal (Chroma, 3C 240 green). In order to allow the medium to penetrate more easily, I put the appendages into a mixture of one part 70% ethanol and one part Euparal. After an hour the objects are transferred into pure Euparal for at least another hour before mounting them onto slides.

If only single appendages are mounted on separate slides and the appendages are rather small there are no difficulties. If the appendages are thick then put the appendage into a large droplet of Euparal and let it dry for a while, put another drop on the cover slip you want to use and let it rest (Fig. 1b: arrow). If the resin gets gluey put the cover slip on the object. It may be that the cover slip is raised on one side if the object is very thick, here put some extra Euparal. During the drying process the mounting medium may contract somewhat. Resulting gaps can be filled from the side.

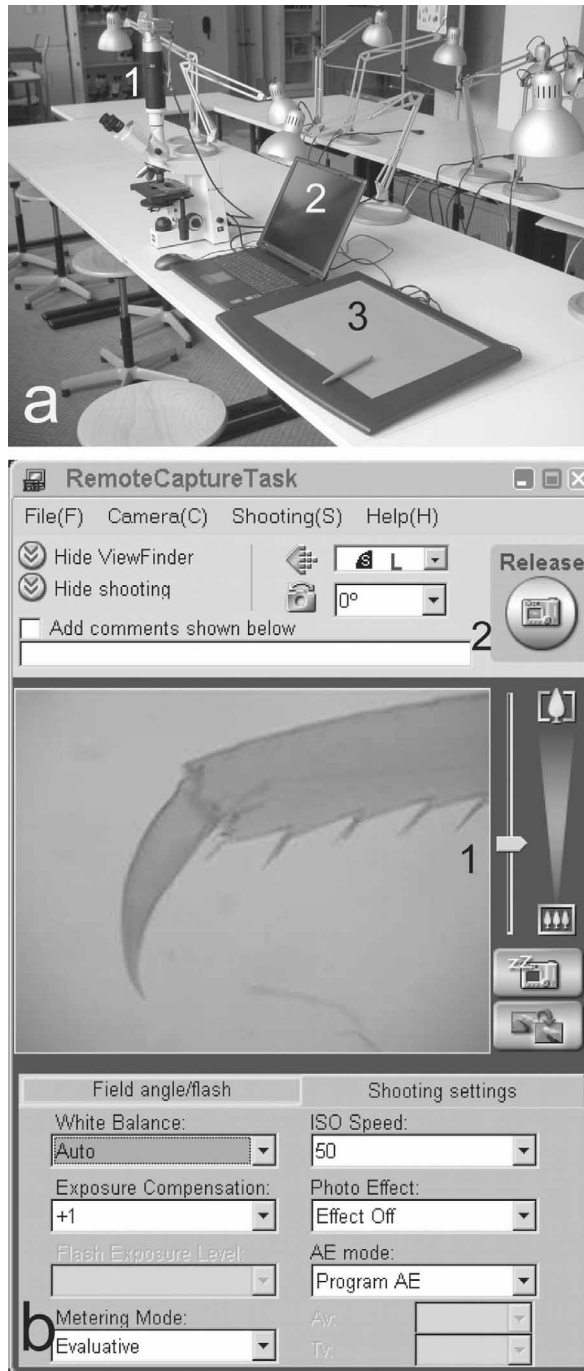


FIGURE 2. Making of micrographs. a, stacks of photos are taken with a camera mounted on a microscope (1), directly transferred onto a computer (2) and drawn with a WACOM Intuous 2 DIN A3 drawing tablet (3); b, all camera functions are controlled by the remote capture task software, in the preview window of this software where focus of the microscope is being controlled. Moving the lever 1 up will zoom out the dark margins; pushing the button 2 will expose a picture and automatically transfers it onto the computer.

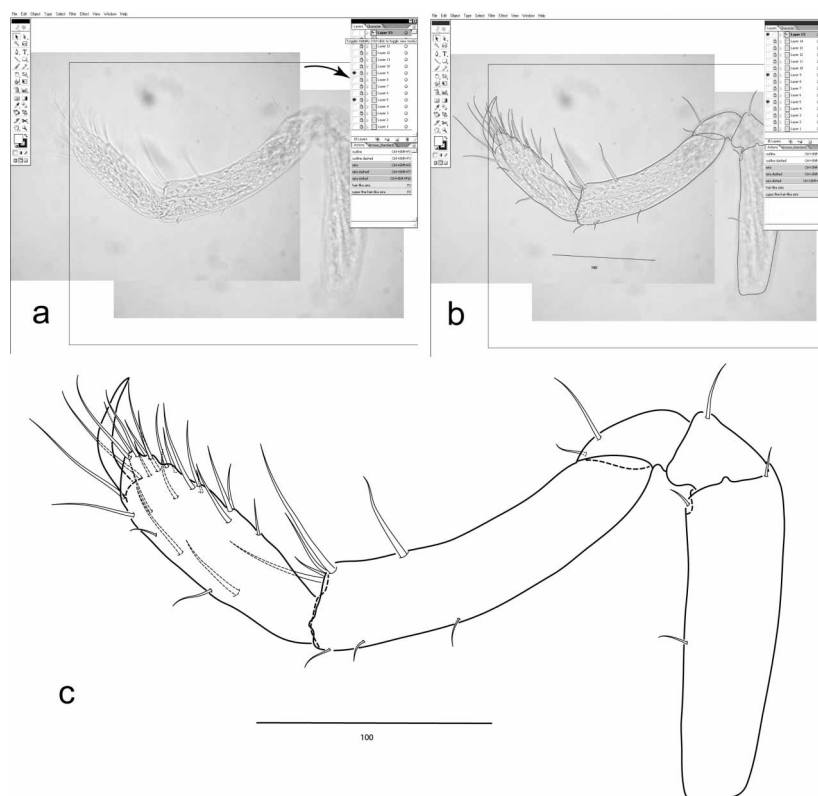


FIGURE 3. Creation of the drawing with the WACOM board using a crustacean appendage as an example. a, stacks of micrographs are imported in a drawing software and arranged into one picture; focus levels can be selected by clicking on the level selector (arrow); b, the outlines of the photos are made using the levels of the stacks that are in focus; c, once all details are drawn, the photos are deselected and the drawing can be used.

It is also possible to mount several small appendages onto one slide. In order to prevent the appendages from moving around when putting them on the cover slip, put a droplet of Euparal on the slide and use a cover slip to tease out the drop. Put the appendages in this layer of Euparal in the required order and let them rest until the medium becomes gluey. Then, put another droplet on the cover slip and put it on the slide after the resin became gluey. Let the slide dry for some days in a dust-free environment before making the micrographs.

Photography and drawing technique

For making photos we used a Canon Power Shot A 80 digital camera mounted with an adapter tube on a Zeiss Axiostar plus microscope (Fig. 2a: 1). Any other microscope and digital camera can be used instead. From each article of the appendages or, if small, whole appendages, several micrographs are made varying the depth of focus. Then the data are transferred onto the computer with a link cable.

Software that allows the remote control of the camera is very useful. For example the Canon Power Shot camera is delivered with the Zoom Browser EX software which allows, among other features, to control all camera settings and transfer the files directly onto the hard-disk of the computer (remote capture task, Fig. 2b). Other types and brands of cameras use similar remote control software from the manufacturer or as shareware (see for example www.breezesys.com). There is a small preview window, this gives a live picture from the microscope. So there is no need to look through the microscope to control the focus; the whole process can be done on the computer screen.

Three settings should be adapted before taking the photo with the remote capture option: 1) adjust the zoom with the lever on the right side of the window (Fig. 2b: 1), so that the dark margins disappear; 2) select in preferences artificial light and 3) open the f-stop as much as possible.

With such kind of software it is much quicker to make the micrographs compared to the direct adjustments on the camera. Click on the button up in the right corner of the window (Fig. 2b: 2) to shoot a picture. Make a stack of micrographs of an article of an appendage and save these files in their own subdirectory. Then move to the neighbouring article of the appendage and produce another stack. Repeat until the appendage is completely photographed.

Open the drawing software. Each picture from a stack is transferred on separate layers of a drawing software (for details see Coleman, 2003). Then add the first picture of the next stack of micrographs and place it next to the already placed stack, so that the corresponding parts overlap. Without changing the position put the next pictures from the stack on separate layers. When all picture stacks are combined, it is possible to move through the different focus layers by clicking on the layer selectors (Fig. 2a: arrow). Only one level is shown at a time. There is no addition of all focus levels.

Draw on a newly created layer on top of all the microphotographs and trace the photographs using a drawing tablet (e.g. WACOM Intuous 2 DIN A3; Fig. 2a: 3) (cf. Coleman, 2003 for details) and select the appropriate focus level with the layer selector. During drawing the permanent slide should be examined through the microscope in order to avoid mistakes. Only the layer containing the digital drawing is used to arrange the single drawings of the appendages on plates.

Discussion

The digital drawing method (Coleman, 2003) let the taxonomist speed up the creation of line drawings. Moreover, just as word processors revolutionized our writing, the use of drawing software allows to experiment until we are satisfied with the result. Selecting special settings in the software perfect lines without any signs of shakiness can be made. Small structures can be enlarged dramatically and the drawing of these structures is much easier compared to traditional ink on paper. Different thicknesses and styles of lines can be

defined and retrieved using macros. With this technique everybody can quickly make perfect line drawings.

But this digital inking is only the second part of the illustration process. The basis for this digital drawing method is usually a conventional pencil drawing, made with a microscope and a camera lucida. In order to really save time, the pencil drawing must somehow be avoided.

Myers (2004) tried to use modern microphotography and images at different depths of focus combined into one photo in order to avoid the time-consuming drawing process completely. However, this method has some fundamental disadvantages: 1) muscles inside the appendages sometimes block the view on details on the lower focal plain; 2) it may be difficult to see whether details are on the upper or lower face of the appendage, as the focal planes are condensed into one picture; 3) artefacts on the cuticle can not be corrected as in a drawing and 4) it is time-consuming to erase disturbing background artefacts in a photography using image-processing software. With a digital camera, Harvey (1998) captured images of decapod crustaceans and prepared them for publication using Adobe Photoshop and Adobe Illustrator.

On the other hand a drawing is much clearer than a micrograph and even small, inconspicuous details, such as fine structures of setae, that are relevant for systematics can be shown. A drawing is not only a documentation of the morphology but also an interpretation, showing the important characters some of which are difficult to see in a photo, e.g. sometimes fine lines between articles of appendages. These cuticular lines are very important for measuring the length of articles; length width ratios are often used as a taxonomic character. Unlike a photo, structures that are surpassed by others can be indicated in a drawing using dashed lines. Another advantage of the digital drawing method is that most of the illustration procedure can be made by technical staff. Later the systematist checks the results and, if necessary, corrects mistakes.

Acknowledgements

I would like to thank Christoph Held, Jason Dunlop and Jörundur Svavarsson for their valuable comments which helped to improve the manuscript.

References

- Coleman, C.O. (2003) "Digital inking": How to make perfect line drawings on computers. *Organism, Diversity and Evolution, Electronic Supplement*, <http://senckenberg.de/odes/03-14.htm>, 1–14.
- Dallwitz, M.J., Paine, T.A. & Zurcher, E.J. (1993 onwards) User's Guide to the DELTA System: a General System for Processing Taxonomic Descriptions. 4th edition. <http://biodiversity.uno.edu/delta/>

- Dallwitz, M.J., Paine, T.A. & Zurcher, E.J. (1998) Interactive keys. In: Bridge, P. Jeffries, P., Morse, D.R. & P.R. Scott (Eds.), *Information Technology, Plant Pathology and Biodiversity*. CAB International, Wallingford, pp. 201–212.
- Dingerkus, G. & Uhler, D.L. (1977) Enzyme clearing of Alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology* 32, 229–231.
- Harvey, A.W. (1998) Rediscovery and range extension of the Galapagos endemic *Pachycheles velerae* (Decapoda: Anomura: Porcellanidae). *Journal of Crustacean Biology*, 18(4), 746–752.
- Myers, A.A. (2004) A new species of amphipod of the genus *Neomegamphopus* (Crustacea, Amphipoda, Neomegamphopidae) from Rodrigues, Indian Ocean. *Journal of Natural History* 28(23-24), 3138–3142.
- Niederklopper, P. & Troxler, M. (2001) *Knochenpräparation. Handbuch für Praktiker*. Bauer Handels GmbH, Waberg, pp. 153.
- Sars, G.O. (1895) *An account of the Crustacea of Norway*. Christiania, Copenhagen, Alb. Cammermeyers Forlag, pp. 701, 248 plates.
- Stebbing, T.R.R. (1888) *Report on the Amphipoda collected by H.M.S. Challenger during the years 1873-1876*. London, Eyre & Spottiswoode, 3 volumes, pp.1570, 210 plates.