

Digital Imaging of Beetles (Coleoptera) and Other Three-Dimensional Insects.

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Introduction

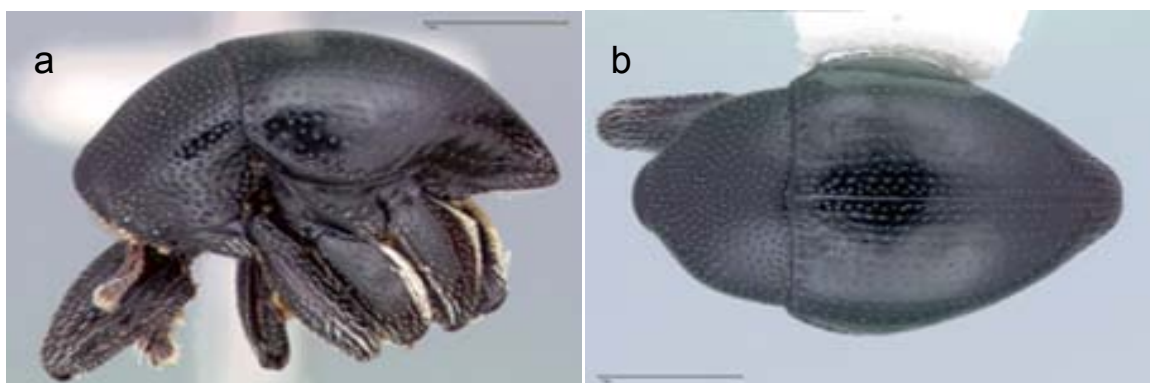
This chapter focuses mainly on imaging techniques for beetles. Usually, beetles impose special problems on the photographer, such as a highly reflecting surface and / or a body that is too convex for the depth of field available. In this respect they are quite different from the rather two-dimensional (when set) Lepidoptera but similar to other groups of insects, such as ants, certain Heteroptera, or some Diptera. The general aspects of photography of such insects will be covered here; however, only the beetles will be treated in depth.

Beetles exhibit a considerable range of body size. Members of the Ptiliidae may be as small as 0.25 mm, whereas *Titanus giganteus* (Cerambycidae) may exceed a length of 160 mm. Thus, the photographic techniques recommended will need to vary with the subjects. As I have mainly worked on beetles of intermediate size I will concentrate on describing the imaging techniques employed for beetles between 1 mm and 10 mm. This size-range forms the bulk of beetle diversity. For photographing beetles of truly microscopic size (such as some Ptiliidae), some useful hints may be drawn from other chapters of this manual, such as “Imaging Soil Mesofauna: The Land in Between” by ARIÑO, BAQUERO & JORDANA.

Mounting of specimens and choice of the background

There are two different traditions of how a beetle is mounted. Most Anglo-Saxon Coleopterists prefer to pin beetles, generally down to a relatively small body size. Specimens too small for pinning are mounted on of cardboard points, with the legs hanging more or less unarranged below the body (figs. 1a-b). In continental Europe the preference is to glue specimens onto rectangular pieces of cardboard. Legs and antennae are arranged symmetrically around the body, so that a rather natural appearance of a sitting beetle is achieved. This method is usually applied to specimens smaller than 20 mm, but occasionally larger beetles are prepared this way. Collectors of beetles as large as *Carabus* may use this method of card-mounting to avoid piercing the specimen with an insect pin and to prepare them in an aesthetically appealing manner. Furthermore, this method provides increased protection to the specimen because its appendages are protected by the card to which it is attached. The point-mounters however, reject this method because the card conceals the entire ventral surface of the beetle. Moreover, it requires tedious manipulation, that may damage the specimen. Both procedures have their merits, and it is impossible to recommend one over the other.

The different mounting methods have implications for imaging and post-processing. While it is possible to choose any kind of background for a pinned or a point-mounted specimen, no such choice is possible for a card-mounted beetle once attached. Correct exposure and imaging of delicate hairs and setae of a specimen will



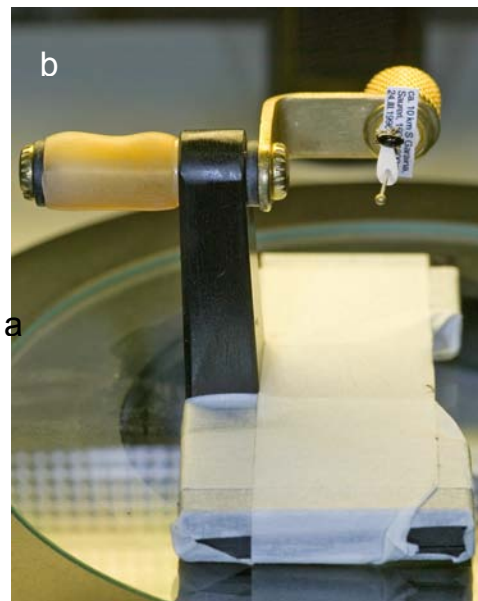
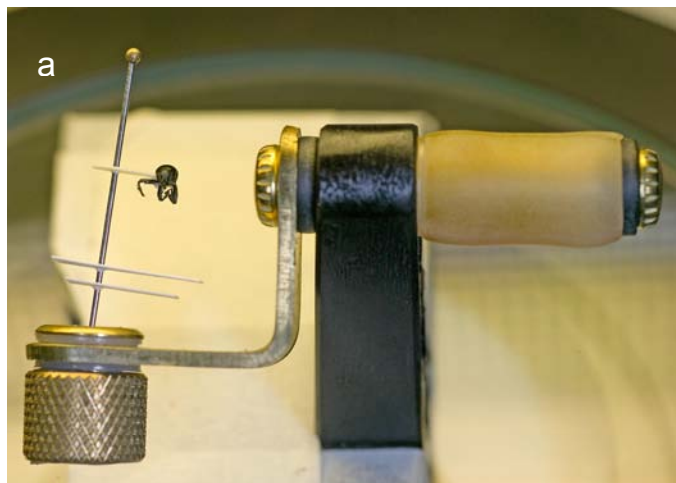
Figs. 1 a-b. Example of a point-mounted weevil (Curculionidae, *Idotasia* sp.) in lateral aspect (a) and in dorsal aspect (b).

be easiest if it is photographed against a neutral grey exposure and imaging of delicate hairs and setae of a specimen will be easiest if it is photographed against a neutral grey background. Thus, if pinned or point-mounted beetles are the subject, it is recommended that they are photographed against a neutral-grey card (fig. 1). On the other hand, the white background of a card-mounted specimen can be turned to advantage. It is quite easy to 'cut' a beetle's image from its white background during digital post-processing. And, if the beetle is mounted adequately, its dorsal aspect will reveal more characters than a specimen that is point-mounted. Furthermore, it is much easier to position it into a precisely horizontal plane. Thus a comparison of characters and body-proportions is much easier when based on images of card-mounted specimens.

The photographer usually has little choice between the two methods. Museums in the US largely hold pinned and point-mounted specimens while those in continental Europe have the specimens mounted on white cards. If the purpose of the imaging project is to create a large scale image-library, manipulation of the specimen should rarely be considered a viable option, especially if type specimens are involved. The situation is different if a specialist taxonomist is undertaking imaging as part of revisionary research. In such a situation the specimens will have to be handled anyway and it is appropriate to use the preferred method of preparing the specimen.

My personal approach is to point-mount the majority of the specimens I am working on but I mount one or two specially selected and carefully cleaned specimens of a series on relatively large white cards for the specific purpose of imaging. These card-mounted specimens can be photographed in an aesthetically pleasing manner that makes more characters visible than the point-mount method could.

A card-mounted specimen is usually photographed dorsally and it is sufficient simply to use a piece of Styrofoam for holding the insect pin. Point-mounted or pinned specimens can be photographed from various aspects. For this purpose, it is very useful to place them on a microscope stage (fig. 2) that allows their rotation around three spatial axes.



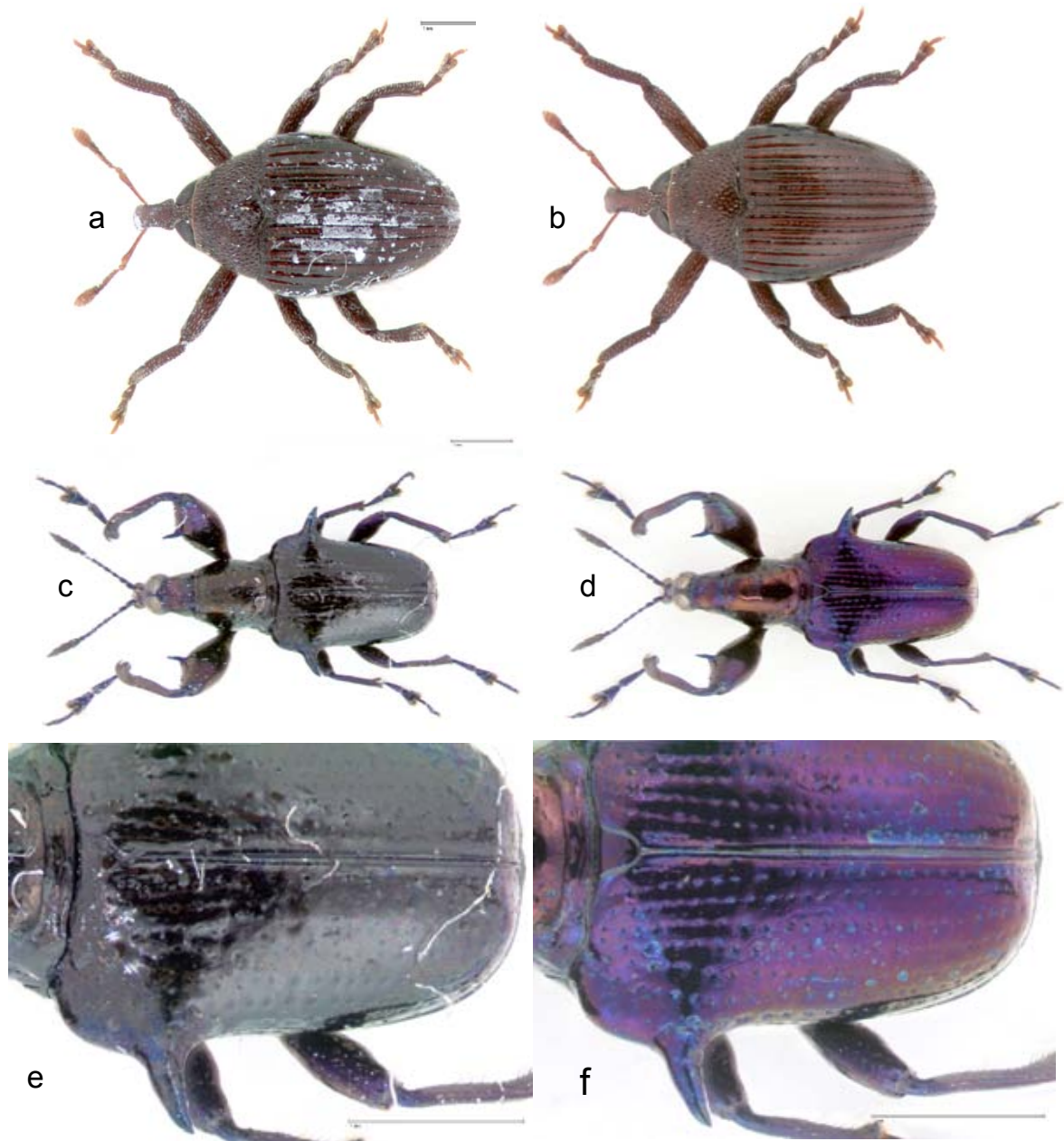
Figs. 2 a-b.
A point-mounted specimen on a microscope stage moved into different positions.

Cleaning specimens

Unless cleaned by the collector, certain beetles may still be covered with original layers of soil, wood dust etc. even after years in a collection. Also, stored specimens inevitably collect dust - even in dust-proof drawers. Grease may emerge from the body of a stored dry beetle and accumulate in layers (often thick) on its integument, thus concealing microsculpture and colour. In some cases, white crystalline substances accumulate on the beetle's surface.

The question arises as to whether specimens should be cleaned before imaging. Here again, we face a dilemma. Excessive cleaning may severely damage a specimen. Also, potential information on the environment of the beetle (in the case of adhering soil / wood dust) will be removed during the cleaning process. Yet some specimens may be covered by such quantities of dirt, dust or grease as to render them hardly recognizable. Two examples illustrate this, each one a weevil covered by white exudate (fig. 3a) and grease (figs. 3c, d), before and after the cleaning (figs. 3b, d, f).

A hands-off approach is recommended for large scale imaging projects, unless the specimen is in a barely recognizable condition. In such a case the curator should decide on adequate measures. In smaller scale projects, such as revisions, where the scientist is handling the specimens, it is recommended that specimens are



Figs. 3 a-f. Examples of beetles before and after cleaning with organic solvents. (a-b) unidentified weevil with white exudate that appeared after 12 years of storage; before and after cleaning. (c-f) specimen of *Euops yali* with grease covering the surface after 13 years of storage; before and after the cleaning.

carefully cleansed of dust and grease if necessary. This should be done with a very fine artist's brush and an organic solvent such as ethanol or ethylacetate. The legs and antennae should not be touched without first relaxing the entire specimen since these structures break all too easily. The same comments apply to beetles with hairy or setose integuments. Oil from the body of glabrous

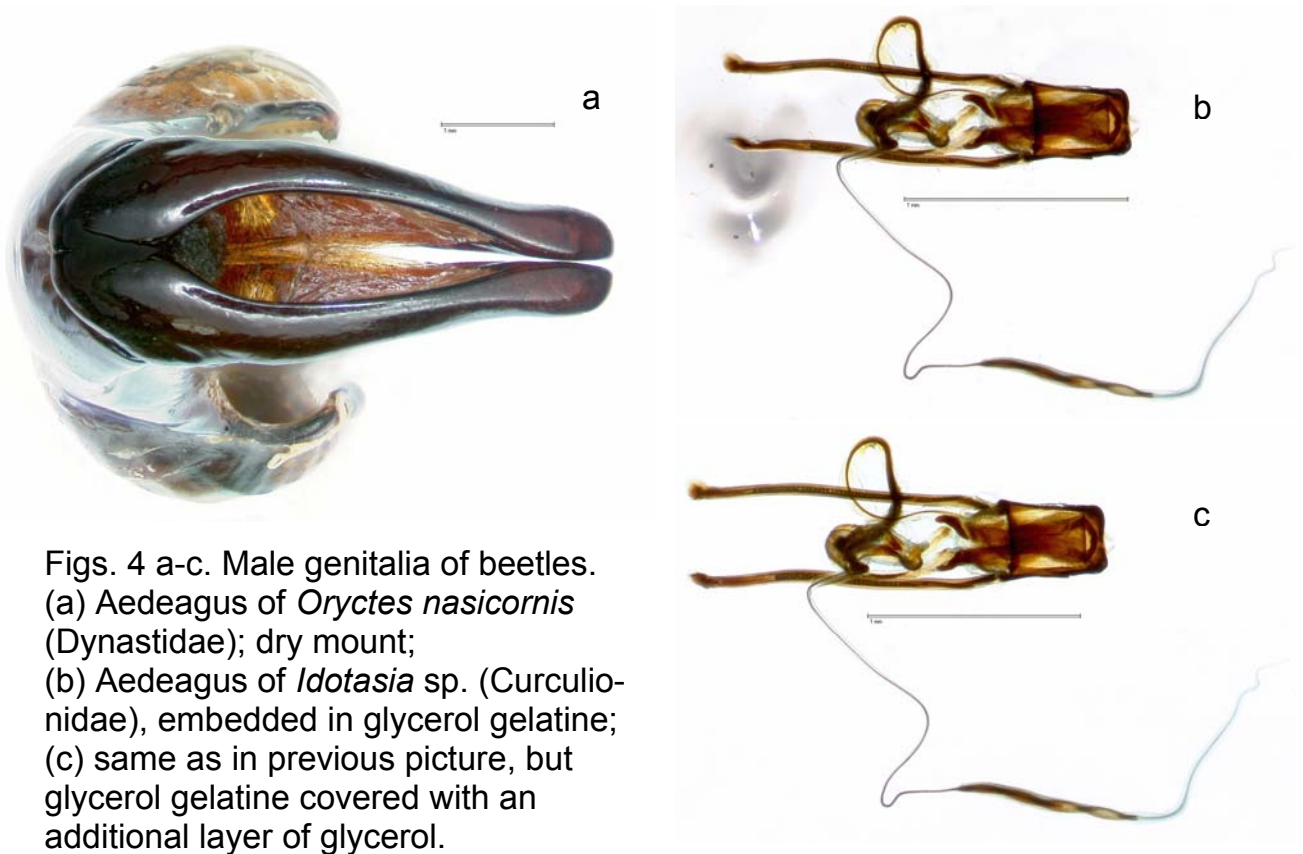
specimens should be carefully removed from dry, card-mounted specimens. The effects of successful cleaning are immediately obvious in specimens that exhibit metallic colouration. Even when just a thin layer of oil is removed from the surface, the change is dramatic with the original appearance restored.

Preparation of beetle genitalia for photography

The diagnosis of many beetle species relies heavily on characters of the genitalia. It may, indeed, be more important to photograph the relevant genital structures than to provide an illustration of the beetle's habitus. There follows therefore some comments on the final preparation of the genitalia for photography. Appropriate techniques for extracting and preparing genitalia depend significantly on the taxon in question. An expert for the specific group should be consulted for advice and help. Dissecting specimens for the extraction of the genitalia should only be done by persons with extensive experience.

In some groups the genitalia are heavily sclerotized and the diagnostic characters are mainly external (e.g. members of the Dynastidae). In this case, the genital can be mounted on cardboard and photographed in dry condition. Lighting and imaging techniques are more or less the same as applied for photographing the entire insect (fig. 4a).

In other cases also internal structures are relevant, i.e. structures of the endophallus that are within the body of the aedeagus. The same applies if membranes cover parts of the genitalia. These membranes while transparent in water, will conceal the view when they are dry. In most such cases it is necessary to clear, in KOH, the genitalia of adhering tissue and examine them in glycerol. A specialist in the relevant taxon should be consulted for precise instructions on dissection and staining. In most cases transmitted light microscopy is best for observing and illustrating the relevant characters. If the size of the specimen is not too great, a compound microscope is preferable to a dissecting microscope. Unless the structures are sufficiently flat for a cover-glass to be used, they are best examined on cavity slides. The main problem that arises when taking photographs of ob-



Figs. 4 a-c. Male genitalia of beetles. (a) Aedeagus of *Oryctes nasicornis* (Dynastidae); dry mount; (b) Aedeagus of *Idotasia* sp. (Curculionidae), embedded in glycerol gelatine; (c) same as in previous picture, but glycerol gelatine covered with an additional layer of glycerol.

jects stored in glycerol is the difficulty of fixing them in a desired position. This is especially true for genitalia of a curved shape, with appendices etc. The best way to overcome this problem is the use of glycerol gelatine. There are various recipes, but the most suitable seems to be the one given by KISSER (1934), which contains less water than the others. The beetle genitalia are fully prepared and stored in glycerol. A small quantity of glycerol gelatine is then placed in the cavity of a slide and heated until it melts and the genitalia are placed into this drop; it is left to cool down while the genital are arranged and held in the desired position with forceps and / or pins. When the glycerol gelatine has coagulated the instruments should be carefully removed (fig. 4b). If necessary, the mount should be covered by a quantity of glycerol to create an even surface (fig. 4c). Such a mount can be photographed most easily and is stable enough to create stacks of images. Later, the genital can be removed easily from the block of glycerol gelatine by placing it into water for some time.

In some cases structures of the endophallus are so complex that even the optical properties of glycerol are inadequate to resolve all details. In such cases another medium should be chosen, such as Canada Balsam or Euparal. The latter is most suitable if the structures are flat and a full mount with a cover glass is made. Unfortunately, this medium dries very slowly, so the objects may be drifting for quite some time. Canada Balsam dries faster and preparations without a cover glass are sufficiently stable after 2-3 days. The position of the object can be carefully corrected during the hardening process and thus it is also suitable for curved objects that are otherwise hard to position. Care must be taken to ensure that the Canada Balsam is not acidic, otherwise it may adversely affect the embedded genital.

Photography of larger beetles

A few general comments should suffice on digital photography of beetles larger than ca. 15 mm. They are usually pinned and comparable in size to butterflies so the chapter on Lepidoptera should be consulted for additional information. A standard digital camera seems most appropriate for the purpose of photographing such species; the best being a digital SLR camera equipped with a 50 to 100 mm macro lens. The camera should be attached to a restand or a focusing rail. At the time of writing, the Canon EOS 20D would appear to be a good choice with its 8.2 Megapixel sensor. Another interesting option with this camera is the Canon macro lens MP-E 65, for the range of natural size to 5X magnification. This lens bridges the gap between macro- and micro-photography. The MP-E 65 will work only for specimens smaller than 20 mm. For larger specimens an ordinary macro lens is preferred. Lenses of longer focal length give a greater distance between insect and camera, which is helpful for manipulation. But, it should be kept in mind that the distance necessary to fit a large beetle into the frame might exceed the height of the restand or the tripod. Therefore, the decision as to whether a 50mm lens or a 100mm lens is used will depend on both the setup and the size of the beetle. A universally appropriate recommendation cannot, therefore, be given. Unlike the

situation in butterflies, getting even illumination can be a challenge for beetles, particularly those many species with a smooth, highly reflective surface. In such cases, indirect, diffused lighting must be applied. The use of softboxes attached to the lights / strobes is one option for these larger specimens.

Photography of medium-sized and small beetles

The majority of beetles measure between 1 mm and 10 mm. Usually, a digital camera attached to a high-end dissecting microscope will be the best approach to this size-range. However, the use of microlenses combined with camera bellows (fig. 5) is a little-known alternative.

This equipment will be especially attractive in those cases where a digital SLR camera is available but not a dissecting microscope. Micro lenses, such as the Zeiss Luminar, or the Leica Photar can be connected to most digital SLR cameras through suitable adapters. With the Leica Photar 25mm/f2.0 magnifications of 5X to 22X can be achieved with an optimum performance at 6.6X. The camera must be mounted on a precise focusing rail. Such a combination will require some practise to work with and is certainly not as easy to handle

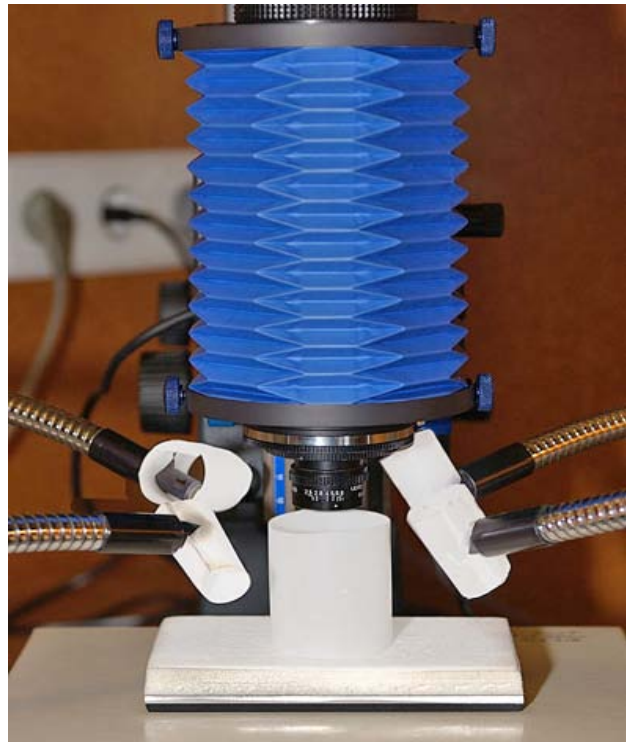


Fig. 5. Setup of camera bellow and microlens. Courtesy of H. SCHILLHAMMER (Naturhistorisches Museum, Vienna).

as is a camera mounted on a microscope. The quality of the resulting images, however, can be outstanding, so such an arrangement is an option worth considering. This technique has been applied by H. SCHILLHAMMER (Naturhistorisches Museum Wien) and samples can be seen at http://www.pbase.com/rovebeetle/mostly_beetles.

The depth of field in photographs increases with decreasing (smaller) aperture (higher aperture number); but, at the same time, lens artefacts caused by diffraction increase, becoming more pronounced when the magnification increases. For magnifications higher than 1.5X, apertures smaller than 8.0 or 11 should be avoided when using an SLR camera. The same is true of a dissecting microscope where the iris should be kept open. In digital photography of still objects the depth of field should be expanded by using adequate computer software (See below "Montage software").

Some general aspects of digital photography of insects are covered by ASHWORTH & FOGARTY (2003); thus in the following, only some additions to the cited publication are given and some idiosyncrasies of beetle-imaging are described in greater detail.

Capture device

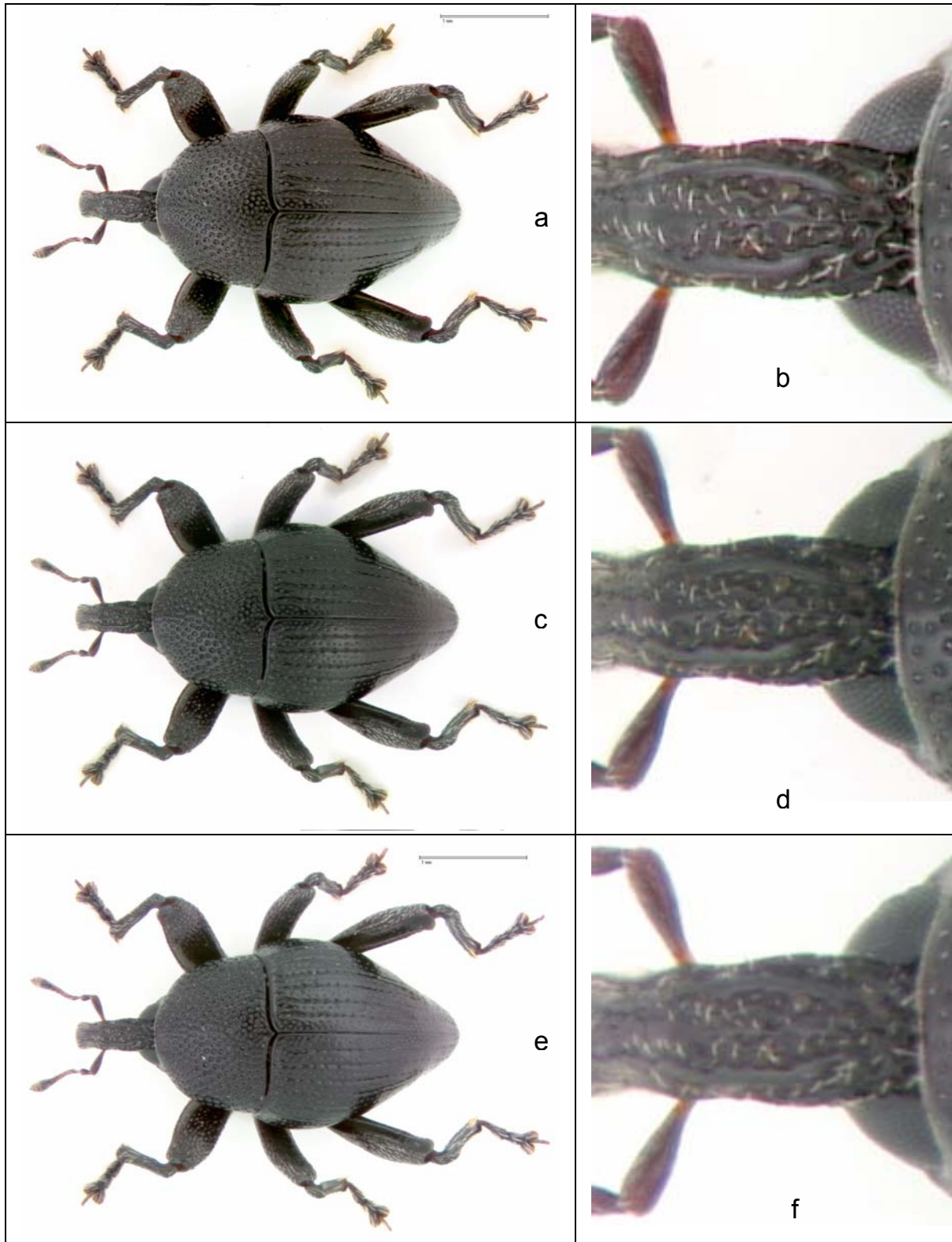
Digital camera technology is currently in a state of rapid improvement and change. It is difficult, therefore, to give specific recommendations that will be valid for more than a few months. Important general aspects are of course image quality and the availability of a live image on the computer screen, so that precise focusing is possible. I am currently using a JVC KY70, as recommended by ASHWORTH & FOGARTY (2003). Another good option is a Leica digital camera such as the DFC 320 (3,2 MP) or the DFC 480 (5,0 MP). With this system image data are transferred to the computer via fire-wire or USB2. Thus, it also provides a live image, just like the JVC KY70. The main drawback of such a Leica camera is that Syncroscopy does not provide the driver software to operate it directly from their Automontage© program. This option is only provided for cameras that are distributed through Syncroscopy, such as the JVC KY70. However, it is only of minor inconvenience to create and save the images with different software than that which is used to create a montage-image.

A mistake to avoid is concentrating on the image quality of the camera, while neglecting the quality of the optical system. The weakness in optical performance of a given microscope is not

necessarily obvious when looking through it. Test images of the same subject should be made and compared. It is also important to realize that some expensive products of manufacturers with an excellent reputation may not necessarily produce high-quality results in a given setup. Each option should be carefully tested for the purpose of digital imaging. Test images (figs. 6a-f) taken with the same camera but with different optical systems illustrate the effects of the optical system on the resulting image. One important point is a suitable video adapter to ensure that the full microscope image reaches the camera sensor. Attaching a 0.5' sensor camera without a suitable video adapter will result in higher magnification - but concurrently in poor image quality of a subject photographed at the same scale (figs. 6e-f). Apochromatic lenses should be used whenever possible. As a rule of the thumb, lenses with a shorter zoom range will have better contrast and resolution compared with those with a longer zoom range. And, lens systems with a shallow depth of field will have a higher resolution than those with a longer depth of field – deciding on which to use will depend on the availability of the Automontage© software. Most will agree that the Leica Z6 Zoom-system provides outstanding image quality and is surely worth trying (figs. 6a-b). A significant advantage of the construction of the Z6 is that it possesses only one single optical axis, eliminating any shifting of images (parallax) through changes in the focal plane. Some other macroscopes / dissecting microscopes may work equally well, especially if they are equipped with an axial carrier, that allows the user to shift into one single optical axis. In any case, it should be borne in mind that it may not be the fault of the digital camera if the resulting images lack contrast or resolution. The optical system in front of the camera deserves some attention for the best results.

Colour adjustment

Colour management is a complex subject, and no comprehensive treatment will be given here. However, a few points should be noted. Whatever camera is used a whitebalance should be performed, and when any changes are made to the lighting a fresh whitebalance is necessary. With the whitebalance, the camera is calibrated, so that it “knows” at what wavelength the colour white is being transmitted into



Figs. 6 a-f. Comparison of different optical systems using the same camera (JVC KY70U) and the same weevil specimen (*Idotasia* sp.); left: overview; right: image detail. (a-b) Leica Z6 plus 0.63X video adapter; apochromatic lenses; (c-d) Zeiss SV11 plus 0.5X video adapter; achromatic lenses; (e-f) Zeiss SV11 without video adapter; achromatic lenses.

the camera.

To optimize settings it is critical that the monitor is properly calibrated, for otherwise, wrong colour-casts may be introduced into the picture based on a false image perception through an incorrectly calibrated monitor. Monitor calibration tools, such as the “Spyder” have become affordable in recent years and are strongly recommended.

Sometimes, the auto-whitebalance function of digital cameras is far from being perfect. The auto-whitebalance of the JVC KY70 can be used as a starting point, but the white-balance must be fine-tuned using the manual setting. If the JVC KY70 is operated through Automontage©, it offers two different options of whitebalance adjustments. One is through the software, *i.e.* through the „Adjust Camera Settings“-Window. The latest version of Automontage© offers a “tool” that can greatly assist in finding a suitable whitebalance (fig. 7). The values shown for each of the three colour channels should be about equal.

Another option for setting whitebalance is through the camera menu that has to be set through the pin-buttons of the camera. The latter option is critical if the following problem is observed: depending

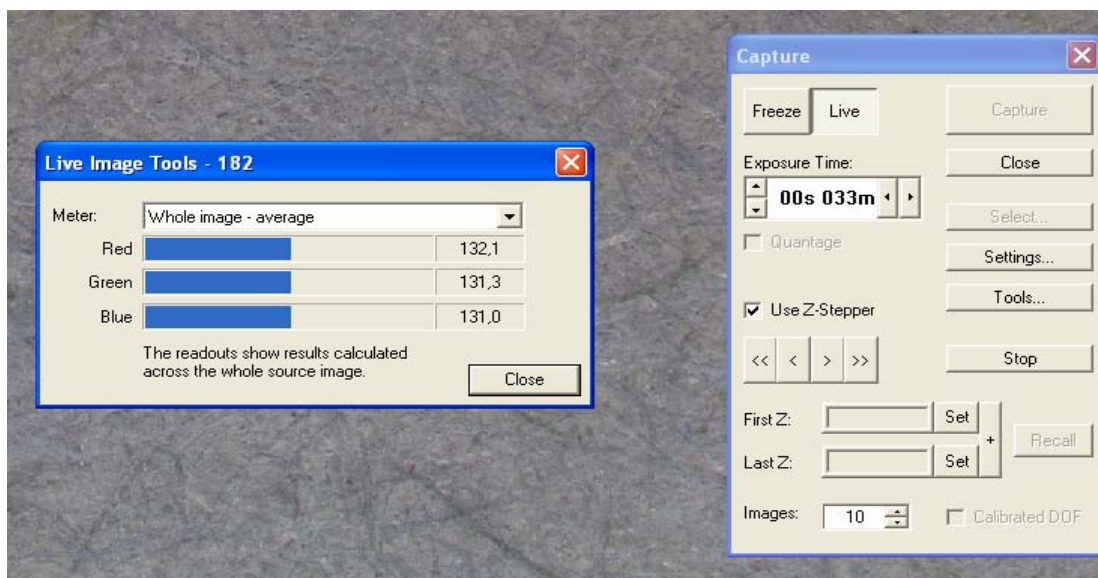


Fig. 7. Live Image Tool of Automontage© being used for improving a whitebalance based on a grey card. The values for the Red, Green and Blue channels should be about the same.

of what microscope / adapter combination is used, the field of view may show an uneven colour cast, despite even and neutral lighting. That means, on one side there is a red cast, towards the middle it becomes neutral, and on the opposite side there is a bluish or greenish cast. It is not possible to get rid of these colour casts by a regular whitebalance. However, if we navigate through the camera menu to the point "whitebalance" we can activate the „Shading mode“ of the camera by filling in RGB values in the "Adjust" submenu. I found this effect most pronounced when working on a Leica Diaplan compound microscope. To compensate, I need to put in the following values: R: -50; G: 30; B: -30. When using the Leica Z6 macroskop, different values are required: R: -15; G: 0; B= -20. It requires quite some attention to fine-tune these settings.

During postprocessing a correct whitebalance should be verified and if necessary adjusted. If parts of the image include white or neutral grey cardboard, then their RGB values should be about the same for each channel. In any case, the photographer should get as close as possible to a perfect whitebalance before imaging. For removing colour casts of images already taken see below under "Postprocessing".

Lighting

Most beetles are a photographer's nightmare from the viewpoint of lighting. They are smooth and highly reflecting. Thus, if a strobe or a cold-light source is used without any attempt to "soften" this harsh light, the beetle will show the blown-out reflection of the light source and the remainder of the insect will be a dark blot without any detail. The only solution to this problem is to spread the surface area of the light source and to diffuse it. This can be accomplished in various ways. When imaging larger beetles with a macro lens, the use of softboxes is an option. When working with a microscope cold-light sources are commonly chosen to provide a strong enough permanent light without burning the specimen. These light sources can be equipped with various glass-fibre arms. The most popular options are a ring-light to attach in front of the microscope lens, and a goose-neck light guide with two branches. The ring-light gives good results -

provided the beetle is non-reflecting. If the beetle is somewhat polished, a ring-shaped reflection with a "black hole" in the centre will appear on the beetle's dorsum (fig. 9c). This artefact can be alleviated by using a vellum cylinder that fits between the lens and the ring-light, so that the light shines through the vellum (fig. 8d). The vellum should reach down as far as possible and surround the beetle. Although this arrangement makes handling the specimen a bit cumbersome, the illumination of surface details is fairly good. The main draw-back is still the artificial ring-shaped reflection (fig. 9d). This method may be useful for scaled or non-reflecting beetles, but for shiny specimens there are better methods.

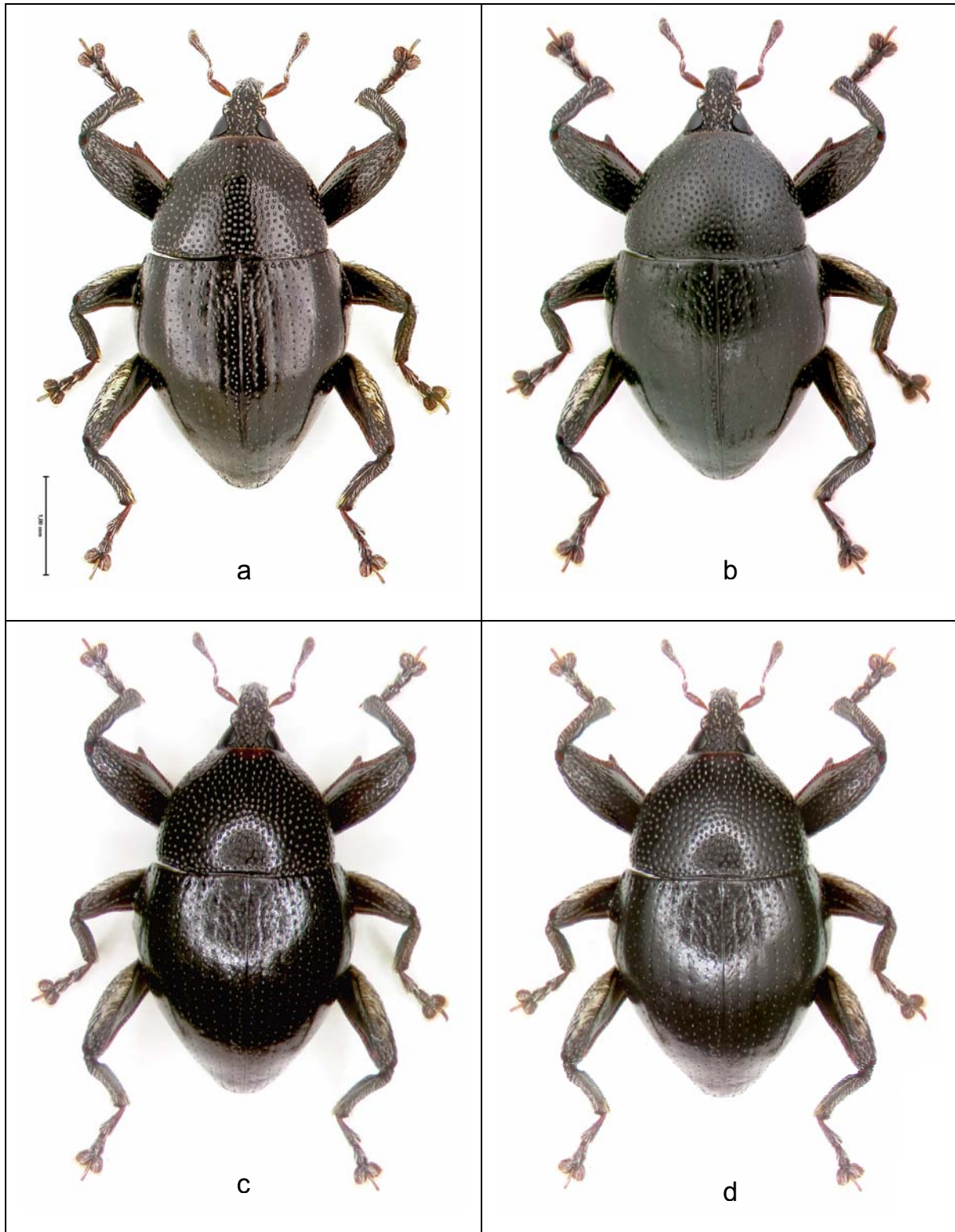
A technique described by LONGINO (2002) gives a more "natural" image: the ringlight is replaced by another set of double goose-neck fibre optic lights (fig. 8b) and the vellum cylinder with a Styrofoam cup with a cut off bottom (fig. 8c). A Styrofoam cup with two double goose-neck lights gives better results for diffuse lighting than does a set-up with a vellum cylinder (fig. 9b). With this method it is critical that the light from the fibre optics does not shine directly at the specimen, but only onto the Styrofoam wall above it. From there it is bounced and effectively diffused. The four light beams may enter the cup from above, or through small openings in the cup's upper third. This technique has been employed successfully for an imaging project of ants (C. KLINGENBERG, Staatliches Museum für Naturkunde, Karlsruhe).

BUFFINGTON et al. (2005) developed the styrofoam-cup-technique further by the use of a construction made of two Styrofoam soup bowls; they dubbed this light chamber "the spaceship". Good results were also obtained for small, shiny Hymenoptera. I have not tested this technique personally.

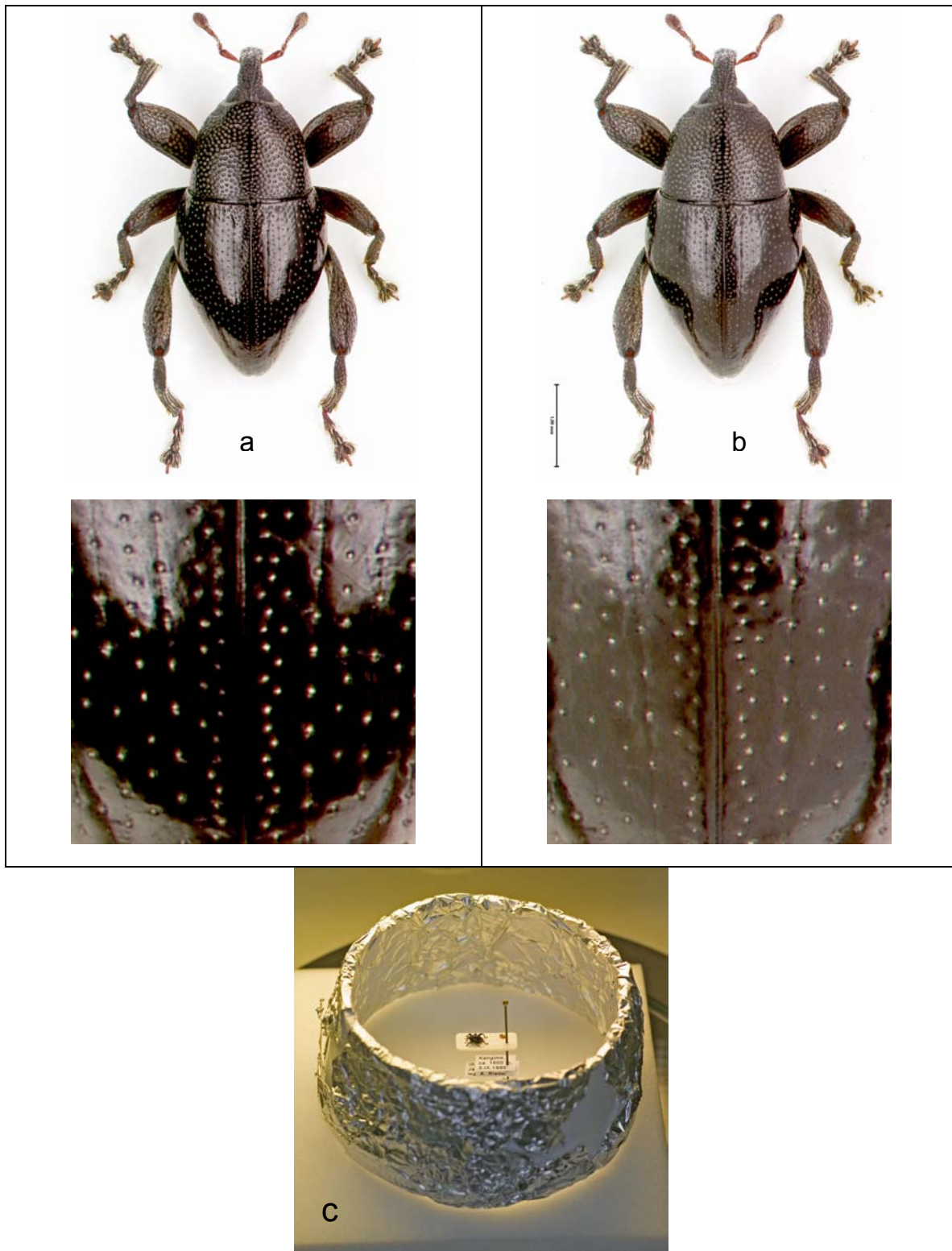
Probably, the method best suited for shiny beetles with delicate surface structure is the use of fluorescent desk lamps (fig. 9a). The set-up (fig. 8a) is both cheap and provides excellent illumination. Two facing desk lamps are arranged around the specimen. Such lamps are usually provided with a silvery reflector about 22 cm long. The precise properties of the lighting may vary with the reflector chosen. The Philips PL-11 W light tube, with a length of about 20 cm



Figs. 8 a-d. Different lighting setups. (a) Leica Z6 and a pair of energy-saving desk lamps; (b) Leica Z6 and setup of four goose-neck fiber lights and a styrofoam cup; (c) detail of styrofoam cup; (d) setup of ringlight and vellum cylinder.



Figs. 9 a-d. Example of a card-mounted weevil (Curculionidae, *Idotasia* sp.) in dorsal aspect illustrating different ways of lighting. Length from tip of elytra to apex of rostrum 4.0 mm. (a) with a pair of energy-saving desk lamps; (b) with four goose-neck fiber lights and a styrofoam cup; (c) with ringlight, without vellum cylinder; (d) with ringlight, with vellum cylinder.



Figs. 10 a-c. Example of a card-mounted weevil (Curculionidae, *Idotasia* sp.) in dorsal aspect illustrating the effect of a reflector of aluminium foil. In both images a pair of fluorescent desk lamps was used for lighting. (a) without the reflector; (b) reflector in use; (c) the reflector used in (b). Note that more fine detail is retained in shadow areas.

yielded good results. Shorter and brighter light bulbs are available, but the lighting is more even with the longer ones, which are preferred despite the added difficulty of positioning them around the specimen. Shadowed areas on the specimen can be better overcome by placing a shallow reflector around the specimen (figs. 10a-b). A reflector can be made by cutting a ring from a Styrofoam-cup and wrapping it with aluminium foil (fig. 10c).

The method selected depends on the characters of the specimens and the purpose for which the image is required. And it should be noted that that the resulting images may differ significantly depending on the method applied.

Montage software

Digital imaging combined with the appropriate computer software has overcome the limitations of depth of field for photography of museum specimens. There exist a number of computer programs that receive stacks of images, examine each image for areas that are in focus, and then stitch all the in-focus components together to make one single perfect image. Examples are CombineZ5©, Astro-Stack©, and Stack Focuser©. Some of these are freeware, many others cost little. Unfortunately the excellent Automontage© by Syncroscopy, the market leader comes with a considerable price-tag. The cheap programs have serious limitations at present, so they are not discussed in greater detail herein. However, some of the montage programs are still being developed and improved and it is worth tracking them in the hope of getting a good yet cheap solution. The following comments are intended to provide a few practical hints on Automontage©. A more comprehensive treatment can be found in ASHWORTH & FOGARTY (2003) and in the user manual of Automontage©.

Automontage© can be used for all stacks of images: either stacks taken manually (i.e. triggered image by image) with any camera, or for stacks taken by automatic capture (i.e. where a z-stepper-device moves the focus and triggers the camera). I found that the use of a z-stepper is only satisfactory if the microscope is equipped with a motor-focus. The construction of a microscope with manual focus

and an attached motor is problematic since the connection between the focus knob and the motor can slip. If a microscope with a manual focus is available only, it is easiest to move it by hand and to trigger the camera manually.

With a fully automatic setup and an average-sized beetle, I capture typically 40-70 steps per montage-set. I could not detect any improvement in image quality if the precision-option is used for scan-montage, thus I make use of the standard settings of “Speed” and “Fixed” (fig. 11). Usually, there will appear some artefacts in the montage-image, i.e. “dead areas” that are not resolved by the montage process. One way to eliminate these artefacts is to enlarge the “patch size”. However, with larger patch size, fine details will be lost from the image, so I prefer to perform the scan montage with a patch size of 10 and remove the resulting artefacts in a subsequent step. The “edit brush” of Automontage© is a useful tool to remove “dead areas” from a montage image by cloning the respective areas from an appropriate single image (fig. 12). The problem of such artefacts can be further limited if a stack of images is divided into two parts. Partial stacks can be processed individually and subsequently montaged manually in Photoshop. This is especially useful if extremities of the insect are sticking out and overlap with other parts. The full stack should be divided into two parts, one with the extremity, the other with the remaining body.

Automontage© has sometimes a problem to identify maximum sharpness of a contour in a stack of images. It can be useful to check such edge areas, e.g. the sides or the declivity of the elytra for maximum sharpness. If necessary, the montage image can be improved manually with a small sized “edit brush”.

My personal settings as I use them at present can be seen in fig. 13. I apply the “contour mode” (which provides a sharpening) to some degree to assist the software in calculating a precise image. But, in general it should be kept at a low level to avoid early over-sharpening of the image (see below “Post-processing”).

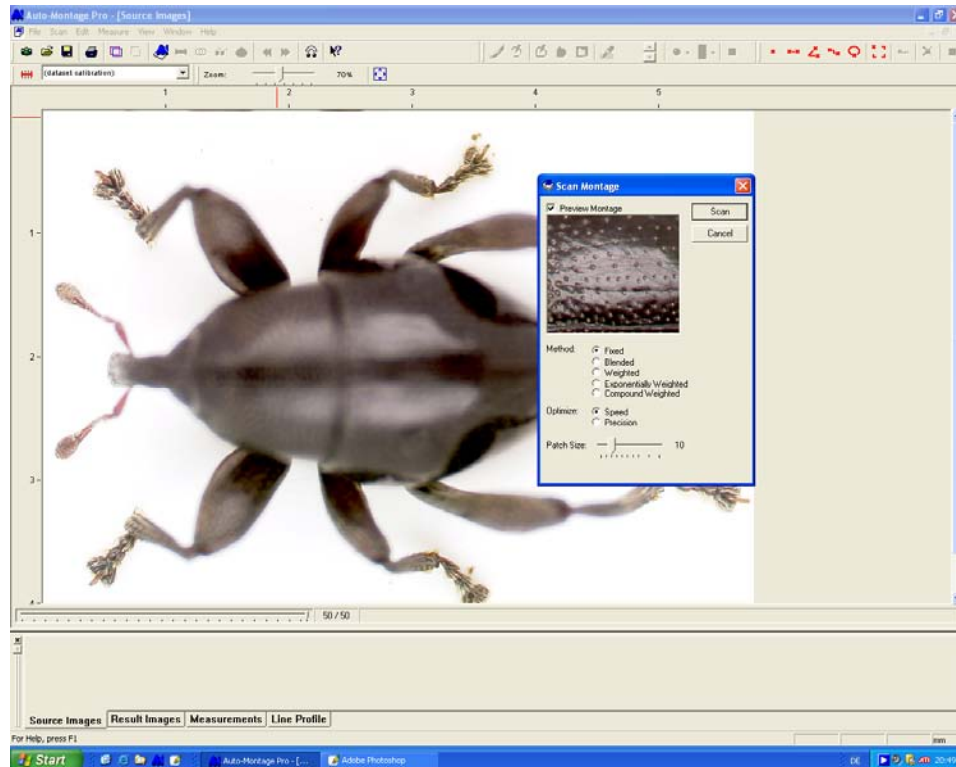


Fig. 11. Screenshot of the Scan montage window of Automontage©. Settings as they were used by the author for the previous images.

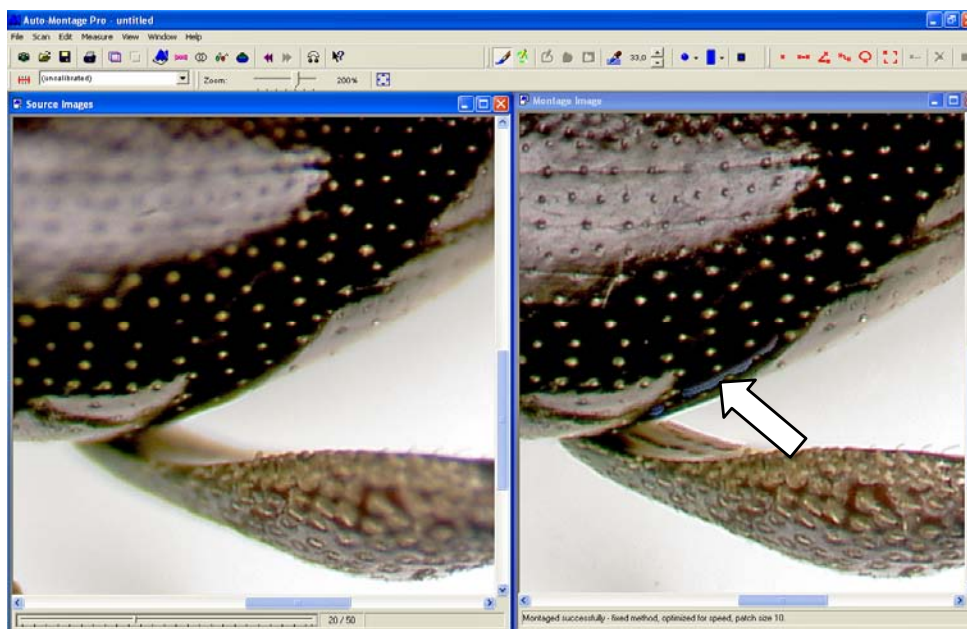


Fig. 12. Screenshot of Automontage© during the editing process of the montage image. On the left a window with a stack of 50 original images. On the right the montage image. Note the artefacts marked with the arrow. They can be removed by cloning the respective area from one of the stack images.

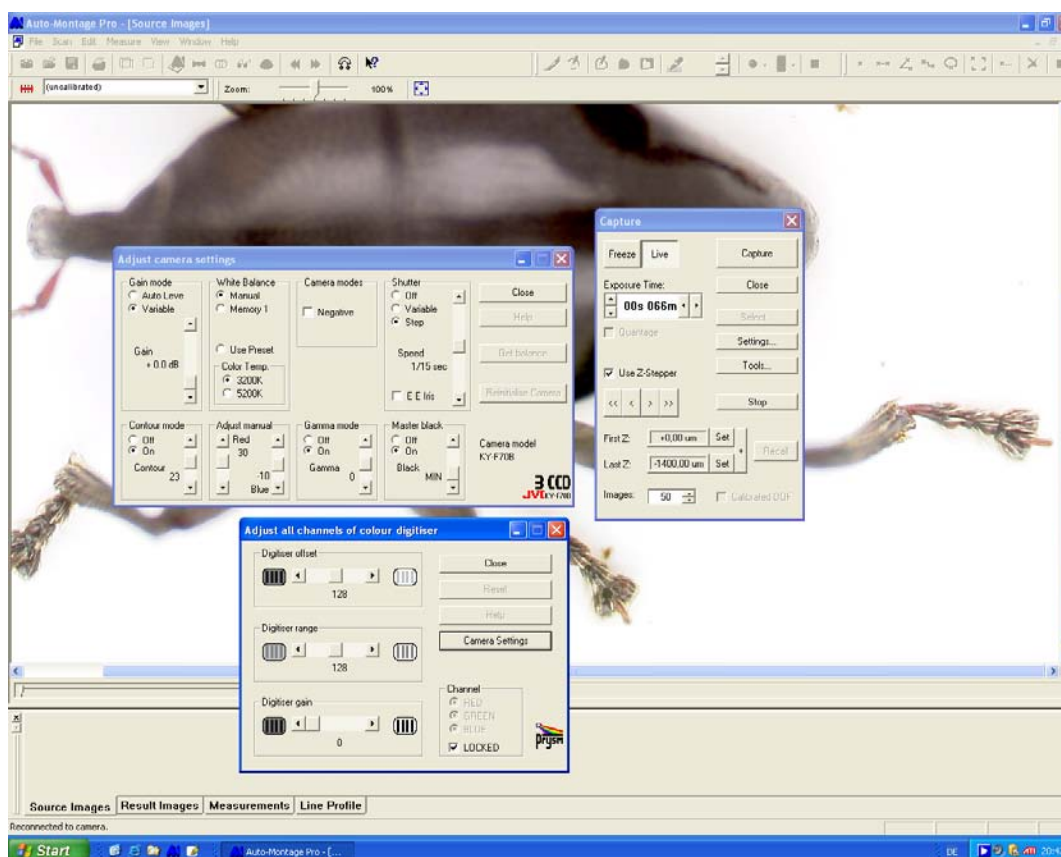


Fig. 13. Screenshot of with the main setting-windows of Automontage©. Settings as they were used by the author for the previous images.

Scale

Since most specimens are very small it would be hard to place an original scale bar next to each specimen that is recorded as a part of the image. Thus, a scale bar needs to be added during post-processing of the image. Automontage© offers convenient options for calibrating and drawing the scale bar. The first option is to use fixed magnifications for which the corresponding scales can be selected from the menu once calibrated. This is convenient, especially if the images of a given taxon are at the same scale. However, this method rarely takes full advantage of the frame of the camera. Personally, I prefer the method of calibrating the scale for each image individually. After taking a montage set of images the specimen is removed and a sheet of millimetre-square-grid paper is placed under the microscope. The magnification must not be changed at this stage, the focus may need some adjustment. The

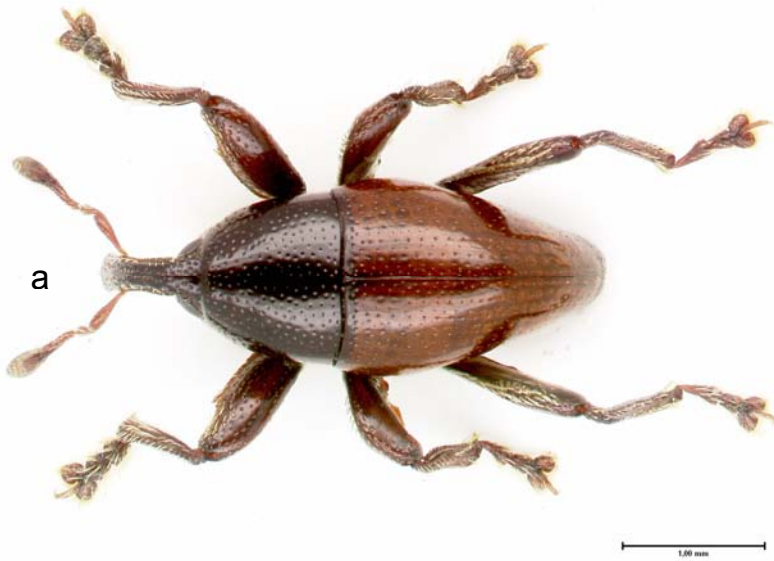
entire length of the frame or a major portion of it is measured. After closing the capture menu, a scale is calibrated in Automontage© using this distance, *i.e.* a line is drawn over the full length of the frame. Automontage© will calculate a suitable scale bar based on this value and place it at a chosen position. The "engrave scale bar"-option must be activated when exporting the montage image as a TIF file from the montage data set.

Post-processing of images

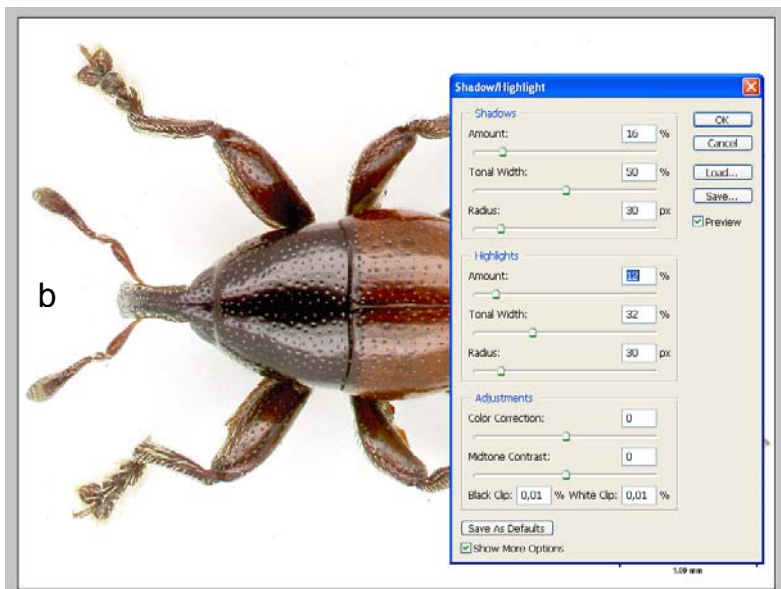
An image can be greatly improved to meet its purpose by post-processing with programs such as Adobe Photoshop© or Corel Photopaint©. A few general hints are given here that will apply to all image processing programs. Some tools of Adobe Photoshop are described in greater detail because this software is most widely used today.

Many digital cameras still suffer from a narrow dynamic range. This means that the tonal range of an image from light to dark may not be covered by the camera without clipping some information. This problem occurs if a black beetle mounted on a white piece of cardboard needs to be photographed. Sometimes, there is only the choice between a correctly exposed body and overexposed legs; or, correctly exposed legs and an underexposed body that may not reveal any detail. There are two different ways to resolve this problem.

The first option is to take a picture with a slightly underexposed body and with overexposed legs and antennae. Subsequently, these artefacts are corrected using the "Shadow/Highlight" option of Adobe Photoshop© (Version 8 or higher). Possible settings and the resulting effects are illustrated in figs. 14a-c. Generally, it is easier to handle a somewhat overexposed image (unless the areas are completely blown out) since the brighter tones contain more data than the darker ones. Usually, underexposed areas contain more digital noise, too. So, unlike the situation in conventional photography where it is easier to handle somewhat underexposed images, the opposite is true for digital images.



Figs. 14 a-c.
Use of the “Shadow/Highlight”-tool of Adobe Photoshop®.
(a) A card-mounted weevil (Curculionidae, *Idotasia* sp.) in dorsal aspect; before the procedure.



(b) example of the settings being used.



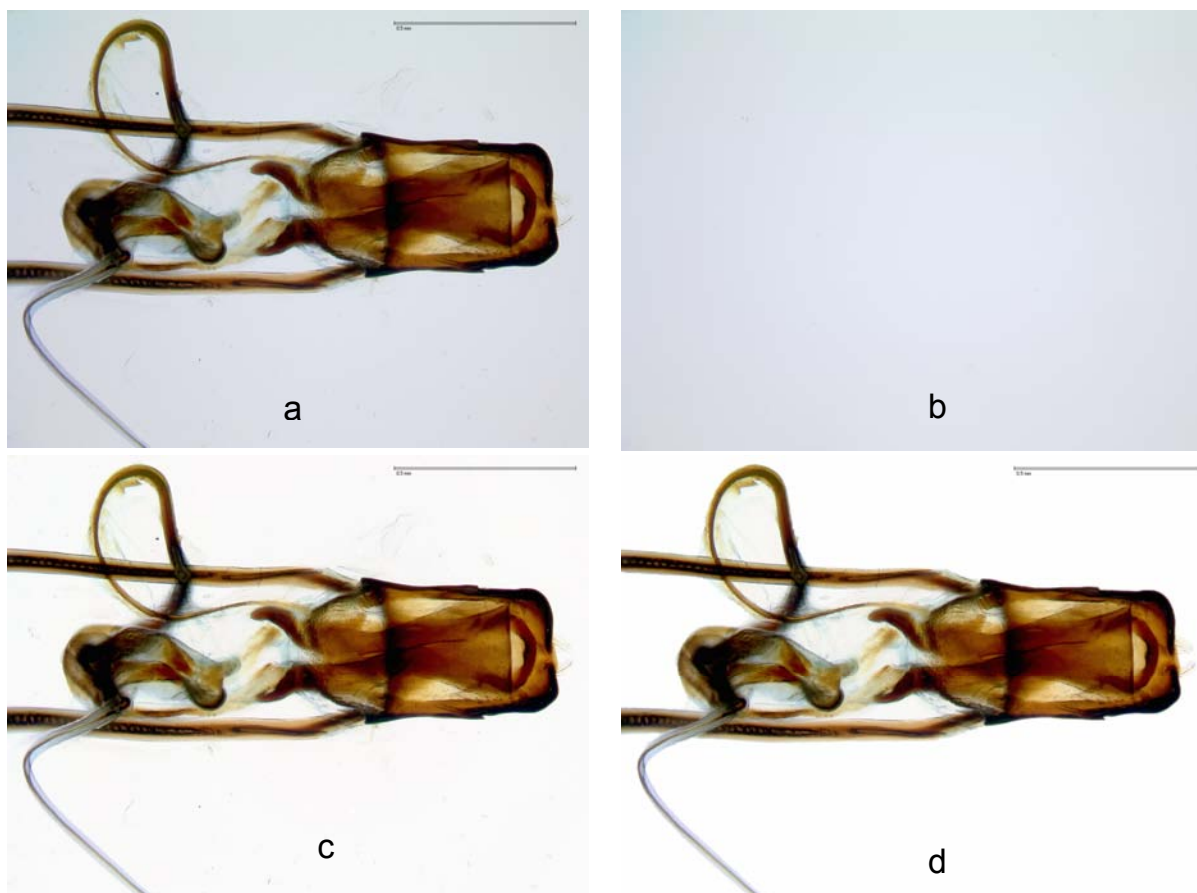
(c) resulting image; more detail can be seen in shadow areas; the tarsi and the antennae are darker and look more natural.

The second option is to take two images, with different exposure settings. Subsequently, these can be merged into one perfect image. This may require some practise, but is in fact quite simple: the two images should be opened on two separate layers and brought into perfect congruence. This can be done by switching between the layers and checking if the beetle "shifts" during the switch. Then, the eraser tool is used to remove parts of the image lying above, so that the better parts of the image lying below shine through. Various degrees of feather and transparency can be applied to the eraser for smooth transitions. Finally, both layers are merged into one image.

Other problems occur when parts of beetles, such as genitalia are photographed with a camera attached to a compound microscope. Images taken with transmitted light microscopy sometimes suffer from vignetting or some shading of the background. This problem can be overcome easily with a technique used by S. SCHMID (Zoologische Staatssammlung, Munich): Along with each photograph of a subject (fig. 15a), a second one is taken of the background (fig. 15b). For this purpose the subject is moved slightly to the side so that it is just outside the frame and a second picture is then taken. Magnification, illumination etc. must not be changed. The picture of the background can then be used in Adobe Photoshop to cancel out uneven lighting by the following steps:

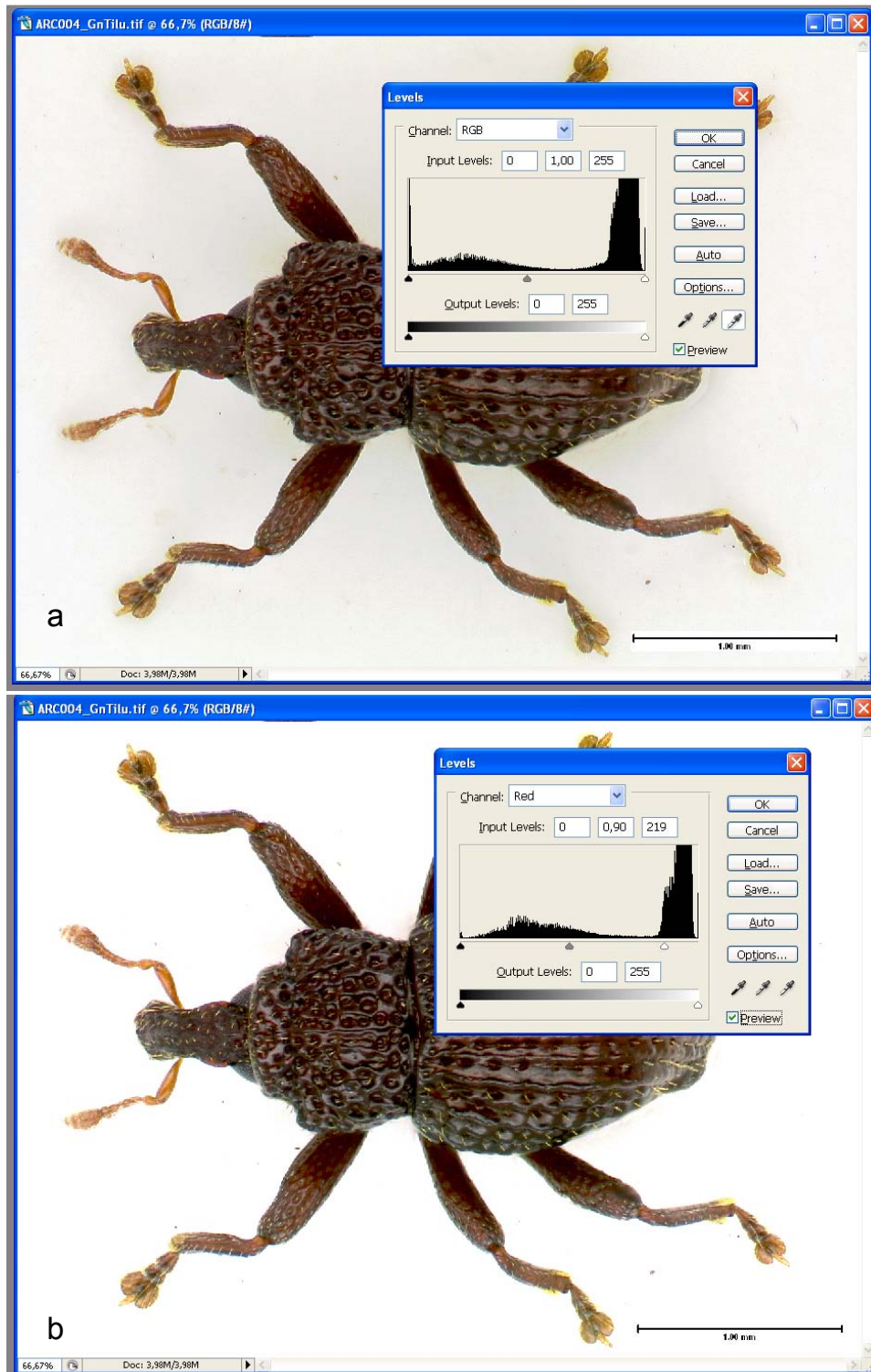
1. Open the image of the subject in Adobe Photoshop.
2. Create a new layer on top of it.
3. Open the background image, and invert it by using the Ctrl-I-shortcut.
4. Select everything (Ctrl-A) and copy it (Ctrl-C). Then close the file of the background-image.
5. Add the copied image into the new layer (Ctrl-V).
6. Chose in the menu of the "Layers"-window "Color dodge". The effect should be visible now (fig. 15c).
7. Combine the layers.

Finally, contrast and tonal levels of an image should be adjusted, so that the entire tonal range is made use of, i.e. that white is white and black is black. It is a common mistake to use the "adjust contrast - brightness" options of such programs. Information could be irrever-



Figs. 15 a-d. Aedeagus of *Idotasia* sp. (Curculionidae), same as in fig. 6c; explanation of postprocessing procedure. (a) image of the subject; (b) image of the background; (c) background image was used to cancel out shading of background in Adobe Photoshop; (d) final, further improved image.

sibly lost from the image without this being noticed. A much better way is to make use of “levels” (figs. 16a-b) or of the “tone curve”. Slight colour casts can also be removed in this way. Usually the picture will contain some portion that should be white, such as the cardboard to which the specimen is glued. Such an area can be used to set a “Whitepoint” with the eyedropper-tool of the “levels”-menu in Adobe Photoshop. The tonal distribution of the image will be adjusted in a way that brings the selected area to a pure white. Such adjustments can also be made manually by pulling the slider for the shadows to the right, and the one for the highlights to the left. Clipping certain tones can be monitored precisely by pressing the “Alt” and “Ctrl”-Keys simultaneously.



Figs. 16 a-b. Use of the “Levels”-tool of Adobe Photoshop©. (a) A card-mounted weevil (*Curculionidae*, *Idotasia* sp.) in dorsal aspect; before the procedure. (b) preview of the resulting image; the white eyedropper was used to set a whitepoint and brighten up the background; subsequently, a remaining reddish cast was removed by changing the midtone-value of the red channel.

Colour casts can be corrected by selecting the respective colour channel in “Levels” and then adjusting the slider for the mid-tones. For example, if there is a bluish cast, the blue channel is selected and then the value of the mid-tones is somewhat decreased. Similar corrections can be done using “tone curves”. For an in-depth treatment of such techniques the relevant literature should be consulted, e.g. EVENING (2005).

Finally, some degree of unsharpen mask can be applied. However, this must be done very carefully. As the settings differ depending on the final purpose of the image (e.g. print size), no general recommendation can be given. It is a good idea NOT to apply any sharpening effects to an original image, not even to a copy that has been edited and adjusted with lots of efforts. Sharpening should be applied as the very last step, and the sharpened image should be saved as a separate file. Sharpening may result in artefacts such as visible halos on edges and this should be avoided in any case. Certain plug-in programs for Adobe Photoshop are available. Most of them achieve better results than the regular “USM” of Adobe Photoshop. I found that “Focal Blade” is useful for sharpening while keeping artefacts at a low level.

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