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A Portable, Low-cost Approach for Photographing Fluid-Preserved Snake Specimens: Recommendations with Comments on Optimizing Specimen Photography in Natural History Collections

Access to preserved specimens in museum collections is one of the key needs of those engaged in systematics research (e.g., Bi et al. 2013; Rocha et al. 2014; McLean et al. 2016). Yet, sometimes the constraints of research budgets and time prevent the optimal use of this critical resource, resulting in project delays, incomplete information, or flawed scientific conclusions. With many natural history museums now digitizing information related to specimens in their collections, imaging of specimens is a logical next step, and one of critical importance to make holdings available electronically to a broader audience (Baird 2010; Lister et al. 2011; Knight-Davis et al. 2015; Page et al. 2015).

A complete 2D image library of all specimens in a collection may appear utopian at the moment, given the millions of specimens and lack of financial support for collections (e.g., Paknia et al. 2015). However, outside of visiting each collection to study individual specimens, or requesting loans of unique and valuable specimens, the lack of suitable specimen images means that some data may simply remain unavailable to researchers who cannot afford to obtain them. We wish to emphasize that the approach we advocate herein in no way negates the need to maintain and make accessible physical specimens in a collection. Although in rare cases where the lack of specimens is unavoidable (e.g., Marshall and Evenhuis 2015; Pape et al. 2016), there is no replacement for examining a well-preserved specimen. Our method should be regarded as an ancillary technique, useful when it is necessary to obtain preliminary data or when it is not possible to examine the specimen in person, and for archival purposes.

CHRISTINE M. KAISER*
AG Evolution und Systematik der Tiere und Zoologische Sammlung Marburg, Fachbereich Biologie, Philipps-Universität Marburg, Karl-von-Frisch-Straße 8, 35032 Marburg, Germany; and Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20013, USA; e-mail: lirellus@yahoo.com
HINRICH KAISER*#
Department of Biology, Victor Valley College, 18422 Bear Valley Road, Victorville, California 92395, USA; and Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20013, USA
KAITLIN J. RICKERL
Department of Biology, Victor Valley College, 18422 Bear Valley Road, Victorville, California 92395, USA; e-mail: kaitlinrickerl@yahoo.com
MARK O'SHEA
School of Sciences, Faculty of Science and Engineering, University of Wolverhampton, Wulfruna Street, Wolverhampton WV1 1LY, United Kingdom; and West Midland Safari Park, Bewdley, Worcestershire DY12 1LF, United Kingdom; e-mail: m.oshea@wlv.ac.uk

**Joint first authors, listed in alphabetical order*
#Corresponding author; e-mail: hinrich.kaiser@vvc.edu

Here we describe a simple, flexible, low-cost methodology for 2D imaging, which two of us (CK, HK) have tested extensively during our research at various institutions whose research space (in terms of size, quality, and access) and supportive equipment (lighting and suitable backgrounds) ranged widely. Our cost-conscious approach produces an image that is adequate for research and publication, although better equipment and alternative set-ups may provide images of higher quality but at higher cost in time and materials. While we realize that many of our more experienced colleagues may have their own protocols according to their experiences and equipment, our method is designed as a practical, entry-level compromise designed to make good 2D imaging accessible to most researchers.

The process we have developed is also appropriate for use on any taxon for which two-dimensional images can assist in data collection, including many fish species but also for the head scalation in reptiles, whose deviation from a flat surface can be shown to be mathematically insignificant (< 1% measurement error; pers. obs.). Our recommended approach makes 2D imaging easy for visiting researchers or collection staff, thereby facilitating the establishment of image databases in a relatively rapid and economical manner. Furthermore, the technique would provide a permanent record of a specimen as it existed at the time it was imaged, a hedge against the loss of all or part of the collection, as regretfully has happened in years past and present (e.g., Staatliches Museum für Tierkunde in Dresden, Germany in 1945; Museo Bocage in Lisbon, Portugal in 1978; Instituto Butantan in São Paulo, Brazil in 2010; National Museum of Natural History in Delhi, India in 2016; Museu Nacional, Rio de Janeiro, Brazil in 2018). In terms of providing a public service, these images could provide instant online access to an image of a specimen by any researcher who may need to take a first look, perhaps as a preliminary step before placing a loan request or deciding on a visit to examine the actual specimen.

Collection abbreviations used herein conform to the listing of Sabaj (2016).

Equipment

Camera and Camera Accessories.—Exceptionally high quality camera equipment with exchangeable lenses and external flash units would likely produce better quality research images, but can easily exceed the budget of the average photographer or systematist. Given that most biologists, especially graduate students, operate on modest budgets, costly equipment can be a significant deterrent. Furthermore, there can be complications during air travel with valuable camera gear, whose weight may exceed even the most generous carry-on allowances of major airlines (MOS, pers. obs.). In our own work (with the exception of MOS, who uses a high-end DSLR system), we prefer the use of advanced cameras in the point-and-shoot category (specifically

the Sony DSC-HX300 and DSC-HX400V, with possible resolution > 20 megapixels; Fig. 1) that were purchased in refurbished condition from online retailers for just under USD 300 each.

Even though the Sony brand is the one we used in developing our protocols, any of the major camera brands (e.g., Canon, Leica, Minolta, Nikon, Pentax) produce camera models that would serve equally well in a low-cost 2D imaging scenario. Any mirrorless camera with suitably high resolution (> 20 megapixels) and a macro lens can also provide similar or better image quality at similar weight and dimensions. Examples of these include the Sony 6000 series and the Fujifilm X-T20 or X-A3 models (using APS-C sensors; crop factor 1.6) or the Olympus Pen-F and E-M1 II (using an MFT sensor; crop factor 2.0). As long as the main requirements of portability and image quality can be met, there clearly are many options. Thus, our methodology should not be considered as an endorsement of one specific manufacturer over another. While we are not photography experts in this camera range and cannot predict shifting markets and technologies, we do not recommend the use of point-and-shoot cameras at the lower end of the spectrum because they do not produce images of appropriate quality (in terms of resolution, light capture, and sensor quality) to be considered research-grade or publication-quality.

As with any camera, users are cautioned to test their system in advance of a photo session to determine which settings work best with their particular equipment. Given the many different lighting conditions one may encounter, there is no single series of settings we can recommend as reliable. However, the camera must have a manual focus setting because light reflection on some specimens may fool a camera's autofocus sensor(s). Furthermore, two (or more) spare batteries, a battery charger, and a set of international adapters should be part of any standard equipment setup (Table 1). If the camera has a hot shoe, it will allow the use of one or more external flash units, which may provide better lighting (see Light Source, below), but will also raise the cost, weight, and battery requirements.

Light Source.—One of the most difficult parts of any photography setup is lighting. In the case of 2D specimen imaging, one option is the built-in flash on the camera. We have found that this flash serves well for images taken from a distance (e.g., whole body shots), but not for close-ups because the protrusion of the lens will cast a shadow at close range. The problem is worse for macro shots at distances < 5 cm. Even for the shots at greater distance, it is better if the built-in flash has adjustable settings so that images are not over-exposed and bleached. Several trials using different flash settings are necessary preparation for the type of photography we propose. We recommend taking images using the flash at its default settings

Table 1. Proposed equipment list for low-cost 2D macroscopic imaging of scientific specimens. This list is comprehensive and includes items reflecting our personal preferences.

Equipment type	Item	Comments
photography	camera	20 MP resolution, manual focus, hot shoe for external flash
	batteries[1]	three batteries recommended, need depends on flash usage
	charger	standard battery charger to fit camera batteries
	adapter	to allow charger to fit into outlets in other countries
light source	LED flashlight	adjustable focus recommended
	batteries[1]	three batteries recommended
	charger	standard battery charger to fit flashlight batteries
background	crochet frame	plastic and wooden models exist
	T-shirt	black is best to reduce staining from preservative spills
	nylon thread	to flatten specimen for 2D imaging
dissecting tools	fine-tip curved forceps	
	fine-tip straight forceps	
	regular forceps	
	fine dissection scissors	
	regular dissection scissors	
	straight probe	
	gauge 0 insect pins	
measuring tools	plastic measuring tape	to determine body length
	non-elastic string	to make length measurements
	digital or dial calipers	to measure head scale dimensions
	manual counter	for counting scales
supplies	1-inch painter's masking tape	attachment of light source
	clear tape	to attach measuring tape to the workspace
	personal fans	optional, to disperse fumes, with batteries
	fine-tipped felt pen	for labeling and note-taking, waterproof ink

[1]There are airline restrictions for the most common type of rechargeable battery (lithium ion). Whereas equipment with batteries installed may be stowed in checked baggage, any spare batteries must be carried in carry-on baggage to avoid unobserved battery ignition that could trigger a fire.

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Fig. 1. The Sony DSC HX-400V, one of the high-end point-and-shoot cameras we have used for 2D imaging of snake specimens. The camera has a manual focus option and a hot shoe for an external flash, as well as GPS capability.

at 10-cm intervals beginning at a distance of 100 cm. This will allow production of a default series, based on which flash settings can be optimized and the minimum flash distance can be approximated (i.e., the distance without a shadow from the lens).

We have experimented with a variety of dual goose-necked illuminators (0–2000 or more lumens, 5000K OEM white light) as external light sources (Fig. 2A) that allow the adjustment of the intensity and the direction of the light beam. Select a goose-necked illuminator with a bulb type that provides neutral white light—we have encountered some illuminators that produce an unnatural, blue tinted light at the highest power. During various trials, we have found that a single illuminator is adequate to shine sufficient light on a specimen, that the chosen light level can be relatively dim if the camera is adjusted properly, and that it is best to shine the light onto the specimen's surface at an angle that does not reflect a glare into the lens. Positioning of the specimen's head is invariably improved by a manual hold (as opposed to placement on a flat surface) because this allows movement of both specimen and camera within the light beam to optimize the image. Holding the specimen also improved steadiness for the shot because the camera can be rested on the hand holding the specimen. We always take multiple images for each surface, and we check each image immediately after shooting a photo to determine whether it is in focus and if it has the correct exposure. Some aspects of exposure and color can be improved later through careful editing in suitable software (e.g., Adobe Photoshop, Aperture, Lightroom, Apple Photos) or online systems (Google Photos).

When a goose-necked illuminator or comparable light source is not available, we have found it expedient to use small flashlights (i.e., torches in British English) capable of producing relatively high intensity light. After much trial and error, we found that the LED Lenser M5 (Zweibrüder Optoelectronics GmbH, Solingen, Germany) provides an optimal solution for this issue. These lightweight flashlights are powered by a single AA battery, allow focusing of the light beam (with a power range of 40–140 lumens), and are easily attached with masking tape to furniture, boxes, or plastic jugs at an angle suitable for photography (Fig. 2B). AA batteries are generally available even in remote locations, which makes this option affordable and practical[1]. Other solutions include the use of a rechargeable LED Lenser, an LED ring light, or a tabletop light tent (which can be built using PVC tubes and white rip-stop nylon, or which can be ordered online, such as the Cubelite series by Lastolite). We have not yet experimented with light modifiers (the simplest of which could be to shine the light through a thin, white plastic bag) to add diffusion and reduce harsh shadows and light reflection. This technique has been used to great success, especially in macrophotography.

In a discussion of lighting, we would be remiss not to mention the so-called exposure triangle (e.g., Judge 2012; Peterson 2016), where photographers optimize the interaction of shutter-speed, aperture, and ISO (gain applied to the output of the digital sensor). The latter is also influenced by the signal-to-noise ratio of the sensor. For 2D imaging as described here, it is essential to provide suitable lighting. Low lighting conditions can, to some extent, be mitigated by increasing the camera's ISO value, but at high ISO values, noise in an image may be produced. Technical limitations in mid-level cameras will generally not allow compensation for this higher ISO by opening the aperture more (which needs to be small to obtain best depth of field; see below), nor can speed be lowered for fear of resulting image blur (from camera shake). Optimization of the exposure triangle therefore

[1] It is preferable to use rechargeable NiMH (nickel metal hydride) or disposable alkaline AA batteries. Lithium-ion AA batteries should not be used in flash units as they cannot withstand the recharging cycle of the flash, and may overheat and malfunction. There are also obvious problems of flying with large numbers of batteries due to weight constraints, and readers should note that spare Lithium-ion rechargeables are not allowed in checked baggage.

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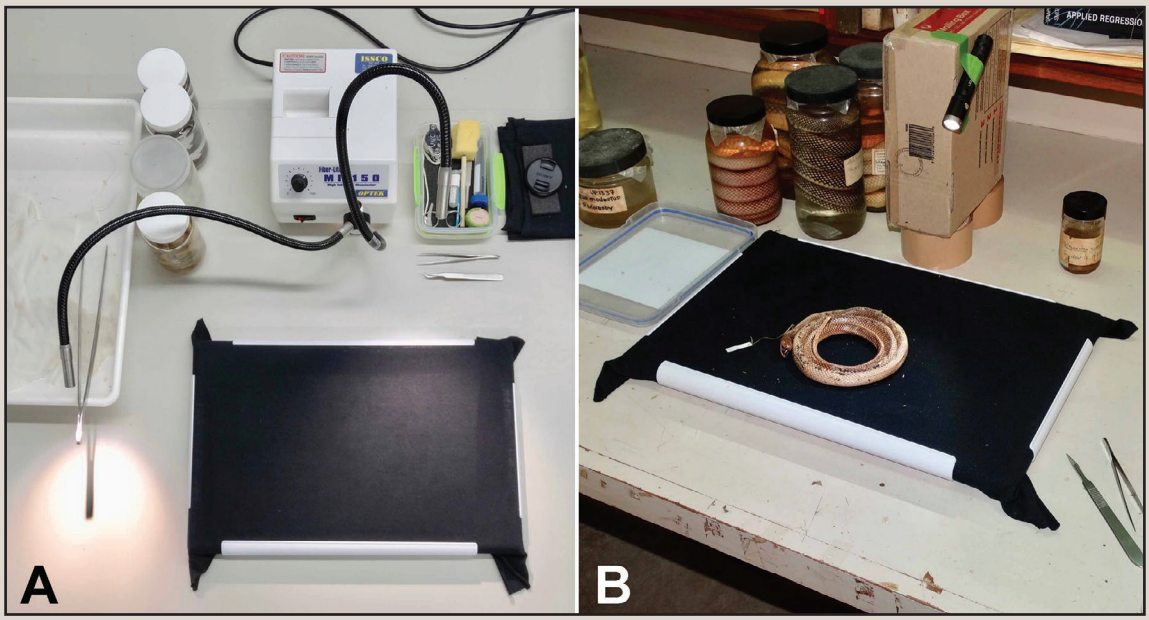

FIG. 2. Two options for a portable, budget-conscious 2D imaging setup. A) An ideal setup at the Australian Museum in Sydney, Australia. Goose-necked illuminators are available, and the working space is smooth and uncluttered. The setup shown includes a specimen tray and specimen jars on the left, with illuminator and dissecting tools in the upper center, and with the background closest to the photographer. Note the position of the two light sources of the illuminator, one to shine directly on specimens held over the dark background material, the other off to the left to allow illumination for dorsal scale counts. B) A makeshift setup on a narrow lab bench at the University of Papua New Guinea in Port Moresby. Note the position of the LED Lenser flashlight taped to a cardboard box, supported by two rolls of packing tape.

requires proper lighting and, whereas goose-necked illuminators will always provide this, it may in certain conditions require a second or stronger flashlight (i.e., torch) to obtain the desired results.

Background.—In order to standardize 2D images of snake bodies and heads, it is necessary to consider the type and color of the background. In photography, a key issue is background reflectance (i.e., how much light is able to reflect from the background back onto the object), which ranges from ca. 90% on a pure white background to near zero on a pure black background. A standard for this has been "neutral gray," which produces a reflectance of 18%. Selecting a suitable background is an important aspect of standardizing photographic images, but just as with the specifics of camera equipment, individual preferences play a role. For example, if high reflectance is desired to reduce background shadows on the object, a white surface (such as a PVC sheet) could be used. In addition, a reflector could be set up opposite the light source to further ameliorate shadowing. As a general reminder, and depending on the background color, adjusting the exposure compensation on the camera may be necessary. In our own setup, we have preferred a uniformly dark background, which produces less obvious shadows. We have found this to be suitable and editable (e.g., using the magic wand tool in Adobe Photoshop), and we always cut out our snake images to place on a black background for creating figures for publication. Of course, there is a trade-off: the edges of very dark specimens on a dark background may be difficult to detect, and masking out such a specimen may be tricky. We always continue to explore alternatives and we encourage our readers to find the solution that best suits their objects and setup options.

A key requirement in our approach is portability so it is easy to set up and break down. Because most of our imaging involves fluid-preserved specimens that must be removed from the preservative shortly before photography, we needed a background that would dry rapidly or could be wiped dry, would be minimally discolored by liquid draining from specimens, would not reflect light excessively (especially important when drops of preservative may be present), and would allow us to eliminate any wrinkles or imperfections beneath and adjacent to a specimen. Whereas a white PVC sheet fulfills these requirements, our solution is a 17 in × 14 in (43 cm × 35 cm) plastic crochet frame, which costs less than USD 20 and is easily assembled (Fig. 3, top) and disassembled (Fig. 3, bottom). We place a cutout from a plain black 100%-cotton t-shirt in the frame (one t-shirt will yield at least two cutouts) and stretch it to eliminate wrinkles. This system provides a uniform, dark, non-reflective surface to allow standardization and eliminates the need for lighting or color adjustments that may be necessary with variable backgrounds. With a specimen in place, the stretched material sinks down slightly, allowing the whole specimen to rest in the fabric (Fig. 2B) and thereby reduce shadowing around the specimen and stabilize its position. When manually manipulating parts of the specimen for close-ups, the background can be moved to accommodate the particular angle needed for the best light. The cotton fabric dries quickly, and can be dried out easily by placing it in the air stream of a fan or air conditioner. We much prefer plain cotton to some other types of fabric (such as short-nap velvet) because it is less reflective and retains less moisture from specimens. We have been able to photograph snakes up to 1800 mm in total length, displayed in coils, with this setup. Because of its uniformity and stretched appearance, this background is also very effective for fishes, including smaller specimens and larval stages (e.g., tadpoles, larval eels, invertebrates). We recommend having two frames available so that one may dry while the other is in use.

The crochet frame setup also allows photography of some specimens that have unusual kinks that prevent them from being naturally be imaged in two dimensions. We use strong nylon thread (such as thick monofilament fishing line), which can be tied tightly across the frame to hold down uneven specimens. Two such threads spread across the frame, if tightened properly, can hold down a specimen to dramatically improve photography. They can be easily removed from the image, using photo-editing software, if desired. The advantages of the crochet frame setup over a solid PVC sheet include the frame's greater portability (i.e., by dismantling it), the fabric's flexibility and the resulting ability to manipulate specimens, and the fabric's ability to hide background imperfections. Again, we arrived at what we prefer to use by trial and error, and we encourage others to develop their specific *modus operandi*.

Ancillary Equipment.—Based on our experience, we have found it useful to bring as much of our own equipment (Fig. 4) to aid in specimen examination as feasible, including measuring tools (e.g., calipers, tape measures), fine-tipped forceps, and scissors. We use brass- or stainless steel-headed steel pins (e.g., gauge 0 insect pins) to facilitate scale counting, pin skin flaps, temporarily pin coils together, flatten out a twisted specimen, or use as a pointer to identify particular features in an image. Because proper ventilation is not available in some facilities, we also carry battery-operated fans to reduce inhalation of preservative fumes, especially when working in conditions

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FIG. 3. Our preferred portable background for 2D imaging of museum specimens, a 17 in. × 14 in. (43 cm × 35 cm) plastic crochet frame, purchased at a craft supplies store. (Top) The assembled frame, with white clips holding the black cloth taut on all edges. (Bottom) The disassembled frame, showing all plastic components separated and the folded T-shirt material.

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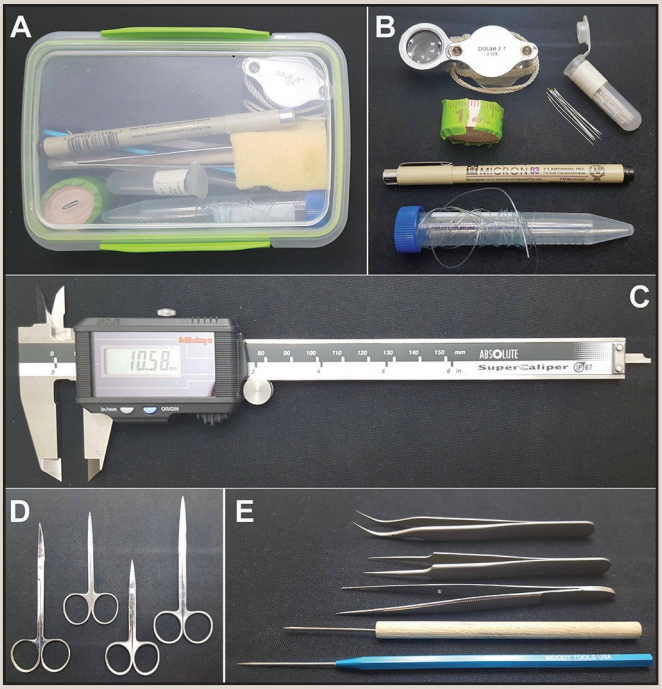

FIG. 4. Ancillary equipment for use with our standardized photography method. A) The toolbox, measuring ca. 7 in × 4 in (ca. 18 cm × 10 cm), allowing most of the materials to be kept in a compact set, protected by padding. B) Equipment we have found useful for specimen examination and positioning, including (left side) a small hand lens, plastic measuring tape, fluid resistant nylon tipped pen, nylon thread wrapped around a plastic test tube, and a set of narrow-gauge insect pins (Gauge 0). C) Our preferred pair of digital calipers (Mitutoyo brand). D) Four different pairs of scissors with cutting tips of different strength, shapes, and sizes, including a curved model. E) Two probes and three sets of forceps, the latter with tips ranging from fine to ultrafine.

where there are no exhaust fans or air conditioning. The specific rules and security restrictions for each country should be checked before importing scientific equipment or placing pointed objects into airline carry-on bags. For example, whereas it is not a problem to bring most small scientific tools onboard aircraft in the United States (as of this writing), scissors of any size are prohibited as carry-on in most other parts of the world.

SETUP

A reliable setup to obtain 2D images of snakes is one that provides sufficient space for the lighting and background, and is sufficiently stable to avoid unnecessary vibrations. In order to obtain properly focused images under varying light conditions, a vibration-free surface is important. Not only does a stable surface make photography easier, it also allows for bracing the user's arms while holding the camera and the specimen. In our experience, gripping the head of a snake specimen firmly with the fingers of the left hand will allow the user to reliably line up the head with the light source. Because most camera bodies are designed to be held with the right hand due to the position of the shutter release, the alignment of specimen, light, and camera forms a dynamic system that allows manipulation of the specimen and the camera at multiple angles. Holding the specimen independently of the camera may introduce inadvertent shaking into the process. A free-moving left hand holding a specimen and a free-moving right hand that is weighted down by the camera body are not easily stabilized. It is our practice to rest the right hand with the camera on the left hand with the specimen while lining up the shot, with both elbows placed on the table. With smaller-sized snakes, this introduces greater stability while retaining the ability to move the specimen and the camera, perhaps to find the "sweet spot" for the light and the autofocus mechanism. There is, of course, a limit to the size of a snake for which this protocol will work because the dimensions of hands, the snake's head, and the camera lens's focal length will make it impractical outside of macro photography. With snake head sizes > 5 cm (an approximate value, based on some trials we conducted), holding head and camera independently provides good results, as the increased size of the head mitigates the need for perfect stability.

In some cases, such as when imaging very shiny specimens, the autofocus mechanism of point-and-shoot cameras fails as the lens continually searches for a focal point. In such situations, manual focusing can be useful. During manual focusing, it is obviously not possible to hold the specimen in one hand, the camera in the other, and focus manually. We therefore approximate the focal length required and preset the manual focus at a specific distance, then vary the distance between the lens and specimen until focus is achieved. Some electronic viewfinders may not be able to detect whether an image is in focus using this method, hence manual viewing through the optical viewfinder may be needed. It may be useful to set the camera to automatically take a burst of images, then move the specimen towards or away from the camera during the burst, in order to make it more likely that at least one of the images will be in focus. This approach is not possible if a flash is used due to the time required for the flash to recharge, but for a well-lit close-up, this method can work well. Manual focus is also important, for example, when taking multiple head images at virtually the same scale without subsequent digital manipulation. Whereas autofocus will cause the camera to hunt for focus, this could be slightly different for every image, producing images at slightly different scales—a nuisance when trying to display several specimens at an identical scale in a publication.

WORKFLOW

The most important features of a snake that may be readily determined from 2D images include overall body morphology, color pattern, ventral and subcaudal scale counts, and the number and distribution of head scales. While these are not the only features important to snakes (others include the morphology of the cloacal region, the tail tip, hemipenes, prey items in the gut, as well as internal and external parasites) these images would be suitable for obtaining most of the data needed for a minimal assessment of a snake specimen, often including identification using automated keys (Hsu et al. 2017). Our efforts to image specimens important to our research have focused on these areas, with additional characters imaged as appropriate. We recognize that some specimens are preserved in a state that does not lend itself to 2D imaging or to the determination of some of the desired characteristics, but we have found that most specimens can be manipulated to allow visualization of most characteristics, particularly head scales.

The First Image.—It is essential for the subsequent management of specimen data to ensure that the images can later be linked to specimens. The first image of any photographic series

therefore must be of the specimen with its tag or, if tags are not attached to a specimen, of the specimen container and any labels present to allow identification of the specimen with the tag. If containers have multiple untagged specimens, we sort the specimens by size from largest to smallest and take photographs in that order, including a hand-drawn letter in alphabetical order with each specimen. We have also requested that collection managers begin tagging specimens so that we are later able to augment our records as appropriate.

Entire Specimen, Dorsal View (Fig. 5A).—One key aspect of overall snake morphology is to determine the relative sizes of head, body, and tail, as well as any visible color patterns. The dorsal view of the specimen provides some of that information, although length measurements still need to be made using string and a tape measure (Natusch and Shine 2012). In order to obtain a dorsal 2D image, we position the specimen on the background in a way that allows it to lie as flat as possible (using nylon string if necessary), with the head visible. Some specimens are bent in ways that do not allow manipulation because a specimen may have hardened or its body may have been preserved in a twisted position (e.g., Fig. 8A). In these cases we simply take photos of the two opposing positions in which the specimen will allow themselves to be set up. After positioning the specimen, we take the photo from directly above the specimen (i.e., directly perpendicular to the surface on which the specimen lies). We avoid tilted angles, and should some features on the specimen not be visible, we obtain additional images as needed.

Entire Specimen, Ventral View (Fig. 5B).—Aside from the ventral coloration and patterning, a critical aspect of snake morphology are the counts of ventral and subcaudal scales. The key goal for taking a photograph of the ventral view of a specimen therefore includes the ability to count the scales from the image. We have found that while some specimens, especially those collected since the use of formalin as a fixative became a standard (beginning in the early 20th Century) do not readily allow placement so that scale counts can be made, a significant majority of specimens (more than 85% in our experience with more than 2000 specimens) can be placed so that scale counts can be made from the resultant images (Fig. 5B). While ventral and subcaudal counts on many specimens can be obtained from the photographs, the same is not the case for the dorsal counts (around the body at three places—one head length posterior to the head, at midbody, and one head length anterior to the cloaca), so these counts should be recorded manually during specimen examination.

We gently flatten specimens preserved in coils, and the pressure of the coil itself tends to allow the specimen to lie flat temporarily. We use small insect pins as needed to hold parts of very smooth snakes' bodies in place when parts of the specimen repeatedly return to an overlapping position before a photo can be taken. When necessary, we take separate images of the ventrals and subcaudals, which can also be useful so that the photo of the subcaudals can be taken from closer range in order to increase the resolution of the image. Sometimes several images are required along the venter to capture all the ventral scales in a twisted specimen. Pins placed at strategic points can be used to indicate where a count starts or ends so that the full count can be obtained from multiple images without error. Although, *in extremis,* simply counting the scales with the specimen in hand may be just as quick. It may be necessary to change the angle from which the image is taken away from straight to oblique in order to accommodate the body of the specimen. It is strongly recommended to take these photos using the largest f-stop

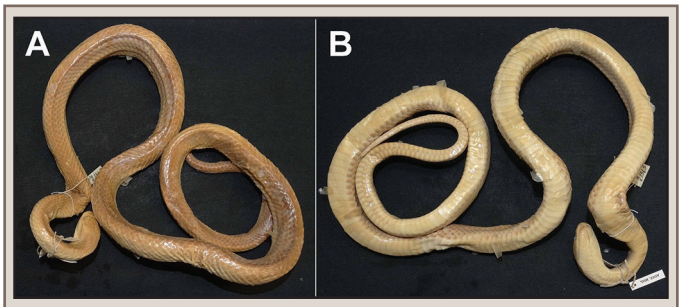

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FIG. 5. Two whole-specimen views of the holotype of *Stegonotus plumbeus* Macleay, 1884 (AMS R31895). A) Entire specimen, dorsal view. B) Entire specimen, ventral view. Placement of the specimen in (B) is designed to allow ventral and subcaudal scale counts. For both images, exposure = 1/40 s, aperture f = 2.8.

possible (e.g., the smallest aperture) in order to obtain the greatest depth of field (see *Depth of Field* discussion below). Ventral and subcaudal counts can be made in photo editing software by placing black points on each scale as it is counted, or by adding numbers as needed to indicate the progress in the count (various methods, including adding lines to indicate groups of 10 or 20, and varying the color of the spot depending on the color of the scales, could be employed). This makes counting ventral and subcaudal scales very reliable, eliminates the need to place pins in specimens, and saves significant time and money because the visit to the collection can be shortened or more specimens can be processed in the time available.

Head, Dorsal View (Fig. 6A).—The critical aspect of the dorsal (and ventral) head views is to ensure that they are as clear as possible given that snake skulls are kinetic, and there are many preserved snake specimens that are not flat. For such specimens, only qualitative information can be gleaned from 2D images. However, for many specimens, reliable measurements can be made, enabling morphometric analyses that might not be possible when working with calipers. We have found that both the accuracy of measurements and the reproducibility of making these measurements are much greater when made from images than when free-handling specimens while making measurements using calipers[2]; this accuracy extends to having multiple measurers make the measurements.

Head, Ventral View (Fig. 6B).—Photography of the ventral view is identical in principle to that of the dorsal view, with special attention paid to ensuring that the head is level. One difference is lighting, given that the top of the head in a majority of snakes is dark on top, whereas it is light underneath. As a consequence, the photographer will have to determine whether the

[2] To explain this statement, it is necessary to state clearly that our need is for relative measurements, not absolute measurements. Relative measurements are made to allow comparisons of relative lengths (e.g., via calculation of length ratios) and do not require the presence of a calibration scale in the image, whereas records of absolute lengths would be of their actual dimensions (in SI units) and with a calibration scale to obtain the measurement. Thus, our measurements are substantively different from measurements using calipers. Taking accurate, absolute measurements from images is tricky, as with macrophotography lens errors and distortions can play a role (see Muñoz-Muñoz and Perpiñán [2010] for a discussion of possible additional error sources). In the case of our images, we use free software called AnalyzingDigitalImages (Pickle & Gould 2011; https://www.umassk12.net/adi/), and ten measurements of the same length by three different measurers returned an error of 0.9%.

same camera settings are suitable for both views. We have found this generally to be the case, but it has meant moving the head in dorsal view closer to the light source, and the head in ventral view farther away from it to reduce glare.

Head, Lateral Views (Fig. 6C).—Lateral head images are made by holding the snake in the left hand and the camera in the right, which readily allows photography of the right lateral view of the head, whereas imaging the left lateral view requires a dramatic twist of the photographer's left hand. Based on the position of the head in the preserved specimen, this can be very difficult to accomplish. As a consequence, and in the interest of time savings, we have not always taken images of both lateral sides unless there were obvious differences between them. This compromise is one we find generally acceptable, albeit not ideal, given that the features on the left and right should be the same, and a morphometric analysis would usually use only one set of measurements. This is, of course, also a common practice when taking measurements of herpetological specimens in general, with most methodologies evaluating one side of a specimen or the other, unless features span both sides or one side differs from the other. Nevertheless, if at all possible under the constraints of time, we recommend obtaining images for both sides of a specimen's head.

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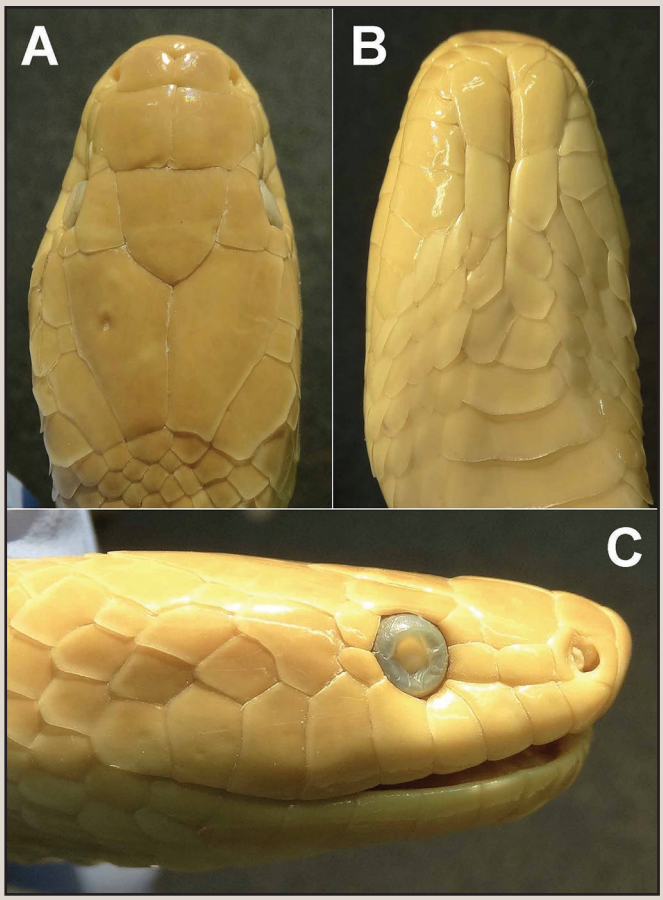

FIG. 6. Three head views of the holotype of *Stegonotus plumbeus* Macleay, 1884 (AMS R31895). A) Dorsal view, taken to include the head plates and the first set of neck scales. B) Ventral view, taken to include the scales leading up to the ventral scales. C) Right lateral view, taken to allow enumeration of supralabial scales. Photographs of the ventral and lateral views will, when carefully assessed, allow enumeration of infralabials scales. For both images, exposure = 1/50 s, aperture f = 3.5.

When examining lateral head scales of snakes, we focus on the number of supralabials, the scales surrounding the eye (e.g., preoculars, supraoculars, postoculars), and the dimensions of the scales anterior and posterior to the eye (e.g., loreals, temporals, pre- and postoculars; Fig. 6C). Recognition of these is generally easy from photographs of the lateral aspect of the head, but it may not be possible to view supralabials in their entirety due to the movement of the upper jaw against the lower jaw, which in a snake's kinetic skull sometimes renders the lower portions of the supralabials positioned in a shadow. We do not believe it is reliable to use the height of a supralabial scale from a photograph in a quantitative analysis (because it may be to bent or its lower edge invisible), but it is possible to determine what the order of height of supralabials is. The number of infralabial scales can be determined using lateral and ventral views together. The last infralabial lies below the last supralabial, and counting towards the tip of the snout usually allows recognition of most infralabials. The specific morphology of the three anteriormost infralabials can be easily confirmed by looking at the ventral head view.

Other Views.—Additional views we have imaged include aspects of both external (e.g., cloacal region, tail tip) and internal anatomy (e.g., hemipenes, *m. retractor penis*, stomach contents, endo- and ectoparasites *in situ*, eggs). Aspects of internal anatomy are not usually included in our photographic repertoire because more often than not, specimens are not fully dissected, and obtaining suitable views would require a more specialized setup of the specimen involving dissecting trays and pins (the exception are visible prey items, eggs, or parasites). We have taken images of the cloacal region (Fig. 7A) to document the characteristics of the cloacal scale (single or paired; see O'Shea

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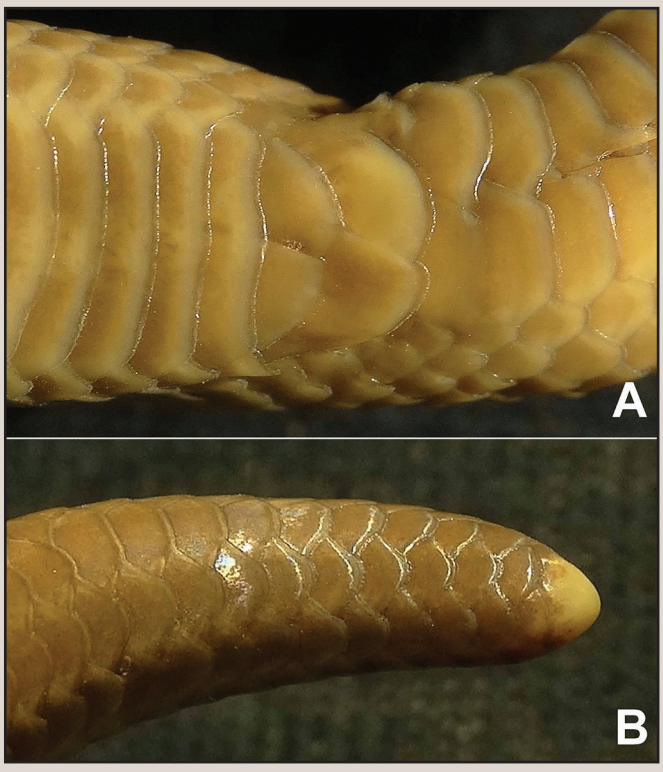

FIG. 7. Images of other relevant structures that may need to be photographically documented, including (A) a set of paired precloacal scales and (B) a keratinized tail tip. Both photos were taken from a specimen of *Toxicocalamus* cf. *longissimus* (AMS R5038). In both images, exposure = 1/40 s, aperture f = 2.8.

et al. 2018) and the presence of paired precloacal scales, to show variations in the subcaudal sequence (some individuals have mixes of single and paired subcaudals), and to document both dorsal and lateral aspects of the tail tip to show spiny tips (Fig. 7B) or lateral tail compression.

Image Processing and Data Management.—Our photographic tasks while visiting museums generally involve dozens of specimens and hundreds of images, roughly 40 snakes per day. We realize that this number may be on the low end for researchers, whose goal could be obtaining the image itself and not the data from it. If someone were taking thousands of images from hundreds of specimens on a daily basis, then the recommendations we make here may be inappropriate. Our approach has allowed us to balance the need for sifting through images during or after a trip.

We understand that data storage has become cheap, and some photographers never delete files—an activity that does not result in any net gain to them. For us, reducing the image load makes subsequent data management easier. For example, our approach to imaging is two-pronged: (1) Obtain research-quality images for our own work, and (2) document a collection photographically and disseminate the images. This makes it necessary to select and edit a set of images to be shared with the collection—it would not be useful to simply dump everything into a collection database and leave the curatorial staff to sort it out. Thus, readers are advised to determine early on what their estimated image number will be, what type of data storage would be required to handle these images, and what their desired outcomes are.

At the end of a day's photography session, we strongly advise downloading the images to a computer and backing them up immediately to avoid the loss of data. We take time at the end of the day to review our images and perhaps complete some basic image processing, at least deleting those files that obviously cannot serve as a permanent record of a specimens (such as out-of-focus or dark images). This review allows us to identify any specimens for which the sequence of images may be incomplete, due to an inadvertent omission or flawed image; a reshoot may be possible the following day. One of us (MOS) keeps a separate notebook listing date, species identification, specimen number, and the order in which photographs were taken to aid in this process.

At the end of a weeklong museum trip, it is not inconceivable that several thousand workable images will have been produced. Our workflow balances time and quality, and we perform our image processing in Photos or Aperture software (Apple Inc., Cupertino, California, USA) on a MacBook Pro laptop. CK and HK upload images directly from the camera's memory card into Photos using the computer's memory card slot and begin editing the images directly from the Last Import panel. MOS creates a folder for each day's images and for each camera used and then uploads them to Aperture[3], resulting in two sets of saved images. Processing invariably involves cropping the images, and most images are rotated to bring them into a suitable arrangement (landscape or square format for whole body photos, vertical according to midline sutures for ventral and dorsal shots, and landscape format to keep the supralabial scale row horizontal for the lateral images). A second level of processing is sometimes necessary to adjustment other parameters (including exposure, color tint[4], reduction of shadows or highlights, etc.). Individualized means of obtaining properly processed and backed up images can vary.

Great care must be taken during the sorting of images to ensure that the specimen number remains associated with the series of images. After editing in Photos software, we move the images out of Photos and into folders, assigning a different folder to each specimen series (copies can be retained in the software as well). For example, images of the holotype of *Stegonotus modestus* Schlegel, 1837, accession number RMNH.RENA 324 (housed at Naturalis, Leiden, The Netherlands) reside in a folder named RMNH_324. In this folder there could be at least six images, named BD_RMNH_324 (body dorsal), BV_RMNH_324 (body ventral), HD_RMNH_324 (head dorsal), HV_RMNH_324 (head ventral), HRL_RMNH_324 (head right lateral), and HLL_RMNH_324 (head left lateral). These files are stored on two computers, two external drives designated solely as backup, and in cloud storage. Given that the taxonomy of organisms is a dynamic study during which name changes may occur, we leave it up to each photographer to determine whether to add taxon names or localities to their images. Sets of images are also offered to museum collections if they are desired.

We acknowledge that the hierarchical approach to image storage is traditional and will be seen by some as outdated. We also understand that for someone needing to handle tens of thousands of specimen photographs, a hierarchical approach like ours is unworkable. However, when a student is beginning a research project involving specimen photography, it is difficult for him or her to simultaneously master and pay for the few database software options (e.g., Media Pro) in addition to all the other photography needs. This is especially the case for students from developing countries and/or those whose native language is not English. While in an ideal world, anyone wishing to embark on a specimen photography project would be able to work extensively with metadata (keyword tagging to allow scientific queries) in a professional grade software environment, we believe our approach is more realistic at the entry level.

Problems

We already alluded to some of the following problems, but wish to provide a more detailed discussion of some problems that are not insurmountable and not a reason to exclude a specimen from imaging.

Light Reflection.—While our method does minimize the impact of reflected light on the imaging process, shiny snake scales, which may still have a moist surface after removal from preservative, make this essentially an impossible task. Furthermore, a balance needs to be struck between the need for speed, to avoid drying out a specimen, and the need for image optimization. Some level of reflection is unavoidable. To address this issue we first try to minimize the glistening of moisture on the surface of the specimen by wiping it down with a paper towel before placing it on the background, but with preservative continuing to seep from the specimen, this is not always successful. Secondly, using the dynamic system of two

[3] Unfortunately, Apple no longer maintains further development of Aperture. Alternatives in use by professional photographers include Capture One and Adobe Lightroom.

[4] While color is not the most important parameter in preserved specimen photography, color correction can be achieved by calibrating the setup with a color card (e.g., X-Rite ColorChecker) and processing images using this calibration.

hands to hold the specimen and camera, we try to optimize the angle with which the specimen catches the light from the light source. Very minor adjustments may make a big difference, even while retaining the overall two-dimensional presentation of the specimen to the camera.

Another secondary issue, particularly encountered during dorsal head photography, is not the shininess of the light source but an uneven reflection: the anterior end of the snake (especially the rostral and internasal scales) can appear brighter in many images than the rest of the head because the anterior end of the snake presents an increasing angle to catch and reflect the light from the light source. While this is not a significant problem, it is an artifact of our setup.

Poorly Preserved Specimens (Fig. 8).—General problems with imaging specimens include the way the specimen is held, but a little practice and a good grasp of the specimen will produce good results. Many specimens have twisted anterior body parts that can make holding a specimen tricky, but we have not found any specimen whose head could not be photographed, although it has taken considerable effort on occasion.

When imaging hundreds or thousands of specimens, one may ask why obtaining images of a particularly poorly preserved specimen is necessary. The answer is that the specimen has intrinsic importance that we may not realize during the imaging session. In addition, each specimen *can* yield *some* data, and when the opportunity presents itself to work through an entire series of specimens, leaving out one for esthetic reasons (as opposed to scientific ones) is not adhering to best practices. Imaging all specimens in a particular group should be the rule, if for no other reason than completeness.

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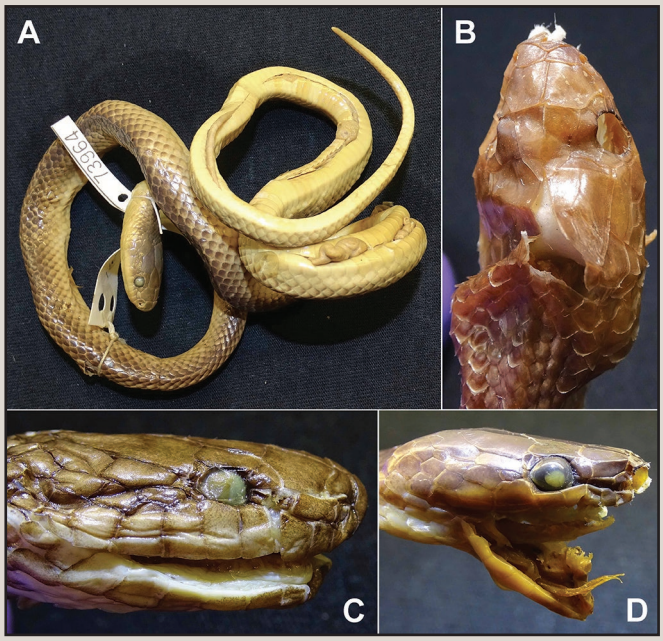


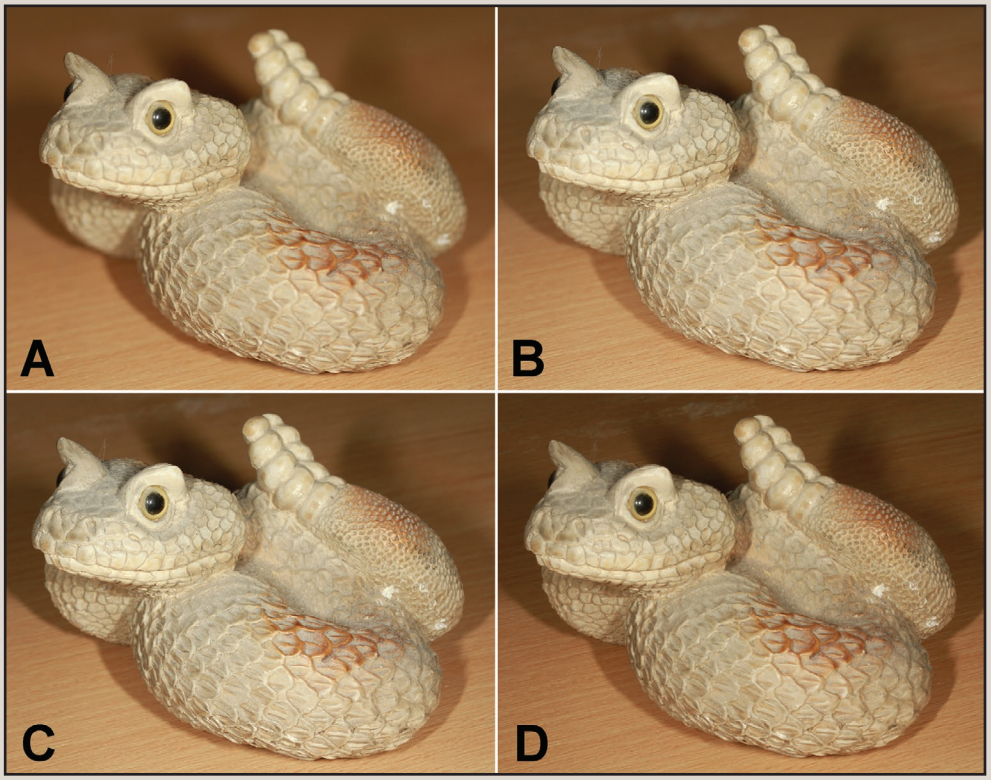
FIG. 8. Four examples of poorly preserved specimens. A) A specimen of *Stegonotus guentheri* Boulenger, 1895 (AMNH R-73964) with excellent preservation overall, but with twists that do not allow 2D imaging. Note that the *m. retractor penis magnus* is exposed and lifted partially out of the tail. Exposure = 1/40 s, aperture f = 2.8. B) A specimen of *S.* cf. *parvus* (AMNH R-75026) whose skull was removed for further study, rendering the remaining skin flexible and crumpled. We added a small amount of wadding to stabilize the skin. Exposure = 1/40 s, aperture f = 2.8. C) A specimen of *S.* cf. *cucullatus* (AMNH R-98872) whose scales and skin have softened considerably, and where this softening is beginning to cause direct damage (e.g., at the level of the second supralabial). Exposure = 1/20 s, aperture f = 3.2. (D) A specimen of *S.* cf. *parvus* (AMNH R-100044) with damaged anterior. Exposure = 1/25 s, aperture f = 2.8.

Poor preservation comes in a variety of guises, some related to the three-dimensional state of a specimen (Fig. 8A), some related to age or type of preservation (Fig. 8B–D), and some even to the shape of the container in which the specimen was preserved. Specimens collected in the early days of explorations were generally not deliberately laid out and fixed but were immersed in preservative while still alive or freshly killed. The movement of the preservative, which sometimes consisted of vats of chemicals as opposed to level specimen trays, would then fix the specimens in no particular position. The preservative itself may have taken its toll, producing imperfections ranging from scale flaking to discoloration, or specimen damage (e.g., deterioration of tissues) if the quality of the preservative was suboptimal. Some specimens may have been preserved in suitable positions but the lack of a sufficient amount of preservative subsequently may have led to hardening of the tissues. Nevertheless, most of these specimens still yield the basic scale counts and overall head scale morphology even if though they no longer look much like a living snake.

Depth of Field.—One key aspect of specimen photography that may be second only to lighting is the ability of a camera lens to produce a greater or lesser focused depth in an image. The photographer's goal should be to angle the shot and/or the specimen to maximize Depth of Field (DoF). The often strongly three-dimensional state of preservation of many snake specimens (i.e., when they were not preserved as neat, nearly two-dimensional coils) makes this an important issue, where shallow DoF leaves more blur in the image, which makes it hard to obtain information. Whereas shallow DoF and the resulting blurry background may be esthetically pleasing in an artistic context, they are not suitable in a scientific context.

In basic terms, to form an image a lens allows light to enter the camera at a certain shutter speed. The diameter of the lens opening (lens aperture) is determined by the f-stop setting (i.e., the width to which the shutter opens to receive light). A larger aperture (smaller f-stop) allows more light to enter but produces a shallower depth of field and vice versa (Fig. 9). When the aperture is changed, adjustments to shutter speed and/or ISO (sensor sensitivity, a.k.a. ASA or DIN) may be required to counteract the change in aperture to ensure proper exposure. Increasing the ISO, for example from 100 to 400, makes the camera's sensor more light sensitive and allows the use of a faster shutter speed to obtain a desired exposure with a given f-stop. Lastly, it is also possible, and practical when using digital cameras capable of high-resolution photography (i.e., high megapixel count), to achieve a higher DoF by increasing the distance between the camera and the subject. If it is desired that shutter speed or ISO remain unchanged at a larger aperture, shooting from a greater distance and cropping the image may provide a high DoF alternative.

It is important that the photographer familiarize himself or herself with the camera's output in order to identify optimal settings to achieve a well-lit image with maximum DoF. For specimens preserved with twists and turns or with uneven surfaces, this is especially important. Specifically, we recommend experimenting with an inanimate object before the museum trip, photographing it at various settings and examining the results. This can be very helpful when trying to maximize output on location.



PHOTOS BY MARK O'SHEA

FIG. 9. Four views of a snake model to illustrate the relationship between f-stop and depth of field. We selected a model and not an actual specimen for this demonstration, and took well-lit photographs using a tripod, to ensure we were able to take four identical images that differed only by the change in f-stop. A) f = 4. With the focus aimed at the area just behind the eye, which is the center of the model, both front and rear of the model are blurry. Even the eye itself is not exactly crisp. B) f = 8. The blurriness has become reduced, but the rattle and the forward coil of the model are not quite focused. C) f = 16. With all parts of the model now in focus, the background now appears slightly darker. D) f = 32. The model and the wooden background now appear in focus, but the background now appears even darker.

Distortion from the Use of Wide-Angle Zoom Lenses.—The cameras we use are high-end point and shoot gadgets that include various functions such as stabilizers, image enhancement settings, variable burst modes, with a high-power zoom lens. While this plethora of features is welcome, the high-power zoom lens doubling as a wide-angle can produce distortion when used for close-up (especially macro-) photography. The phenomenon emerges at two levels. In one, the wide angles produce abnormal compression towards the edge of a macro image. In the other, so-called "barrel distortion," the wide-angle lens is used to feed signals into a sensor field that is smaller than the wider fields of view that the lens can accommodate. This becomes noticeable when photographing straight lines and close distances (including well-delimited snake heads) and becomes important for some statistical analyses at focal distances < 2 cm as straight lines may appear as slightly bent in an image. It is possible to correct for the problem by obtaining images at high resolution (> 5 MP), using a lens of focal length > 35 mm, and photographing from directly above a specimen (e.g., Muir et al. 2012). There is no effect on enumerating scales, and the effect on qualitative descriptions is negligible. Non-camera based corrections for distortions are available in many types of photo-editing software (e.g., Photoshop), where use of a lens-profile specific plug-in allows eliminating the distortion.

Grip and Strength.—During a day's uninterrupted photography session of 8 h, including time for the positioning of oddly shaped specimens, compensation for reflection of moist bodies, or other similar adjustments, we feel that the number of specimens that can reliably be photographed at optimal quality is probably between 30 and 40 (this does not include time allotted for measuring length or counting dorsal scale rows). If for each specimen view at least three images are taken to ensure quality control (i.e., by ensuring that a suitable image was recorded), then the total number of images will be at least 750. With the weight of one of our Sony cameras including battery and media at 660 g (23.28 oz), this activity may place a strain on muscles and joints that should not be underestimated. Prospective photographers should be aware of the limitations imposed simply by handling the equipment and the specimens, and the time required for imaging should be scheduled so that fatigue or strain does not set in.

Grip and strength issues could, in principle, be resolved by the use of a lightweight, tabletop tripod. A tripod-mounted camera creates no strain on the operator and also improves overall consistency and stability during a photo session. A tripod allows for longer exposure times, enabling the photographer to shoot using smaller apertures (higher f-stops) to obtain better DoF. However, a camera positioned on a tripod is inflexible in situations where dynamic positioning of a specimen and/or the camera is necessary. This may occur with particularly twisted specimens, where the firmly planted tripod could become a hindrance. Whereas the camera hand can assist (even by pushing with the camera itself) to position the camera into the best position on a specimen, use of a tripod would negate this. Once again, stabilizing the specimen so that the images can be obtained vis-à-vis preserving one's endurance may end up being a judgment call.

DISCUSSION

One may wonder why there has not been a set of recommendations for photographic practices to produce standardized 2D images of preserved snakes, let alone other reptiles, amphibians, or fishes, formally published previously. In part, this is because technological advances now bring fairly high-end equipment within the financial reach of individuals just starting out in systematics research. Furthermore, broad discussions regarding workflows and standards for the digitization of natural history specimens (e.g., GBIF 2008) and the need to improve global access to collections (e.g., Baker 2011) are contemporary and ongoing. There is now a concerted publicly

funded effort in the United States in the form of the National Science Foundation's Advancing Digitization of Biodiversity Collections initiative, led by iDigBio (www.idigbio.org; Page et al. 2015) to support the creation of databases for metadata and images (2D, 3D, CT-scans) and the concomitant standards. For us personally, our visits to natural history collections around the globe and our encounters with international colleagues pursuing similar research goals, often with limited equipment and at great personal or institutional cost, brought the need for some guidance into focus. For the sake of both researchers and collections, the availability of properly produced, publicly available photographs of specimens would be a significant asset.

Some of the key questions regarding 2D photography in the pursuit of standardization have not yet been answered. For example, there is no universal recommendation for the resolution at which 2D images must be taken (for publication quality photography, the resolution must be 300 dpi at the desired image width, with most journal page widths < 20 cm), and a common presumption has been that higher resolution is better. While it is true that sensor capacity has improved dramatically year upon year (akin to what has happened in computers according to the famed Moore's Law; Moore 1965) it would appear that the resolution of 20–24 megapixels in the APS-C and MFT sensors of the equipment we mention is suitable for most applications. Simultaneously, in-camera storage capacity (in terms of SD card space) has reached 512 GB, so there really is no limit on the camera side. There is, however, a potential limitation of hard drive space at the collection end if 2D, 3D, and CT scan images of all accessioned specimens were to be produced and required storage. Thus, the limits of storage in the short term may, to some degree, dictate the resolution of image files.

One of the limitations of the cameras we use is their inability to save files in RAW format. They produce files in JPG format, which is not a "loss-less" format (as opposed to RAW) and requires transfer to TIFF format to allow copying without image disintegration. For reasons of both dynamic range and color RAW is the most desirable format. However, on some online discussion boards, users have described experiences with some lower-cost cameras that did generate RAW files, but with output very similar to that achieved by JPG compression. Thus, in the range of camera we promote for our low-cost approach, the lack of a RAW file option may not be a significant loss.

We consider our photography of specimens a mutually beneficial relationship between researcher-photographer and collection curator, whereby use of the specimens provides output useful to both parties. However, even while photography by a researcher focused on a particular suite of taxa can be a boon to the hosting institution, in a fashion similar to citizen science and crowdsourcing (e.g., Endresen 2014), the institution must be ready to receive the information. This includes "future-proofing" the received images (to include consideration of data migration and data integrity as well as suitable diversification of storage media and their location in a global context).

Lastly, we wish to emphasize that the process of producing or using 2D images is not by any means an attempt to steer researchers away from the examination of actual specimens. Such direct examination is one of the key best practices for taxonomists (see Kaiser et al. 2013), and personal examination of character states, especially those found in type specimens, should always supersede literature findings and accompany any taxonomic revisions. 2D imaging of snake specimens is designed so that information about specimens can be shared more readily across the globe, so that researchers are able to make an initial determination regarding their need to actually view the specimen – not to obtain the best possible, high-resolution images of the specimens under examination but the best possible images given the circumstances. We believe that in many instances, the photos taken by the experts in the field, when taken in observance of the few provisions we list above, will suffice to help solve taxonomic questions rapidly and in a manner that protects time and money, two of a taxonomist's scarce resources.

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