

species of *Necturus* were incorrectly placed in the family Plethodontidae.

Conservation status is mentioned in the accounts for most taxa in the form of a sentence or two on Federal or State listing status. There is no mention of status in IUCN rankings. A table would have been a more efficient way to display this information because there are few amphibians on these lists. For information such as identification keys, diagnostic features, and natural history, regional field guides or recent tomes such as those for salamanders (Petranka, 1998) or frogs (Dodd, 2013) of North America should be consulted.

I found myself using *North American Amphibians* for a quick check on the distribution of several species, and it may serve well as a handy desk reference for distributional information. Some may purchase this book in hopes of having a field guide, which it is not. Biologists who have a firm grasp of species identification and taxonomy can use it as a handy overview of distributions. Nonspecialists may struggle to find a particular species they are interested in learning more about. I recommend that potential buyers first examine a copy (e.g., at a book store) to be sure this volume fits their needs.

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Collecting and Preserving Genetic Material for Herpetological Research. T. Gamble. 2014. Society for the Study of Amphibians and Reptiles, Herpetological Circular No. 41. ISBN 9780916984885. 50 p. \$11.00 (soft cover).—Sampling, preserving, and storing genetic material has become routine in many subdisciplines of herpetological research, yet a one-stop resource for how to best perform these methods has been lacking. As such, most beginning investigators have to learn and refine their techniques from mentors, peers, or through personal experience, sometimes with painful lessons. This fine little guide aims to summarize the currently accepted best practices for sampling, preserving, and storing herpetological genetic material. As noted by the author, the guide complements Simmons's (2002) *Herpetological Collecting and Collections Management* by providing more and up-to-date detail in collecting and storing tissues. Although some of the methods are specific to herpetology, such as skin swabbing for the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) or using autotomized tails of salamanders, lizards, and snakes as tissue samples, most of the methods covered in the guide are also applicable to other vertebrate taxa. The author achieved his aim to

write the guide at a level appropriate for those with a basic background in biology, including advanced undergraduates and graduate students, professional scientists, and wildlife resource managers.

The brief Introduction rightly points out that the field of herpetology has been at the forefront of using molecular genetic tools in biological research. Collectors should appreciate the author's plea to properly preserve and store tissue samples, as an enormous amount of effort and money may have gone into obtaining these samples, many of which are irreplaceable owing to population declines and extinctions. In this era of online museum collection databases, when one can search for and request to use a tissue sample in only minutes, it is easy to forget that weeks or more of effort, thousands of dollars, and personal sacrifices by the collector may have gone into getting that sample from the field to the collection freezer. In addition to the effort, cost, and irreplaceability, I would add that we have an ethical responsibility to properly care for and maximize the utility of these samples after, in most cases, having euthanized the animals for this purpose.

Chapter 1 asks why we need to collect genetic material. Some parts are redundant with the Introduction, such as how molecular data have revolutionized biology, including herpetology, and these should probably have been combined. The author identifies and discusses three emerging research trends in herpetology that are rapidly increasing the amount of available genetic data and the number of studied individuals. These are genomics, the study of amphibian pathogens such as *Bd*, and species identification with DNA barcodes such as the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. The author did not mention the generation of large, multilocus datasets for resolving evolutionary relationships (e.g., Frost et al., 2006) or delimiting species (e.g., Leaché and Fujita, 2010). He makes an important point that field herpetologists should consider opportunistically sampling genetic material other than the taxa that he or she may currently be studying. When permitted to do so, this is particularly important when encountering species that are difficult to find, such as fossorial species, or when working in geographic areas that are difficult to access for logistical or political reasons. After all, one never knows how useful these opportunistic samples may become to future researchers.

My favorite part of this chapter is Box 1, which asks if DNA can be obtained from fluid preserved museum specimens of amphibians and reptiles. Based on my experience (e.g., Stuart et al., 2006), I agree that it is sometimes possible to obtain useable, but usually degraded and fragmented, DNA from formalin-fixed specimens or specimens that have been stored in ethanol at near room temperature for a long period of time. I also agree with the author that these successes, including very recent and exciting advances using next generation sequencing technology, do not justify neglecting newly collected tissues because degraded DNA can be rescued later. I will add that the process of extracting and amplifying degraded DNA from fluid-preserved museum specimens is prone to contamination by exogenous DNA, and that these efforts need to be undertaken with the same precautions as required in ancient DNA protocols. I usually recommend only trying to extract DNA from fluid museum specimens when no other options exist, such as the when the species has gone extinct. The effort and cost to obtain fresh samples, even if very challenging, will often still be lower than if working with formalin-fixed samples, and of course, the yield and quality of DNA will be much higher.

Chapter 2 provides an overview of the science of preserving genetic material. Unfortunately, this chapter occupies less than one page in length, and the content is redundant with Chapters 3 and 4, making it unnecessary.

Chapter 3 describes sources and methods for collecting genetic material from amphibians and reptiles. The chapter title is "Methods for Collecting and Preserving Genetic Material," but sample preservation is actually covered in Chapter 4. In a brief section on permits and ethics, an important point is made that genetic material sampled from wildlife may also be regulated by wildlife laws, as it is technically a part of the animal, even if a voucher specimen is not taken. A few years ago, I queried a law enforcement officer of the U.S. Fish and Wildlife Service and was told then that even amplified DNA (PCR product) of protected species is regulated in the same manner as the animal itself, as technically a very small portion of the PCR product consists of the original, sampled DNA. Thus, I concur with the author that caution is warranted with permitting of genetic samples, and that this discussion on permits is relevant to readers of the guide. In a brief section on humane euthanasia, the only new information for experienced collectors might be intracoelomic injection of tricaine methanesulfonate (MS-222) in reptiles.

Most collectors and curators of genetic material have experienced the heartbreak of encountering a tissue tube with the identification information lost due to smudging of the writing, ethanol dissolving ink, an adhesive label falling off, or a myriad of other reasons. The greatest methodological variation among researchers who handle samples of genetic material may be in how tubes are labeled, and so I read this section with great interest. The author was thorough here, and covered all of the labeling methods that I am aware of, although there are undoubtedly others. I appreciated his recommendation to include an internal tube label as insurance against losing the external label. The author promotes VWR lab markers (VWR International, Radnor, PA) as being ethanol resistant, but I would urge users to first test any marker on multiple surfaces of the tube while also rubbing ethanol onto the writing. Be aware that even if a tube contains a non-solvent preservative, such as a high-salt solution, other tubes housed adjacent to it in a genetic resources collection might contain ethanol that spills. I agree with the author that etching an identification number onto the outside of the tube with an engraving drill, diamond-tipped pen, or, in my preference, a very heavy gauge hypodermic needle, may be the only way to ensure long-term identification.

There are some gems of information in a section on sampling tissues from specimens. For example, the author cautions against breaking the gall bladder when sampling liver tissue, which is commonly used, as the bile contains nucleases that degrade DNA and RNA. This leaves me to wonder how much bile nuclease I have detrimentally introduced into my tissue samples over the years. The author gives the important, but often neglected, recommendations of cutting tissue into small fragments so that preservative fluid can fully permeate the material, and ensuring that the ratio of tissue to preservative fluid in a tube is not too high. I will add that a secondary benefit of mincing the tissue at the time of sampling is that it enables one to later rapidly retrieve only the small amount of tissue that is needed for an extraction or tissue loan, without having to handle (and potentially contaminate) the remainder of the sample.

Non-lethal sources of genetic material of amphibians and reptiles are also reviewed, including buccal and cloacal

swabs, blood, biopsies, tail and toe clips, shed skin, and some rather novel sources such as dried snake venom and mosquito blood meals. Missing here is mention of handling samples for the rapidly emerging field of environmental DNA analysis (e.g., Thomsen et al., 2012). The author briefly reviews how sources of genetic material described above also enable the study of DNA of the sampled animal's pathogens and parasites. Figure 7 illustrates a live treefrog having its skin swabbed, but unfortunately shows the frog being swabbed on its dorsolateral surface rather than on the ventral surface, as recommended in the *Bd* sampling protocol in Box 3. (Also, a spacing error places the title of Box 3 against the box outline, and there are problems with word wrapping in three lines of the second footnote.) The final section of this chapter, and Box 4, are devoted to sampling tissues of amphibians and reptiles for the purpose of initiating tissue cultures. The author uses tissue cultures in his research on the evolution of lizard sex chromosomes, and some potentially helpful tips based on the author's personal experience are provided in Box 4.

Chapter 4 covers preservation, storage, and transportation of genetic material. The take-home message of this chapter is the colder the storage temperature, the better, even for samples that are in fluid preservative. The author suggests that cryopreservation (flash freezing) in liquid nitrogen is the best method for preserving genetic material, but rightly recognizes the difficulties in finding liquid nitrogen in the field and transporting samples preserved in liquid nitrogen. Tissues are more commonly preserved in fluid preservatives, and these can also serve as backup to flash-frozen tissues in case the liquid nitrogen supply becomes exhausted (such as delays in customs during transportation) or a freezer fails. Ethanol (95–100%) is regularly used to preserve genetic material, but I have wondered why this is still the case when, as pointed out by the author, ethanol poses noteworthy challenges to both collectors and curators. These include the need to replace the ethanol a day or two after preserving the tissue because water extracted from the tissue will dilute the ethanol concentration, difficulties in purchasing full-strength ethanol in many countries, and shipping regulations resulting from ethanol being considered a hazardous (flammable) material. In my experience, ethanol can evaporate from cryovials with internally threaded caps and a silicone gasket. Fortunately, the author provides alternative fluid preservatives to consider, including high-salt solutions such as RNAlater (Life Technologies, Grand Island, NY) and salt-saturated DMSO/EDTA solution. Useful and up-to-date suggestions for packing and shipping tissue samples are also given. Finally, the author identifies real challenges that museum tissue collections face that differ from traditional museum collections. These include the difficulty in maintaining a link between tissues, vouchered specimens, and published DNA sequences, and the fact that tissues are consumed when they are used, making them finite resources.

The Literature Cited accounts for more than a quarter (28%) of the length of the guide, but is thorough and worth the space. The author assembled numerous useful references that support the methods presented in the guide. I was unaware of many of the references until reading the guide, and am keen to track them down.

My primary criticism of this short guide is that it should be made available in electronic format, such as pdf, rather than only in hardcopy. The text length is not longer than that of many scientific journal articles. Availability only in hard copy seems rather non-progressive for a book in such a

modern and rapidly advancing field. Perhaps the publisher will consider also distributing it electronically. On a more positive note, the short text length keeps the guide small and lightweight, and easily transportable into the field. I hope this useful guide is only the first of more editions to come. Future editions might be even better served as an online resource, such as the list of standard symbolic codes for institutional resource collections in herpetology and ichthyology that evolved from hardcopy published in *Copeia* (Leviton et al., 1985) to a regularly updated Internet version (Sabaj Pérez, 2014). Readers will find places where the methods can be supplemented or improved based on their personal experiences or new publications, and online availability would allow the resource to be easily updated. After all, this science has not been around long enough to know for certain which methods will best preserve genetic material in the long term, and the sooner that challenges can be remedied, the better for all.

In conclusion, this guide provides a fine summary of current best practices for sampling, preserving, and storing genetic material of amphibians and reptiles. The redundant text, errors, and missing points identified above are relatively minor, and do not seriously detract from the guide. Beginners and non-beginners alike should read this guide, as I suspect even the most experienced collectors and curators of genetic resources will find useful information contained within it. The low cost of the guide makes it readily accessible, even to students. Hopefully, a future electronic version will make it even more readily available. Properly preserved and curated genetic samples benefit us all, and future researchers, and the author should be commended for providing this service to the herpetological community.

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Medical Care of Turtles and Tortoises—Diagnosis • Surgery • Pathology • Parasitology. J. Hnízdo and N. Pantchev (Eds.). 2011. Edition Chimaira. ISBN 9783899734935. 559 p. €128.00 (approximately \$173.50) (hardcover).—*Medical Care of Turtles and Tortoises* is the first new book on the veterinary care of turtles and tortoises since McArthur et al.'s (2004) *Medicine and Surgery of Tortoises and Turtles*. The new book was originally published in Czech, later translated into German, and is now available in English (translation by Donald W. Stremme). The book's eight chapters cover anatomy and physiology, biology and husbandry, general medicine, parasites, medication administration, anesthesia and surgery, turtle diseases, and an appendix. Contributors with expertise in their respective field have authored each section. This new title provides a good starting point for any veterinarian or herpetologist beginning to work on tortoises and turtles. It delivers short and concise sections that are useful for quick reference and everyday use. The book's primary focus is on freshwater and terrestrial chelonians, and even brings in examples and pictures of rare species; however, it does not include any specific sections on sea turtles. Fortunately, comparable information is available on sea turtles (e.g., Wyneken et al., 2013; and chapters in Mader, 2005).

The first chapter covers anatomy and physiology and provides brief descriptions of each organ system with color pictures and black-and-white illustrations. The intent of this chapter is to review the differences between chelonians and other reptiles. This section focuses on gross anatomy. More in-depth descriptions of chelonian gross and microscopic anatomy are beyond the scope of this book. Each subsection has basic physiological mechanisms integrated into the anatomical descriptions, such as blood flow through the heart; however, the only section to focus specifically on physiology is dedicated to body temperature. This section briefly reviews behavioral thermoregulation and how species have adapted to specific temperature requirements; however, this section would benefit from a more in-depth discussion of the different thermoregulatory strategies employed by chelonians to exchange heat.

One of the highlights of this text is the second chapter, which reviews the general biology and husbandry of turtles. Topics covered in this chapter include chelonian evolution, taxonomy, biology, keeping turtles, hibernation/brumation, and protecting turtles. The taxonomy section within this chapter covers each family and the genera included within each family, in addition a detailed description of anatomical variations among families, family-specific characteristics, life-history traits, and biogeography are provided. Understanding species-specific traits is beneficial when treating a diversity of species. The section about keeping turtles covers the basic husbandry requirements of captive chelonians, but does not delve into the specifics of each species.