University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Faculty Publications from the Harold W. Manter Laboratory of Parasitology

Parasitology, Harold W. Manter Laboratory of

1-1-2012

Collecting and Preserving Parasites during Reptile Biodiversity Surveys

Scott L. Gardner
University of Nebraska - Lincoln, slg@unl.edu

Robert N. Fisher
USGS Western Ecological Research Center

Sean J. Barry
University of California-Davis

Follow this and additional works at: http://digitalcommons.unl.edu/parasitologyfacpubs

Part of the Parasitology Commons

Gardner, Scott L.; Fisher, Robert N.; and Barry, Sean J., "Collecting and Preserving Parasites during Reptile Biodiversity Surveys" (2012). *Faculty Publications from the Harold W. Manter Laboratory of Parasitology.* Paper 738.
http://digitalcommons.unl.edu/parasitologyfacpubs/738

This Article is brought to you for free and open access by the Parasitology, Harold W. Manter Laboratory of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications from the Harold W. Manter Laboratory of Parasitology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Gardner, Fisher & Barry in Reptile Biodiversity (2012), ch. 7: Preparing Reptiles as Voucher Specimens.
Foster & McDiarmid, eds. Published y the University of California-Davis.

CHAPTER SEVEN

Preparing Reptiles as Voucher Specimens

MERCEDES S. FOSTER
Chapter Coordinator

Collecting and Preserving Parasites during Reptile Biodiversity Surveys *Scott L. Gardner, Robert N. Fisher, and Sean J. Barry* / 114

Reptile Biodiversity: Standard Methods for Inventory and Monitoring, edited by Roy W. McDiarmid, Mercedes S. Foster, Craig Guyer, J. Whitfield Gibbons, and Neil Chernoff. Copyright © 2012 by The Regents of the University of California. All rights of reproduction in any form reserved.

From Mercedes Foster and Roy McDiarmid, Reptile Biodiversity, Berkeley and Los Angeles: University of California Press, 2012.

Gardner, Fisher & Barry in Reptile Biodiversity (2012), ch. 7: Preparing Reptiles as Voucher Specimens.
Foster & McDiarmid, eds. Published y the University of California-Davis.

This article is a U.S. government work, and is not subject to copyright in the United States.

Collecting and Preserving Parasites during Reptile Biodiversity Surveys

Scott L. Gardner, Robert N. Fisher, and Sean J. Barry

Obtaining parasites from reptiles that are collected during biological diversity surveys or inventories is time consuming, and in the past such collections were rarely made (Gardner 1996). However, parasites and other symbionts are integral parts of the ecological characteristics of organisms and provide important information on biodiversity from the population level to the species level. For example, recent biogeographic analyses of the skinks, geckos, and anoles of some Oceanic Pacific Islands (Bertrand and Ineich 1986, 1987, 1989; Goldberg and Bursey 1991, 2000, 2002; Hanley et. al 1995; Goldberg et al. 2005) could not have been accomplished without parallel studies of the parasite/symbiont faunas that they host. The latter studies were possible only because the parasitic and symbiotic organisms had been properly collected and subsequently deposited in recognized museum collections where they were available for study. We strongly recommend that investigators sample the parasites and symbionts of reptiles whenever they are encountered.

Most researchers are reluctant to assign individuals from an often stressed and overworked field crew to process parasites when the specimens and associated data are considered "auxiliary" or "collateral" to the main focus of the study (i.e., reptiles). On the other hand, the most scientifically valid, efficient, and cost-effective method of obtaining data on the parasite fauna of a group such as reptiles is to preserve the parasites when host animals are being collected and processed in the field. Examples of the types of data that can be obtained when a host is collected include *prevalence* of the parasite among individuals within a population (i.e., proportion of individuals with the parasite), *intensity of infection* on individual reptiles (i.e., number of parasites per individual), and distribution of parasites in or on individuals and among populations of reptiles.

Studies of the systematics and ecological characteristics of hosts and their parasites require proper identification of both groups. When the parasites associated with a host are not collected and preserved properly, species-level diagnostic characters (i.e., morphological characters) are usually destroyed. In addition, improper preservation of parasites severely limits, or more likely prevents, studies based on DNA from those specimens.

Proper handling of parasites is also critical for the correct designation of the host individual for a specific sample of parasites, as parasites may accidentally transfer from one host to another at time of collection. Anthropogenic host-transfer, especially difficult to avoid in the field, can occur from as early as when an animal is collected until, and subsequent to, its final deposition in a recognized collection or museum. For example, *ectoparasites* (organisms that live on the external structures of a host organism) that are attached to a host individual may release their holds and fall from the host after it is collected, remaining in a trap or collecting bag long after the host animal has been removed. Those parasites may then

transfer to another host of a different species or of the same species but a different demographic group (e.g., sex, age, breeding status); transfer may occur at the same collecting locality or even subsequently at a different one. Anthropogenic host-transfers occur more frequently when investigators place more than one host animals in a single container or place a host in a collecting bag or container that has been used for another host previously, but not properly cleaned.

In this section, we outline methods for collecting parasites that maximize the amount of morphological and molecular information available from each specimen obtained. The procedures are also designed to minimize the likelihood of anthropogenic host-transfers. An excellent, thorough protocol for sampling parasites of reptiles and amphibian has been published on the Web (Goater and Goater 2001), although it focuses mostly on Canadian species of parasites and techniques used there.

Specimen data are often collected in a field laboratory in assembly-line fashion, with different members of the field crew assigned to different tasks. When an individual finishes his or her task, she or he passes the organism to the next person in line for the next step in processing. Generally, one or two persons are assigned to process parasites. We have oriented our presentation toward assembly-line processing. However, individuals working alone can use the same protocols. In our procedures, every part of every animal that is collected is processed in some way. Pritchard and Kruse (1982), Anderson (1965), and Gardner (1996) have provided additional information on collecting techniques for parasites in general. One general rule applying to the collection of both external and internal parasites as well as host tissues is that investigators wear latex or nitrile gloves while sampling and wash their hands thoroughly afterward to avoid potential infection, particularly from pentastomids (Jacobs 1982).

Collecting Ectoparasites and Ectosymbionts

1. The collector places each live host in a new (i.e., *never* used) bag made of thin (1.5–2.0 mil) plastic. She or he also immediately places any dead host removed from a trap or found as road kill in a new plastic bag to prevent loss of ectoparasites and cross-contamination of hosts. Depending on the size of the host, a 200–350-mm bag is best. We recommend that standard cloth snake bags *not* be used, as they often harbor ticks and mites from previous occupants for extended periods unless thoroughly cleaned, which is almost impossible to accomplish in the field. Without such cleaning, investigators must assume that host transfer has occurred.
2. Depending on the study, hosts are either euthanized (see "Euthanasia," under "Preparing Scientific Specimens," earlier in this chapter) or maintained alive for future release. In the latter instance, the investigator will need to subdue the animal or lightly anesthetize it so that it can be examined carefully.
3. After a host is processed (either euthanized or weighed and measured), the researcher examines its ears, eye areas, skin folds, under-scale spaces, mite pockets (skin invaginations where mites concentrate, found in certain families of lizards; see Arnold 1986), and the rest of the body for external parasites, especially leeches, ticks, and mites. If the animal is examined over a sheet of white paper using a lighted 10X hand lens or a dissecting microscope, parasites that fall from the body are more easily located. All parasites encountered are removed and stored appropriately (see below). Live tortoises are particularly difficult to examine because they reflexively draw their legs into their shells when handled, yet the legs must be extended for the investigator to examine all the skin folds where the shell meets the skin (Fig. 27, in Chapter 6). Parasites collected from different parts of the body are placed with a label in individual 1.5-mm vials of 70 percent ethanol (ETOH) for storage. Subsamples can be placed into cryotubes and frozen directly in liquid nitrogen. The investigator repeats the procedure with the next animal until all animals have been processed. Labels should be made of good quality, 100 percent cotton-rag, acid-free material, and all writing is done with permanent India ink (e.g., Higgins Eternal) or disposable, permanent-ink pens. The label includes the host-specimen identification number or the field collection number of the collector. It also indicates the part of the host body from which the specimens were collected and may include a count of the number of ectoparasites collected.
4. The preparator should carefully inspect an animal that has been euthanized for additional (especially small) ectoparasites. Scales with small mites under them tend to stick up and can be detected by holding a lizard or snake at eye-level and observing it from the tail end forward. The investigator lifts each raised scale with jeweler's forceps and a dissecting needle and examines it for 'suspicious objects' using a dissecting microscope at low power (20–40X); he or she then increases the magnification (50–70X) to see if the object has legs. Fly larvae (bot flies or warbles) can also occur subcutaneously, particularly in the cloacal region of turtles. At present, however, larval bot flies cannot be identified to species using morphological characteristics. Collecting live larvae and rearing them to adulthood in an artificial brood chamber is the only way to obtain a reliable identification based on morphology. The larvae can be kept alive in cheesecloth-covered uncapped vials or .45-caliber polystyrene cartridge boxes until they pupate and emerge. Adult flies are preserved in 70 percent ethanol (Gardner 1996).

   Parasites and other symbionts can be removed from a host with forceps, a swab, or a needle dipped in ethanol. A leech, tick, or mite that is embedded in the skin of the host should be carefully pried from the skin, rather than scraped, to ensure that mouthparts are included. Small mites that are attached under scales are collected by lifting the scales and carefully teasing out the small arachnid. Parasitic mites often inhabit the oral cavity, lungs, and trachea, as well (Reichenbach-Klinke and Elkan 1965).

   The locations on hosts of all ectoparasites collected are recorded in the field notebook of the collector (Fig. 34). The field notebook should contain a record of the numbers of ectoparasites collected from each body region of the host and the type of vial used for storage (to facilitate locating the organisms years later during museum searches). Ectoparasites and other ectosymbionts are preserved and stored in 70 percent ETOH.

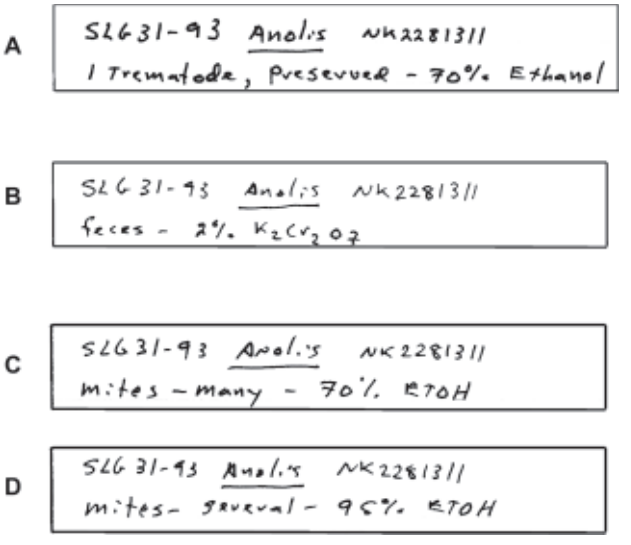


FIGURE 34 Examples of data labels to be placed in vials or plastic bags with parasite specimens. (A) Sample label to be placed in a vial with a single trematode from an *Anolis* lizard. The collector's number (SLG31), the genus of host (*Anolis*), field identification number (93), kind of parasite, and type of preservative in the vial are included on the label. (B) Sample label to be placed in a vial of potassium dichromate with lizard feces from specimen SLG31-93. (C) Sample label to be placed in a vial or plastic bag containing ectoparasites. The collector's number, host genus, field identification number, type of preservative, and general kinds and approximate numbers of parasites in the vial are recorded on the label. (D) Label with the same data as in "C" but also noting that the specimens were preserved in 95 percent ETOH for future molecular and morphological analyses.

Parasites from each individual host must be stored in separate vials.

Live reptiles can be kept for a few hours in wire-mesh cages over pans of water, and ectoparasites that drop through the mesh into the water can be collected with a spoon, forceps, eyedropper, syringe, or filter paper. Ectoparasites can be removed post-mortem by euthanizing a specimen and placing it immediately in an appropriate-size plastic bag with a cotton ball soaked in chloroform or ether. After a few minutes, the bag is shaken to dislodge the parasites and the parasites are recovered and preserved in 70 percent ethanol. The drawbacks of these methods are that the original locations of the dislodged organisms on the host remain unknown and that fumigation does not dislodge all the ectoparasites.

5. After a reptile is removed from a plastic collecting, holding, or kill bag, the preparator collects any ectoparasites that may have fallen from the animal into the bag by squirting a small amount (2–6 ml) of 70 percent ETOH into the bag, washing down the sides so that the alcohol and any bag contents accumulate in a corner. The collector holds the bag so that one corner is over the opening of a small Whirl-pak (plastic bag with integral wire twist-ties), cuts off the corner, allowing the ectoparasites and ethanol to drain into the whirl-pak, and adds a label. This method quickly produces an uncontaminated sample of ectoparasites and ectosymbionts and ensures that no plastic bags will be reused, which is critical for avoiding contamination. Whirl-paks, are small, leakproof, and generally unbreakable—ideal for transporting specimens back to the laboratory, where the contents should be transferred to vials as soon as possible.

Collecting Endoparasites and Endosymbionts

In this section, we discuss only the endoparasites and endosymbionts most commonly collected from reptiles: Protozoa (coccidia), Acanthocephala (thorny- or spiny-headed worms), Cestoda (tapeworms), Trematoda (flukes), Nemata (nematodes), and Pentastomida (tongue worms). Field collectors should keep in mind, however, that novel organisms are sometimes discovered during a dissection.

1. The researcher collects endoparasites from a host specimen after the animal has been euthanized but before other host tissues are removed for genetic analyses. Scissors, forceps, probes, and other implements are always rinsed with 70 percent ethyl alcohol (ethanol, ETOH) and wiped dry with a tissue between animals to avoid cross-contamination with blood or other sources of foreign DNA.
2. Blood smears that are well prepared and of even thickness are important for detecting and documenting protozoa and microfilariae (juvenile filarioid nematodes) that may have infected the reptile being studied. Blood smears can be made from live or freshly dead (i.e., the blood has not yet coagulated) animals. Blood can be collected from any convenient area of a recently dead animal, usually while taking tissues. With a live animal, blood is collected from a toe or tail clip or from the postorbital sinus of most lizards, using a hematocrit tube. Tubes with blood can be sealed with putty and stored. Alternatively, the blood can be blown out onto a slide.

   Typically, an investigator collects blood with a disposable plastic pipette and places a drop (approximately 250 µl) in the middle of a microscope slide. He or she holds another slide at an angle, placing its edge on the first slide in contact the blood drop, and pushes the inclined slide evenly and rapidly away from the blood drop, drawing the blood out into a thin (one cell layer thick) smear (Fig. 35). The field identification number of the host is written on the slide with a diamond-point pencil, and the slide is allowed to air-dry for at least 10 to 30 min, depending on the humidity. New blood smears are fixed at the end of each day (within 24 h of preparation), or sooner if the temperature and humidity are high, by immersing them in 100 percent methanol or 100 percent ethanol for from 2 to 5 min. Slides are stored and transported in standard slide boxes and may be stained and examined after return to the laboratory, or they can be stored and then stained later.
3. To examine the digestive system for endoparasites, an investigator opens the abdominal cavity and severs the esophagus, just above the stomach, and the colon, just anterior to the rectum. Care must be taken not to perforate the organs during this procedure to avoid transfer of parasites from one organ to another, thus invalidating information on the distribution of the parasites within the individual host. The investigator removes the digestive tract intact and places it in a clean plastic or glass Petri dish or beaker with a generous amount of water or saline and a label bearing

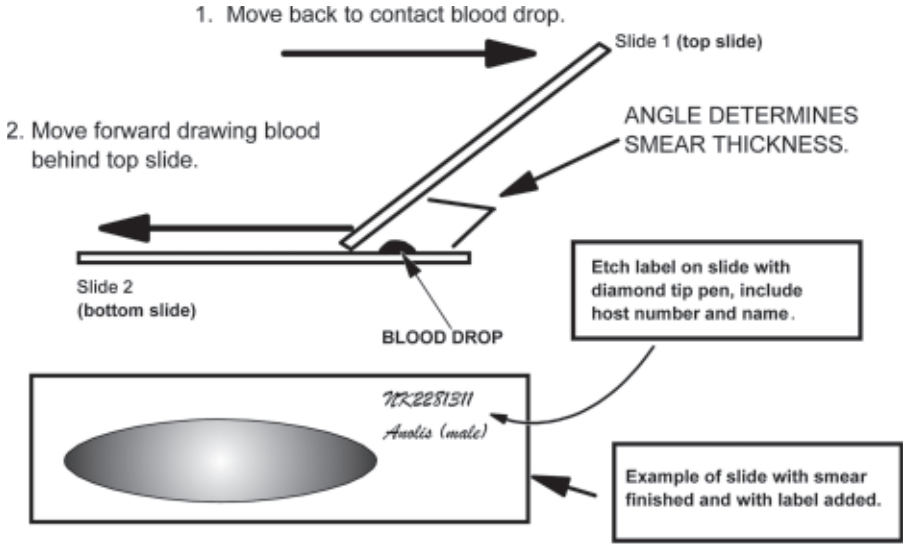


FIGURE 35 Preparation of a blood smear. The smear must be made from fresh, uncoagulated (nonclotted) blood. The angle of the slide above determines the thickness of the smear; a steeper angle creates a thinner smear, whereas a shallow angle provides a thicker smear. Approximately 45 degrees is a good general angle for the top slide. The collector's number (SLG31), host genus (*Anolis*), field identification number (93), kind of parasite, and type of preservative in the vial are included on the label.

the field identification number of the host. If the organs are large, buckets and porcelain pans are used as holding containers. If organs are very large (e.g., from a python, monitor lizard, tortoise, or crocodile), subsamples can be taken from the organs.

4. At this point, the researcher examines the body cavity, liver, kidneys, and lungs for helminth cysts, filarioid worms, and pentastomids. All organs to be removed from the host for future molecular or biochemical-genetic work must be carefully examined for parasites, which are then collected. The presence of parasites in these tissues must be recorded in the field notes that accompany the host. However, because the primers for the host will not pick up the parasites' DNA, the presence of the parasites is not a problem for molecular or biochemical-genetic work. Filarioid nematodes may be encountered in the heart, aorta, pleural cavity, mesenteries, or subcutaneous tissues. Larval or juvenal acanthocephalans, pentastomes, nematodes, cestodes, and trematodes can be found in the liver, mesenteries, or any other tissue. In addition, protistans may be encountered in any of these tissues. Samples should be taken for subsequent investigations for histology and DNA analysis; again, any parasites found should be recorded, even at the most basic level, e.g. "cysts found, protista?" Cysts found should be preserved separately, some of the sample in 10 percent buffered formalin (37% formaldehyde = 100% formalin for the 10% dilution; buffer = 4.0 g mono- [NaH_2PO_4] and 6.5 g anhydrous dibasic [Na_2HPO_4] sodium phosphate/liter 10% formalin) and some in 95 percent ETOH.

   If cestode cysts are encountered, some are fixed *in situ* in the host tissue. The investigator removes a part of the organ with the cyst intact, preserving it in 10 percent buffered formalin. If more than about 10 cestode cysts are encountered in one host, some cysts are removed and carefully cut open. The cestode strobila is placed in distilled water to relax it and then is fixed in 10 percent buffered formalin. If available, some strobili are also stored in 95 percent ETOH, and some are frozen in liquid nitrogen. These various methods of preservation ensure that adequate material will be available for future investigations of both morphological and molecular traits.

5. After examining the body cavity and other host tissues for parasites, the collector frees the intestinal tract from attached mesentery and straightens it, which facilitates using scissors to cut open the intestine along its length. The stomach, small intestine, cecum, and large intestine are placed in separate Petri dishes or other containers, each with a tag bearing the field identification number of the host. Petri dishes must always be cleaned with alcohol or detergent and water and dried between uses. Each organ is opened and examined for parasites. It is important to use scissors with blunt ends, because scissors with sharp points perforate the organ while cutting, making it difficult to open the organ quickly. For small specimens iris scissors are appropriate. An enterotome (special scissors for opening the digestive tract, especially the intestine) is appropriate for opening the intestines of large animals.

   Helminths are often embedded in the mucosal lining of the intestine, which can be removed by scraping the mucosa from the submucosal layer with a microscope slide, tongue depressor, or popsicle stick. If water is abundant, intestinal contents and the intestinal mucosa can be suspended in saline, washed in a fine-mesh soil screen, and then placed in a Petri dish and searched. If water is limited, the intestinal contents are covered with water and gently stirred; the worms sink, and the lighter materials (plant parts and other food items) either float or settle slowly and can be decanted. This procedure is repeated several times, and then the remaining material is searched for helminths with either a dissecting

microscope or a 10X jewelers magnifying visor. If helminth specimens are too numerous to count quickly, the intestinal contents should be preserved in 10 percent buffered formalin (with aliquots going into 95% ETOH for DNA work) so that the parasites can be counted accurately in the laboratory.

Organs such as skin, eyes, urinary bladder, trachea, lungs, and gall bladder must also be examined, preferably with a dissecting microscope. Small trematodes commonly occur in the bile ducts and gall bladder of the liver and in the mesenteric veins; small nematodes can be found in the urinary bladder and lungs.

6. Cestodes, trematodes, and acanthocephalans relax and die when placed in distilled water. Tap water or filtered river water can also be used, but *not* saline. Osmotic imbalance causes water to move into the body tissues of the worm leading to osmotic shock and death. The increasing pressure within the body tissues or cavity (depending on the phylum) also may cause the scolex or proboscis to evert. The cestode strobila relaxes when an animal dies from osmotic shock. It is especially important to leave a specimen in water long enough for eversion of the scolex, rostellum, or proboscis, and for relaxation of the strobila or body. Relaxation can take from 10 min to more than an hour, depending on the size of the worm, the ambient temperature, the amount of salt in the water used, and the species. After being relaxed and killed, helminths to be saved for morphological study are fixed in 10 percent buffered formalin and placed in vials with both the field identification number of the host and the location of the parasite in the host; standardized abbreviations (e.g., SI = small intestine, C = cecum) can be used, but a key to abbreviations must be recorded in the field notebook. Parasites from each organ must be preserved separately, in their own vials. Saline is never used to kill cestodes or other platyhelminths, because it prevents osmotic imbalance and subsequent relaxation of the body or strobila.
7. While working with a host, nematodes can be placed in saline temporarily and then transferred directly to a vial filled with 10 percent buffered formalin. Distilled water should be avoided; the osmotic imbalance it produces causes nematodes to burst, and in many cases the specimens are destroyed. Some investigators kill nematodes in nine parts very hot water, brought to volume immediately after immersion with one part formaldehyde to make standard 10 percent buffered formalin. Alternatively, if nematodes are placed in glacial acetic acid (GAA) for a few minutes, they will uncoil and straighten and then can be transferred to and stored in either 10 percent buffered formalin or 70 percent ethanol. Specimens that have been straightened are much easier to identify than those fixed without straightening, because the morphological characters are more readily distinguished. Specimens to be saved for molecular analyses should be washed in saline and then placed in a 1.5-ml nunc (cryo) tube and stored in liquid nitrogen or placed in a vial of 95 percent ethanol. Material being collected for molecular analyses should never be exposed to formalin.
8. If sufficient numbers of helminths are available, investigators should preserve representative individuals in liquid nitrogen or 95 percent ethanol for genetic analyses. Because glacial acetic acid and formalin destroy DNA, individuals preserved for DNA studies must not be treated with either chemical.
9. Investigators should preserve fecal pellets or material from the cecum for later examination for coccidian (protozoa) parasites. Specimens are placed in a vial half filled with 2 percent potassium dichromate ($K_2Cr_2O_7$). A label bearing the field identification number and the generic name of the host is added. We recommend using Wheaton snap-cap vials (15 mg) because one vial half filled with 2.0 percent $K_2Cr_2O_7$ contains sufficient oxygen to keep the coccidia alive for a very long time (morphological structures of coccidian oocysts are visible and can be studied only in living specimens). Recently we found that coccidians of the genus *Eimeria* remained alive after 10 years in 2 percent dichromate stored in a 15-ml snap-cap vial under refrigeration at 2°C (SLG, unpubl. data). The vials rarely leak and can be reused many times.
10. If many conspecific hosts are available, investigators should preserve from two to five entire gastrointestinal tracts individually in 10 percent buffered formalin. This will allow for future examination of the morphological characteristics of the intestines and any associated worms *in situ*. In fact, because of the vagaries of transport of processed specimens from remote field locations, researchers should preserve parasites using many different methods, thus ensuring availability of adequate material for future studies.

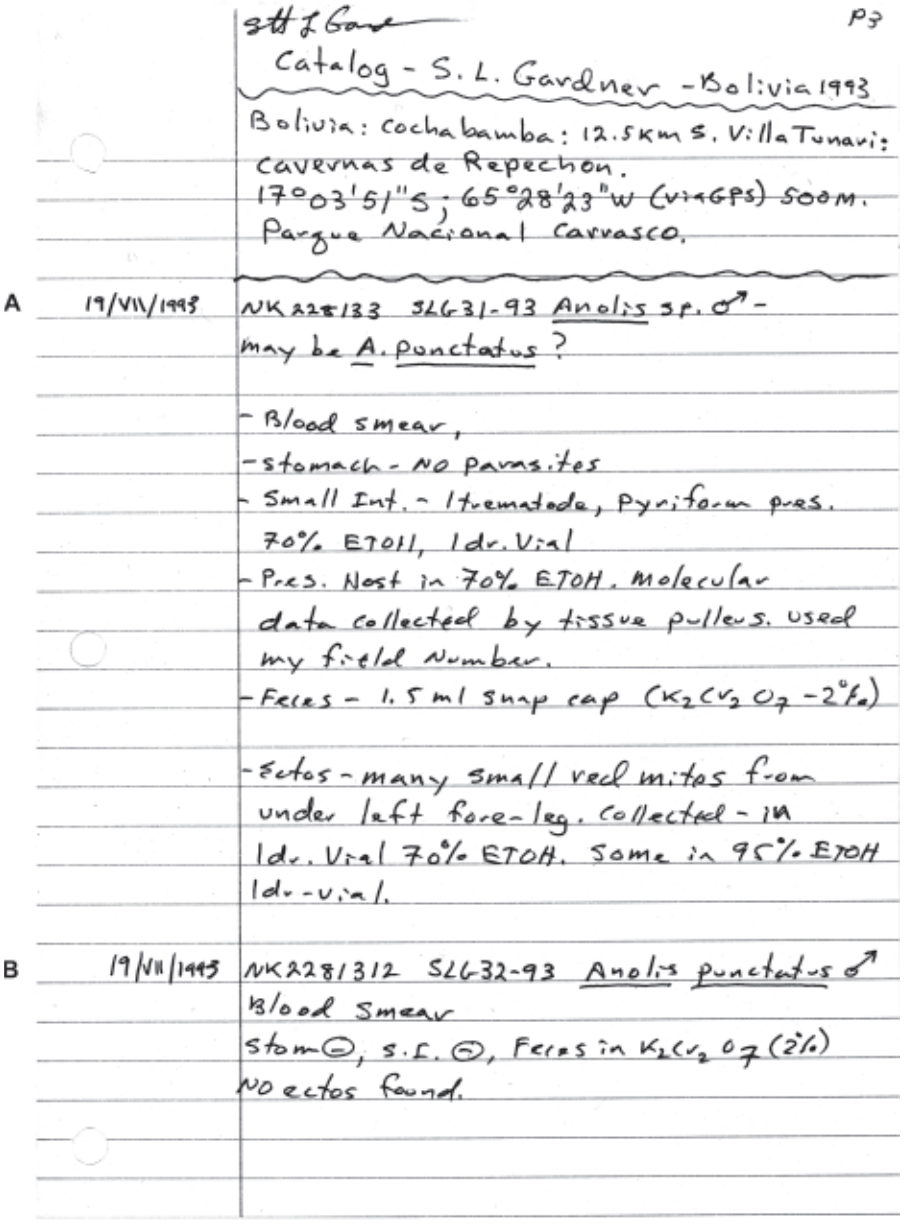
Recording Data

Each researcher should maintain a field notebook (Fig. 36) for recording data on his/her parasitology collections and preparations. The notebook should be made of 100 percent acid-free cotton-rag paper. Only permanent black ink (e.g., Higgins Eternal) should be used to record data. Data can also be recorded in a PDA (see "Handheld Computers for Digital Data Collection," in Chapter 4). However, data stored in an electronic format are much more labile than those recorded in permanent ink on archival paper, and we urge investigators to maintain backup files and printed copies of all data. Collectors should number their specimens sequentially, beginning with 001 and continuing indefinitely, rather than beginning a new sequence each year (e.g., 2008-001, etc.) or each collecting trip (e.g., SLG-001 through SLG-12500).

Each specimen record should include the field collection number of the host to ensure accurate cross-referencing of a parasite sample with the host voucher or symbiotype specimens. A specimen number is always preceded by the collector's initials so that she or he can be identified in the future. In addition, specimen records should include the collecting date and locality, the species name of the host (although acknowledging the provisional nature of field identifications), and the location of the parasite on or in the host. The collector should record the general kinds of parasites encountered in each organ as well as negative searches (Fig. 36).

Materials Needed to Collect Parasites

The materials needed to collect parasites in the field are listed in Table 11. The quantities required will vary with the numbers



~~Stt L Gard~~ P3

Catalog - S. L. Gardner - Bolivia 1993

Bolivia: Cochabamba: 12.5 km S. Villa Tunari: Cavernas de Repechon. ~~17°03'51"S; 65°28'23"W (via GPS) 500m.~~ Parque Nacional Carrasco.

A 19/VII/1993 NK228133 SLG31-93 Anolis sp. ♂ - may be A. punctatus ?

- Blood smear,
- stomach - NO parasites
- Small Int. - 1 trematode, Pyriform pres. 70% ETOH, 1 dr. Vial
- Pres. Nest in 70% ETOH. Molecular data collected by tissue pulleus. used my field Number.
- Feces - 1.5 ml snap cap ($K_2Cr_2O_7$ - 2%)

- Ectos - many small red mites from under left fore-leg. Collected - in 1 dr. Vial 70% ETOH. Some in 95% ETOH 1 dr - vial.

B 19/VII/1993 NK2281312 SLG32-93 Anolis punctatus ♂
Blood Smear
Stom ⊖, S.I. ⊖, Feces in $K_2Cr_2O_7$ (2%)
NO ectos found.

FIGURE 36 Sample field notebook entries. (A) Record of examination of *Anolis* SLG31-93 for parasites. Note that the name of the collector and the locality are always included at the top of each page or at any point where the collecting locality has changed. The date is always included with each individual record. The field identification number, followed by the field collector's number is next, followed by the field identification of the host (often, species names assigned in the field are incorrect if new or unknown species are collected). Next, the investigator notes that a blood smear was made; lists the parasites collected from different organs and how they were preserved or stored; records the fate of the host (in this case the whole animal was preserved in 70% ethanol); notes that feces were preserved; and lists the ectoparasites found and how they were preserved. The present entry also indicates the absence of parasites from the stomach. (B) Record of examination of *Anolis* SLG32-93 for parasites. A blood smear was taken, and feces were collected and preserved in potassium dichromate; no other parasites were found. Negative data are always recorded to verify that the host was examined and to ensure accurate estimations of prevalence, intensity of infection, and other ecological parameters.

TABLE 11

Materials Required for Collecting Parasites

Materials[a]	*Amount*[b]
Equipment and Glassware	
Dissecting microscope, 0.5–30× magnification	
Magnifying visor, jeweler's 10× or 10× lighted hand lens	
Light source, bright, for working at lab bench; headlamps (e.g., Justrite) requiring 4 size-D batteries work well	
Jeweler's forceps (100 mm), 2 pair	
Blunt-nose gross dissection forceps (120 mm and 140 mm), 1 each	
Copelin staining jar for fixing blood smears	
Porcelain dissection trays, small (30 cm × 20 cm) and large (40 cm × 30 cm)	
Scissors, both sharp-nosed and blunt-nosed of different sizes:	
100-mm iris scissors for fine work	
120-mm blunt-nose scissors for coarse work	
120-mm sharp-nose scissors for cutting tissue	
Rapidograph (or other) pens and indelible India ink (Higgin's Eternal), or disposable Black UNIBALL Deluxe (Faber-Castell) permanent ink pens	
Scalpel and disposable blades (#21) for cutting through host tissue	
Soil sampling sieve, no. 325, USA standard sieve series, 45-µm mesh; 20-cm-diameter small-mesh sieve that catches nematodes but allows colloidal particles in the water to pass through	
Diamond point pen for scribing on glass microscope slides	
Workshop light, fluorescent, for detailed microscope work or work with a magnifying visor	
Liquid nitrogen tank with a static holding capacity of 2 weeks (or sufficient for the length of the collecting trip)	
Squirt bottles of several sizes for the various solutions	
Expendable Supplies	
Plastic bags, 1.5–2.0 mil, rectangular (200 mm × 350 mm)	200
Whirl-pak or other plastic bags with twist tie closures	400
Latex and nitrile gloves	
Microscope slides, standard pre-cleaned (not frosted)	2 boxes
Tongue depressors or popsicle sticks for scraping intestinal mucosa	
Petri dishes, plastic, small, medium, and large	
Insect-pin probes, small	
Dissection probes and needles	
Plastic pipettes, many disposable	100
Field notebook paper, 100% cotton rag, acid free	200 sheets
Vials, Wheaton snap cap, 15-ml	1 box of 144
Vials, Wheaton snap cap, 20-ml	1 box of 144
Vials, screw-cap with Teflon inserts in cap, 1-dram	1 box of 144
Vials, screw-cap with Teflon inserts in cap, 15-ml	
Cryotubes (nunc), with colored caps (or lid inserts); brown tops for parasites	100
Labels (or label paper) for wet preparations inside vials; 100% rag notebook paper or archival (museum)-quality label stock	
Cheesecloth	
Tissues or Kimwipes for cleaning equipment	10 boxes
Paper towels	2 rolls

Materials[a]	*Amount*[b]
Reagents	
Methanol (MEOH), 100%	500 ml
Ethanol (ETOH), 95%; (can be used to make 70% ETOH)	500 ml
Ethanol (ETOH), 70%	1000 ml
Formalin, 100% (= 37% Formaldehyde)	500 ml
Buffering salts (monobasic sodium phosphate, NaH_2PO_4,), either anhydrous, premeasured in 4-g packets, or in a water solution premeasured	5 packets
Buffering salts (dibasic sodium phosphate, NaH_2PO_4), either anhydrous, premeasured 6.5-g packets, or in a water solution pre-measured	5 packets
Potassium dichromate ($K_2Cr_2O_7$), 2% solution	500 ml
Glacial acetic acid	

a. See Appendix II for a list of vendors.
b. Estimated quantities of some disposable supplies needed for collecting parasites from approximately 100 reptiles.

of collectors, the duration of the fieldwork, and the number of animals collected. We have estimated the quantities of various disposable supplies listed in the table needed for collecting parasites from approximately 100 reptiles. For general collecting, researchers will need approximately one large plastic bag, two Whirl-pak bags, two 1-dram and two 15-ml snap-cap vials per host animal. Actual use will probably be lower, because not all hosts are infected with parasites.

Acknowledgments

We thank Agustin Jimenez-Ruiz, Robert L. Rausch, Robert M. Storm, and Donald W. Duszynski for long-term support and encouragement during the development and implementation of the techniques we outline.