collection and preservation of amphibians and reptiles

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COLLECTION AND PRESERVATION OF AMPHIBIANS AND REPTILES FOR SCIENTIFIC STUDY

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INTRODUCTION

While going around animal collections in Biology Museums in different education institutions in Pakistan, author has observed several reptilian specimens ruined due to improper preservation techniques. Invariably abdominal region of the specimens is destroyed by the bacterial activity, and thick parts of the limbs are abnormally swollen, despite the specimens are surrounded with ample amount of preservative.

Preservation of reptiles for museum display or research work involves special methodology. Present paper outlines special instructions necessary for collection, preservation and storage of the herpetological material for scientific study and museum display.

Unfortunately there is yet no adequate reference depository in Pakistan where reference specimens can be deposited or available for comparison and new material can be deposited with an assurance that it will not be lost. Recently established (1976) Pakistan Natural History Museum, Islamabad, is still in formative stages (Mufti, 1997), and presently is in shambles. Thus these working conditions necessitate building and maintaining a personal collections by individual workers, who collects according to his or her research interest throughout life and wants to maintain it healthy for long time. While moving to United States in 1999, my life long collection of amphibians and reptiles (1963-1999) was requested to be deposited in zoological Museum of Government University College, Lahore. I hope somebody would be studying it now…..?

Collection

Before starting collection of amphibians and reptiles from an area:

A. **Familiarise yourself with the already known species** from it. It is advised to collect and read pertinent literature, meet people already working on the animals in the area. You can gain important information by discussions and know the types of animals you are expected to encounter in the field. A timely advise may change your planning altogether, guiding you in your fruitful collections.

There are three ways to build a good representative herpetological collection:

a. By Picking road-killed animals, which may be rare and most wanted species. The picked animal should be cleaned of the debris and adhering ants and preserved by the method suggested in this chapter.

b. Acquaintance with personnels of departments of Wildlife, Agriculture, Forest, and students returning to their homes for holidays are of great help in building a good collection. They may be provided containers with preservative, requesting to put in the animals killed in their locality. In this way, material from far flung areas can easily be obtained. Otherwise, time and financial constraints jeopardise year-round collecting from remote areas.

c. Field collecting trips are the most desirable way to obtain first hand ecological information regarding specimens. To ensure a large collection of animals a team of three to four members should be arranged. During field-trips first-hand knowledge on habits and ecological data can be recorded, and life activity and ways of life are noted.

However, a field trip entails careful planning, pin pointing the areas from where collection is intended. All potential needs that may arise in field should be anticipated. A badly arranged and planned collection tour always ends in disappointment for the participants.

The collection should be as representative of an area as possible. While engaged in field activity, **diurnal** and **nocturnal** as well as **dawn** and **dusk** periods of animals activity should be kept monitored to ensure collection of as many species as possible.

Field ---is a great teacher, one learns practically the collection techniques, where to look, how to bag the animal. However, a few tips for beginners are given in the following section.

Collection tips

Amphibians are best collected at night at their breeding sites. The calling males are spotted by torch-light which does not disturb them. They are caught by hand or a hand-net. Small species like *Microhyla ornata, Uperodon systoma, Limnonectes limnocharis* and *L. syhadrensis* are best collected by spotting the calling male that perches well away from water, in the marginal grass or other vegetation and are very agile hoppers (Khan and Malik, 1987).

In day light amphibians are a bit harder to find and capture. They may be found by turning stones, logs, debris and leaf-litter, searching along marginal grass along ponds, puddles, and streams, and looking into holes, crevices and fissures in ground. However, at night frogs gather under light posts feeding on photophilic insects and can easily be captured. Care should always be taken not to leave the captured animal in a net, since they may widen net-mesh and sneak away.

Reptiles: Majority of lizards and snakes are diurnal, are active 3-4 hours after dawn. Are exceptionally agile, a slight disturbance around may drive them deep into burrow. To extract an animal from burrow is really a difficult and laborious job.

Lot of careful surveillance is needed to locate a reptiles in field without making any noise, practically walking on tip-toes. In field, always keep in mind, your movements should look as non-threatening as possible, so move slowly and deliberately. Use your full visual field, observe without turning your head. Most reptiles are located under bushes and grass or under rocks, logs, leaf litter, tree bark, trash and leaf litter. May be found sneaking in crevices and holes in walls, among stones, and in hollow logs.

When an animal is located, keep it in your view without directly looking at it. Look unconcerned while approaching it as close as possible. Now you must decide quick, how best to get it-

- by grasping with hand, or giving it a blow with stick. Grasping with bare hand through branches of thorny bushes should always be avoided. Nocturnal lizards (mostly geckos) are spotted in torch light on walls, boulders, rock outcrops, or holes in the ground, from where it can safely be caught by hand.

Remember, while in field look as unobtrusive as possible, make no extra movements, walk slowly, looking for any animal movement around you, use your full visual field without turning your head. All the animals hiding from you are keenly observing your movements. Any provocative movement, on your part may derive them in their hides. More agile reptiles are located from a distance, preferably before they see you. Observe keenly any movement among vegetation including branches of bushes touching ground. When you have located an animal, keep it in view and approach it as closely as possible. When it is within striking range, give it a powerful blow with the stick. The most active lizards that do not allow a close approach can be shot by an air-gun or stunned by a strong rubber band or rubber sling. To catch one with hand requires a good control of one's wits and takes time. Collection of diurnal reptiles requires lot of patience and some luck. In one locality, a species may be hard to collect, while in another area it may be an easy catch. While turning over rocks, stones, logs, leaf litter etc., care must be taken to use some tool, or protect your hands with heavy gloves. Scorpions and poisonous snakes often hide under these objects.

Nocturnal geckos and snakes are active just after sunset. Geckos are located by looking in lighted parts of the walls with bulbs at night, catching light attracted insects. In torch light, they may be located under bridges and on walls of inhabited houses. Snakes are found along road sides, around human habitations in urban areas, and around ponds and puddles in warm nights. Areas recently watered are often visited by snakes in warm nights.

Things related to animals are of great importance in subsequent taxonomic and ecological studies. They include eggs, egg-shells, larvae, juveniles, broken parts of body (tail etc.), squats, and photograph tracks on soil and if possible record the vocalisation.

Most reptiles are best collected by grasping with bare hand. However, large varanids are caught in such a way that animal’s sharp teeth and claws are best avoided. They are grasped from neck from dorsal side with one hand, and with the other hand from pelvic region so that mouth and claws of the animal face away from your body. Turtles and tortoises are caught from the sides of their shell, avoiding contact with claws. Large live lizards and turtles are carried in sacs of firm-material.

Most lizards autotomize their tail during capture, do not discard it, tie it securely with the specimen, since tail characteristics are of great help in specific determinations.

Snakes: Extra care should be taken in handling snakes. Colubrids are rarely encountered in field since they are very quick to escape. However, when cornered, mostly they coil and attack and hiss loudly. while except cobra, all venomous snakes prefer to lie coiled to escape detection. A coiled snake is easy to handle.

For the beginners it is advised to kill the snake, by giving a strong blow at mid-section of its body, and wait until it is motionless. Do not smash its head, its characteristics are important in specific determinations. To ensure death of a snake, turn it in supine position (belly up) with a stick, if it remains so for 5-10 minutes than it is dead. Handle it with a forceps, do not touch with hand, often apparently a dead snake, may bite in reflex which persists for some time, so do not handle an apparently dead snake carelessly, always avoid its head.

Catching a live snake is always risky. Even experts are bitten, so avoid it in field. Special sticks, tongs, to catch live snake are not available in Pakistan.

I use the following technique: press snake's head against ground with a strong, half a meter long stick, then grasp it firmly from just behind its head, with your right thumb and fingers, so that the snake cannot turn its head sideways to bite. I do not recommend this to beginners, they should handle only a dead snake. Catching a live wild cobra is avoided even by experts, it should always be killed first.

Putting a live snake in a collection bag, demands extra care. The cloth bag is held open in left hand, the snake is lifted in right hand, well above so that its tail could first be lowered into the bag. As hand holding snake's head is well down in the bag, the grip on snake is released and hand is quickly withdrawn from the bag and is snapped close tightly with both hands, firmly tied with a strong cord. Extra precaution is taken by bending over the tied end of the bag, and tying it double. Bags with living snakes should be handled with care, body contact should be avoided. The bag preferably should be carried by holding it at the tied end or by the free ends of the cord, for snake may bite through the bag.

Small lizards are put into plastic bags and tied. In hot weather animals should not be kept for long in plastic bags as they will die and start rotting

Field Collecting Equipment

Every team member should be provided with a set of equipment in a handy sling bag, which should include:

1. A strongly bound notebook for recording data.
2. Good quality pencils, ball-point pens, sharpeners, erasers, measuring tape, a penknife, a spool of thread and needles.
3. Good quality torches with spare dry cells and bulbs.
4. A strong walking stick 1.5 m long and 20 mm thick.
5. A hand-net and tea strainer for catching aquatic animals.
6. A Plastic jar with capacity of about 500 ml for frog tadpoles and soft-bodied small frogs and reptiles.
7. Strong cotton sacs of different sizes with strong strings.
8. Strong 40 mm long and 20 mm wide clear plastic bags with double sealed end, for keeping small reptiles. These bags are handy and weightless and are secured by knotting the open end.

9. A pair of Large forceps or tongs to handle obnoxious animals and extract animals from crevices and holes.

10. A whistle that can help team members to locate each other when they become separated. At night a torch may also serve this purpose.

11. A field camera with flash gun.

12. A thermometer and a hygrometer.

Recording field Data

Following methodology, to record information pertaining to every collected animal, is important. A specimen without field-data is scientifically useless. The information should be recorded on the spot. Memory should not be trusted as you may later fail to recall important information.

1. Assign an identification code number to each collection tour and mark a part of your notebook accordingly.

2. Assign a collection number to each specimen. Write it on a tag with lead pencil and tie it to a leg of the specimen or around midbody in case of snakes.

3. Enter the following data in notebook referring to its tag number:

i. Date of collection: day-month-year.

ii. Time of collection: using a 24-hour clock.

iii. Locality where collected. Be as specific as possible. Give district, province, distance and direction from nearest town on standard maps. Give name of mountain, highway, river, or canal if appropriate.

iv. Record ecological data: habitat where animal was found, elevation above sea, temperature/humidity.

4. Name of collector.

4. Take a colour photo of every specimen collected. The habitat in which it was found should be shown. Some times a series of photos may illustrate locomotion, breeding, feeding habits of the animal.

Killing, Fixing and Storage of Material

Preservation of scientific specimens involves killing and fixing and storing it properly. Specimens must chemically be treated to ensure proper preservation of their different body parts in a natural shape as possible, to facilitate later detailed scientific study. In the field it is essential to carry all liquid chemicals in well stoppered, strong plastic bottles.

Concentrated (40%) formalin buffered with calcium carbonate is a good, economical preservative for field work.

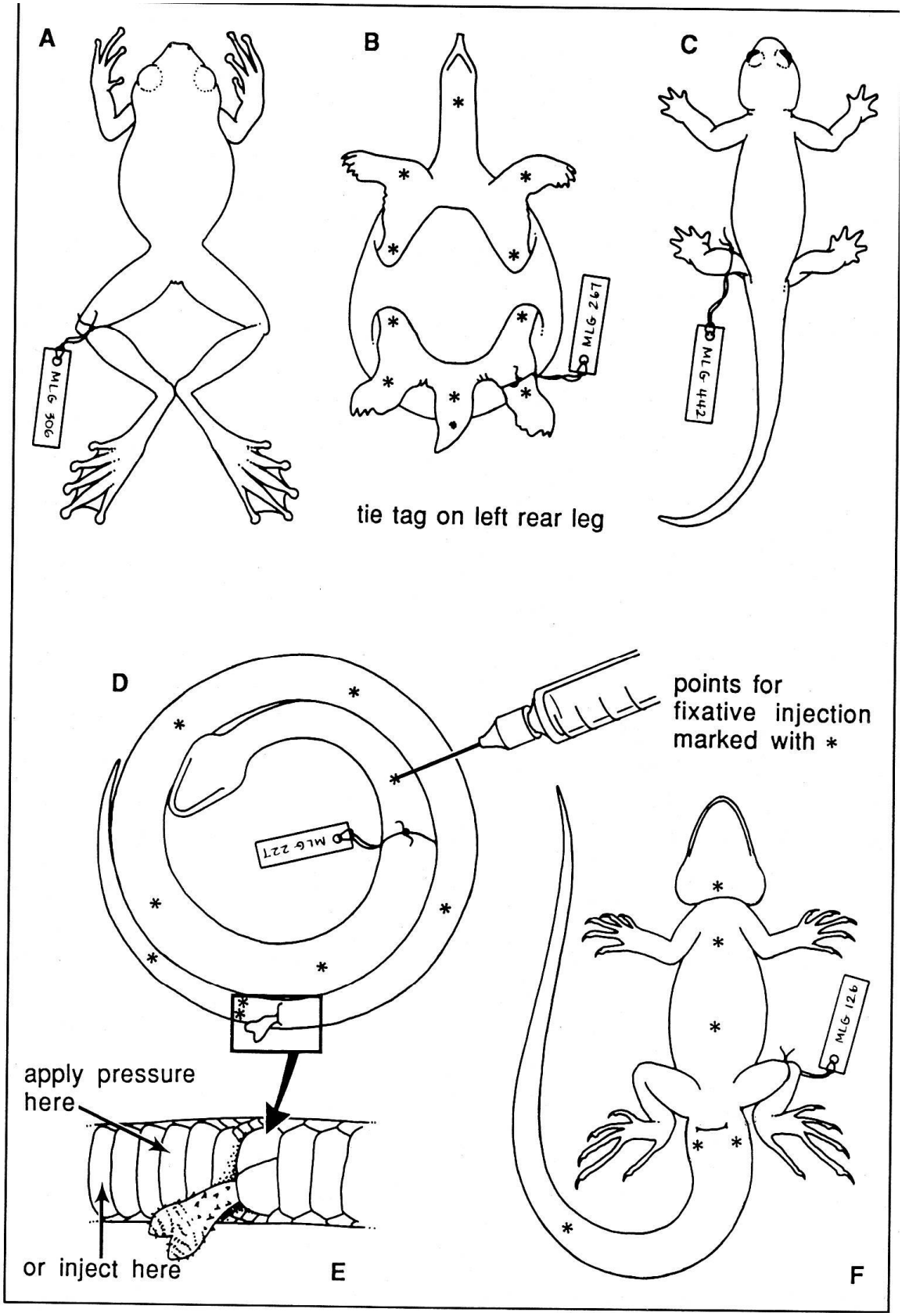
Plastic trays with lids are needed for fixing and hardening specimens.

A two meter length of muslin cloth twice the width of the tray is needed.

Syringes (20-50 cc) and needles (16, 20 and 25 g) are required injecting specimens.

Amphibians: Amphibian skin is permeable and readily absorbs killing agent. Adults, eggs and larvae are put directly in 10% formalin until dead (Khan, 1965, 1982a, 1982b). There are several other killing agents such as chloreton, procaine hydrochloride and barbituates that as equally good but are not readily available in Pakistan and are more costly.

Reptiles: Lizards and snakes are killed by injecting concentrated formalin at the site of heart, which is at mid-chest in lizards, and about two head lengths from the anterior end of the body in snakes (Fig. 1 A, B, C). Turtles are injected with concentrated formalin at sites indicated ((Fig.1B). Moderate pressure on shell will cause these parts of body to come out. To preserve viscera, formalin is injected deep in the body using special long needles.


A
B
C
MLG 306
MLG 267
MLG 442
tie tag on left rear leg
D
points for
fixative injection
marked with *
MLG 227
apply pressure
here
or inject here
E
MLG 126
F

Legend to the figure

Injection sites (*) of preservative, and hardening postures of animals desired for scientific study: No. Tag with specimen number; E. Extruded hemipenes. Frog; B. Turtle; C. Lizard; D. Snake.

Fixing

A plastic tray is prepared for fixing by folding and spreading half of the piece of muslin cloth at the bottom of the tray. Sufficient 10% formalin is poured into the tray to soak the cloth.

Amphibians: The frog is taken out as soon it is dead and placed belly down in the fixing tray. Limbs of the animal are folded in natural position. The animal is covered with the other half of the muslin cloth, wetted with 10 % formalin, then the tray is covered. The specimen is left for hardening for 6-12 hours.

Reptiles: The scale-covered, hard skin of reptiles is impermeable to preservatives. So that the animals are fixed by injection of preservative. Concentrated formalin is injected in the body cavity to preserve viscera. Large lizards require injection into other parts of the body also (Fig.1A). If a syringe is not available, a cut in the mid-abdomen of lizard helps penetration of the preservative. Snakes are injected at 4-5 cm intervals along the whole length of belly and tail (Fig.1D). Moderate pressure at the base of the tail of a freshly killed lizard or snake everts its hemipenes. Hemipeneal morphology is very helpful in taxonomic determinations. Injection of formalin at the tail base also serves to put pressure to evert hemipenes and harden them at the same time (Fig.1D).

Dead lizard is placed belly down, in the fixing tray. Its legs are folded in natural position, soles facing down and digits are evenly spread. The tail is bent on the left side. The cloth is folded over the animal and sprinkled copiously with concentrated formalin and is left for 6-12 hours. Fixing a snake involves coiling its body after injection according to the size of the storage container. After formalin injection it is covered, as usual, with formalin-wetted muslin cloth and left for 12-24 hours depending on the size and bulk of the snake.

Storage

Fixed and hardened specimens are stored in air-tight plastic jars. Caution should be taken not to pack too many specimens in one jar. Over-crowding and too little preservative in the container may distort and ruin the specimens for ever.

Jars with specimens should be transported from field carefully. Each jar should be marked with a number, which should be recorded in the data-book. While loading the jars on transport, ensure that every jar is loaded in a secure place. During journey to laboratory, care should be taken, that none of them is damaged. On reaching destination make sure that every jar is unloaded and placed safely.

Museum display

Adult animals with all body parts in contact and in good condition, are to be selected for museum display. A clear transparent glass jar, 20-30 mm longer and wider than the specimen, should be selected as container. It should have fairly tight stopper. If the body of the animal is weak, i.e., it cannot stay vertical in the jar, support it by tying it to a glass strip. The jar is filled with 7% formalin, the animal is lowered in it and sealed. The displayed jars should be surveyed time after time. Evaporated preservative should be maintained regularly.

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