Instructions for Collecting Bird Parasites

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Bird collectors and preparators may make important contributions to the study of avian parasitology. Arthropod ectoparasites and protozoan and helminth (worm) endoparasites are still imperfectly known. A better knowledge of their occurrence and host specificity may shed light on the cause and transmission of disease, delineate relationships of various groups of birds, and provide a better insight into different aspects of zoogeography. Arthropod ectoparasites are generally easier to find and preserve than internal parasites, but both are of vital taxonomic and biomedical interest to specialists.

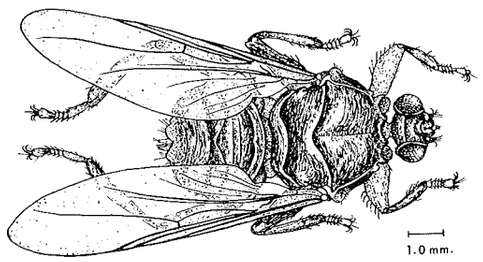
It is advisable for field collectors planning expeditions to coordinate their parasite-collecting activities with microbiologists, parasitologists, acarologists, and entomologists prior to the actual trip. Some parasite systematists have specialized requirements for fixation and preservation of materials for study or have urgent need for parasites of particular species. By prior consultation, the collector will be able to direct his efforts in the field toward groups most likely to produce worthwhile results, and to make arrangements for processing the material after it is collected. Information about active specialists working on various taxonomic groups of parasites is available through the Smithsonian Institution.

Equipment

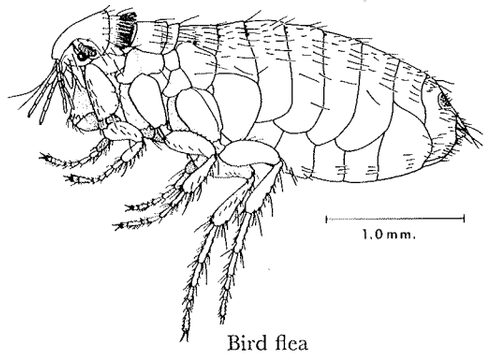
The field equipment listed in the table on page 12 includes all the necessary tools and supplies one ordinarily needs for collecting and preserving parasites in the field. A few of the more specialized laboratory techniques described in these instructions demand equipment bulkier than is generally suitable for use under field conditions.

Arthropod Parasites

Parasitic arthropods (phylum Arthropoda) belonging to two classes, Insecta and Arachnida, are found associated with birds throughout the world. The majority of insects and mites occur on the host's body, where they suck fresh blood, although most lice and some mites feed on the host's body fluid, tissue, dead skin, or feathers. Some parasitic arthropods are found only in the host's



Louse fly



Bird flea

nest. Different bird species and individuals may be parasitized to varying degrees; parasites may teem all over one bird's body, while another individual may be free of them.

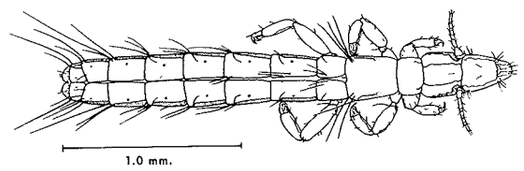
Birds trapped or netted in banding studies may be examined live for ectoparasites and then released. More ectoparasites are found on recently killed birds, however, because natural cooling of the host's body will usually bring them to the surface of the feathers, where they may be picked off.

It is essential to keep different host species separate before removing parasites in order to maintain the original host-parasite relationships. Put birds in closed plastic or cloth bags immediately after collection. A piece of cotton soaked in chloroform placed in the bag will kill bird flies and fleas, which might otherwise escape. Although it is preferable not to use the same bag twice, if the bag must be reused, it should be reversed, shaken, and washed thoroughly to prevent contamination.

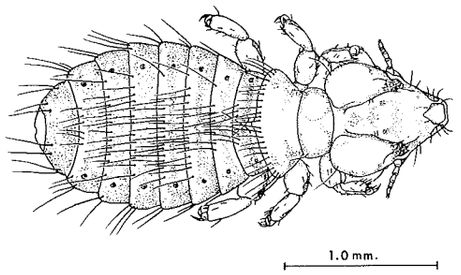
Many different techniques are available for obtaining ectoparasites. Examination of the skin and feathers with a dissecting microscope, although time-consuming and normally limited to laboratory use, produces excellent results. A 10- or 15-power hand lens is useful in the field. A simple field method for obtaining ectoparasites involves shaking a bird specimen over a white pan or piece of paper; the dark parasites are conspicuous when they fall on the white background. This method is improved by use of a dessicant such as pure silica gel without a propellant (available in powder form under the name "Dri-Die") which, when dusted on the host in a plastic bag, causes arthropods to lose water and detach in 5 to 10 minutes. This method is also effective on live birds. The Dri-Die particles are small and leave on the feathers a fine, dusty bloom which is difficult or impossible to remove in the field. Consequently, such treatment is not recommended for bird specimens to be made into study skins.

It is essential that ectoparasites be handled carefully, since rough treatment may damage delicate legs and setae. Fine-tipped brushes, probes, and jewelers' forceps, moistened in alcohol, are excellent tools for picking up ectoparasites. To avoid mixing parasites from different hosts, brushes, probes, forceps, dishes, and pans should be thoroughly washed before processing a new individual.

Combing bird feathers or washing whole birds with soap or household detergent may increase the yield of ectoparasites, but



Feather louse (wing type)



Feather louse (head type)

since these methods may ruffle or damage plumage they are recommended only for birds to be made into skeleton or spirit specimens. The soap or detergent is used merely as a wetting agent, and abundant lather is undesirable, since the arthropods are difficult to find in the suds.

Washing techniques are highly effective, although more suitable for laboratory use. In one method, a refrigerated or frozen bird is placed in a jar of warm water and detergent, then shaken vigorously. The sudsy water, along with several rinses, is poured into a tall glass container and allowed to settle. After the clear water is decanted, the residue is placed in a petri dish for examination. A separatory funnel may be substituted for the tall glass container, and, after settling, the residue at the bottom of the funnel may be drained for examination. This washing-and-settling method is 70–90% effective in obtaining the total ectoparasite population on the host.

By filtering the wash and rinse water through a no. 2 filter paper or paper towel the parasite yield is increased so that a near-total count is possible. Filtration through a Buechner funnel speeds up this process. Several changes of filter paper may be necessary, however, since residue tends to clog filter pores. Parasites may be picked from the filter paper under a microscope.

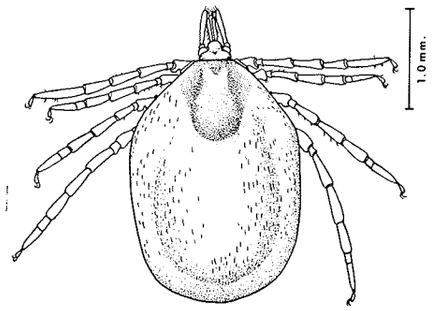
An electric paint mixer may be adapted for shaking the jar. If standard speed, time, liquid level, and detergent are used, yields from several hosts will be quantitatively comparable.

Another ectoparasite-collection procedure is the complete dissolving technique, which guarantees recovery of the total parasite fauna but is feasible only if skin and feathers are to be discarded as in skeleton preparation. The dissolving solution consists of:

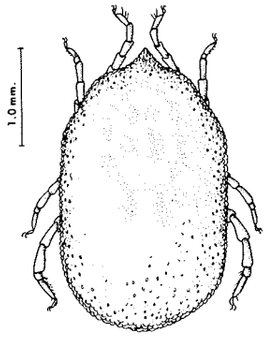
500 ml. distilled water;
15 gr. dibasic anhydrous sodium phosphate ($NaHPO_4$);
6 gr. trypsin (4× U.S.P. Pancreatin).

This will make sufficient solution for six or seven starling-size birds.

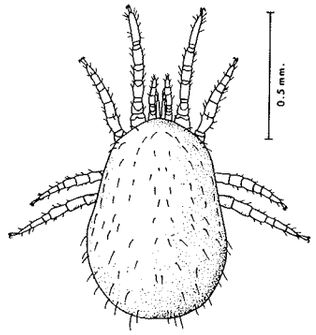
Remove the skin and all feathers from the bird and place them in a container of solution in a 38° C. oven for at least 12 hours. Then add 15 gr. of potassium hydroxide (KOH) and boil until the solid material is completely dissolved. If the bird is heavily pigmented, the resulting fluid will be a dark "soup." Cool the mixture and pour through a 22-mesh bronze screen. Wash the residue by running distilled water through the screen, and examine the screen



Hard tick



Soft tick



Bird-nest mite (Dermanyssidae)

surface for ectoparasites. *Caution:* the fumes from the dissolving solution are toxic, and the boiling operation should be carried out under a hood.

Preserve all arthropod parasites in small screw-cap or procaine vials with natural- or butyl-rubber stoppers containing 70% ethyl (grain) or isopropyl (rubbing) alcohol. Methyl (wood) alcohol is not recommended, and Formalin should always be avoided since it hardens specimens. If specimens are to be stored in alcohol for a long time, a small amount of glycerine (5 ml. in 100 ml. of 70% alcohol) will keep them from hardening. Keep parasite specimens from different host specimens and species in separate containers. A label written in India ink or soft pencil on sturdy paper should be placed in the vial (never on the outside). It should show host species (or, if yet unidentified, specimen field number), geographic locality, location on host's body, date, and collector. If the parasite was not on a host, the circumstance of its capture (e.g., in a bird nest) should be stated.

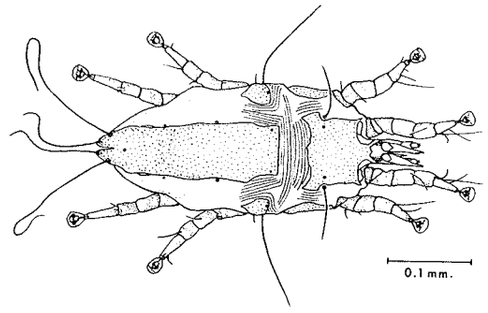
Arthropod parasites of birds belong to several different groups which occur on different parts of the host's body. Each group necessitates specialized methods of collection.

INSECTS of three types, louse flies, fleas, and feather lice, are frequently found on birds or in their nests.

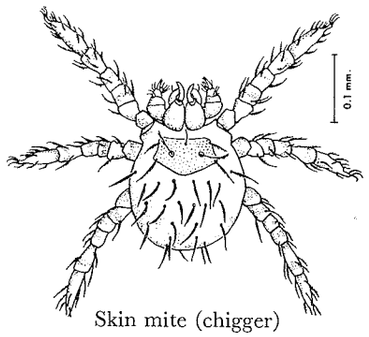
Louse flies (order Diptera, family Hippoboscidae) are the largest and most conspicuous bird ectoparasites. They look superficially like house flies, but are flattened and live under body and wing feathers where they feed on blood. They are most easily secured immediately after the host is collected. Within seconds after it is dead, place the bird specimen in a plastic bag with a piece of cotton saturated in chloroform (use a cloth bag in the humid tropics). When the flies are dead, transfer them to a vial of 70% alcohol.

Fleas (order Siphonaptera) are probably the most difficult of all bird parasites to collect because they are inconspicuous and can leap many times their length. Special effort should be made to secure them because they are poorly known. They are small, wingless, laterally compressed (flattened) insects which suck blood. Use the same bag technique as for louse flies. The nests of birds, especially burrowing species, may be far more productive of fleas than the hosts themselves.

Feather lice (order Mallophaga) feed on various parts of feathers and skin, and a very few



Feather mite



Skin mite (chigger)

feed on blood or eye fluid. The pale nymphs resemble the adults, and the life cycle is spent entirely on the host. Those lice found on the head are small, round, and flat; those on the breast and back are somewhat more elongated and move actively over the plumage. Those found on the wings are long and slender, and some, as nymphs, live inside the wing quills. Other highly specialized species feed on blood, tissue, and debris in the throat pouches of pelicans and cormorants. Feather lice are best collected during skinning or shortly thereafter. Cooling of the body of the host following death generally drives the lice to the surface of the feathers, where they may be picked off with a fine-tipped forceps or a small brush dipped in ethyl alcohol. They may also be found on the cotton wrapped around bird-skin specimens during drying. Be sure to look along the shafts of wing quills for the long, slender species. Mallophaga eggs when attached to feathers sometimes look like small, oblong scales.

MITES (Arachnida, order Acarina) of several sorts, including ticks and feather, skin, and nasal mites, live on, and in association with, many birds. Adult and nymphal parasitic mites have four pairs of legs; larval stages have three pairs.

Ticks (superfamily Ixodoidea) are frequently found on areas devoid of feathers, such as underwings, feet, brood patches, bare throat, and eyelids, or on the head, where they can attach to suck blood. The abdomen is distensible in soft ticks (family Argasidae) and in female hard ticks (family Ixodidae), so that after engorging on a meal of blood a tick may be quite large. Male hard ticks, however, are frequently overlooked because they do not become distended. With forceps dipped in alcohol, carefully pick the tick off the host, avoiding destruction of its mouth parts if it is attached for sucking, and place in a vial. Ticks are often abundant in large seabird colonies, and may also be found in solitary nests. They use the host only for a food source, passing their life cycles nearby in the nest or litter, so that they should be looked for in piles of organic debris under the bark and in the foliage of trees, on the ground, and under stones near nesting sites.

Feather mites (superfamily Analgoidea) include several families which feed on plumage or dead skin and are so small that they may look like dirt particles on body, wing, and tail feathers. Examine especially the undersides of wing and tail quills and between the barbs. Some mites may even be found inside of the feather shafts. Use a fine brush wet in alcohol for transferring mites from the

feathers to the collecting vial. Certain stages of some feather mites are known to burrow just under the skin. When the bird is turned inside out during skinning, these appear as yellow spots on the inside of the skin in the neck and breast regions.

Skin mites (superfamilies Cheyletoidea, Trombidioidea, and Cytoditoidea), which feed on blood or tissue, are usually harder to see than other mites. The head and brood patches of adult birds are important places to examine. On nestlings, any area devoid of feathers, such as the neck, belly, and underwings, is important. Pick the mites off the host with a fine brush or probe dipped in alcohol and transfer them to a vial. Some skin mites, especially chiggers, may be attached to the host, appearing as a small red or yellow dot in the middle of a swollen area of skin or at the base of a feather. Pick or scrape the mite off carefully, using a needle or fine-tipped forceps dipped in alcohol. Chiggers (family Trombiculidae) may also be collected by suspending the host over a pan of water; these mites (and possibly other parasites) drop off in a few hours. Some skin mites burrow into the skin of the host's body and legs, where they may cause tumors or cysts which must be opened to obtain the parasite.

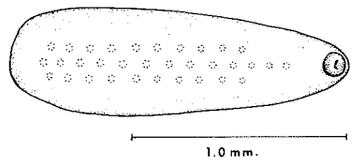
Nasal mites (superfamilies Laelaptoidea, Tydeoidea, Psoroptoidea) are endoparasites which live in the nostrils of birds and feed on mucus, blood, or tissue. They may be collected by holding the host with its head pointed downward over a small dish. Flush the nasal cavity several times with a fine stream of water from a hypodermic syringe or large-bulb eye-dropper pipette. Pick the fairly large reddish or white mites from the water in the dish with a brush or probe. If an intact bill is not vital for the bird specimen, nasal mites may best be removed by splitting the bill between the nostrils and examining with a hand lens. The culmen can be kept intact for a study skin by removing the palate of the host at the time of bird specimen preparation so that the nasal mucosa and associated mites are exposed. True nasal mites (family Rhinonyssidae) may also be found in the trachea, lungs, and air sacs.

Bird nests frequently are heavily infested with the immature stages and adults of louse flies, fleas, moths (Lepidoptera), beetles (Coleoptera), bugs (Cimicidae, Hemiptera), and ticks and other mites. Many of these are conspicuous enough to be seen with the naked eye, and some mites (Dermanyssidae) will crawl onto a person handling a nest. Many parasites in bird nests only feed on the host while it is in the nest, and thus are rarely found on the host elsewhere. Parasites from nests are best obtained with a special Berlese funnel used in the laboratory. Nests may be collected in the field, however, and placed with a label in a sealed plastic bag for later processing. A Berlese funnel is a steep-sided cone with an attachment at the base for a collecting jar and a light source (25-watt bulb) suspended over the large upper end. Litter or nest material is placed on a wire screen inside the funnel. Heat from the light causes the nest material to dry out, driving the arthropods down the funnel and eventually into the jar, which contains a small amount of alcohol. Portable Berlese funnels have been developed, using the sun and other sources as drying agents.

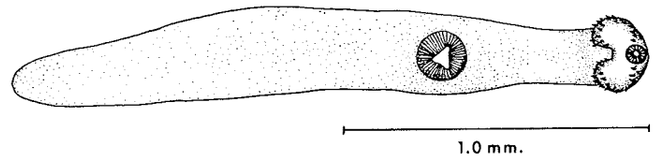
For further information about specific ectoparasitic insects and mites, consult: W. B. Hermes and M. T. James, *Medical Entomology* (New York: MacMillan Co., 1961), and E. W. Baker, T. M. Evans, D. J. Gould, W. B. Hull, and H. L. Keegan, *A Manual of Parasitic Mites of Medical or Economic Importance* (New York: National Pest Control Association, Inc., Technical Publication, 1956).

Helminth Endoparasites

Helminths of four classes are found in the internal organs of birds, where they feed on the host's blood, tissue, or food in the intestines. Trematodes (flukes) and cestodes (tapeworms) belong to the flatworm phylum Platyhelminthes, thorny-headed worms to the phylum Acanthocephala, and nematodes to the roundworm phylum Nemathelminthes. Helminths vary greatly in size, often in relation to the bulk of the host, but they are generally large enough to be conspicuous when present.



Trematode



Trematode

Trematodes are small, flattened worms which attach to the host by two large suckers, one surrounding the anterior mouth and the other on the ventral surface. Adult flukes occur in the digestive tract, liver, lungs, heart, blood vessels, and occasionally in the eyes; subadult stages may be found throughout the host's body, and eggs may be numerous in the lungs or lining of the intestine.

Cestodes occur as long, many-segmented adults in the intestines, where they attach firmly to the gut wall by suckers or hooks (rostellum) located on the head (scolex); larval stages are tissue parasites and may be found between skin and body musculature. Species identification is largely based on the intact scolex.

Nematodes are unsegmented, active roundworms which are pointed at both ends and which may coil into tangled masses in heavily infested hosts. They occur in nearly all visceral organs as well as in musculature.

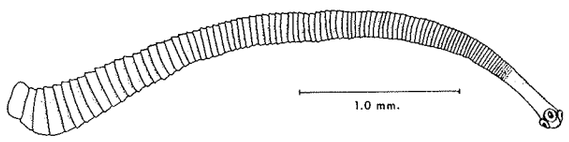
Acanthocephala, adult thorny-headed worms, are intestinal parasites which attach firmly to the wall of the gut by hooks on an eversible proboscis, or head region, and thus are difficult to remove. Immature worms are commonly found encysted in the liver, mesenteries, and intestinal wall.

Autopsy

Hosts should be examined as soon after death as possible, since intestinal parasites deteriorate rapidly and some species of worms migrate within the body of the host after it dies, so that their normal organ location is uncertain. If feasible, dead hosts should be refrigerated until they are skinned and examined. In general, internal autopsy is performed when the carcass is removed during skinning.

If the host has not been skinned, however, start with a ventral incision through the abdominal wall and around the vent to leave the cloaca intact. Before removing the viscera, look for parasites which are free in the body cavity or hidden beneath the

peritoneum or in the mesenteries, and check the surface of the liver for light-colored cysts. Remove the heart, lungs, oviduct, liver, gall bladder, bile duct, kidneys, ureters, esophagus, stomach, small intestine, caeca, large intestine, and cloaca. Also examine the mouth, eustachian tubes, and



Cestode

Nematode

eye sockets. Place each organ or organ part in a separate small dish (a petri dish is ideal) of physiological saline (8.5 gr. table salt in 1 l. of distilled water). If time and circumstances discourage preparation and use of physiological saline, clear spring, well, tap, or sea water may be used.

Organs should be torn open or cut apart with a probe or sharp scissors. This will allow exposure of a number of parasites and will cause the least damage to specimens present. Frequently, attached parasites will free themselves if organs are allowed to stand in water for an hour or so. After removal of free parasites, tissues should be cut into smaller pieces or preferably teased apart with fine-tipped forceps. Time permitting, tissues should be washed in several changes of clean water. Tissues which have been teased apart may be placed in a screw-cap, wide-mouthed jar to allow shaking. After 15 seconds of vigorous shaking, the jar should be allowed to stand for 3 to 10 minutes to allow sedimentation of parasites. The excess fluid should be poured off, and the process of cleaning with fresh water repeated two more times. This helps remove blood, mucus, fecal matter, and bits of tissue which frequently obscure small parasites. The take of small parasites may be increased 20–25% by employing this technique.

Time permitting, worms should be transferred to shallow dishes with clean water for a few minutes to allow relaxation of parasites and removal of remaining debris before fixing.

In the case of tapeworms, it is essential to obtain the scolex which is imbedded in tissues of the intestinal wall. Thorny-headed worms should be allowed to sit in clean water for several hours so that the proboscis, which frequently is withdrawn or imbedded in tissues, may relax into a position that will allow identification.

Killing and Fixation

Improper killing and fixation of helminth parasites frequently render them practically useless for identification purposes. Worm parasites have a tendency to contract, curl up, or otherwise become distorted prior to death. Flattening of trematodes and cestodes is highly desirable, but this process is usually too time-consuming for field collectors. Consequently, specimens should be fixed in a position and condition that will allow reasonably flat mounting at a later date prior to study. This can be accomplished by the use of hot fixatives.

Helminths should be placed in a petri dish (or heat-resistant container) with just enough water for half immersion. Specimens are killed by pouring hot (almost boiling) water or fixative into the dish. If hot water is used the killed worms must be transferred to cold fixative *immediately*. Most, but not

Acanthocephala

all, worms tend to straighten as they are killed.

The fixatives most often used for parasites of vertebrates are the following:

10% Formalin:

Commercial Formalin (40% formaldehyde solution)	1 part
Distilled water	9 parts

Formalin-alcohol-acetic acid (FAA):

Ethyl alcohol (85%)	85 parts
Commercial Formalin	10 parts
Glacial acetic acid	5 parts

(Some parasitologists believe that this solution is unstable when the acetic acid is added and that it should be prepared only as needed.)

Bouin's fluid:

Picric acid, saturated aqueous solution	75 parts
Commercial Formalin	25 parts
Glacial acetic acid	5 parts

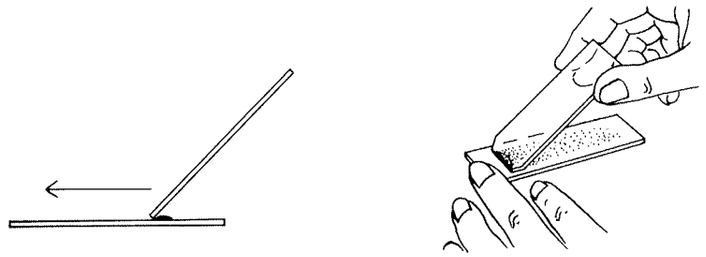
70% ethyl alcohol

Flukes should be killed and fixed in heated 10% Formalin, FAA, or Bouin's fluid. Small tapeworms are killed in a dish of distilled water heated to 75° C., and then fixed in 10% Formalin, FAA, or Bouin's fluid. For killing longer tapeworms, the water should be agitated when hot water or fixative is poured onto the immersed specimens, causing relaxation and insuring uniform instant killing of the worm. Cestodes may also be fixed in a semiflattened and relaxed condition by grasping the large end of a tapeworm with forceps and dipping it up and down in a container of hot water. If time and conditions permit, two more complicated laboratory methods are available for flattening specimens. Flukes and shorter tapeworms may be placed in a few drops of water on a microscope slide under another slide or a large cover slip. Add hot fixative between the glass layers, drawing it past the specimen with a small blotter or paper towel applied at the other end. Larger tapeworms may be relaxed by storing them overnight in an ice-box in a flat dish of distilled water. The worms are then removed from the water, stretched on a glass plate, and fixed by drawing an eyedropper filled with fixative along their sides. After the fixative has penetrated, the specimens are placed in a container of fixative. Live Acanthocephala are immobilized in a little distilled water, until they no longer evert the proboscis when stimulated. They are then fixed in heated FAA, 10% Formalin, or 70% alcohol to which a few drops of glacial acetic acid has been added. Nematodes are best fixed in one of the same heated fixatives used for live Acanthocephala, but it is essential that they be transferred immediately to cold fixatives.

Preservation

After fixation for 3 to 24 hours, helminths should be transferred to a vial of preservative until stained and mounted. Do not use cork stoppers because the tannin in them

leaches out, darkening the worms and thus interfering with subsequent staining. Use 70% alcohol if fixed in alcohol, Bouin's fluid (wash out the yellow color in several changes of alcohol), or FAA; use 5% Formalin if fixed in Formalin. For specimens stored in alcohol, a small amount of glycerine



Correct method for making a blood smear

(5 ml. in 100 ml. of 70% alcohol) will keep specimens pliable, even if the alcohol evaporates. Be sure to include a sturdy paper label inscribed in India ink or soft pencil in each vial, showing host (name or specimen number), geographic locality, location in host, fixative, date of autopsy, and name of collector.

Protozoa

Protozoan parasites occur in the digestive system, blood, and tissues. Collection usually involves use of a microscope and methods beyond the facilities normally available to a field collector of birds. Blood smears, however, are not difficult to make, and fecal material and tissue cysts may be preserved for future study.

Birds are frequently infested with malaria-like protozoan parasites which may be detected and studied by examination of peripheral blood smears. To prepare a thin smear, place a single drop of fresh blood on one end of a glass microscope slide which is absolutely clean and free from finger prints. Quickly touch the underside of a second spreader slide, the corners of which have been cut away, to the drop of blood, which will spread along the edge of the spreader slide in contact with the lower slide. Then, with the spreader slide held at a 45° angle, rapidly draw (do not push) the spread drop over the horizontal lower slide, making a uniform thin smear. A well-spread smear will appear very pale. Allow the blood to dry in the air until it changes color. Host numbers may be scratched on the slide with a diamond-point stylus, or numbers may be written directly on a corner of the blood smear with a sharp lead pencil. The smear should be fixed within five hours of smearing. Pour a few drops of absolute methyl (wood) alcohol over the smear, and dry in the air before storing in a slide box for subsequent staining and examination.

The intestinal protozoa of birds are very poorly known. Samples adequate for study may be obtained by fixing and storing approximately 1 gr. (volume of three garden peas) of fecal material from the lower part of the intestinal tract in 10 ml. of 5% Formalin.

Tissues containing protozoan parasites or cysts should be fixed in 10% Formalin, FAA, or Bouin's fluid. The volume of fixative should be at least five times the volume of the tissue. After fixation and labeling, the tissues may be stored in vials of 70% ethyl alcohol until returned to the laboratory for sectioning and more detailed study.

Other special techniques for collecting and preserving helminths are covered in: M. C. Meyer and L. R. Penner, 1962, *Laboratory Essentials of Parasitology* (Dubuque, Iowa: W. C. Brown Co.); and R. M. Cable, 1958, *An Illustrated Laboratory Manual of Parasitology* (Minneapolis, Minnesota: Burgess Publishing Co.). A good general book on bird parasites is M. Rothchild and T. Clay, 1952, *Fleas, Flukes and Cuckoos, a study of Bird Parasites* (London: Collins Clear-Type Press).

Many persons have aided in the preparation of these instructions by suggesting special techniques or by criticizing the manuscript. We are especially grateful to W. T. Atyeo, W. W. Becklund, J. F. G. Clarke, R. E. Crabill, Jr., K. C. Emerson, R. E. Kuntz, N. L. Levine, and E. E. Wehr. We also wish to thank Elaine R. Taylor, who prepared the illustrations.

SHIPPING INSTRUCTIONS

The Smithsonian Institution will be pleased to receive any bird parasites and will provide prompt identifications. Specimens sent from domestic or foreign sources by either surface or air mail should be addressed:

Division of Birds
Smithsonian Institution
Washington, D.C. 20560

Specimens from foreign sources packed in large containers (as they frequently are when combined with host specimens) should be addressed:

EAST COAST:

U.S. Despatch Agent
45 Broadway
New York, N.Y. 10006 U.S.A.
For: Division of Birds
Smithsonian Institution
Washington, D.C. 20560

or

U.S. Despatch Agent
U.S. Customs Building
South Gay Street
Baltimore, Md. 21202 U.S.A.
For: Division of Birds
Smithsonian Institution
Washington, D.C. 20560

WEST COAST:

U.S. Despatch Agent
555 Battery Street
San Francisco, Calif. 94111 U.S.A.
For: Division of Birds
Smithsonian Institution
Washington, D.C. 20560

USEFUL FIELD COLLECTING EQUIPMENT

	Ecto-parasites	*Endo-parasites*	*Blood Protozoa*	*Intestinal Protozoa*
Cloth or plastic bag	x			
Cotton and chloroform	x			
White pan	x			
Hand lens	x			
Dessicant	x			
Comb	x			
Brush or fine probe	x			
Sharp probe		x		
Fine-tipped forceps	x			
Scissors		x		
Syringe or pipette	x			
Vials	x	x		x
Jar		x		
Petri dishes		x		
Slides		x	x	
Labels	x	x		x
India ink and pen	x	x		x
Pencil	x	x	x	x
Diamond-point stylus			x	
Berlese funnel	x			
Field stove		x		
Formalin		x		x
Ethyl alcohol	x	x		
Isopropyl alcohol	x			
Methyl alcohol			x	
Acetic acid		x		
Bouin's fluid		x		
Glycerin	x	x		
Physiological saline or water		x		

SMITHSONIAN INSTITUTION
Museum of Natural History
WASHINGTON, D.C.

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