

THE COLLECTION AND PRESERVATION OF PHTHIRAPTERA (INSECTA)

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The order Phthiraptera includes small, flattened ectoparasitic insects, popularly referred to as the 'biting-, bird-, or chewing-lice' (*Mallophaga s.l.*) and the 'true-, or sucking-lice' (*Siphunculata* or *Anoplura auct.*). The order is now considered to contain four suborders viz., *Amblycerophthirina* (= *Amblycera*), *Ischnocerophthirina* (= *Ischnocera*), *Rhynchophthirina*, and *Siphunculophthirina* (= *Siphunculata*) (Clay, 1970; Lakshminarayana, 1970, 1976). The first three suborders (or superfamilies of some) constitute the erstwhile *Mallophaga*. While the *Amblycerophthirina* and *Ischnocerophthirina* are parasitic both on birds and mammals, the *Rhynchophthirina* and *Siphunculophthirina* are exclusively parasitic on mammals.

The chewing-lice have usually biting type of mouthparts with dentated mandibles, thoracic segmentation, two segmented tarsi with single or paired claws, and ventrally placed thoracic spiracles. In contrast the sucking-lice have highly modified mouth parts for piercing and sucking, with obliterated or fused thoracic segmentation, single segmented tarsi with unpaired claws, and dorsally placed thoracic spiracles. The *Ischnocerophthirina* and *Amblycerophthirina* normally feed on the plumage or pelage, and in addition may ingest blood also, while the *Rhynchophthirina* feed on sebaceous matter and blood like the *Siphunculophthirina*.

The lice normally cause considerable annoyance to their hosts by their movement and feeding. The damage caused to the wool by the sheep infesting chewing-lice and fall in egg production and even death in heavily infested poultry birds are well known. The chewing-lice are known to transmit typhus, rickettsias, and some are definitive vectors of bird filaria, dog tape-worm, infectious equine anaemia, and produce dermatitis. Because of the sucking habits, the sucking-lice definitely inject toxins and are known to transmit typhus, trench-fever, relapsing fever and probably trachoma in man and tularaemia, murine typhus, surra, hog-cholera, rat trypanosomiasis, canine Leishmaniasis and the dog filariasis.

I. METHODS OF COLLECTION

The Amblycerophthirina are not normally restricted to any part of the host body, but the Ischnocerophthirina have selected ecological niches. The lice of mammals are tightly attached to the hairs, and often it is difficult to extricate them. A list of references on the methods of collection and preservation were given by Kéler (1939) and Eichler and Zotorzycka (1969). Hopkins (1949), and Lakshminarayana (1977) discussed in detail the collection techniques for Phthiraptera and the same procedure more or less universally followed with slight modifications. Phthiraptera can be collected by any of the following methods :

(1) MECHANICAL

(a) *Hand Picking*

Using a fine watch-repairer's forceps (BB or NN) one can conveniently pick up the lice. Plucking the barbs of the feathers, or hairs, holding a little below the louse will avoid crushing of the specimen in the process. Though laborious, the method can be used for collecting the lice from a live host.

(b) *Brushing Technique*

The host (a mammal or bird) should be placed in a separate polythene bag, and the lice which can be seen actively moving about, should preferably be killed by Chloroform, Ether, or Carbon disulphide, sprinkled over a cotton swab kept inside the bag. Care should be taken so that the fumigant should not come in direct contact with the host body, with the head always kept outside of the fumigating jar or bag (when a live host is to be fumigated), and that each host is fumigated in a separate bag to avoid contamination. After about thirty minutes, the live animal or the skin should be vigorously tapped or rubbed with hand over a clean (preferably white) sheet. The dropped scales, hairs, feathers, and the dust should be examined for the ectoparasites. Some parasites also leave the host body when fumigated, and therefore, the empty bag may also be examined for the parasites. The specimens can be preserved in 70-95% alcohol.

Dusting the host animal with pyrethrum powder, sodium flouride (Rao *et al.*, 1954) or silicon dioxide and ammonium silicon dioxide and ammonium silicoflouride or 4% ammonium flourosilicate and commercially available as 'DRI-DIE or DRY-DIE' (Biswas, *in litt.*) (this method is particularly useful for collecting the parasites on migratory birds), and spraying with diluted DDT (Mani, *in litt.*) also yield the parasites.

(2) CHEMICAL EXTRACTION (DISSOLVING TECHNIQUE)

This procedure is useful for extracting parasites from a dead host and cannot be used, if the skin of the animal is required for identification or permanent preservation.

Hopkins (1949) described it in detail and it is more useful for mammal infesting forms. Briefly, the procedure is as follows : Pieces of freshly killed or dried host skin should be placed in 5-10% potassium or sodium hydroxide (KOH or NaOH) solution, until the hair becomes soft and can be scrapped off. The scrapped and partly softened hairs were then heated with additional quantity of 5% KOH or NaOH solution over a water bath, till the hairs dissolve completely. The contents of the beaker were then filtered, while hot, through a fine mesh of stainless steel gauge. The solid residue on the gauge was washed well into a petridish and examined for the parasites, which if present, can be preserved in 70-95% alcohol.

Hopkins' method was used by several workers for extracting bird parasites also (Ash, 1960 and Ledger, 1970). Recently, Hilton (1970) slightly modified the technique. The modifications involve essentially the following : the entire host animal can be kept for about 12-24 hrs depending on the size in KOH solution ; heating the scrapped hair or feathers for about 1-2 hrs ; keeping the solution for about 12 hrs till the solid material like the parasites settle down ; discarding the supernatant liquid and centrifuging the solid residue with a little KOH solution for about 5 minutes at about 1200 r.p.m. ; discarding the supernatant solution in the centrifuge tubes and refilling them with ZnSO₄ solution (386 gm of Zinc sulphate in 1000 ml. of water) ; centrifuging the solution a second time ; decanting or removing with an aspirator the bodies of the parasites that float over the ZnSO₄ solution.

In this process care should be taken to avoid handling the KOH or NaOH solution with bare hands and also inhalation of the hot fumes.

This method of collection is useful for making the whole mounts and for section cutting purposes this method is not suitable (*vide infra*).

II. METHODS OF PRESERVATION

The material collected can be conveniently preserved in a liquid medium or mounted on slides.

(1) STORING IN A LIQUID MEDIUM

The material can be preserved in small glass vials containing 70-95% alcohol, plugged with cotton or air tight plastic stoppers (cork stoppers should not be used), which are in turn placed in a large jar containing 95% alcohol. Hopkins (1949) quoted Werneck's technique in which the mouths of the vials were sealed over a blow pipe flame.

(2) WHOLE MOUNTS

(a) Resin mounts

The freshly collected material (if not already treated with 10% KOH or NaOH during extraction) should be heated for about 20 minutes, or even less (depending on the size) over a water bath (or the specimens were left overnight) in 10% KOH or NaOH solution. With the help of entomological pins No. 16 the gut should be punctured through the intersegmental membrane. The specimens were then gently pressed with the head of the pin, so that the inner contents drain out. The specimens were washed well through three or four washing with water, and gradually passed through the ascending series of alcohols, viz., 70% to Absolute alcohol. Alcohol-Xylol (50 : 50), Xylol, and finally mounted on glass slides in Canada balsam.

Elbel (1967) quoted Emerson's procedure of mounting. In this process, after treating in KOH or NaOH solution, the specimens were washed well with water (about 20 min.) and then placed in 40% alcohol (15 min.). They were then transferred to a solution of Ziehl Nielson's Carbol-Fuchsin (see below) for about 30 minutes. The material then passed through 70% alcohol (30 min.), 95% alcohol (15 min.) washed in Abs. alcohol, and then placed in Beechwood creosote (1 hr to overnight), and finally mounted in Damar gum or Canada balsam.

The present author found an alternate method most suitable where in, the processing through the ascending series of alcohols and xylol could be avoided by the present author (Lakshminarayana, 1970, 1977). This method was originally used for mounting aphids, and was equally good for Phthiraptera. In this process, after clearing the inner contents in 10% KOH or NaOH solution, the specimens were washed well with water, and then transferred into a cavity block (embryo dish) containing glacial Acetic acid for about 15-30 minutes (two or three washings would be ideal), and then transferred to another cavity block containing Clove oil. The material should be left in Clove oil till it attains sufficient clearance (which normally takes about 10 min.), and then mounted in Canada balsam.

(b) *Gum chloral mounts*

Kéler (1960) used Faure's Gum Chloral mountant (Hopkins, 1949). The essential ingredients of Faure's medium are : Chloral hydrate (50 gm), gum Arabic (30 gm), Glycerine (30 ml), dist. water (50 ml), which are mixed well and filtered through bolting silk. The treated specimens either from water or alcohol were placed in a mixture of equal volumes of Chloral hydrate and Lacto-Phenol and then mounted in the mountant.

Symmons (1952) suggested the use of Swan's mountant. She treated the specimens with Lactic acid for some time over a hot plate and when sufficiently cleared, were transferred into liquid Phenol for 30 min., and mounted in Swan's mountant. Swan's mountant consists of the following : dist. water 20 ml., gum Arabic (15 gm), Chloral hydrate (60 gm), Glucose syrup (10 ml.), Acetic acid (5 ml.).

For Indian conditions, experience shows that Chloral hydrate mountants are not well suited for permanent slides.

(c) *Polyvinyl Alcohol-Lacto-Phenol mountant*

Hopkins (1949) stated that he used Polyvinyl alcohol-Lacto Phenol medium. Boudreaux and Dosse (1963) gave a modified formula following after Heinze for mounting mites. This mountant was found quite suitable for other arthropods also. The mountant can be prepared by the following method : Polyvinyl alcohol (10gm) and dist. water (40-60 ml.) were mixed in a large beaker and then gently heated over water bath, stirring constantly. To this solution Lactic acid (30 ml.) was

added, stirred well, and then Glycerine (10ml.) was also added to the mixture. Another mixture containing Chloral hydrate (100gm) in Phenol-water solution (1.5%) (25ml.) was prepared and both the mixtures were added together. The polyvinyl alcohol-Lacto-phenol mixture was then filtered through a filter paper in a suction funnel. The treated specimens can be mounted directly in this medium. Edwards (1961) states that the disadvantages of using Polyvinyl alcohol is that it does not completely clean the matter in the alimentary tract, the slides must be examined periodically to fill in the vacuities that appear under the coverslip, and the medium occasionally hardens with dispersion of small crystal like objects in it.

(3) SERIAL SECTION MOUNTS

Symmons (1952) described section cutting and mounting of the serial sections. In this method, live specimens of Phthiraptera have to be fixed in hot Bouin (60°C.) or Zenker's solution and the lice were dehydrated and cleared for few hours to 1-2 days in Supercedrol. Celloidin mixture (2-6%) is prepared with equal volume of absolute alcohol and ether, and then diluted further in Supercedrol so as to make $1\frac{1}{2}$ celloidin solution. The dehydrated specimens were then transferred into celloidin solution in which they were left over for a day or two. The specimens were then placed in a saturated mixture of Chloroform and wax at 37-40°C., for about 12 hours. They were then removed to melted wax (M 60°C., and previously heated to smoking point and filtered) in a vacuum embedding bath. Impregnation of wax under vacuum may take about 2 hours. Embedding of the specimens in watch glass, embryo dish, or paper boat, section cutting, and mounting can be carried in the conventional way.

(4) STAINING

Normally, the phthiraptera are heavily sclerotized and therefore, staining of the specimens is not required. However, the following stains would be useful wherever required.

(*Ziehl Nielson's Carbol-Fuchsin*

Elbel (1967) quoted Emerson's technique in which this stain is employed. The stain consists of the following : basic Fuchsin (1 gm), acid Carboic (5 gm), Alcohol (10 ml.), and dist. water (100 ml.).

(b) *Acid Fuchsin*

The present author used acid fuchsin (B. D. H.) satisfactorily (Lakshminarayana, 1970, 1977). After processing the specimens through KOH, water, Acetic acid, the specimens were kept for few seconds in acid fuchsin in 70% alcohol. The specimens were then differentiated to the desirable degree in acetic acid and cleared in Clove oil. Mounting was done as given above.

(c) *Symmon's modified Heidenhain's Azan stain*

This stain is useful for staining microtome sections. Symmons (1952) suggested the modified method using Heidenhain's Azan stain. In this process, after removing the wax from the serial sections and the conventional way of passing the sections through the descending grades of alcohols and water, the slides were placed in Azocaramine solution for few minutes at room temperature. The sections were slightly overstained, and if necessary differentiated with aniline water. They were then rinsed in water, and mordanted in 10% Phosphotungstic acid in water, for the Aniline Blue, and fixed for the red dye for about an hour. The slides were then washed and placed in Azan solution containing Aniline Blue and orange G for about 30 mins. The excess stain was removed with 90% alcohol, followed by changes with Absolute alcohol, Absolute alcohol and xylol mixture (50 : 50), cleared in xylol, and mounted in Canada balsam.

(5) SCANNING ELECTRON MICROSCOPE PREPARATIONS

Attempts to utilize scanning microscope for the study of Phthiraptera have been made elsewhere (Clay, 1959, 1960). The material for the study has to be processed through a special technique and is not discussed here, since the scanning microscope is not usually available for many workers. However, those interested may refer to Grimstone (1970).

III. LABELLING

For all parasitic groups the host name is necessary, hence *proper identification of the host is a must*. The following data have to be provided both for wet collections as well as for the slides *viz.*, Tube No., Host, Locality, Date of collection, and Collector's name. The name of the species, its sex along with the name of the person who determined it

has to be provided on another label. It is preferable to have a uniform labels for the slides. The left hand label should bear the collection particulars, and the right hand one, the name of the species etc., and the registered number. It is always desirable to mount single specimen on a slide and the labels be pasted on either side of the cover slip, parallel to the anteroposterior axis of the specimen in the centre.

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