

COMPENDIUM OF ENTOMOLOGICAL METHODS

PART I

COLLECTING MAYFLIES (Ephemeroptera)

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Foreword

This is the first of a series of papers on methods of collecting the various groups of insects. Each paper will be contributed by a recognized authority in a particular group and it is hoped that when the series is complete, the resulting compendium may be published in book form.¹ The current publication on *Collecting and Preserving Mayflies*, written by Dr. Jay R. Traver of Massachusetts Agricultural College, will shortly be followed by one entitled *Notes on Collecting and Studying Orthoptera* by Dr. Irving J. Cantrall of the University of Michigan. This has already been published serially in *Ward's Natural Science Bulletin*. Another manuscript has been received from Dr. F. H. Wilson of Tulane University entitled, *The Mallophaga or Biting Lice*.

A series of brief contributions on collecting, which have previously been published in *Ward's Natural Science Bulletin*, will be

assembled and printed in another booklet in this series. These contributions will be of interest chiefly to professional entomologists but Ward's has not forgotten the needs of the beginning or amateur entomologist. A new booklet entitled *How to Collect, Mount and Store Insects* is soon to be published. It will be profusely illustrated, concisely and interestingly written, and we hope will be instrumental in stimulating a greater interest in Entomology on the part of the general public.

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COLLECTING MAYFLIES (Ephemeroptera)

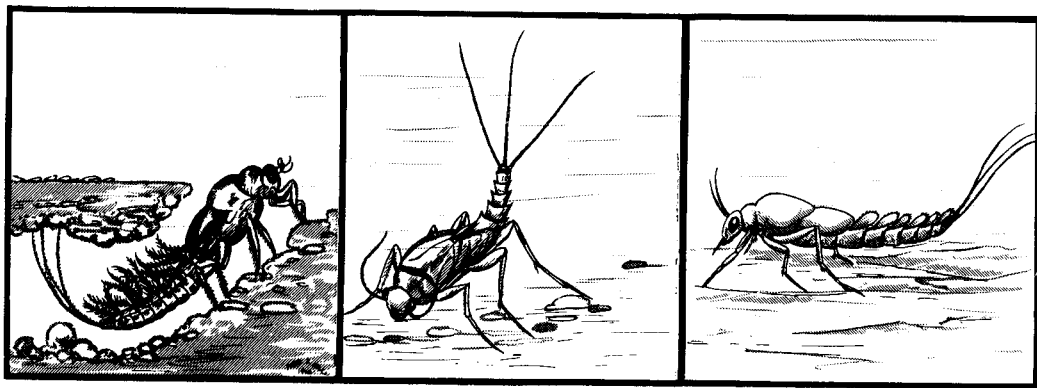
Anyone who has lived near a lake or large river in summer is familiar with the winged stages of mayflies. In great swarms and hordes, these insects take possession of the landscape for a period of two or three weeks. In late June or early July in the north, somewhat earlier in the south, the subimagos rise from the surface of the water and settle by hundreds of thousands on bushes and trees along water courses; on buildings and bridges; and even on any humans who happen to be present. The array of common names by which mayflies are known in various parts of the country indicates the familiarity of the layman with these aerial stages. Shadflies, willow flies, cisco flies, day flies, river flies, trout flies, eel flies, Canada sailors or soldiers,—thus the dwellers near pond and stream have dubbed them. As spinners, drakes, duns and mackerels, and as models for many of the finest "dry flies," they are widely known to all who follow the gentle art of Izaak Walton.

To the entomologist who would add some of these fragile winged beauties to

his collection, the following suggestions may prove of value.

The Immature Stages

The life history of a mayfly consists of egg, nymph and adult. There are two winged stages in the *Ephemeroptera*: the first, an immature form known as the subimago, and, second, the mature form called the imago. The immature stages of all known species live in fresh water. Eggs are deposited in the water by the fertilized female where they hatch into young nymphs which are very small, with large heads, and either with no gills or with mere rudiments of gills. Complete life histories are known for very few species. Indeed, the complete length of time required from egg to adult is known in a few species only. Recent work by Spieth² seems to show that at least some of the family *Ephemeridae* do not require two or three years for development, as has been the common opinion regarding this group, but may reach maturity in one year. Ide³ has



From left to right: *Hexagenia*, a shallow water lake-dweller; *Tricorythodes*, a sprawler in silt and mud; *Isonychia*, a climber and swimmer.

shown that in certain species of mayflies the nymphs may pass through forty or more instars in the course of their development. Murphy⁴ found that the life cycle in one species of *Baetis* varied from six to nine months, according to the seasons of the year in which most of it was spent.

Several ecological groupings of mayflies are recognizable, based on the portion of the stream in which they live and on their structural fitness for their particular environment. The two principal groups are still water and rapid water forms. Of the former group, some are diggers and spend most of their lives burrowing in mud or silt. The lake-dwelling forms are probably never found in the deeper parts, but in shallower stretches near the shores. It is this group which, as adults, are best known to humans who live near lakes or large rivers. Adaptations of the nymphs of this group to their mode of life include fore legs fitted for digging, plumose gills held over the back, narrow heads and mandibles that are usually tusked.

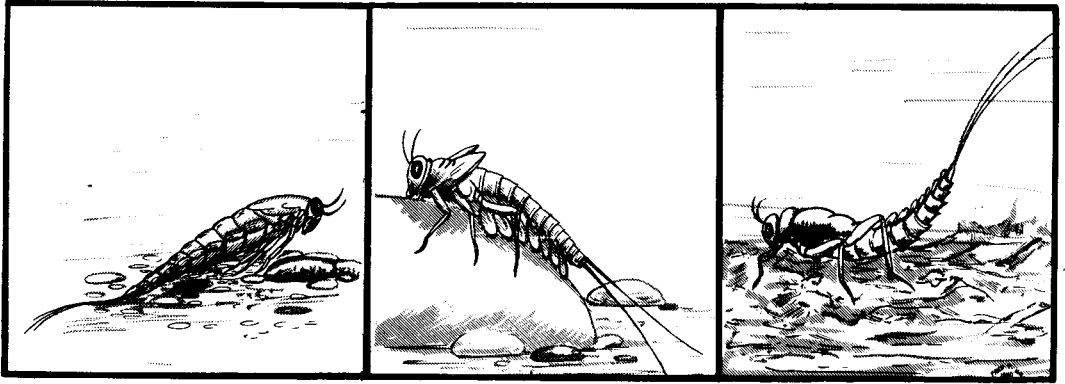
A second group of still water nymphs are sprawlers upon the bottom, often in the silt. They are hairy, stiff-legged, stout-bodied forms with the gills often completely protected by gill-covers. Still a third group climbs about in the vegetation. The bodies of these are stream-lined, tails heavily fringed, gills large and plate-like and held at the sides of the abdomen. They are fairly rapid swimmers and strong, agile jumpers.

Among the rapid water forms are found other agile, stream-lined nymphs very similar in general appearance to the climbers of quiet waters. Some of these live in the

open or between stones on the bottom of the stream, darting like minnows when disturbed. Other rapid water species have flattened bodies and various adaptations for clinging to surfaces of stones. They are found beneath (sometimes on top of) stones in rapid waters. A third group clings to trash or inhabits moss-grown rocks in swift-flowing portions of the stream and are stiff-legged nymphs, slow-moving and awkward, rather like the silt dwellers of quiet stretches.

How to Collect and Preserve Nymphal Stages

The collector of nymphs must search in each and all of the above-mentioned habitats if he would become acquainted with the mayfly population of a given pond, lake or stream. For the collection of burrowing forms, in lakes or rivers, some sort of dredging apparatus is essential. For shallower waters, the *Ward Scraper Net* (after Needham) is very useful. In small streams, areas of mud may be scooped up with the hand and placed, like mud pies, on the bank or on flat-topped stones. If any nymphs are present, they will soon come wriggling into view and may be captured. For silt dwellers, the same technique is also useful. To capture climbers among the weeds, the *Ward Apron Net* (after Needham) is well-nigh indispensable although a water dip-net may also be used, both for these and for rapid-water inhabitants. Sweep among water plants for the first group; drive the others into your net as you would pursue minnows. Some of the stream-lined nymphs may be taken on



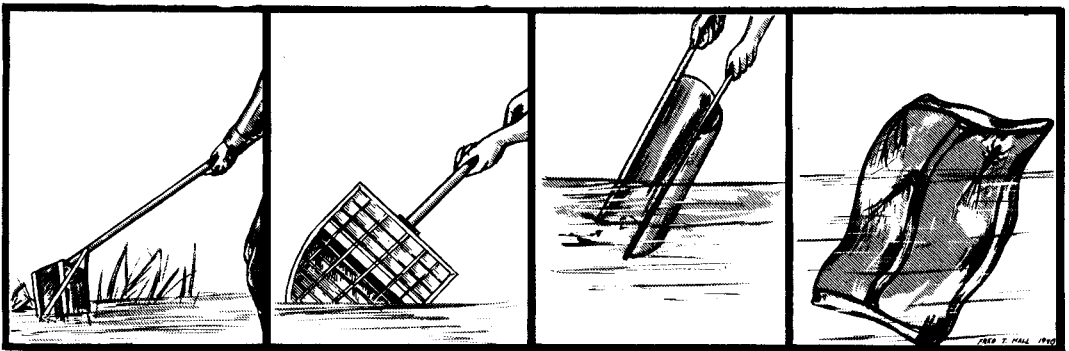
From left to right: *Ameletus*, a rapid water form; *Iron*, a rapid water dweller who clings to stones; *Ephemerella*, slow moving swift water dweller clinging to trash and mossy rocks.

the hand-screen as indicated below. Still others may be scooped up with the hand when they congregate in shallow water just previous to emergence.

Trash-dwellers may be obtained by removing sticks, leaves and other trash from the water, also by setting out on the bank the big stones overgrown with moss. Soon slow movements here and there will indicate the presence of the nymphs. It may take ten minutes or more for all of them to begin to move, so that it is well to have patience. Two methods have been found useful in capturing the flattened forms clinging to stones. Hand-picking from the stones after the latter are quickly lifted out of the water is productive of very good results. Be careful not to injure the nymphs, as many of them cling so closely to the stones as to lose legs and tails when fingers attempt to remove them. In such cases, a knife blade will cause the nymph to loosen its hold, or a blade of grass

slipped beneath its body is effective.

Another method is the use of the hand-screen⁵ which is held in the water below a series of riffles whilst the stones above are thoroughly stirred about. For such stirring the writer uses a three-pronged garden tool of convenient size. This work can be done by one person, but it is much easier if two take part; one to hold the screen, the other to manipulate the rake. To the above methods, add the following: by hand-picking from stones in each part of the stream, one is pleasantly surprised to find how many kinds of nymphs will cling to the stones which are lifted from the water. Try flat stones, round smooth stones, any and all kinds of stones that are present. Use the hand-screen also in several areas of any given stream. Don't forget to beat along the bank, among over-hanging roots and plant fibers, holding an aquatic net down-stream the while to catch anything loosened above. This is particu-



From left to right: Ward's Scraper Net; Ward's Apron Net; Hand Screen; Wire Pillow Cage.

larly important in early spring and late fall and winter, as many forms, which in summer are found on or near rocks in the stream bed, seem to migrate to vegetation along the bank to spend the winter months.

If nymphs are to be preserved as specimens, they should be put at once into 75% to 80% alcohol. After a few days, this alcohol should be changed for new, clean 70%, and the nymphs then stored in permanent vials. The writer prefers 4-dram homeopathic vials, using the same size for all specimens. Be sure to label each vial fully, using strips cut from some good quality rag paper. Each slip should be slightly shorter than the vial and is inserted in the vial after all necessary data is entered on it with India ink or medium to soft (not hard) pencil.⁶



If one wishes to rear nymphs, they must be placed at once in some container which will keep them in good condition for transportation. For this the writer prefers a wire pillow cage⁷ into which one or more smallish stones from the stream are inserted, putting the nymphs in as they are caught. If there is a possibility of two closely-allied species being placed in the same cage, sort the nymphs carefully and use separate cages for different forms. Keep one end of the cage in water until ready to leave, then place it in a pail half-filled with water for transportation to the laboratory or the stream where the nymphs are to be reared. By changing the water in the pail occasionally, most nymphs

will withstand a journey of several hour's duration and apparently be unharmed.

Rearing Life History Material

Many species of mayfly nymphs can be reared only in running water. Specially prepared troughs may be made for this purpose, or cages containing nymphs may be placed in a stream. Most species will become acclimated to tap water, and troughs in which the water supply is constant and can be kept at a certain depth are to be preferred for rearing purposes. Cages in streams out-of-doors often become tempting to roving small boys who can always use a nice wire cage for purposes of their own.

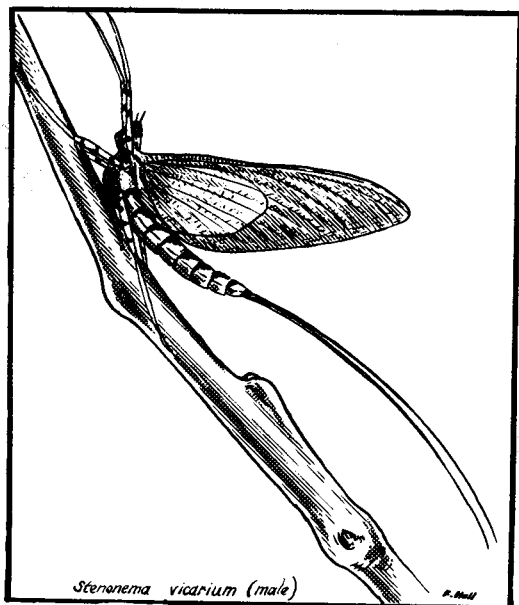
If immature nymphs are to be reared, suitable food must be supplied to them. This is usually in the form of diatom-coated stones or leaves from their native stream which must be replaced with fresh ones when the nymphs have eaten off all the plant growth. Water plants must be supplied for climbing nymphs.

If one wishes to obtain only a so-called "life history," i. e., nymph skin, cast skin of subimago, and imago, bring in from the field only those nymphs which are nearly mature. In both sexes the wing pads of such nymphs will be darkened; in males the eyes will be prominent, sometimes reddish; and will usually show the divisions into upper and lower eyes. Provide at least one rock which projects above the water surface as nymphs of some species crawl out of the water for transformation. If kept in wire cages, these nymphs will crawl up on the wire instead. Slant the cages to provide a slope up which nymphs may crawl and upon which subimagos may rest. If cages are kept in an upright position, subimagos may fall back into the water and drown.

As a nymph emerges, take out its skin and place it in a properly labeled vial of alcohol. The just-emerged subimago may be left in the wire cage until its final molt, or transferred to a covered container which is kept cool and moist (see notes under winged stages). When it has shed its cuticle and become an imago, the subimaginal skin should be put in the vial with the nymph skin. After a few hours (to permit the colors to darken, etc.) preserve the imago in the same vial. This vial now contains "life history" material of the spe-

cies at hand. If several nymphs of a given species are reared, mount some of the imagos thus obtained on pins also.

The Winged Stages



Stenonema vicarium (male)

The subimago is the first winged stage, and one in which the insect is dull-colored, immature, and more or less inactive. Its entire body and wings are covered with a thin cuticle, which will presently be shed, and from it will emerge the shining, mature, active imago. As entomological specimens, subimagos are utterly worthless and should not be collected since legs, genitalia and color patterns are but imperfectly developed. The temptation to collect them is almost overwhelming at times, for, as they rise with feeble flutterings from the water, the collector's net seems to gravitate toward them of its own accord. If sufficient care is used, subimagos may be captured in the net, very gently removed from it and placed in a closed or covered container, there to undergo their final molt. A word of warning will not be amiss here, —any injury to legs, wings, or tails will result in a malformed, valueless imago. Furthermore, the container *must* be provided with footholds for the insects, preferably on sides or top, where they can cling and into which their claws may be fastened to

enable the imago to pull itself free from the enveloping cuticle. The container must also be kept *damp* and *cool*, else transformation will be unsuccessful. Medium-sized paper bags, obtainable from any grocer, may be used as containers, if a supply of fresh, succulent green leaves, or a piece of dampened cloth or cotton, is placed in the bottom of the bag. The writer's preference is a wire pillow cage into which is inserted a short branch bearing green leaves, and over which is laid a similar mass of leaves. In such a container, subimagos are likely to transform normally. Those caught in the wild, or those reared from nymphs, do equally well in this type of cage. After the final molt, *but not before*, the specimen is ready to be added to your collection.

It is much better, also much easier, to wait until the subimagos have transformed to imagos in their native haunts, and to capture the mature forms of both sexes as they engage in their nuptial flight. They will be found near or over a body of water such as a lake, pond or small stream, the location depending upon the species of mayflies concerned. Often a meadow or hillcock some distance from the water will be chosen by the swarm as the site of the marriage dance. The time for the dance varies with the different species. Some dance from about 10:00 to 11:30 a. m., others at high noon; certain species perform this flight as the last rays of the sunlight fade away; many others do not appear until twilight has fallen, and these may continue to dance after it is too dark for the human eye to distinguish their forms. It is but seldom that subimagos will appear among the dancers and if present, the general dull coloration will distinguish them from the imagos.

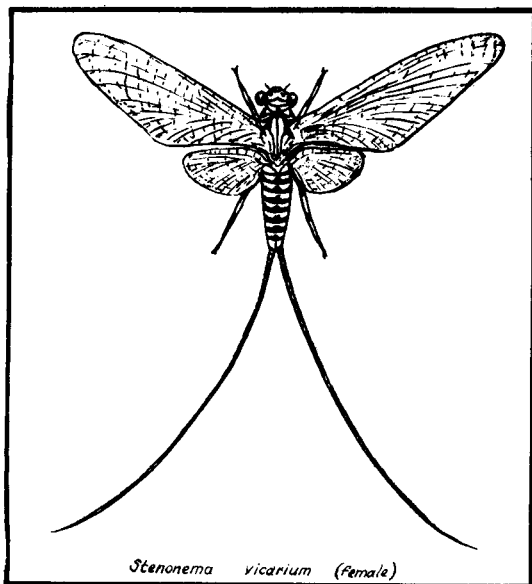
Provided with a light weight net having a small mesh bag and cyanide jars, present yourself at the dance. The dancers are often high above your head, in swarms like dark clouds against the sky. Be patient and some mayflies at least will come drifting down within reach of your net, only to rise swiftly again, sink again, rise again. Often one sweep of the net will capture several fine specimens which will be mostly males. Occasionally a female, or a pair in copulation, will be taken. Many females can be captured later, as they fly low along the water to oviposit, touching the tip of the abdomen again and

again to the water's surface. *Carefully* transfer each specimen from net to cyanide jar, being sure that legs, wings and tails are intact. Do not put too many specimens in one jar, as the claws of one may close on the legs of another, and the latter organ be torn off as you try to disentangle them. Do not leave the insects in the killing jar for long, as over-exposure to cyanide fumes tends to change the coloration of some specimens. Ordinarily it is well to sort over your catch within an hour, laying each specimen by itself on a board or paper to dry. Only very small forms may be pinned at once, for the wings, tails and abdomens of the larger ones will droop unless dried for several hours before pinning. It is even well to dry for a short time those specimens which are to be added to your collection of alcoholics. The relative merits of pinning vs. immersion in alcohol will be discussed later.

Another way to obtain imagos is to go to the nearest restaurant or other store having neon lights, at any time from 10 p. m. to midnight or after. Hand-pick from the windows those specimens you desire, putting them in the killing jar at once. A warm, quiet evening after a hot day usually is most productive. Hundreds of both sexes swarm to the lights, settling on windows and window casings, sidewalks, and passersby. Brightly-lighted billboards, theatre signs, unusually bright street lamps, even automobile headlights, attract scores of mayflies, both subimagos and imagos. However, more specimens are found near the neon lights. Still other imagos may be obtained by searching the vegetation along streams, and examining bridges and culverts, not forgetting to look carefully in all spider's webs for spiders are expert collectors of insect life.

Preserving the Winged Stages

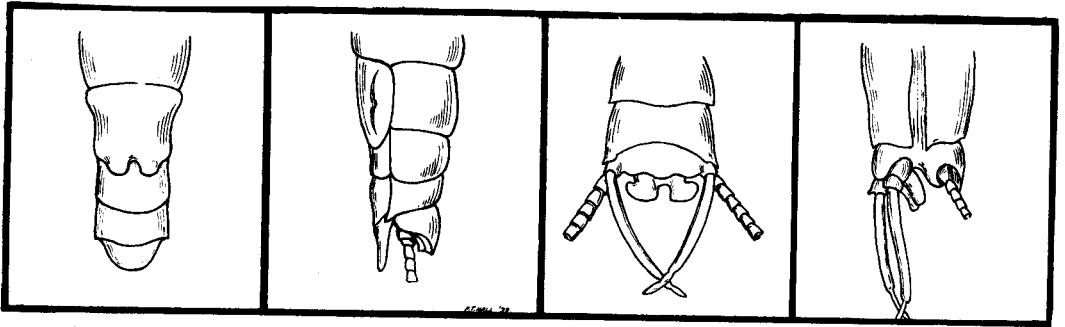
Pinned mayflies are very fragile. A draught of air from an open window may destroy a dozen in half a second. Off come wings, tails and even abdomens. The small size of some mayflies prevents direct pinning and these should be mounted on points by means of a drop of shellac (or Dupont's Household Cement) touched to the venter of the thorax and then to the



Stenonema vicarium (female)

point. They may also be fastened directly to the side of a pin with a drop of shellac applied laterally to the thorax. Specimens so mounted may fall off the pin later, unless the shellac is of the proper consistency which must be determined by experiment.⁸ In spite of these disadvantages, however, pinned specimens retain their original color, and color or color patterns are often important in the determination of species. It must be emphasized at this point that the criterion of species determination, at least in some cases, is based largely on the structure of the external genitalia of the male imago. Hence it is often necessary to remove the tip of the abdomen from the pinned male specimen, treat it with KOH⁹ to soften the parts and avoid distortion and mount it permanently in balsam or gum damar. If the tails and remaining apical abdominal parts are to be preserved, they must be placed in a vial of alcohol and lettered or numbered to correspond with the pinned specimen and the genitalic mount.

The writer feels that the following types of specimens are necessary for a complete collection: pinned specimens, alcoholic specimens, and a representative series of slides for each species. The latter series should contain mounts (preferably dry) of one pair of wings; permanent mounts (balsam or damar) of male genitalia treated in KOH; one set of legs; and in certain cases, mounts showing unusual structural



From left to right: End of abdomen of ♀ imago (ventral view); side view of ♀ imago; Genitalia of ♂ imago (ventral view); side view of ♂ imago.

features of abdomen, head or thorax and even color patterns of certain abdominal segments. Should it happen that but one specimen was available to the writer, it would be preserved in alcohol, after first preparing permanent slides of the parts indicated above. If one has two specimens, pin one and put the other in alcohol. It is hoped the above advice will prove helpful to both the amateur and professional in preparing a useful collection of *Ephemeroptera*. For additional information the following references contain many valuable suggestions.

Needham, Traver, Hsu—*The Biology of Mayflies*, Comstock Pub. Co. 1935.

Morgan—*Field Book of Ponds and Streams*, G. P. Putnam's Sons 1930.

¹ Acknowledgment for this suggestion is made to Mr. R. L. Post, formerly of the Entomology Department of this Establishment.

² Spieth—*Canad. Ent.* 68:263-266. 1936.

³ Ide—*Canad. Journ. Research* 12:433-478. 1935.

⁴ Murphy—*Lloyd Library Bull.* 22 (Ent. Ser. 2): 1-46. 1922.

⁵ See Needham and Needham, *Guide to the Study of Fresh Water Biology*, pg. 65.

⁶ Excellent suggestions for storing and caring for a collection of insects in alcohol will be found in an article by Dr. F. Earle Lyman, *Ward's Combined Natural Science and Entomological Bulletin*, XIII:1: Sept. 1939.

⁷ See pg. 230, *Biology of Mayflies*—Needham, Traver, Hsu—for directions as to the making of such a cage.

⁸ For a useful adhesive see *WARD'S Entomological Bulletin*, VI:7: April '39.

⁹ Hold the male specimen on its side in the dissecting dish (if an alcoholic specimen; otherwise carefully snip off the latter third of the abdomen and proceed as with an alcoholic one). With sharp scalpel or dissecting knife, cut along the lateral line of the abdomen on segments 9 (or 8) and 10, thus separating the forceps and penis from the upper parts of the abdomen. This cut is made *below* (i. e., ventrad of) the tails. Another cut, along the division between sternites 8 and 9 removes the external genitalia. Place these at once in 10% KOH; allow to remain for several hours (for large specimens, over night). When genitalia appear softened, transfer to water. Wash for several minutes. Thence into the series of alcohols (35%, 50%, 75%, 95% are usually sufficient). Transfer to carbol-xylene, thence to a drop of thick balsam or damar. Arrange genitalia carefully. Add a small cover glass—12 mm. diameter is a good size and allow at least three weeks to harden at room temperature.