Rapid Methods for Obtaining Permanent Mounts of Radulae

by

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Introduction

The taxonomic value of the radula is an established fact and will not be discussed here. Its general morphology will only be touched upon. For both subjects readers are referred to the more advanced textbooks of zoology (an excellent account is, e.g., given by Cooke, 1896) and to Thiele's „Handbuch" (1929). A historical review was given by Bowell (1928). Popular accounts in the Dutch language will be published elsewhere (Meeuse, 1949a, 1949b, 1950).

The study of radulae, necessary when one is dealing with taxonomic problems, requires good mounts, preferably permanent ones, for later reference and for illustration purposes. As was pointed out by Verdcourt (1948) the mounting of radulae is not even mentioned in the standard works of microtechnic and, judging by the scarcity of contributions on the subject, most malacologists seem to rely on the old-fashioned usage of mounting the unstained radulae at once in glycerin jelly in spite of the excellent though laborious methods indicated by Bowell (e.g., 1928).

The author studied the mounting of radulae in some detail, the main purpose being to find methods which yield satisfactory results in the shortest possible time, because the existing procedures were rather tedious, which may well account for the fact that many people are discouraged from taking up this kind of work. The suggestions described in this paper are probably not all new, but they proved to be very useful and it seems worth while putting them on record. The main improvements are time-saving devices, because it was not necessary to search for entirely novel staining procedures, Bowell's techniques leaving hardly any room for improvement. The author sincerely hopes that these notes may contribute towards making this really fascinating study more popular among those already interested in Mollusca, so that it may widen their scope and aid in improving their methods of investigation. Incidentally, the labour involved in making good mounts is amply rewarded by the aesthetic pleasure of examining these beautiful structures.

Some general remarks

Before discussing the various techniques for mounting radulae, a few introductory remarks for those who are not yet conversant with the anatomy of Mollusca would not be out of place.

The radula is one of the most characteristic organs associated with this group, lacking only in the Lamellibranchiata, in which the head is vestigial. The radula (from neo-latin radula, a scraper) is a chitinaceous ribbon- or tongue-shaped organ, consisting of a thin basal membrane bearing rows of minute teeth and situated in a sac (the odontophore) on the floor of the mouth. The teeth are laid down with an almost mathematical regularity by a definite number of groups of epithelial cells. As the teeth grow older a layer of enamel is deposited on their surface, so that the older teeth consist of chitin covered by a very hard coating. The arrangement of the teeth in the rows and their shapes vary with the groups of Mollusca, but is constant for any given group, hence the great value of the radula for the taxonomy of Gastropoda. When used in association with other characters, the structure of the radula is often of great importance for generic and even specific determinations. One of the most striking features of the radula is its perfect lateral symmetry. Each transverse row consists of one central (or rhachidian) tooth (which is missing in only a few groups), bordered by a number of lateral teeth on either side. The outermost teeth are known as the marginals. In Prosobranchia the laterals and marginals are quite distinct as a rule, but in Pulmonata a gradual transition prevails. In a single tooth we can distinguish a flat, mostly approximately squarish, basal part, the basement, and a number of usually pointed protrusions, the cusps or unci. The enamel is only deposited on the cusps. This is important, because, as was pointed out by B o w e l l among others, the affinity of chitin and that of enamel to dyes is different, the chitin showing affinity to acid dyes, and the enamel under favourable circumstances to basic dyes. For certain staining methods either the enamel has to be modified by decalcifying (treatment with dilute acids) or the chitin is „oxidized", the respective affinities to dyes being considerably altered by these treatments, so that they stain more uniformly, the chemically changed chitin (chitosan) having acquired an affinity to basic dyes and the slightly decalcified enamel to acid ones. Details can be found in B o w e l l's paper (1928).

It should be mentioned that the cusps are much more prominent in terrestrial Pulmonata and less defined in e.g., Prosobranchia and, consequently, the radulae of the latter contain less enamel. The radulae of freshwater pulmonates are more or less intermediate in this respect. These differences are the reason why certain staining methods will give excellent results with one particular group and fail altogether in other cases. Chrysoidin staining is an example. Being a highly specific stain for unmodified enamel, it yields excellent results with many terrestrial forms, but it is not very satisfactory for staining radulae of Lymnaeids and this holds a fortiori for the freshwater Taenioglossa.

The various stages in the preparation of radulae

The work involved in obtaining a mounted specimen generally includes the following treatments:

1. preliminary treatment of the animal, depending on its condition (alive, preserved in alcohol or completely dried out);
2. a maceration process to detach the radula from the tissues surrounding it;
3. cleaning, arranging, smoothing out and flattening of the extracted radula;
4. (in some cases) a chemical modification of the chitin as a preliminary treatment before staining;
5. staining;
6. rinsing, dehydrating and mounting.

The order of some of these manipulations may be reversed or some of them can be omitted, depending on the staining method employed. According to the author's experience some of them can be most succesfully combined (e.g., 2 and 5 or 3 and 5). The radulae of certain aquatic forms for instance can be simultaneously macerated and stained, and only need subsequent rinsing, followed by treatments 3 and 6.

The various stages will be discussed in the order given.

1. Preliminary treatment of the animal

It is generally stated that freshly killed material is superior, but in the author's opinion completely desiccated specimens give scarcely less satisfactory results if properly dealt with. According to Verdcourt (in a private communication) Peile has mounted radulae from dry specimens a hundred years old and the author has succesfully extracted radulae from snails collected about 50 years ago. Application of chlorine dioxide (cf. 4) makes these old radulae as soft and pliable as those extracted from fresh material. A disadvantage is of course that the shell has to be sacrificed, because as far as known there is no method of extracting the desiccated, brittle and usually deeply retracted animal from the shell without damaging either the shell or the remains of the animal to such an extent that it is rendered entirely useless.

Specimens put directly into alcohol during or immediately after collecting also contain retracted animals as a rule and although it is possible to draw the animal out of the shell of some of the larger forms and of those having a wide aperture, usually the shell has to be sacrificed in any case.

The best method of saving both is, for pulmonate snails — especially slugs —, to put the live animals in a tube or bottle completely filled with water and closed without leaving an air-bubble under the cork till they are killed (which takes from 8 to 12 hrs.), followed by a hardening

process in 70 per cent. alcohol for 12 to 24 hrs., and for aquatic and larger terrestrial forms (but not for slugs), an immersion in boiling water for about 5 minutes. Extraction of the animal from the shell is essential if the latter is to be preserved because the treatment with caustic (see 2) spoils the shell and even a prolonged stay in the liquid in which the animal is decaying (the alternative method of maceration) causes corrosion or turns hyaline shells (e.g., those of Vitrina) opaque.

The larger forms are dissected and the odontophores taken out. Of smaller snails the anterior part of the body (containing the head) is cut off and of the minute ones the whole body is treated. Killed specimens, heads and odontophores can be preserved in 70 per cent. alcohol if the maceration is to be carried out later.

Dried and retracted specimens are placed in 5 to 10 per cent acetic acid which dissolves the calcium carbonate of the shell and, in addition, swells and softens the remains of the animal. The shells of larger forms can be first carefully crushed or broken to accelerate the process. The treatment is continued till no more carbon dioxide bubbles are formed. The specimen is thorougly rinsed in water to remove excess of acid and the dissolved calcium ions, which would otherwise cause undesired precipitates with the caustic during maceration. The specimen is then ready for maceration.

2. Maceration processes

The purpose of maceration is to dissolve the tissues enveloping the radula, leaving the radula intact. Various procedures have been suggested, viz., treatment with caustic soda or caustic potash, treatment with proteolytic enzymes and a natural decaying process in water.

Treatment with caustic seems to be the most popular and it is certainly the most convenient method, but opinions are divided on the concentration and the temperature. Some suggest 0.5 to 1% sodium hydroxide and treatment at room temperature, others 10 (or even 30) % and boiling. As chitin is attacked by boiling concentrated alkali, the concentration of the caustic should not be too high, but according to the author's experience boiling with 5% caustic solution does not seem to damage the radula to an appreciable extent. It is sometimes said that one should never treat a radula with caustic if it is to be used for delicate staining experiments, but the author has never noticed any difference as far as the affinity to dyes is concerned. A general complaint is that boiling makes the radula brittle, but if the subsequent smoothing out is carried out in a drop of glacial acetic acid, it is sufficiently pliable to stand a great deal of handling. A treatment with chlorine dioxide (cf. 4) makes the radula as soft and pliable as any fresh specimen macerated by a decaying process.

Maceration by means of enzymes (pepsin or trypsin) is quite satisfactory if properly carried out, but it is difficult to adjust the p_H (which should be about 2 for pepsin and about 8.5 for trypsin) unless buffering salt solutions are employed. According to Bowell (1928) a prolonged treatment causes corrosion of the cusp enamel, so that the duration of the process is critical.

Decaying in water is the mildest treatment, but it takes much time (at least several days) and the smell of the rotted specimens is most offensive. If one has plenty of time and does not object to the odour, it is quite satisfactory. After the specimen has become very soft it is washed in alcohol to remove the smell and the radula is dissected out by teasing with needles.

The best method, in the author's opinion, is a treatment in the cold with 1% (fresh material) or 5% (old and dry material) sodium or potassium hydroxide for 12 to 24 hrs., followed by a short boil if the maceration has not yet been complete. If desired the maceration process can be prolonged till the radula has become isolated, but this is not necessary, unless maceration with concomitant staining is desired (cf. 5).

According to Verdcourt (1948), minute species must be treated on a slide, but the author macerated e.g., **Hawaiia minuscula** quite successfully in a small dish, using concomitant staining and maceration (cf. 5), the stained radula being sufficiently conspicuous to be found and transferred to a slide after completion of the maceration.

(to be continued).