DANISH BILHARZIASIS LABORATORY.

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WHO Collaborating Centre for Applied Malacology

A FIELD GUIDE

to

AFRICAN FRESHWATER SNAILS

INTRODUCTION

1998

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Preface

Certain species of African freshwater snails are exceedingly important both from a medical and a veterinary viewpoint, for which reason all African countries are working intensely with them. However, in the majority of countries their efforts are hampered by a lack of adequate literature through which they can identify the snails reliably according to species. In order to counteract this deficiency two Training Courses in Malacology have been held here at the Laboratory under the auspices of DANIDA (Danish International Development Agency) and the World Health Organization. Keys for the identification of the snail species, prepared by the Laboratory, were provided to the participants, but following the second course they strongly urged us to publish these keys since there was such a great need for them. In order that they may be used also independently of the training courses we have revised and extended them in the form of the present Field Guides.

Since new species are still being discovered, however, and since our present knowledge of the individual species' distribution is insufficient, these tables have been reproduced in a way that new and improved editions can easily be printed when warranted by new material and information.

A reliable identification of snails presupposes a certain knowledge of technical terminology and procedures for which reason the present "Introduction to the Field Guides" has been produced to provide the necessary explanation and instruction for their use.

Suggestions for improvements in the "Introduction" and in the "Field Guides" will be gratefully accepted.

WHO SNAIL IDENTIFICATION CENTRE
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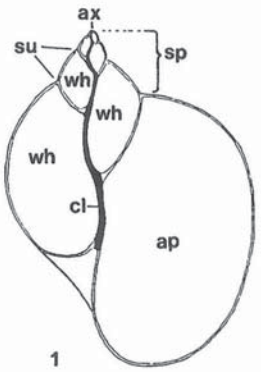
The Gastropod Shell

By far the majority of the approximately 60,000 recent species of shell-bearing gastropods and all of the approximately 200,000 described fossil species can be recognized by the shell alone. Therefore the shell is of the greatest importance in the determination of snails, and as far as the fossil species are concerned, we have only the shell. It is obvious that to differentiate between approximately a quarter of a million shells all built up after the same fundamental principle, it is necessary to operate with quite definite and distinct terminology in the description of a shell.



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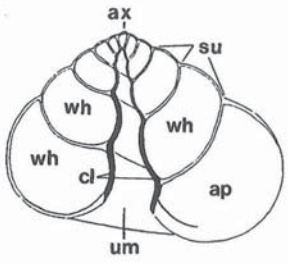

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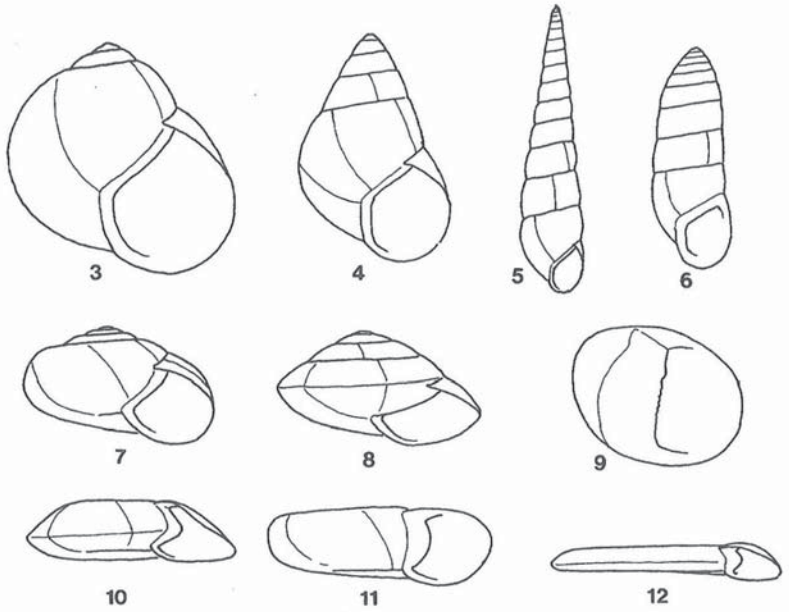
Shell with a massive columella (1) and with a hollow (2) cut in half.

The fundamental feature of the shell (fig. 1-2). A shell of a snail consists in principle of a conical tube, which is spirally coiled. The point of the tube is termed apex (ax). The separate coils of the spiral are called whorls (wh) the opening of the tube is the aperture (ap) and the limit of this the peristome. The last whorl is often called the body whorl because this is where the snail can withdraw. All whorls above the body whorl together form the spire (sp). In the middle of the shell the whorls form an axis, columella (cl), which is a massive column when the whorls are meeting completely, and if not close together, a hollow cone. In the latter case the opening into columella can be seen on the underside of the shell as a narrow or wider hole, which is called umbilicus (um). If columella is massive, the shell is called imperforate and otherwise perforate or umbilicate.

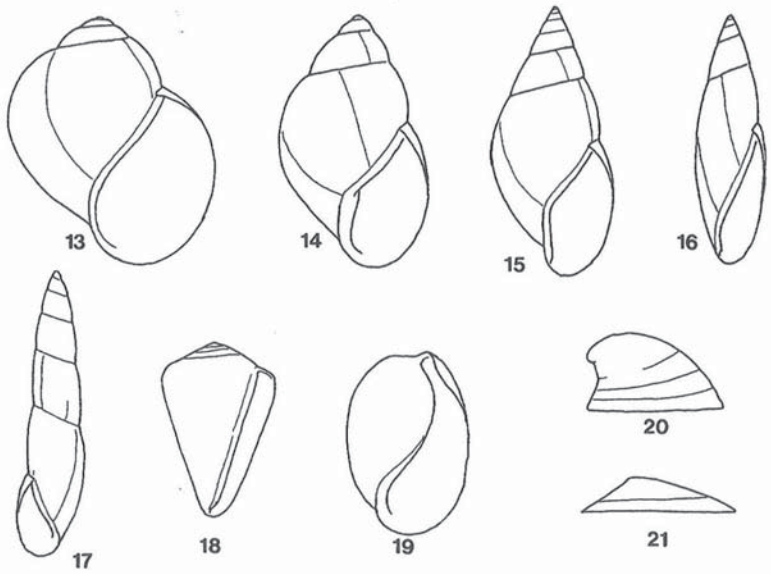
The various terms giving the size of the umbilicus indicate a foregone conclusion, i.e. minutely perforate, perforate, umbilicate, widely umbilicate, etc. It is not unusual that the umbilicus is open in young individuals but in fully grown covered by the reflected margin, and umbilicus is then closed or covered. The line which occurs where two whorls meet is called the suture (su).



3 4 5 6 7 8 9 10 11 12

The form of the shell (fig. 3-21). As a fundamental form from which other forms can be derived, the globose shell (3) may be taken as an example. The height and width of the shell as well as of the aperture are almost the same. If the height of the shell is larger than the width because the spire is higher, the shell is called conical-globose, or if the spire is still a little higher and more cone-shaped, conical (4). When the height of the shell is about twice the width while the height and width of the aperture is still about the same size, the shell is called turreted conical. When the shell forms a very high cone and the height is many times the size of the width, the shell is described as turreted (5) or the more rare term turriform. A special

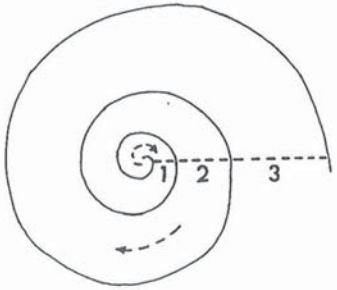
form is the one described as cylindrical where (6) only the upper 3-4 whorls form a low cone while the remainder of the shell has the same width. The opposite way, when the height of the shell is smaller than the width, is called depressed globose, and if still lower, depressed (7) and finally, when the shell is coiled in one plane, called discoid or disc-shaped (11, 12). The so-called lentiform or lens-shaped shell has a convex upper side and angular or carinate periphery. It can develop both from the depressed (8) and from the discoid shell (10). A special form, derived from the globose shell, is the hemispherical with a very short, often partly hidden spire (9).



From the globose shell another evolution line runs with whorls distinctly higher than wide, called ovate (13) and with a rising height compared to width it is described acuminate ovate (14), acuminate (15) and turriculate (17) respectively. In case of an acuminate shell becoming more pointed basally, it is described spindle-shaped or fusiform (16). From the ovate shell a form with a lower spire can be derived and is called short ovate unless the spire is so low that the shell is formed like an inverse cone and in such a case it is called inversely conical (18). If the spire is totally surrounded and hidden by the ultimate whorl, the shell is designated as involute (19).

A special form in which the shell consists of a low cone, at the most with an indication of coiling at apex, is the patelliform. When the patelliform shell is thin and small, it is often called cap-shaped (20) or if very low, shield-shaped (21).

The shape of the whorls also influences the form of the shell. The outer wall of the whorls may be flat, more or less convex or angular. Three different angles can be present on the whorls, namely a shoulder angle (29, sh) placed between the suture and the periphery, a periphery angle (29 pa) following the periphery and finally a basal angle between periphery and umbilicus. If the angle is so protruding that it appears sharp, it is called a keel and the whorls (and the shell) are then carinate (8 and 10). Convex whorls as a rule are separated by deep sutures and flat whorls by shallow sutures.



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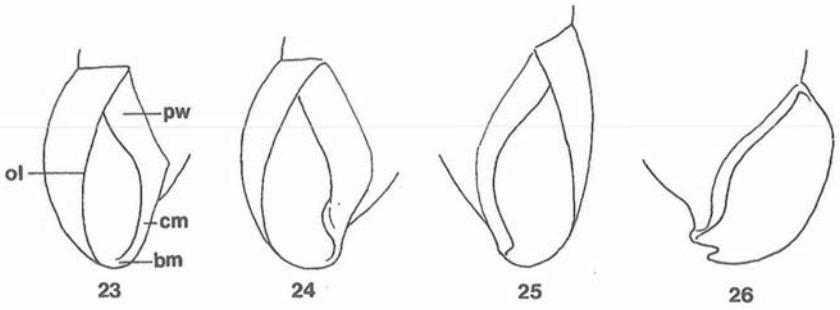
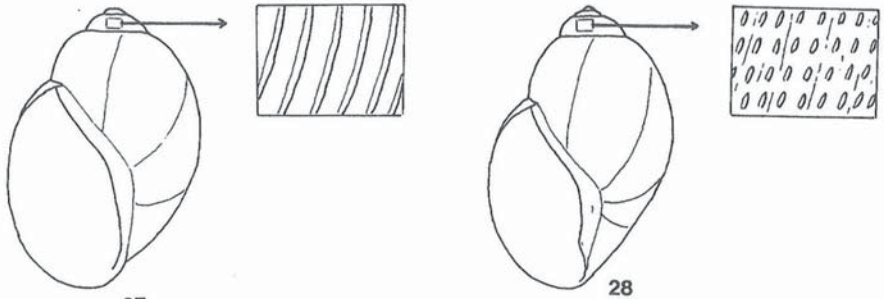
The number of whorls varies considerably. Most frequently they count five to six but may be less than one and rise to about thirty. It is common on very high shells that the upper whorls are broken off, sometimes in several spells, and such a shell is called decollate. Decollation must not be confused with corrosion taking place on the older part of the shell and which is always caused by wear and chemical influence. The number of whorls, which can be counted as shown in fig. 22, can often be of use in separating closely related species because two species with the same shell shape and size but with a different amount of whorls must grow in different ways.

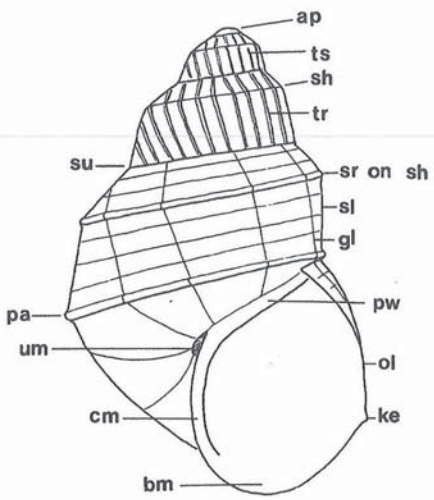


Form and development of the aperture (fig. 23-26) are of great importance to the identification of many snail species. The aperture is the opening leading to the cavity of the shell and is enclosed by the peristome consisting of four parts: the outer lip (ol) formed by the side of the last whorl and entering downwards into the basal margin (bm), which adjoins the columellar margin (cm), or just columella. Finally the aperture is enclosed above by the parietal wall (pw), which more or less projects into the aperture and is often covered by a calcareous layer called callus, which in fact is the upper part of the last whorl. Occasionally the whole peristome is formed by the last whorl and is then described as entire or continuous (6). The basal margin in many marine and a few freshwater snails is notched (26) and drawn out into a shorter or longer spout for the use of the sipho and such a shell is in fact called siphonostom. Occasionally the transition from the basal margin to columella is uneven as only a part of columella is connected to the basal margin (truncate columella, fig. 25). The members of the subgenus Physopsis of the genus Bulinus possess a false truncation caused by a fold on columella (24, 28). While a snail is growing, the outer lip and the basal margin are thin and sharp, but at growth stops and when the shell is fully grown, the peristome is often strengthened by an extra calcareous layer. Furthermore lamellae or toothlike projections can be formed, which will narrow the aperture. Such a shell is described as dentate and there are distinct designations for the individual teeth and lamellae which can be so strongly developed that it seems almost impossible for the snail to pass. Dentation is very rare in freshwater snails.

Direction of coiling. In most snails the shell is dextral, i.e. it has grown clockwise. If such a shell is held with apex upwards and aperture facing, this turns to the right (25-26). In sinistral shells the whorls run the opposite way and the aperture is turned left when the shell is held as before (23-24). By far the majority of snail species are normally either dextral or sinistral and individuals with the opposite whorls are extremely rare, but in a very few species the numbers of dextral and sinistral individuals are more or less equal. Normally it appears that snails with a dextral shell have their genital opening, anus etc. placed on the right side of their body, and snails with a sinistral shell on the left side, but in some cases the shell is sinistral while the animal itself is dextral. This phenomenon is called hyperstrophy and can be explained by the fact that the original upper side of the shell has become the under side. In the few snails which fasten the shell to stones, corals etc., the whorl may appear quite irregular.

The sculpture of the shell (fig. 29) consists in most cases of growth lines which are formed by the shell growth not occurring continuously but from time to time. These growth lines (gl) are always placed across the longitudinal direction of the whorls and are therefore termed transverse lines. These lines are to be found on all shells, but many other sculpture forms exist apart from this. If the sculpture is so delicate that it can be seen only through a lens or under the microscope, it is termed microsculpture. This is an important character for separation of the species groups of the genus Bulinus. (fig. 27 shows the sculpture in Bulinus s.str. and fig. 28 in the subgenus Physopsis.)





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From the orientation of the sculpture a distinction can be made between axial or transverse sculpture, which runs across the whorls' longitudinal direction and longitudinal or spiral sculpture, which runs parallel to the whorls. When the sculpture has no distinct direction, it is termed irregular. Where cap- or shield-shaped shells (20-21) are concerned, terms like transverse or spiral sculpture are not used, but the terms concentric and radial. The whorls which are formed in the ova, the so-called embryonic or nepionic whorls, often have a different kind of sculpture than the other part of the shell.

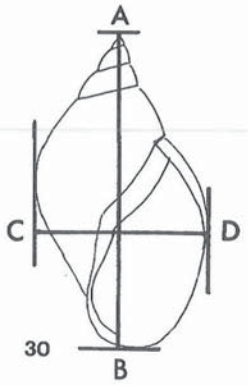
Apart from growth lines, a more or less distinct transverse sculpture consisting of striae (ts) or ribs (tr) may often occur. If such ribs are strongly protruding, the shell is called costate and if less protruding, costulate. Furthermore the ribs may be provided with knots or spines. The spiral sculpture consists most frequently of fine spiral lines (sl) appearing as if they have been scratched into the shell. Sometimes the space between the spiral lines is somewhat raised, resulting in spiral ribs (sr) or spiral cords. When a distinct spiral as well as a transverse striation are present, a network pattern occurs, termed reticulate sculpture. Both a transverse and a spiral striation may be divided up in points and the sculpture is then called punctate.

The most frequent forms of irregular sculpture are corrugation and malleation. Only the first mentioned, consisting or irregular convergent elevations, is a genuine sculpture. Malleation, which has a sort of hammered appearance, has derived mechanically by formation of the shell and is specially found within the freshwater pulmonates. Occasionally the surface of the entire shell is grained and is then termed granulated. The above mentioned forms of sculpture are nearly always formed in the calcareous layer of the shell, but periostracum can also show various forms of sculpture, usually as hairlike extensions or more rarely in the form of scales.

The colour of the shell is usually some shade of yellow or brown, which generally is called a horny or corneous colour. In freshwater snails the shell is often seemingly grey, black or a reddish brown but this, however, is due to the coating deriving from the surroundings. This can usually be removed by placing the shell in a concentrated aqueous solution of Oxalic Acid. In some snails the shell is encircled with bands in a different colour to the ground colour, as a rule darker, and spiral bands are especially frequent. The term "band" is only used of colour, and lines only of sculpture. Various forms of spots are not unusual either. The colours are found partly in the calcareous layer and partly in periostracum. In certain marine snails the various calcareous layers are in different colours and this is employed in the manufacture of cameos.

Dimensions of the shell (fig. 30-32). The height of the shell is the distance between the apex and the basal margin measured parallel to columella, and the width of the shell the greatest distance between the outer lip and the opposite outer wall measured at a right angle to columella. The height of the aperture is the distance between the beginning of the outer lip and the basal margin when measured parallel to columella and its width is the greatest distance between columella and the outer lip measured at a right angle to columella. On shells wider than high, the width is usually measured as the greatest distance between the outer lip and the opposite wall even if this measurement is not at a right angle to columella.

The line A-B indicates the height of a shell and C-D the diameter. E-F shows how the diameter of umbilicus of a planorbid shell is measured and G-H the height of the ultimate whorl.



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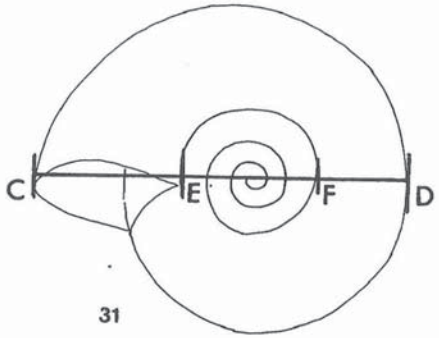

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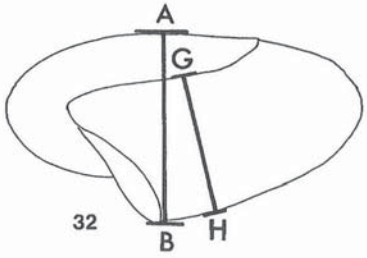

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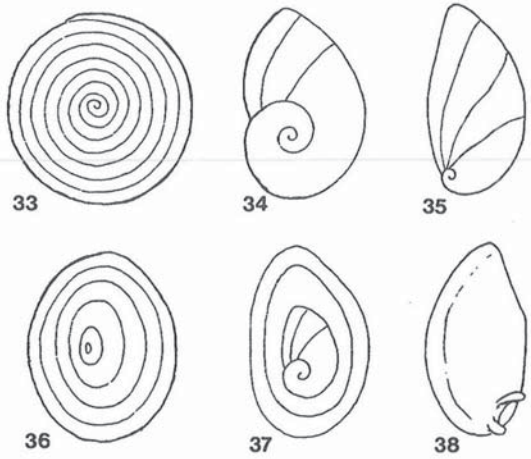
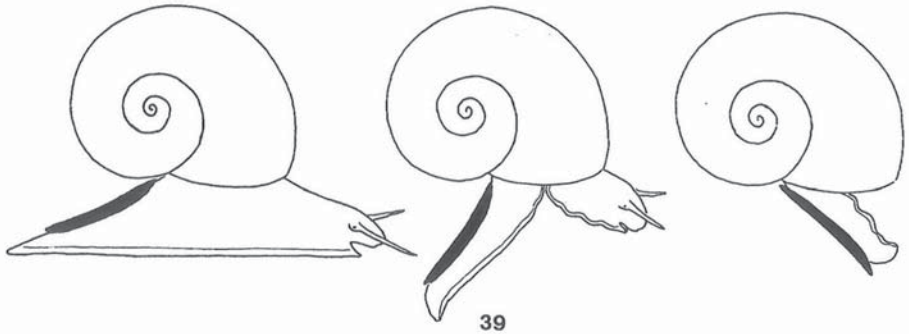
Operculum (fig. 33-38). While the pulmonates during eastivation or hibernation only are able to close their aperture with one or several layers of coagulated mucus, most prosobranchs have a genuine lid, operculum, which is formed from the upper side of the posterior part of the foot and which closes the shell completely when the snail has drawn itself inside. Operculum may be formed completely by conchiolin and then appears horny and supple, or it may be strengthened by a calcareous layer, which is sometimes of a considerable thickness. The growth of operculum follows the growth of the shell, of course, and is effected in either a concentric (36) or spiral way. In the latter case it may consist of a few rapidly increasing whorls and is then called paucispiral (34-35), or of many slowly increasing whorls whereby it becomes multispiral (33).


33 34 35

36 37 38

In certain snails the oldest part of operculum is spiral while the younger part is concentric, and operculum is then called concentric with spiral nucleus (37) since nucleus is used as a designation for the oldest part of operculum. Besides, nucleus may be placed in or near the centre or closer to the margin (35). In a number of snails the operculum fits exactly into the peristome, but in most snails it is slightly smaller than the aperture and fits into the last whorl a certain distance inside the aperture. In some cases the operculum has two processes (apophyses) basally on the inner surface (38).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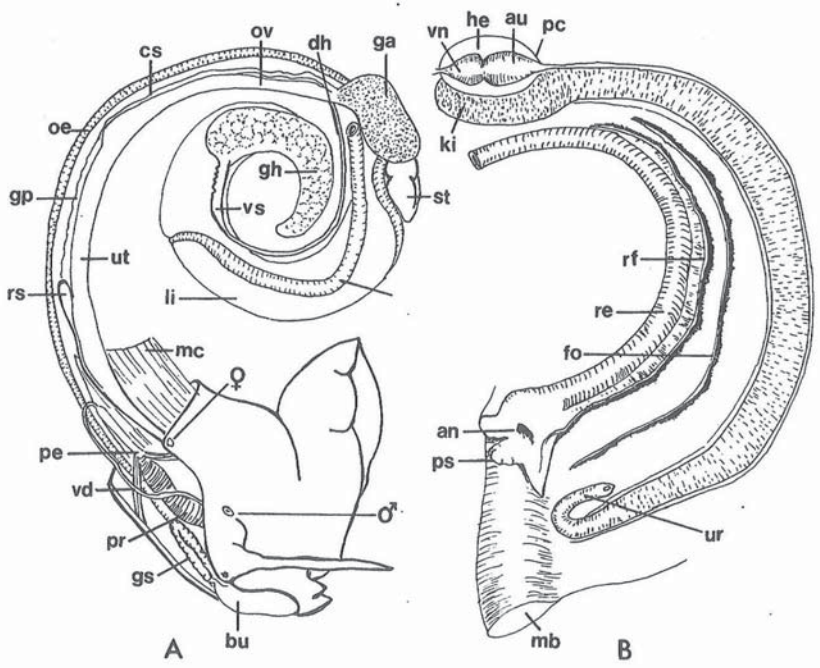
Unlike the pulmonates a prosobranch snail bends its foot (39) when it draws itself into the shell. Operculum is drawn solid black.

The Gastropod Anatomy

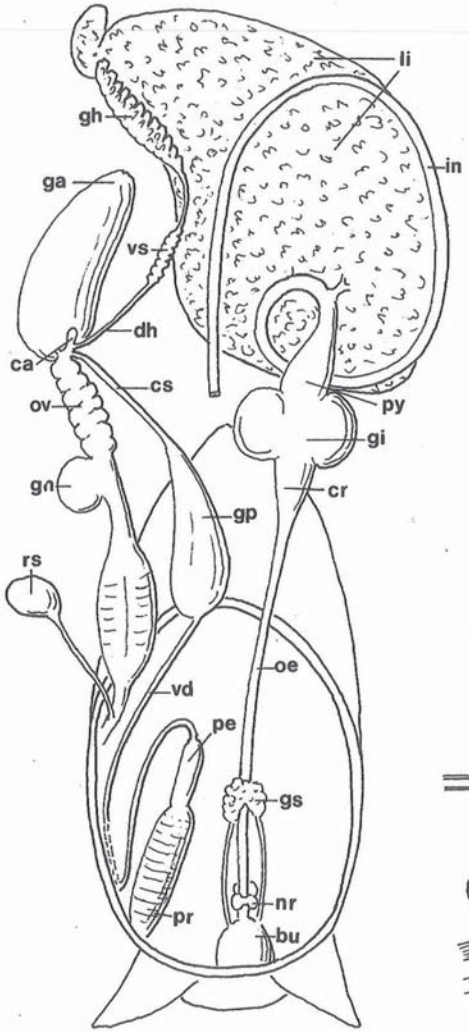
The body of a shell-bearing gastropod consists of two clearly different parts: below is the head-foot, the only part of the snail which normally comes outside the shell, and above is the visceral mass, which always is hidden in the shell. Anteriorly the head-foot carries the head with one or two pairs of tentacles, the eyes and the mouth, as a rule surrounded by a pair of labial palps. The under side of the foot has in most species developed into a muscular pedal sole, which partly serves for the locomotion and partly enables the snail to fasten itself. Also the great retractor muscle radiates from the foot with its upper end attached to columella, and this makes it possible for the snail to withdraw itself entirely into the shell. The visceral mass containing the main part of the inner organs is below surrounded by a large fold of the skin, the mantle or pallium, which results in a large cavity, the pallial cavity between the mantle and the visceral mass itself. A large number of organs are attached to the mantle and these are altogether named the pallial organs. The mantle border takes care of the growth of the shell as cells in the outer part secrete periostracum and the outside calcareous layer, the prismatic layer, which is deposited on the under side of periostracum. The following calcarerous layers, the lamellar layers, are secreted from the upper side of the mantle.

The pallial organs (fig. 40-41B). As consideration of space forbids going into the development of the pallial organs in all the different snail groups, the pallial organs in some medically important families have been chosen as examples. Posteriorly and along the border, the mantle is firmly grown to the visceral mass apart from a short space on the left side where an opening occurs, the pneumostome through which the mantle cavity is in connection with the outside world. To examine the pallial organs in detail, the entire mantle is cut away and viewed from the under side. Seen in this way, we have from left to right the following organs. First the rectum (re), which is provided with a longitudinal fold (rf) and concluded with anus in the pneumostome. To the right of the rectum is another longitudinal fold (fo), which on the live snail

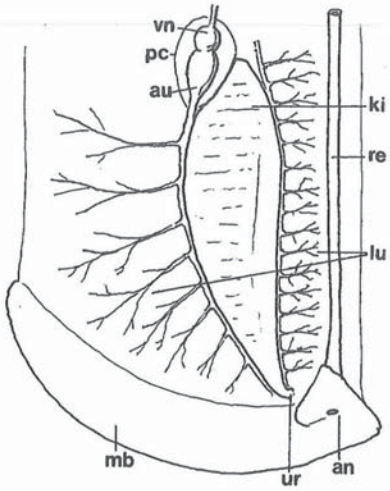
is placed close to the rectal fold. Then follows the elongated kidney (ki), or nephridium, ending through a short ureter (ur) bent backwards. Along the left side of nephridium runs a large vein and on the right side of this another large vein, the pulmonary vein leading the oxygenated blood from the lung to the heart (he), which is surrounded by the pericardium (pc) along the upper end of nephridium. Finally, to the right of this is the lung, being a vascularized part of the mantle. In connection with the pneumostome a large folded vascularized lobe serving as a gill has been developed in the planorbids. Not being homologous with the true gill in the prosobranchs, it is named secondary gill or pseudobranch (ps). In some planorbids a longitudinal ridge being of taxonomical value is present on the ventral side of the kidney. In the dextral Lymnaea (41-41B) the organs are in the opposite order.



40 Anatomy of a Biomphalaria



41 A



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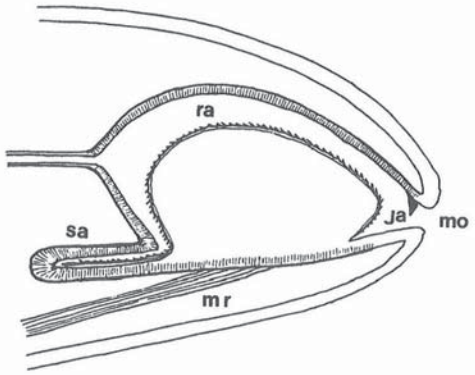

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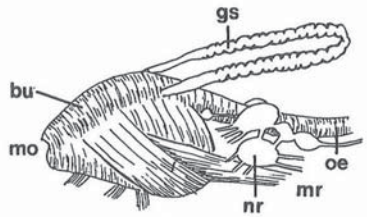
The digestive organs (fig. 40-41A) can be naturally divided into four sections; in front the buccal mass with the oral opening, jaw and radula plus the salivary glands, next comes oesophagus, the tripartited stomach with the blind sac and liver, and finally the intestine itself.

The buccal mass (m and fig. 42-43) is a pear-shaped muscular sack to which the protractor as well as the retractor muscles are attached. Just inside the oral opening is the horny jaw (ja), which in most freshwater pulmonates consists of a larger dorsal part and two smaller lateral parts. In others the three parts have grown together in a single horseshoe-shaped jaw. Further back in the buccal cavity is a ventral pillow-shaped elevation carrying the radula (ra). This basically consists of a basal membrane on which the individual teeth are placed in longitudinal and transverse rows. Each tooth consists of a basal plate and a projecting crown ending in one or usually more cusps. The radula is formed in a radula sac (sa), protruding posteriorly and ventrally from the buccal mass. It is continuously formed from the posterior and is pushed forward in accordance with the wear of the front part. As the radula constitutes a very important organ in classification as well as in identification of the snails, this will be dealt with later. Joining dorsally in the buccal mass are two salivary glands (gs) and these may be combined posteriorly so that they form a continuous loop. Oesophagus (oe) begins just behind the buccal mass and leads to the stomach.



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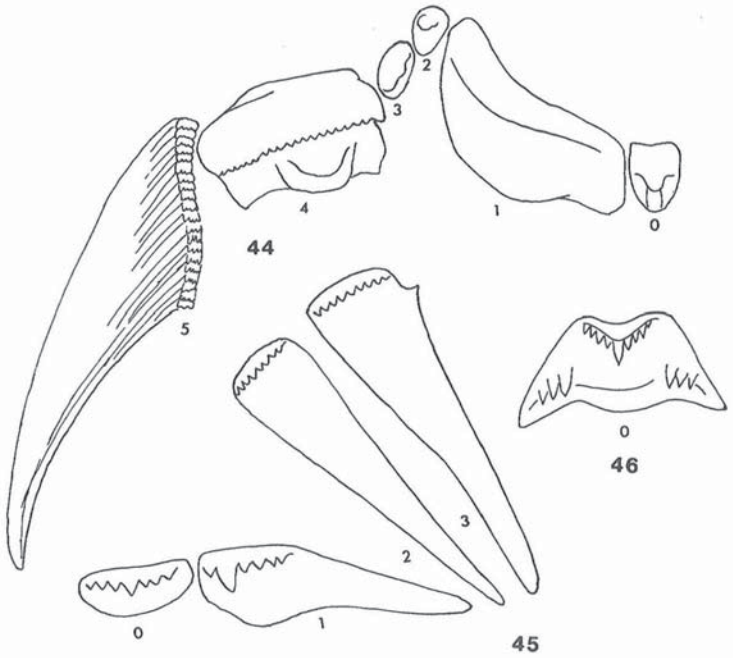
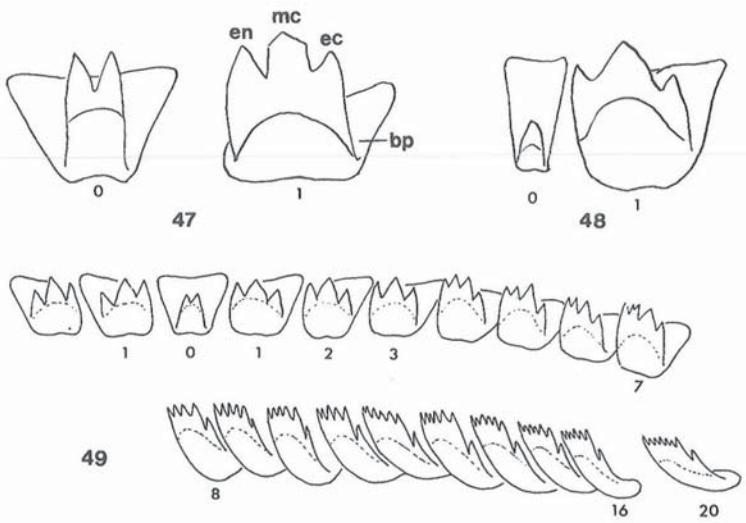
The front section of the stomach, which morphologically is a widened part of oesophagus, is termed the crop (cr), followed by the gizzard (gi) being very muscular and inside provided with folded projections which assist in the treatment of food. The last section of the stomach, pylorus (py), is provided with a blind sac, caecum, that produces a gelatinous enzymatic substance. Furthermore, the hepatic duct is joining in pylorus, connecting the stomach with the large liver (li) or digestive gland (hepatopancreas). The intestine (in) extends from the under side of pylorus and forms a couple of loops and finally as rectum (re) follows along the side of the mantle with anus (an) placed near the mantle border.

Radula (fig. 44-49). Especially in the prosobranchs, the radula is differently developed. There is a number of different radula types, two of which are present in African freshwater prosobranchs.

The rhipidoglossate radula (fig. 44) is found in primitive prosobranchs with each transverse row consisting of a central tooth and on each side of this, five to seven differently formed lateral teeth and a great number of narrow, almost bristle-formed marginal teeth. Snails with such a type of radula are predominantly algae and detritus feeders.



In by far the most freshwater prosobranchs the radula is taenioglossate (fig. 45) with seven teeth in each transverse row, namely one central tooth and on each side of this one lateral tooth and two marginal teeth. In some groups the central teeth are provided with some additional cusps on the basal plate (basal denticles 46). Taenioglossate snails live mainly on fresh or putrid vegetable matter, but several of them must be described as omnivorous.



In all pulmonates (fig. 47-49) the teeth are small, rather conform and present in great numbers, sometimes as many as 20,000 - 25,000. Their teeth can also be divided into the central tooth (0), the lateral (1-7) and marginal teeth (8-20) but the distinction between lateral and marginal teeth is not always clear. In the freshwater pulmonates the central tooth often has one or two cusps and is termed unicuspid (48) or bicuspid (49), frequently the lateral teeth are tricuspid. The cusp closest to the central tooth is called endocone (en), the middle one mesocone (me) and the outer one ectocone (ec). On the outer lateral teeth, the endocone is often divided into two or three smaller cusps while the ectocone only commences to divide into several cusps on the marginal teeth. The mesocone generally remains undivided which also may be the case with the ectocone. Moreover, the individual teeth consist of a basal plate attached to the basal membrane and from the front margin of this the tooth itself is raised in a more or less acute angle.

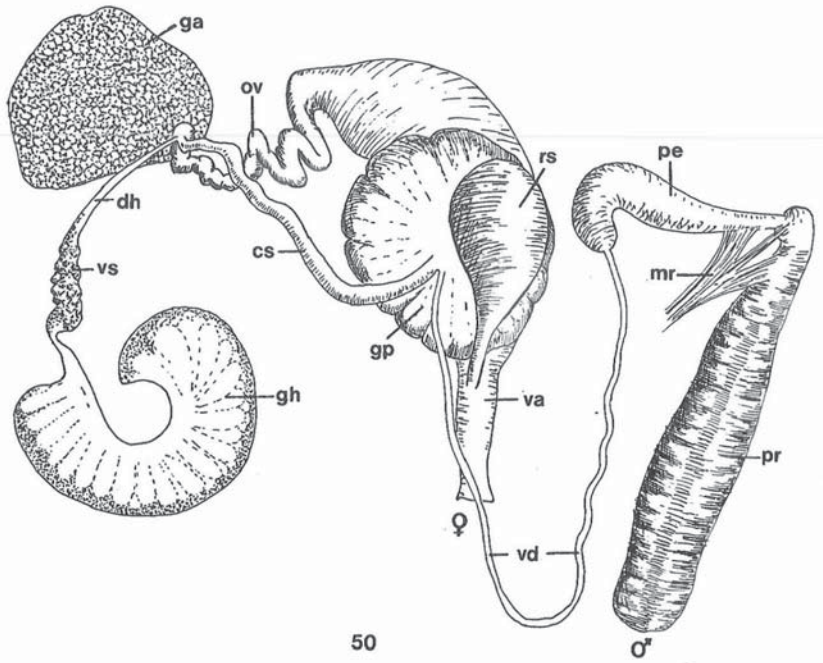
Preparation of radula. To examine the structure of the radula it is necessary to isolate, clean and preferably also stain it. This is done easiest as follows. The whole buccal mass is placed in a 7-8% solution of Potassium or Sodium Hydroxide in which everything apart from the jaw and radula is

dissolved during 24 hours. The process can be speeded up considerably by careful heating, but boiling should be avoided. Hereafter the radula is cleaned for the last bits of tissues and placed in a 15% solution of Acetic Acid in which a small amount of Aniline Blue, that stains the young teeth, basal plates and the outer marginal teeth blue, has been dissolved. After a few minutes the radula is transferred to a saturated aqueous solution of Chrysoidin, that stains the actual teeth bright orange in about five minutes time. The radula is then rinsed carefully in pure alcohol and placed in a drop of Euparal on a slide. The radula is straightened out, placed with its teeth upwards and finally covered by a slip. It is very important that the radula is straightened out carefully because, if not, the shape of the teeth cannot be seen with accuracy.

The reproductive system (fig. 40, 41, 50) consists of the genital organs, which from a taxonomic point of view belong to the most important organs, especially in the pulmonates. In the freshwater prosobranchs the development of a copulatory organ is of a special taxonomic importance. In many species a true penis can be found hidden in the pallial cavity, in others, for instance Bellamya, the male's right tentacle acts as a copulatory organ and in all melanians the copulatory organ is missing altogether. In the females it is of taxonomic importance whether they are oviparous or viviparous, and in the latter case also where the ova are kept until the young are born. In some viviparous prosobranchs the ova are kept in the distal part of the oviduct which then acts as uterus, while in others a special brood-pouch has developed in the foot behind the head.

In the pulmonates the genital organs consist of a hermaphroditic gonad, also named ovotestis, an excretory duct, the ovispermduct, which later divides into a male and female duct, respectively the oviduct and the spermduct to which different accessory organs are attached.

The ovotestis (gh) consists of numerous follicles placed in the upper part of the shell and partly embedded in the liver. The ovisperm duct (dh) is normally rather long and the anterior part provided with numerous vesiculae seminales (vs) for storing the snails own sperma. As a rule the last part of the ovisperm duct is simply tubular and joins the carrefour (ca) where

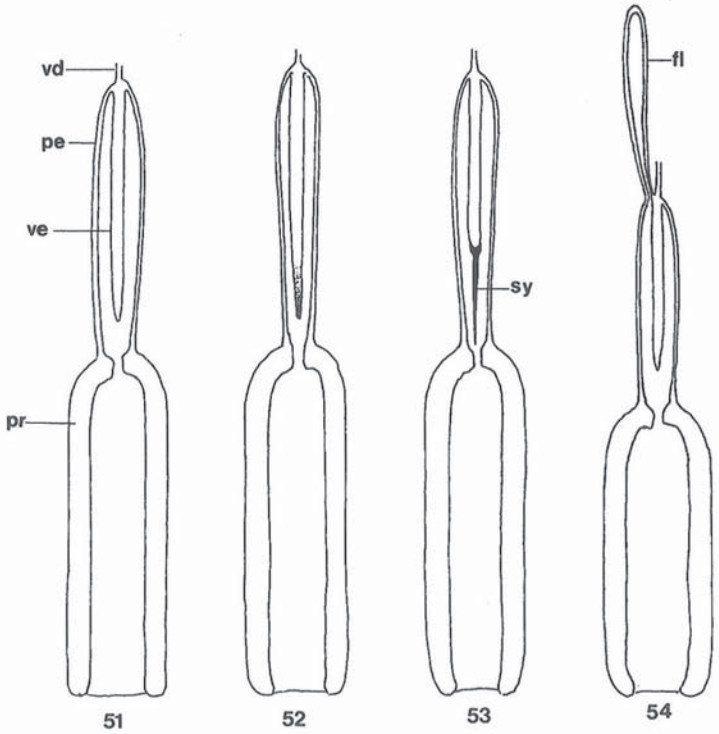


50

the female and male genital duct separate, and where the fertilization of the ova takes place. For this reason it is called the fecundation pouch as well. The duct joins into this from the large albumen gland (ga), normally placed close to the stomach and providing the egg cells with a layer of albumen. The oviduct (ov) is usually a very folded tube with its middle part swollen and thick-walled owing to numerous gland cells that form the shell of the ova (the nidamental gland, gn). The lower part of the oviduct is generally called uterus (ut) and the very lowest part vagina (va). The border between uterus and vagina is agreed to be where the duct to the receptaculum seminis (rs) or spermatheca joins. This is a bag-shaped and more or less pedunculated organ which serves as storage for the sperma from another individual.

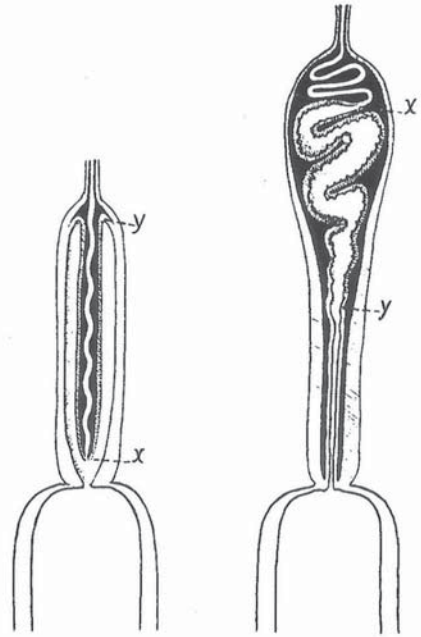
In the freshwater pulmonates the female genital opening (♀) is placed on the side beneath the mantle border, while the male genitals are placed further in front just behind the tentacle. In the land pulmonates the male and female duct end with a joint atrium where the opening is found on the side near one of the tentacles.

The spermduct (cs) is a long curved tube, which in its lower part is provided with a well developed prostate gland consisting of a number of simple or branched diverticula, which either join directly in the spermduct or in a prostate duct finally ending in the spermduct. Having passed the prostate gland (gp), the male duct narrows and is now called vas deferens (vd). In freshwater pulmonates, vas deferens follows along with uterus and vagina and then, hidden in the body wall, proceeds until it again appears near the male genital pore from where it goes in a long curve to the top of the copulatory organ (co). In the land pulmonates vas deferens does not run in the body wall but directly to the copulatory organ. This organ is very differently formed in the pulmonates, but it is always invaginated when not in use contrary to the case of the prosobranchs. In the freshwater pulmonates the copulatory organ consists of two parts: above a narrower vergic or penial sheath (pe) surrounding penis (ve) itself and below a wider muscular preputium (pr) which is an invagination from the surface of the body.



The copulatory organ is of great systematic value in the Planorbidae family because of considerable individual variability in the different genera. Fig. 51-54 show four of the types found in African planorbids with a discoid shell. Biomphalaria (51) represents the most primitive type without a special shape or extra appendage. In fig. 52 the tip of the penis is scleroid and vas deferens stops short of the tip. This type is known only in the genus Ceratophallus. The next type, (53), found in Gyraulus, among others, has developed a stiletto instead of a scleroid tip. Fig. 54 is an example of a planorbid with flagellum, an appendage which sits on top of the vergic sheath. It is found in certain Segmentorbis species and is doubled in other closely related families. Some planorbids have developed a special gland which appears as a projection on the upper part of the preputium's inner wall.

In Bulinus the copulatory organ is completely invaginated when not in use. Fig. 55-56 show it in relationship to the copulatory organ in Biomphalaria. A split between the two layers of the sheath has presumably taken place. In the two figures x denotes the tip and y the base of the penis.



FRESHWATER MALACOLOGY

With a few exceptions all molluscs of medical or veterinary importance live in fresh water. It is necessary, therefore, to go into the development and conditions of these fairly detailed.

Almost all mollusc families living in fresh water are from a geological point of view old families. Most of them are known from the Mesozoic (Jurassic or Cretacean) but are possibly even older as only very few freshwater sediments from Permian or Trias are known. According to their great age several of the families have an almost world-wide distribution. Some are represented on all continents while others are missing only in South America or Australia. Others are only known from the tropics but then, as a rule, are found in the New as well as the Old World. Only a few have a more limited spread.

Freshwater molluscs are generally easy to identify to families. Almost all members of one and the same family of freshwater molluscs are often to a great extent alike, no matter from where in the world they descend. Very often it may be difficult to determine the genus of freshwater molluscs although as a rule it can be done easily, for instance by studying certain anatomical characters. The determination of species, however, is often a very difficult problem, and without sufficient experience with regard to the family and country in question, it is almost impossible. This is due to two circumstances, i.e. partly the great variability found within most freshwater molluscs and partly the lack of good distinctive characters. Both these circumstances are due to the specific conditions applied to most fresh waters.

It is a well-known fact that many hololimnic species are divided into a number of more or less different populations. This can distinctly be observed with molluscs but is also known with fish for instance. If being familiar with the snails and bivalves within a certain area, one can often by only casting a glance at the shell be able to determine from which lake or river an individual originates. This is due to the freshwater molluscs being influenced to a high degree by the environment under which they live.

Most freshwater bodies represent isolated habitats as far as they have no direct connection with other fresh waters. As a consequence of this isolation,

the population of a freshwater species can develop in a certain direction without being affected by interbreeding with other populations of the same species. As commonly known, the fresh waters are of a highly different nature. One has only to think of large and small lakes, ponds, marshes, ditches, rivers etc. to realize this. The animals living in such different habitats, therefore, must have adapted themselves to the particular conditions there. Such adaptation cannot, of course, be perceived as referring to the single individual but rather as an adaptation of the whole population and has come about in such a way that those individuals being best fitted to life under the conditions in question have produced a greater number of offspring while the less fitted individuals have gradually disappeared.

These two aspects in the nature of fresh water - isolation and variation - affect the way of forming new species while a third aspect, their unstable nature and as a rule relatively short duration, works in the opposite direction and will most frequently prevent formation of new species because time available before new changes occur is insufficient. Only in very old and large lakes as for instance Lake Tanganyika has this been possible, but then a considerable number of endemic species are to be found in such lakes.

The conditions under which the molluscs live in fresh water, therefore, will tend to develop microgeographical races and prevent the formation of good, clear-cut species. In most cases the individual species are connected through intermediate forms, which normally are only found within a fairly limited area. It happens therefore time after time that two species are easy to separate in a certain area while it may cause considerable difficulties in another.

Therefore, if consideration is given to the total distribution of the individual species, the mutual limitation is often very difficult. The actual reason for this is, of course, that a single species to a certain extent is an artificial conception with which for practical reasons we have to work, but what we call a species within the freshwater molluscs is in reality a collection of more or less different populations. Whether different populations can be referred to one and the same species depends on a subjective view, which can often be difficult to decide and under all circumstances needs great experience.

Presuming that two species are separated by five characters, and populations are found where only four, three, two or one of these characters are present, it will be necessary in each single case to consider the importance of those characters present and those absent before it can be determined whether the population in question should be referred to either one or the other species.

Occurrence

Most freshwater molluscs prefer stagnant or slowly running water. On the exposed shores of big lakes and in fast-flowing rivers there are few if any pulmonates whereas prosobranchs and bivalves may be present. They are also usually lacking in very acid or alkaline water, but apart from these exceptions they can be found in all types of freshwater bodies from the greatest lakes to small rainwater pools. In the great lakes they are most plentiful in sheltered bays with shallow water, but sometimes they live at greater depths, down to 10 metres or more for pulmonates and 150-200 metres for prosobranchs and bivalves. When these do not occur at greater depths, the reason is lack of oxygen. It is, however, the smaller lakes, ponds and sluggish streams that are the preferred habitat for most of the species. Water lilies are as a rule indicative of good conditions for snail life, while Nile lettuce seems to indicate poor conditions. Papyrus swamps are also bad habitats. Certain species of freshwater pulmonates live preferably or entirely in temporary pools, even in pools that hold water during only a few months of the year. Many populations of freshwater pulmonates are subjected to great fluctuations, which means that a species abundant at one visit might seem to be very scarce a few months later. Repeated visits are necessary to be sure that all species have been found, even in small ponds.

COLLECTION, PRESERVATION AND DISPATCH OF SNAILS

Collection

In Africa it is usually not advisable to collect freshwater snails by picking them up by hand. The risk of becoming infected with *Schistosoma* is too great. Of course, rubber or plastic gloves protect perfectly against such

infection, but they are most unpleasant to wear in a hot climate. The use of a suitable long-handled net is preferable, especially where there is a rich submerged vegetation. The net should be shaken in the water after it has filled with plants. Most snails will then loosen their hold on the plants and drop to the bottom of the net. The net may also be emptied into a suitable white enamel or plastic dish, where it is easy to see the snails and pick them up with a pair of forceps.

For collecting in deeper water a dredge is indispensable. It consists of a rectangular or triangular frame of flat iron to which is attached a bag of nylon net. The frame should have a side length of 25-40 cm. A small piece of rope is fixed to a ring in each of the three corners. The free ends of these ropes are collected in another ring to which a long rope is tied. The length of this rope depends on the depth of the water; it should be about three times the depth. The dredge can be used either from a boat or from the shore (or a bridge). It should be pulled at such a speed that it just scrapes the top layer of the bottom. If pulled too fast it will go above the bottom, and if too slowly it will dig itself into the bottom. With some experience it is easy to judge by the feel of the rope whether the dredge is working properly. As in the case of the hand-net, the dredge should be emptied into a dish for sorting out the snails.

If the snails are wanted alive in the laboratory, it should be remembered that they survive better when transported in a bottle with very little water than they do in a bottle with much water. Small or fragile specimens should never be transported in the same bottle as large, heavy specimens.

Preservation

The easiest way of preserving snails is to put them into 70% ethyl alcohol. For identification this method is sufficient, if the volume of alcohol is at least twice the volume of snails. If the snails are wanted for anatomical purposes, it is better to have them preserved extended. For this the following procedure is recommended.

The snails are placed in a small jar containing sufficient water to allow them to creep freely around. Then a few drops of a saturated solution of

menthol in alcohol are added to the surface; the menthol will narcotize the snails with the lower part of the body extended. When the snails no longer react to being touched, they are transferred to 70% alcohol, which kills them quickly. After 24 hours the snails may be dissected, but if they are to be kept for any length of time, the alcohol should be changed, because the first portion is diluted with water from the snails.

Storage of shells and preserved specimens

Since all preservation fluids after some months or years will attack the shells, it is wise to keep some shells dry and other specimens of the same lot preserved in alcohol. With snails killed and kept in alcohol for at least a couple of days or killed in boiling water it is usually possible to extract the body with the aid of a pin with the point bent in the form of a small hook. A large insect-pin is excellent for the purpose, and when fixed in a wooden handle and bent in a curve corresponding to the body whorl of the snail, it forms a useful implement. It is seldom possible to extract the bodies of small snails, and in such cases the snails are placed in 96% alcohol for 24 hours, after which the soft parts are allowed to dry inside the shells. When the shells are completely dry, they are put in suitable tubes and labelled. The tubes should be plugged with cotton wool and kept in darkness.

The best way to keep preserved specimens is to put them into a tube filled with 70% alcohol, plugged with cotton wool and stored with other tubes in a bigger jar, also filled with 70% alcohol and tightly closed in order to prevent evaporation of the alcohol. Each tube should, of course, be properly labelled, with indication of the exact locality, the date, the name of collector, and, if possible, the name of the species for each lot. As most African localities are difficult to locate on a map, it is advisable that the nearest big village or town be indicated. Labels should be of good paper and written with Indian ink.

Dispatch of specimens

Dry shells and preserved specimens may be sent in any suitable container. Each lot should be properly labelled. Too frequently specimens have been sent

with illegible labels written in pencil on poor paper or with a reference number of no value to the recipient. Of course, reference numbers may be used, if the sender remembers to send the clue. For air mail, light plastic containers are advantageous when placed in a sufficiently strong box or tin. Heavy specimens should never be sent in the same container as small and fragile specimens.

Live specimens may be sent packed firmly between layers of damp, but not wet, cotton wool in a suitable tin. The specimens should always be arranged in a single layer and should not touch each other. Several layers of snails and cotton wool may be arranged in the same tin. Packed this way live snails can usually endure 5-6 days' travel or more. Live specimens should also be labelled properly, but the labels should be placed outside the tin. Of course, all shipments of live specimens should be sent by air mail.

HOW TO USE THE IDENTIFICATION KEYS IN "A FIELD GUIDE TO AFRICAN FRESHWATER SNAILS".

Below follows an example of the type of key in the "Guide" and how to use it. The six animals pictured are found on a farm and it is possible to separate the species from each other by using the key.

The key to identify the animals is extremely simple. In each paragraph two questions are posed. The correct answer leads either to another paragraph or assigns the animal to a species.

1	Two legs	proceed to paragraph	2
	Four or more legs	proceed to paragraph	3
2	Feathers on the animal, a pair of wings		a hen
	No feathers or wings		the owner
3	Four legs		4
	Six legs		a fly
4	Two horns, no front teeth in upper jaw, hooves		5
	No horns, teeth in both jaws, claws		a dog
5	Shoulder height more than 1 meter, a long tail		a cow
	Shoulder height less than 1 meter, a short tail		a goat

List of Symbols

an	anus	nr	nerve ring
ap	aperture	oe	oesophagus
au	auricle	ol	outer lip
ax	apex	ov	oviduct
bm	basal margin	pa	peripheral angle
bp	basal plate	pc	pericardium
bu	buccal mass	pe	vergic sheath
ca	carrefour	pr	preputium
cl	columella	ps	pseudobranch
cm	columellar margin	py	pylorus
co	copulatory organ	pw	parietal wall
cr	crop	ra	radula
cs	seminal canal (sperm duct)	re	rectum
dh	ovisperm duct	rf	fold on rectum
ec	ectocone	rs	receptacle
en	endocone	sa	radula sac
fo	fold between rectum and kidney	sh	shoulder angle
fl	flagellum	sl	spiral line
ga	albumen gland	sp	spire
gh	ovotestis	sr	spiral rib
gi	gizzard	st	stomach
gl	growth line	su	suture
gn	nidamental gland	sy	stylet
gp	prostate gland	tr	transverse rib
gs	salivary gland	ts	transverse stria
he	heart	um	umbilicus
in	intestine	ut	uterus
ja	jaw	ur	ureter
ke	keel	va	vagina
ki	kidney	vd	vas deferens
li	liver	ve	verge
lu	lung	vn	ventricle
mb	mantle border	vs	vesiculae seminales
mc	columellar muscle	wh	whorl
me	mesocone	♂	male opening
mo	mouth opening	♀	female opening
mr	retractor muscle		

Appendix

Methodology for dissection and preparation of freshwater snails

By

Thomas K. Kristensen and Flemming N. Frandsen

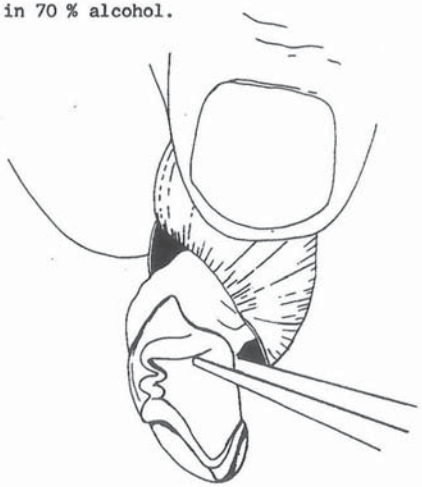
METHODOLOGY FOR DISSECTION AND PREPARATION OF SNAILS.

For all kinds of snail studies, it may be neccessary to dissect the snail body and make preparations of one or more of the organs. The present manual is primarily based on an examination of the pulmonate snails, but most of the instructions can be followed in dissection and preparations of prosobranchs as well.

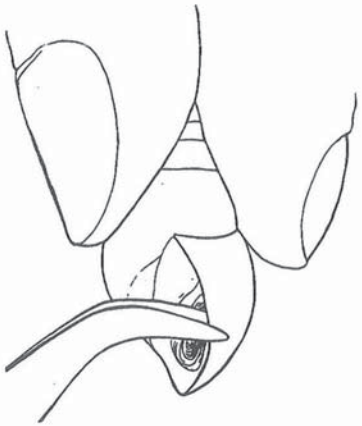
Snails for dissection can be killed in 70 % alcohol, 4 % formalin solution or boiled in water for a couple of minutes. For storage, snails should preferably be kept in 70 % alcohol.

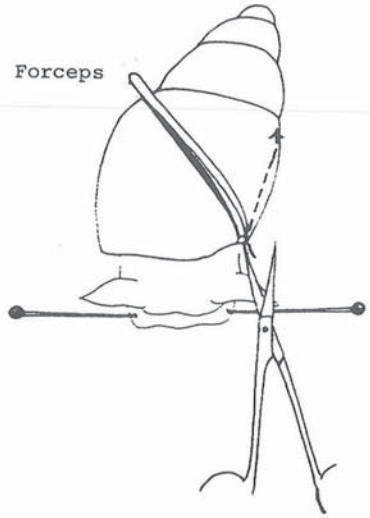
Removal of the body from the shell.

Pick up the shell between two fingers of your left hand. Using your right hand, grip the body firmly with a strong forceps. Loosen the body carefully by screwing it to the left (dextral snails) or right (sinistral snails) out of the shell. Be careful not to pull too much as this will destroy some of the organs.



In some prosobranch snails you have to remove the operculum before you are able to grip the body with the forceps. Likewise, prosobranchs often have withdrawn so far up into the shell that you have to break the last whorls in order to get hold of the body. The whorls can be broken by use of a strong forceps.



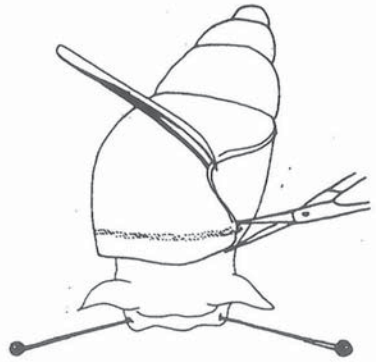


Dissection.

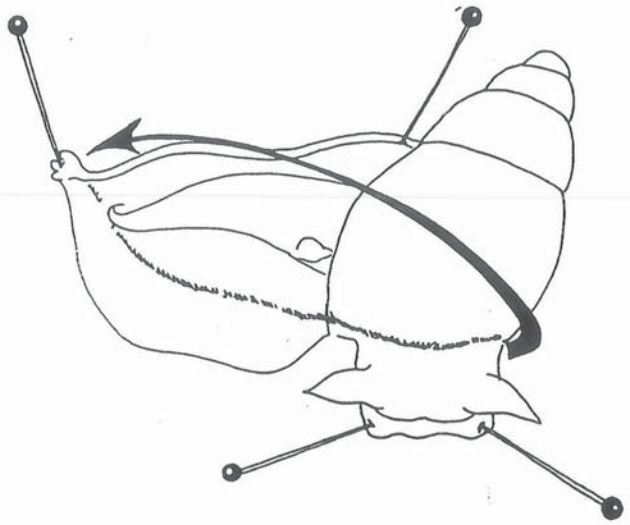
In order to open the mantle cavity of a snail, you orientate the snail, of which you have removed the shell, on its foot so that the head points towards you.Then find the pneumostome (the opening into the mantle cavity) and cut with your scissors backwards following the white columellar muscle.

Now you cut across the body following the connection of the mantle and the body, underneath the mantle border.

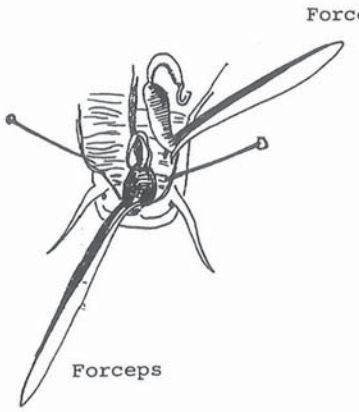
Cut continously and pull the mantle backwards as seen on the figure.



You continue cutting until you have crossed the body and reflexed the hole mantle. Now the pallial organs are exposed. Fix the body with pins.

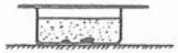


The head region is opened by an incision between the tentacles from the point where the two palps meet, to the line where the mantle border was cut transversely.

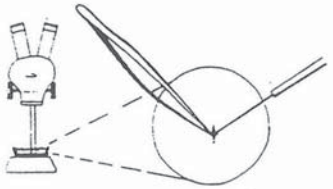


Reflex the body walls and fix with pins. The buccal mass and penial complex are now exposed. These organs are important if you want to make preparations of radula and copulatory organs.

Preparation of the radula.



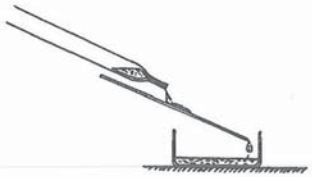
Remove the buccal mass from the head (see above). Macerate it in 7.5 % sodium hydroxide (NaOH) for 2 hours at 60 °C or 24 hours at room temperature.



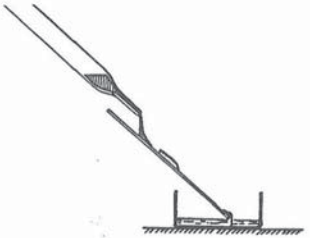
The released radula is washed in water and membranes are removed under dissecting microscope.

The radula is transferred to a drop of glacial acetic acid on a slide. Orientate the radula with its teeth uppermost and straigthened out. Let the acid evaporate.

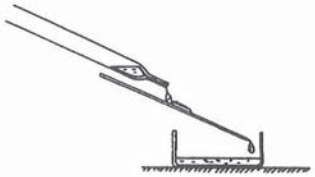
When the radula is completely dry a drop of Mallory-2 is placed on the radula. After 2-3 minutes it is rinsed off with water.



The radula is now rinsed with 2 % oxalic acid.



Then in 96 % alcohol.



Rinsing in kerosine

Now a drop of eukitt is placed on the radula and it is covered with a cover slide.

Prescription of Mallory-2:

Stock solution of Mallory-2:	Anilin blue Orange	0.5 gr
	Orange G	2.0 gr
	Oxalic acid	2.0 gr
	Distilled water	100 ml

Prior to staining, the solution is diluted as follows: 1 part Mallory-2 and 9 parts distilled water. The stock solution may be stored in a refrigerator for up to one year. The diluted solution for a shorter period.

Preparation of the penial complex.

The penial complex is dissected and removed as described above, rinsed in 96 % ethanol, put into xylene for 2-3 minutes for clearing, stretched out on a slide in a drop of Eukitt and covered by a cover slide.

Cleaning of the shell.

Shells are often coated with a dark ferrugenious deposit and have to be cleaned before the sculpture(microsculpture) can be studied. This is easily done in a saturated(10 %) solution of oxalic acid. After a couple of minutes treatment in oxalic acid, the shell is scrubbed with a toothbrush, rinsed in water and dried.

Cleaning of operculum.

Like shells, operculae often need to be cleaned before it is possible to see structures. This can be done in a saturated solution of oxalix acid. Small operculae can be mounted on slide in eukitt after cleaning, covered by a cover slide and studied under a compound microscope.