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A taxonomic study of Oreohelix haydeni in western Montana

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A TAXONOMIC STUDY OF OREOHELIX HAYDENI IN WESTERN MONTANA

BY

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ABSTRACT

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A Taxonomic Study of Oreohelix haydeni in Western Montana (113 pp.)

Director: Dr. Royal B. Brunson

The taxonomy of subspecies in the genus Oreohelix haydeni appears to be based on rather small and arbitrary differences between the populations. Character weighting is also apparent. A more objective basis for description of new subspecies is needed.

Four geographically separated populations of Oreohelix haydeni were located in Western Montana. A total of 155 specimens were collected from these four populations. Each shell was measured for eight different meristic characters. A total of 24 snails were dissected, six from each population, and their radular tooth formulae and denticulation were compared. From these same 24 snails the genitalia were removed, measured, and compared. Color banding was also studied. Serum was obtained from the head and foot tissues of a total of 73 snails from the four populations. Starch-gel electrophoresis was used to compare esterases of these sera.

The results indicated that when each meristic character was compared among the samples, only a few characters were significantly different. Statistical tests, analysis of variance and a modified Duncan Multiple Range Test, performed on these date indicated a significant difference (at .05 level) between some samples. The genitalia comparisons indicated no qualitative differences. Mean radular tooth formulae varied significantly; however, the overlap in number of teeth per row was high. There was no color banding observed in any specimen collected. The electrophoretic results indicated that each population had a distinctive esterase pattern.

Population II was distinctive and appeared to be a dwarf population. Population III was distinct by virtue of an extremely depressed spire. Populations I and IV appeared quite similar to each other but distinctive from populations II and III.

Comparisons of these populations with established subspecies of Oreohelix haydeni indicated significant differences for populations II and III, with populations I and IV differences questionable. However, the comparisons with the established subspecies were based on few specimens from each group, therefore, the question of new subspecies is left for additional study. The weighting of certain characteristics to demonstrate population differences is still a problem.

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CHAPTER I

INTRODUCTION

Description of the Subgenus Oreohelix

The genus Oreohelix (Pilsbry) (Stylommatophora, Pulmonata) includes among its members most of the large land snails of the Rocky Mountain states and the Great Basin. The first species was discovered in 1841 by naturalists of the U S Exploring Expedition, and described by A. A. Gould (1846) who named it strigosa assigning it to the genus Helix. By 1869 a total of three species was described. G. W. Binney (1878) transferred the group to Patula and reduced all of the species to varieties of strigosa. In 1904 H. A. Pilsbry proposed the name Oreohelix. The first catalogue of species assigned 24 species to Oreohelix (Pilsbry, 1916). A member of the family Oreohelicidae (Wartz), the genus is divided into two subgenera: Oreohelix and Radiocentrum.

The subgenus Radiocentrum is characterized by an embryonic shell of one and a half radially costate whorls. The penis is short, widened distally with the internally plicate part very short. The epiphallus is about as long as the penis. In addition, Radiocentrum is oviparous. The subgenus Oreohelix is characterized by an embryonic shell of more than two whorls at birth. The shell is variously wrinkled and striate. The epiphallus is much shorter than the penis. This subgenus is viviparous. Species of the subgenus Oreohelix are differentiated by shell

morphology, reproductive system morphology, radular description, and location.

All species of genus Oreohelix are calciphilous, and "most of them are restricted to limestone outcrops and vicinity" (Pilsbry, 1939). As a general rule they live near the surface, with a single stone, a bit of bark, or a few leaves for cover. Talus seems to be the best place to find Oreohelix, "although they may be found in situations which seem the least favorable. The opaque, whitish and earthy texture of shells of Oreohelix of the semiarid states is a protective adaptation to the strong light of a high country with little shade. It is a character common to snails exposed to strong insolation all over the world" (Pilsbry and Ferriss, 1910).

Description of Oreohelix haydeni

The characteristics of Oreohelix haydeni (Gabb) Henderson are as follows: the depressed shell is solid and white, and has a low-conoidal spire with the umbilicus contained five times in the diameter. The whorls are convex, the first very finely striate the next with two or three spiral threads; the late whorls have coarse irregular striae of growth, and strong raised spiral cords on both upper surface and base, one at the periphery a trifle more prominent; between some of them weak spiral lines appear. The last whorl descends moderately in front. The aperture is slightly wider than high; peristome blunt, crenulated by the spirals, the margin joined by a thick parietal callus (Pilsbry, 1939).

Originally described as Helix haydeni by W. M. Gabb in 1869 the type of Oreohelix haydeni came from Weber Canyon, Wasatch Mountains,

Utah. At this time there are nine subspecies of O. haydeni described (Pilsbry, 1939). The many forms of O. haydeni are found in areas of Colorado, Idaho, Utah, and Montana (fig. 1).

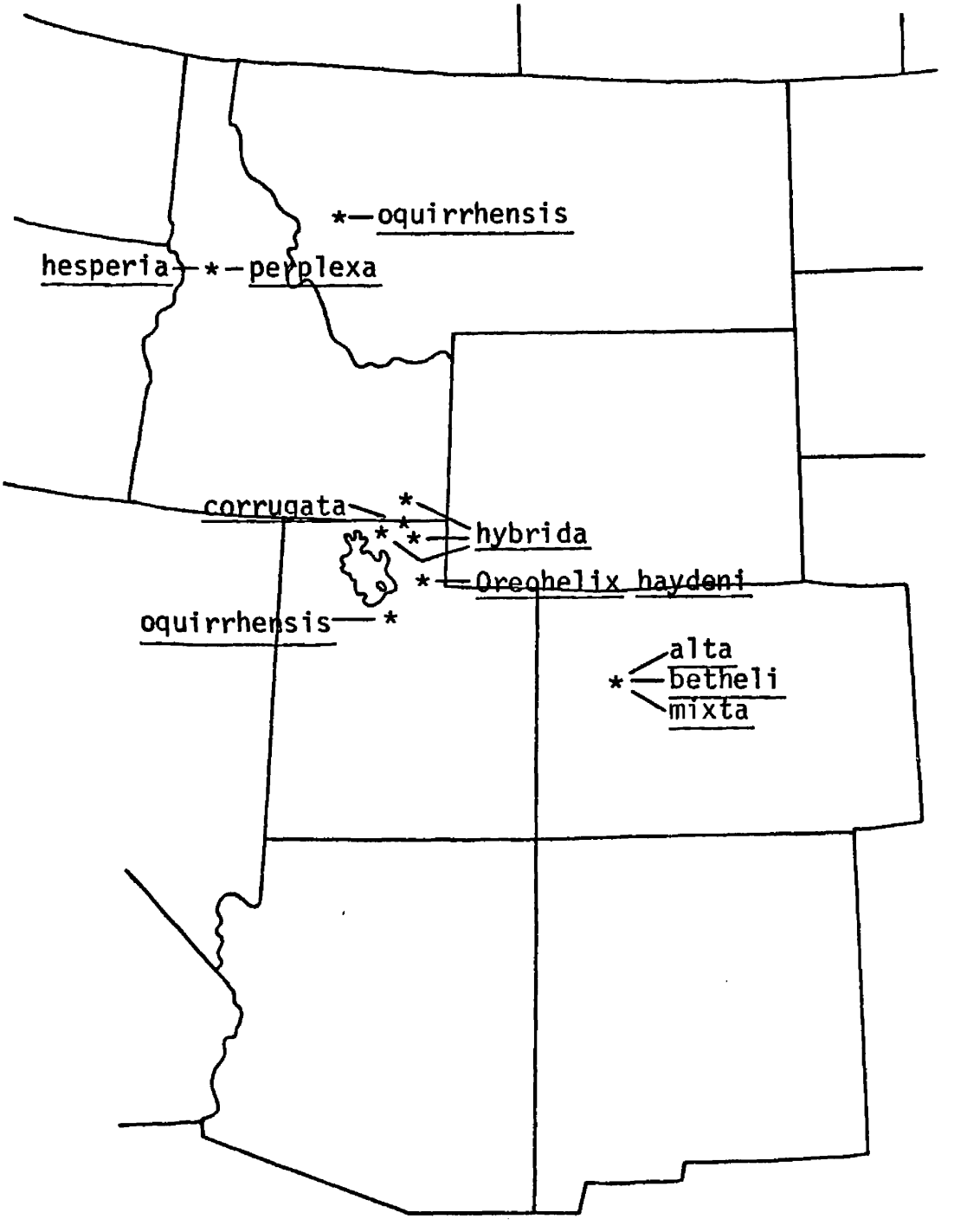
Problems in Taxonomy of Oreohelix

Pilsbry (1916) stated, "The genus Oreohelix is one of the most difficult groups of land snails within our boundaries by reason of the multiplicity of forms, and the strange parallelism of shell characters sometimes existing between species or races not directly related." He further stated, "The shell seems to be especially plastic; not only are there many local races of various grades of differentiation, but in any colony of some of the species one finds a wide range of variation in the features usually depended on for specific discrimination, such as absolute size, height of spire, width of the umbilicus relative to the diameter, and development of the sculpture."

The parts of the genitalia normally used for classification of land Gastropoda, penis and epiphallus, are, in the Oreohelicids, useful only in separating the many species of Oreohelix into three groups: the O. strigosa group which includes O. haydeni, the O. subrudis group, and the O. yavapai group (Pilsbry, 1939).

Despite the plasticity of the shell and the multiplicity of forms, many species and subspecies have been established on the basis of shell characteristics alone. For example, Oreohelix junii Pilsbry (1934) was made a new species because this species "differs by having the umbilicus up to the last whorl decidedly narrower than that of jugulis, though in the last whorl it expands to about the same width (Pilsbry, 1939). Another example is O. eurekensis uinta Brooks (1939)

Figure 1. The locations of Oreohelix haydeni and its described subspecies.



The location of O. h. bruneri is not known other than "Montana". (Pilsbry, 1939).

which was established although "It resembles O. eurekensis closely in shape, texture, color and sculpture" (Brooks, 1939). The only difference was that it "...differs by the somewhat wider umbilicus contained about 3 3/4 times in the diameter of shell" (Brooks, 1939). Of O. haydeni Pilsbry (1916) states, "O. haydeni does not differ from O. strigosa depressa in any important or diagnostic characters of the genitalia or dentition, but the features of the shell seem quite sufficient to give it specific rank."

Shell characteristics are highly variable; so, apparently, is the radular tooth formula. Descriptions of the genitalia that include statements such as "genitalia like other forms of haydeni" and "not materially different from Oreohelix strigosa" (Pilsbry, 1939) point out the inadequecy of these characteristics for taxonomic purposes.

Use of Paper Chromatography and Chromosome Number in Taxonomy of Gastropoda

Other investigators have studied the possible use of paper chromatography and chromosome number as aids in the taxonomy of Gastropoda.

Separation of substances using paper chromatography is based upon the fact that the rate at which molecules in the solvent pass over the filter paper is related to their relative affinities for solvent and paper, and hence possesses a characteristic rate of movement. The application of reagents (usually ninhydrine) produces colored areas in the positions occupied by the separated materials (Burch, 1961a).

There are, however, many problems associated with the use of paper chromatography in molluscan taxonomy. It is difficult to standardize procedure to constantly give precisely identical patterns, and,

in working with smaller taxonomic categories, differences are quantitative rather than qualitative, and therefore more refined methods must be used in characterizing species (Burch, 1961a).

Determining the diploid number of chromosomes in various snails has not been helpful in the classification of Gastropoda at or below the specific level.

Burch (1961b) stated that "since 67% of planorbid species and 75% of the basommatophoran species investigated so far have the haploid number 18 the chromosome number by itself has only very restricted value in species discrimination."

Electrophoresis and Taxonomy

Electrophoretic separation on paper, cellulose acetate, agar, acrylamide-gel, and starch-gel is based on a simple electrophoretic effect, namely, the migration of ions under the influence of an electric current.

Disc electrophoresis has been used to some extent in molluscan systematics. In this type of electrophoresis, the protein components to be separated migrate within a cylindrical column of acrylamide-gel; after separation the various fractions are stacked in the tube like coins and are visible as bands, in side view. After a test is conducted, the gel columns are placed in a stain to fix and stain the proteins (Davis and Lindsay, 1967).

Davis and Lindsay (1964) used disc electrophoresis and the serum of Helix pomatia and three distinct populations of Oncomelania formosana. They determined that "the fingerprint of one species was distinctly different from that of any other species."

On the other hand, Pace and Lindsay (1965) used disc electrophoresis to compare variation between populations of nine different species of Bulinus, and found that "when the protein patterns of all of these taxa were compared, strikingly little variation was found." However, in 1967 Pace and Lindsay again used disc electrophoresis. The snails used in their study were Helix pomatia and four different populations of Pomatiopsis lapidaria. Again the results showed that each species has a distinctive electrophoretic fingerprint, and that "the electrophoretic technique used is sensitive enough to demonstrate population variability in terms of migrational differences in identifiable components and of new or different fractions."

In starch-gel electrophoresis the protein components to be separated migrate in a thin rectangular strip of starch-gel. Starch-gel electrophoresis will separate many more proteins from any given mixture because of the porous structure of the gel (Smith, 1968). An example of the increased capability of starch-gel electrophoresis is the ability of the starch-gel to resolve one band from paper electrophoresis into ten sub-bands after starch-gel electrophoresis.

Wood et al (1959) used starch-gel electrophoresis to separate the components of serum from Loligo pealii and Ostrea virginica. In their discussion they stated, "These findings suggest that starch-gel electrophoresis of serum proteins may be useful in certain racial studies, taxonomic problems, and considerations of biochemical individuality."

Manwell and Baker (1963) used evidence from starch-gel electrophoresis to establish sibling species of the sea cucumber Thyonella gennata.

Manwell, Baker, Ashton, and Corner (1967) found that three species of copepods: Calamus finmarchicus, C. helgolandicus, and C. hyperboreus can be differentiated easily and consistantly by starch-gel electrophoresis of a number of their enzymes.

The conclusions of all of these studies are in general agreement. It appears that:

1. Species have distinct electrophoretic patterns which can be used for identification.
2. These patterns can be used to supplement other morphological characters in taxonomy.

Purposes of This Study

Four populations of Oreohelix haydeni have been discovered in Western Montana. These four populations are geographically and therefore most likely genetically isolated from each other and have, in all probability, been isolated from each other since the retreat of the Cordilleran ice sheet which began its retreat about eleven thousand years ago (Dr. R. B. Brunson, verbal communication, 1974). If the current criteria for subspecific description were used, these four populations could probably be given subspecific status (Dr. R. B. Brunson and Dr. R. H. Russell, verbal communication, 1974).

This study compared statistically these four populations based on several of the measurements currently accepted for the description of subspecies of Oreohelix haydeni. Other characteristics were compared without the use of statistics, ie. radular tooth formulae and reproductive tract morphology. In addition, the use of starch-gel electrophorsis was tested as a tool in the taxonomy of Gastropoda.

The purpose was to determine if this procedure would be a more consistant and reliable method, objectively, to designate subspecies in the species Oreohelix haydeni.

CHAPTER II

METHODS AND MATERIALS

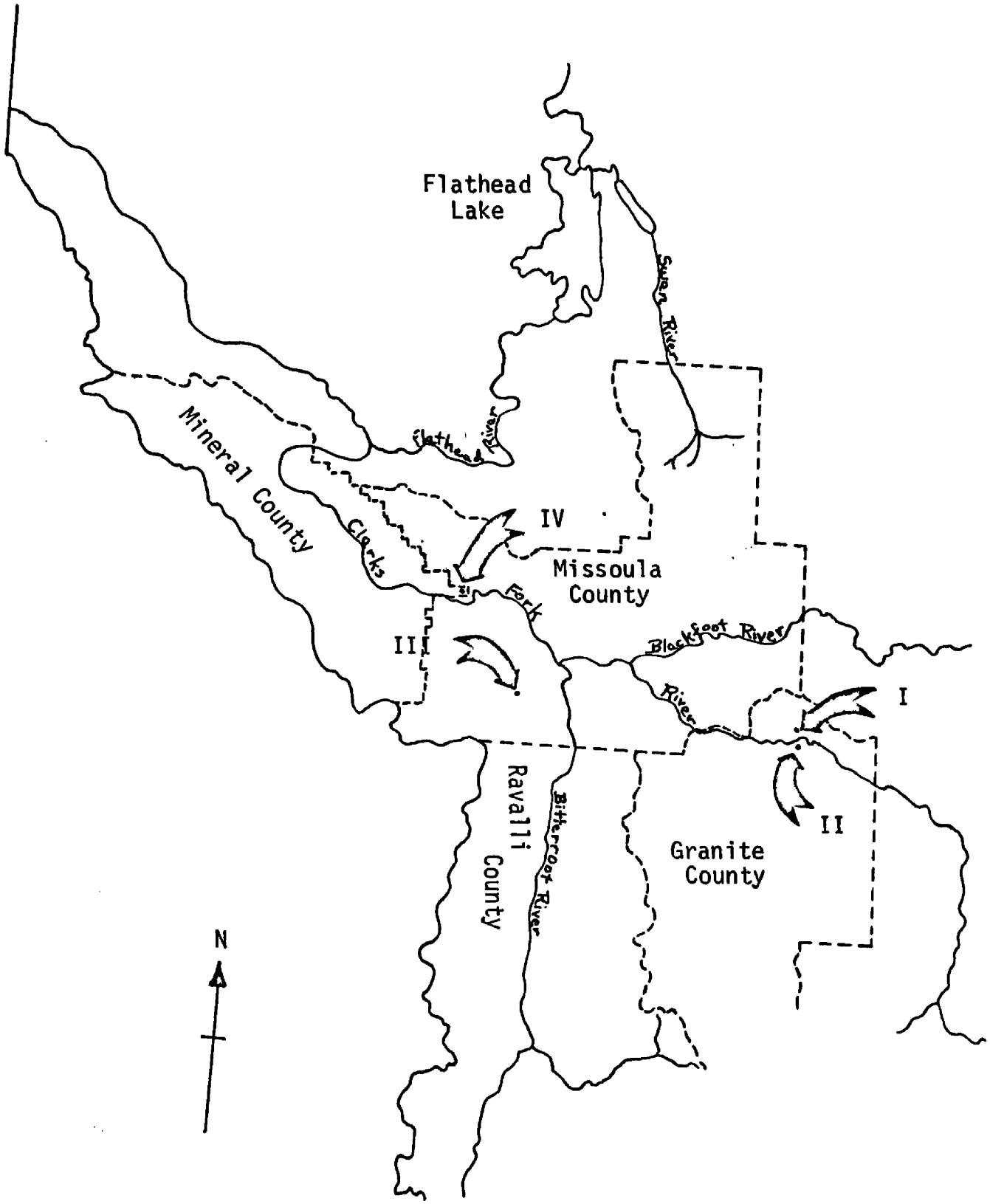
Study Areas

All four of the populations that were used in this study are located in the Rocky Mountains within a fifty mile radius of Missoula, Montana. The Rocky Mountains within this study area are composed of Precambrian, Paleozoic, and Mesozoic sedimentary rocks, including limestone in each era. Populations of Oreohelix haydeni are restricted to small limestone outcrops.

Physical barriers to movement between populations are distance and water. The extreme distance between populations is approximately seventy-five miles, the minimum distance is approximately one mile. The Clarks Fork, Blackfoot, and Bitterroot rivers as well as numerous creeks form aquatic barriers (see fig. 2).

Population I, which is located in Granite County in Rattler Gulch at R13W T11N section 3. This is approximately five miles west of Drummond, Montana, and north of the Clarks Fork River. Located in the Garnet Range, the talus and numerous rock outcrops are composed of Paleozoic limestone. The majority of the population occupies the southeast facing slope of Rattler Gulch. The steepness of the slopes is approximately 45^{o}. Vegetative cover includes Douglas Fir, Mountain Maple, Aspen, Ninebark, and Serviceberry. Talus varies in size from ten to fifty centimeters in diameter.

Figure 2. The location of populations I,II,III, and IV in Western Montana.



Population II, is found below high cliffs at R13W T11N section 22 in Granite County. This site is also located approximately five miles west of Drummond, Montana, but on the south side of the Clarks Fork River. This population occupies part of the Sapphire Range and the long northwest facing cliffs are composed of Paleozoic limestone. The angle of the slopes is approximately 45^{o}. The only cover observed was Douglas Fir and Mountain Maple. There is very little area below these cliffs that does not have a canopy. Talus varies in size from about seven to twenty-five centimeters in diameter.

Population III, which is located above Woodman Creek at R21W T12N sections 20 and 29, is approximately nine miles west of Lolo, Montana in Missoula County and north of US 12. The canyon of Woodman Creek cuts into the Precambrian limestone of the Bitterroot Range. Cover on the 45^{o} slopes consists of Douglas Fir, Ponderosa Pine, Mountain Maple, Ninebark, and Serviceberry. Talus size varies from ten to fifty centimeters in diameter. The slope faces southeast.

Population IV is located at R23W T14N section 1, which is approximately one half mile east of Alberton, Montana in Mineral County. The population is located below the highest set of cliffs on the north side of the Clarks Fork River. The cliffs are part of a Cambrian limestone outcrop in the Squaw Range. The talus slopes appear somewhat steeper than in the other areas. The cover is sparse and what cover there is provided by Ponderosa Pine and Mountain Maple. The talus on these south facing slopes varies in size from ten to fifty centimeters in diameter.

Collection of Snails

A total of 148 snails from the four sites was collected; 48 snails from Rattler Gulch, 27 snails from South Cliffs, 66 snails from Woodman Creek, and 7 snails from Alberton. Ninty snails were prepared for electrophoresis and the remainder were kept for dissection. In addition, 7 shells were collected at the Alberton site and data from these shells were incorporated along with data from the live snails into the statistical tests.

The number of snails collected from the Alberton site was considered too low to give a good indication of the population variation in the electrophoretic tests, and because they were collected in September, 1973, it was neither possible to ascertain the size of the population nor to obtain a larger number of specimens. All snails except those from Alberton were collected on June 12 and 13, 1973.

The approximate range of each population was determined in each area by walking out the limits in a variety of directions. Boundaries were determined by the absence of snails. After the range of a population was determined, snails were randomly collected both vertically and horizontally across the range. Snails were collected at the edges of the range as well as centrally.

Only snails of mating size were collected. The minimum size was determined by observing the size of snails that were in the act of copulation. The smallest size of snail that was observed became the minimum size that was collected.

As each snail was collected, it was placed in a numbered vial. Later, that number was printed, in ink, on the shell of the snail. After the snails were numbered, they were kept in large tubs until

they were preserved or processed for electrophoresis.

Measurements and Statistical Tests

All snails which were collected were measured in the following dimensions: maximum height of shell, maximum diameter of shell, maximum height of aperture, and maximum width of aperture (see fig. 3). The measurements were made to the nearest tenth of a millimeter using a vernier caliper. In addition, a binocular dissecting microscope was used to count the number of carinae above and below the periphery of the shell and the number of whorls in the shell.

These data were compared statistically by analysis of variance tests and a modified Duncan Multiple Range test. The analysis of variance design used was a one-way completely randomized design. A computer program was obtained for this test (see table 1), and comparisons were made in the following manner. By use of the analysis of variance tests each entire sample was compared to the other samples, first in terms of maximum height, then maximum diameter, followed by maximum height divided by maximum diameter. The number of top carinations and number of bottom carinations were compared in the same manner.

Following these tests each sample was divided into groups that had the same number of whorls in their shells. Then by the use of the analysis of variance test, each size group in a sample was compared to that size group in each of the other samples in terms of maximum diameter, maximum height, and maximum height divided by maximum diameter.

Next, an extension of the Duncan Multiple Range Test (Kramer,

Figure 3. Measurements taken of each snail shell.

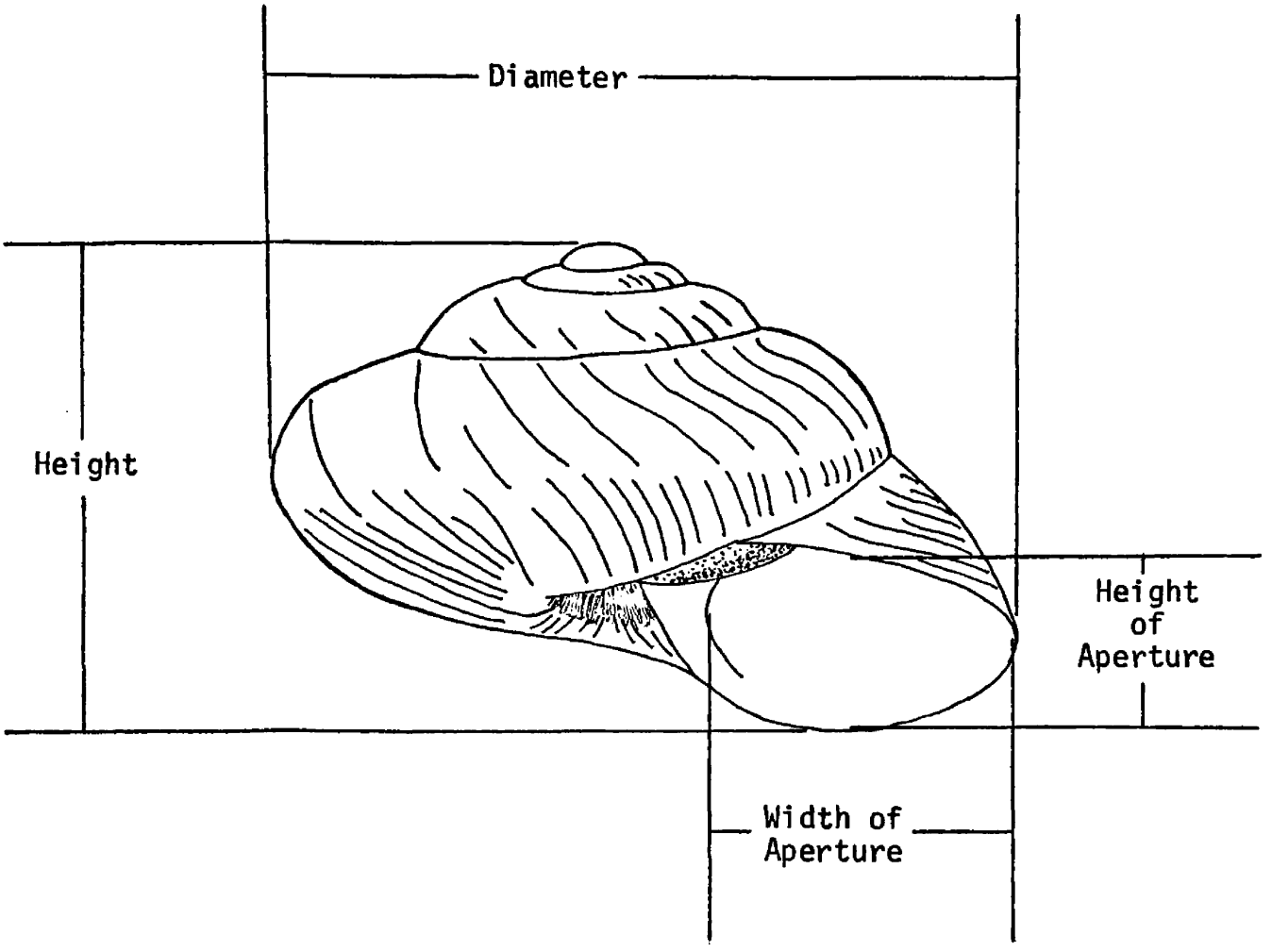


TABLE 1

Computer Program for Analysis of Variance

```
100   Name--Stat13
110
120   Description--Computes the analysis of variance for a
130   One-way completely randomized design.
140
150   Source--Unkown
160
170   Instructions--Enter data in lines 1010 and following.
180   Enter data in the following order:
190
200   1)A, The total number of observations
210   2)M, The number of different treatments
220   3)N(1).......N(M), Where N(J) is the number of
230   Observations in treatment J
240   4) The observations themselves, first for treatment 1,
250   Then treatment 2, etc.
260
270   If any N(J)>20 change the dims in line 340
280   If M>10 change the dims in line 340
290   Sample data is in lines 1010 through 1040
300
310
320   *    *    *    *    *Main Program*    *    *    *    *
330
340   Dim X(99,50),N(50),T(50),S(50)
350   Read A, M
360   Mat read N(M)
370   For J=1toM
380   For I=1toN(J)
390   Read X(I,J)
400   Next I
410   Next J
420   For J=1toM
430   For I=1toN(J)
440   Let T(J)=T(J)+X(I,J)
450   Let S(J)=S(J)+X(I,J)*(I,J)
460   Next I
470   Let U=U+T(J)
480   Let R=R+S(J)
490   Let V=V+T(J)*T(J)/N(J)
500   Next J
510   Let C=U*U/A
520   Let W=V-C
530   Let E=R-V
540   Print "Anova table"
550   Print
560   Print "Item","SS","DF","MS"
570   Print
580   Print "Grand total",R,A
```

TABLE 1 (Continued)

```
590   Print "Grand mean", C, "1"
600   Print "Treatments", W, M-1, W/(M-1)
610   Print "Error", E, A-M, E/(A-M)
620   Print
630   Print
640   Let F=(W/M-1))/(E/(A-M))
650   Print "F="F"ON"M-1"AND"A-M"Degrees of freedom."
660   Let G=F
670   Let N=A-M
680   Let M=M-1
690   Gosub 710
700   Stop
710   Rem the subroutine for computation of the F probabilities was
720   Rem programmed by Victor E. McGee, Psyc. Dept., 646-2771
730   Let P=1
740   If G<1 then 790
750   Let A=M
760   Let B=N
770   Let F=G
780   Go to 820
790   Let A=N
800   Let B=M
810   Let F=1/G
820   Let A1=2/(9*A)
830   Let B1=2/(9*B)
840   Let Z=ABS((1-B1)*F↑(.333333)-1+A1)
850   Let Z=Z/SQR(B1*F↑(.666667)+A1)
860   If B<4 then 900
870   Let P=(1+Z*(.196854+Z*(.115194+Z*(.000344+Z*.019527))))↑4
880   Let P=.5/P
890   Go to 920
900   Let Z=Z*(1+.08*Z↑4/B↑3)
910   Go to 870
920   If G<1 then 940
930   Go to 960
940   Let P=1-P
950   Go to 960
960   Print
970   Let P=INT(100000*P)/100000
980   Print "Exact Prob. of F=";G;"with ( "M;", "N;" ) D.F. is ":P
990   Print
1000  Return
1050  End
```

1956) was used to determine which of the groups compared were or were not significantly different from each other at the .05 level for the parameter compared. If the test did not indicate a significant difference, this meant that there was no detectable difference between the samples at the .05 level. If the test did indicate a significant difference, this meant that at the .05 level there was a detectable difference between the samples.

For each test, the null hypothesis was that there was no difference between the samples being compared. The null hypothesis was accepted if the probability was greater than .05.

These tests were used to determine if there was a statistical basis on which to separate these populations through the use of shell characteristics alone.

Dissection and Preparation of Soft Parts

Snails which were used for dissection were killed in a 2% solution of chlorotone. The snails were then placed in a 4% formalin solution for twelve hours to harden the soft parts. A 5% glycerine in 70% ethanol solution was used to preserve the specimens until they were dissected.

Six snails from each population were dissected. The dissections were done under a binocular dissecting scope at 15X. Microdissection sissors, BB forceps (Dixon Co., Switzerland), needles, and No. 1 insect pins were used in the dissections. The entire reproductive system and the buccal mass were removed from each snail.

After removal from the snail the buccal mass was placed in a 5% KOH solution that had been heated to 80-85^{o}C. The radula was removed

from the solution as soon as the surrounding tissues were dissolved. The average time necessary to dissolve the tissues was 16 minutes. The radula was rinsed in distilled water and preserved in a solution of 5% glycerine in 70% ethanol.

Each radula was mounted on a slide using glycerine jelly in a double coverslip preparation (Mitchell and Cook, 1952). The radula was placed on a 15mm diameter coverslip and flattened as much a possible. A drop of glycerine jelly was placed on the radula and immediately an 18mm diameter coverslip was pressed down into the glycerine jelly to make the mount as thin as possible. The coverslip "sandwich" was placed on a standard slide and balsam was worked around the edge of the larger coverslip, sealing the "sandwich" and gluing the mount to the slide.

A binocular microscope was used to count the number of radular teeth in each of ten rows on each radula, viewed at 450 magnifications. The counts were made near the posterior end of the radula so that new unworn teeth could be counted. Drawings of teeth were made using a Zeiss camera lucida. Not all of the teeth in a row are shown, only obvious differences from those already drawn are shown. The teeth were drawn 650 times natural size.

A watchglass with black wax in the bottom was used during the drawing and measuring of the reproductive tracts. The reproductive structures were pinned so that as many of the parts as possible could be seen. The drawings were made using a Spencer Lens Co. camera lucida on a Spencer binocular dissecting scope. The drawings are 22 times natural size.

After the drawings were completed, measurements were taken of

those parts which have taxonomic significance. These include length of penis, length of internally plicate part of the penis, length of epiphallus, and length of penial retractor muscle. The origin of the penial retractor muscle was also noted. The measurements were made using an ocular micrometer after the parts were straightened as much as possible.

Electrophoretic Preparation of the Snails

Each snail that was used in electrophoresis was prepared in the following manner. The shell was broken away until the mantle collar was exposed. The head, foot, and that part of the mantle up to the mantle collar were removed and placed in a tissue mascerator. An equal amount, by volume, of distilled water was added and this combination was mechanically mascerated.

The tissue mascerator consisted of a thick testtube and a steel rod with a teflon ball on the end of it. The ball just fits the testtube. The specimen was placed in the bottom of the tube, the distilled water was added, and then the rod-teflon ball was pushed in on top. The steel rod was then tightened into a chuck which was connected via reduction gears to a variable speed electric motor. The motor was turned on and the teflon ball was forced down upon the specimen, grinding it between the ball and the inside of the glass tube. The glass tube was held in ice during masceration of the tissue to reduce possibility of denaturing the enzymes and other proteins. The fluid resulting from masceration of the tissues was removed with a micropipette and placed in a numbered centrifuge tube. After each tissue masceration, the tube and the teflon ball were thoroughly cleaned

with distilled water. Each sample was centrifuged at 5000 gravities for five minutes in a Fisher Model 59 centrifuge. The supernatant remaining was removed with a micropipette and placed in a numbered storage tube. All serum was transferred with sterile micropipettes and a different pipette was used for each transfer. All storage tubes were stored in a freezer at -40 ^{o}C until used in the electrophoretic tests.

Electrophoretic Technique

In this study vertical starch-gel electrophoresis, as described by Smithies (1959), was used. Gels were made by mixing 42g of hydrolyzed starch (Electrostarch Co. Madison, WI) with 400 ml of the buffer shown in table 2. The mixture was heated, with constant stirring, until a translucent viscous fluid was formed. Gas was removed from the fluid by application of a vacuum for one minute. The mixture was then poured into a lightly oiled Hiller Plexiglass starch-gel chamber (Otto Hiller, Madison, WI). A plexiglass sheet with two rows of sixteen slot makers each was carefully placed over the chamber in such a way that air bubbles could not form in the gel. Each gel was 268mm x 122mm x 3mm. Each slot in the cooled gel was 4mm x 1mm x 3mm. The cover was held down by weights for at least one hour until the gel had set.

After one hour, the weights and cover were carefully removed and serum samples were injected into the slots. A disposable 1ml syringe and a 25 gauge needle were used to inject the samples. The end slot on each side was not used because samples close to the edge of the gel became distorted. The rows of slots were covered with

TABLE 2

Formula for Gel and Electrode Buffer

Tris(hydroxymethyl)aminomethane (Sigma No. T-1378)	14.52 g
ED2SS Ethylenediamine tetraacetic acid sodium salt	4.92 g
Boric acid grade Crystalline	66.80 g

Dissolve in 4000 ml of distilled water

pH of this buffer was 7.02

all chemicals from Sigma Chemical Co. St. Louis, Mo.

TABLE 3

Formula for Stain for Esterase

0.1M Tris-HCl buffer pH 7.0 6.06 g Tris(hydroxymethyl)aminomethane 4.1 ml HCl (37.0-38.0% HCl) 500 ml distilled water	10 ml
1% alpha-naphthyl acetate in acetone water 0.5 g a;pha-naphthyl acetate 25 ml acetone water to 50 ml	3 ml
Fast Blue RR Grade III (Sigma Chemical Co.)	100 mg

Add distilled water to 100 ml

Incubate gel one half to one hour or until banding is distinct

This is enough stain for one gel

molten vasoline, and the entire gel, except for the ends, was covered with Handiwrap. Although there were two rows of slots, only one row was used so that anodal migration of the esterases could be observed.

Following application of the test samples, electrode buffer (table 2) was poured into the electrode trays. The starch-gel chamber was placed upright in the anode tray. A paper towel "wick" was placed from the end of the gel to the buffer in the cathode tray to complete the circuit for the current flow. Handiwrap was placed over the cathode tray and the end of the gel to prevent evaporation.

All tests were run at 450 volts DC for four hours. Coolant at $4^{o}C$ was continuously circulated through the gel chamber during each test.

After the test was completed, the Handiwrap and vasoline were removed and the gel, after trimming, was carefully lifted out of the chamber with a large spatula. Care was taken to notch the gel prior to removal so as to be able to determine the correct number of each sample. The gel was placed, upside down, in a tray containing the stain (see table 3). The gel was left in the stain for approximately 30 minutes or until banding was clearly discernable. When staining was complete, the gel was rinsed in tapwater and placed in a gel wash (see table 4) for one hour. After washing, the gel was wrapped in Handiwrap and stored in a refrigerator.

TABLE 4

Formula for Gel Wash

Methyl alcohol (anhydrous, acetone free)	1980 ml
Distilled water	1980 ml
Acetic acid (glacial)	40 ml
Allow the gel to sit in the solution for at least one hour	

CHAPTER III

RESULTS

Shell Measurements

The shell measurements of all of the snails that were collected are shown in appendix A. Table 5 consolidates these measurements and shows the mean and range of each parameter measured. It is readily apparent that sample II had a diameter of mean value much smaller than the other samples; 12.1mm as compared to 17.0mm for sample I, 17.1mm for sample III, and 17.9mm for sample IV. The mean height of sample II was also lowest of the four samples at 6.1mm. Sample III had a mean height of 7.1mm, sample IV had a mean height of 8.7mm, and sample I had a mean height of 8.8mm. However, the size of the shell is affected by several environmental factores, such as altitude and exposure (Pilsbry, 1939). Therefore, the height/diameter ratio would appear to be a more consistent indicator to use when trying to show differences between populations. Table 5 shows that the mean height/diameter ratio of sample III, 0.41, was much lower than that of the other three samples. Sample I had a mean height/diameter ratio of 0.52, sample II had a mean ratio of 0.50, and sample IV had a mean ratio of 0.48.

The number of carinations above the periphery of the shell ranged from 0 (in only one snail) to 5. Sample I had a range of 1 to 5 carinations with a mean of 3.0. Sample II had a range of

TABLE 5

Means and Ranges of Shell Measurements

Population number	Number of snails per population	Mean diameter	Range in diameter	Mean height	Range in height	Mean height/diameter ratio	Range in height/diameter ratio	Mean number of carinations above periphery	Range in carinations above periphery	Mean number of carinations below periphery	Range in carinations below periphery	Mean number of whorls	Range in number of whorls	Mean width of aperture	Range in width of aperture	Mean height of aperture	Range in height of aperture
I	48	17.0	12.1 to 20.6	8.8	5.9 to 10.8	0.52	0.45 to 0.59	3.0	1-5	7.2	4-11	5.0	4.5 to 5.5	6.0	4.1 to 7.3	5.2	3.4 to 6.5
II	27	12.1	8.3 to 14.5	6.1	4.3 to 7.5	0.50	0.43 to 0.58	2.9	0-4	5.8	3-8	4.9	4.3 to 5.0	4.2	3.0 to 5.0	3.3	2.3 to 3.8
III	66	17.1	11.8 to 20.8	7.1	4.8 to 9.3	0.41	0.36 to 0.47	1.7	1-3	5.7	3-8	5.0	4.5 to 5.5	5.9	4.3 to 7.2	3.8	3.2 to 5.8
IV	14	17.9	13.8 to 19.6	8.7	6.9 to 10.5	0.48	0.46 to 0.59	2.3	1-3	5.4	4-7	5.5	5.0 to 5.8	6.4	5.0 to 7.0	5.3	4.2 to 5.7

All measurements to the nearest 0.1 mm

0 to 4 and a mean of 2.9. The range of carinations above the periphery for sample III was 1 to 3 with a mean of 1.7. Sample IV also had a range of 1 to 3 carinations, however, the mean value was 2.3

The range in the number of carinations below the periphery was 3 to 11 among all of the samples. Sample I had a range of 4 to 11 with the highest mean value at 7.2. Samples II and III both had a range of 3 to 8. Sample II had a mean of 5.8 but sample III had a mean of 5.7. Sample IV had the lowest mean at 5.4 with a range of 4 to 7.

The number of whorls is a rough indicator of maturity. It is apparent from table 5 that these samples were about equal in the number of whorls; 4.9 whorls for the mean of sample II, 5.0 whorls for the mean of both sample I and sample III, and a mean of 5.5 whorls for the mean of sample IV.

Table 5 shows that sample II, which had the smallest mean diameter and height, also had the smallest mean height and width of the aperture. The mean width of the aperture for II was 4.2mm. Sample I had a mean width of aperture of 6.0mm, for sample III the mean width was 5.9mm, and for sample IV the mean width was 6.4mm. Sample II had the lowest mean height of aperture at 3.3mm. Sample III had a mean height of 3.8mm. Both of these values were considerably below the mean height for sample I which was 5.2mm and sample IV which was 5.3mm.

Statistical Tests

The analysis of variance test was used for five different parameters, with the entire sample treated as a group. The results

of these tests are shown in table 6. All of the tests showed that for each of the parameters tested (mean height, mean diameter, mean height/diameter ratio, carinations above the periphery, and carinations below the periphery) one or more of the samples were significantly different from the others ($P<0.01$).

The results of the modified Duncan Multiple Range Tests are shown in table 7. The significance level for these tests was .05. At the .05 level, sample II was significantly different from all of the other samples in terms of height and diameter. However, sample II was not significantly different from samples I and IV in terms of the height/diameter ratio. Samples I, III, and IV were not significantly different in terms of diameter, but in terms of height, sample III was significantly different from samples I and IV. In addition, sample III was significantly different from all other samples on the basis of the height/diameter ratio. Both samples I and IV were not significantly different from sample II in terms of the height/diameter ratio. In terms of carinations below the periphery, only sample I was significantly different from all other samples. Samples III and IV both were significantly different from each other and from samples II and I on the basis of carinations above the periphery.

Following the tests performed on the entire sample, the samples were divided into groups in which all of the snails had the same number of whorls. Table 8 shows the mean height, mean diameter, and mean height/diameter ratio plus the ranges of these parameters for each group in each sample.

Tables 9 and 10 show the results of the analysis of variance tests and the modified Duncan Multiple Range Tests for groups with 4.5

TABLE 6

Results of the Analysis of Variance Tests Using Each Sample as a Group. Comparisons Were Made on the Basis of Diameter, Height, Height/diameter Ratio, Carinations Above the Periphery, and Carinations Below the Periphery

Populations compared		Diameter	Height	Height/diameter ratio	Carinations above the periphery	Carinations below the periphery
I,II,III, & IV						
	Probability	<0.01	<0.01	<0.01	<0.01	<0.01
	F value	59.76	53.40	113.26	33.77	18.73
	Degrees of freedom	3 & 153	3 & 153	3 & 153	3 & 147	3 & 147

TABLE 7

Results of the Modified Duncan Multiple Range Tests Using Data From the Analysis of Variance Tests in Table 6. Any Means Not Underscored by the Same Line Are Significantly Different At the 0.05 Level

Population	II	I	III	IV
Mean diameter	12.1	17.0	17.1	17.9
Population	II	III	IV	I
Mean height	6.1	7.1	8.7	8.8
Population	III	IV	II	I
Mean height/diameter ratio	0.41	0.48	0.50	0.52
Population	III	IV	II	I
Mean number of carinations above the periphery	1.7	2.3	2.9	3.0
Population	IV	III	II	I
Mean number of carinations below the periphery	5.4	5.7	5.8	7.2

TABLE 8

Means and Ranges of Shell Measurements of Snails With the Same Number of Whorls

		4.3 Whorls	4.5 Whorls	4.8 Whorls	5.0 Whorls	5.3 Whorls	5.5 Whorls	5.8 Whorls
Population I	Number of snails in group	0	6	4	20	14	4	0
	Mean diameter		14.9	13.7	16.6	18.5	19.0	
	Range		13.3-16.4	12.1-14.6	14.1-18.9	16.9-19.8	18.2-20.6	
	Mean height		7.9	7.2	8.5	9.6	9.9	
	Range		6.2-9.5	5.9-7.9	7.0-10.1	8.5-10.8	9.3-10.4	
	Mean height/diameter ratio		0.53	0.52	0.51	0.52	0.52	
	Range		0.47-0.59	0.49-0.54	0.45-0.56	0.47-0.58	0.49-0.56	
Population II	Number of snails in group	2	7	11	8	0	0	0
	Mean diameter	9.2	11.1	12.2	13.5			
	Range	9.1-9.3	8.3-12.4	10.3-13.3	12.6-14.5			
	Mean height	4.9	5.6	5.9	6.9			
	Range	4.8-5.0	4.3-6.4	5.1-6.6	6.6-7.5			
	Mean height/diameter ratio	0.53	0.51	0.49	0.51			
	Range	0.52-0.54	0.45-0.58	0.43-0.55	0.49-0.55			
Population III	Number of snails in group	0	7	12	32	13	3	0
	Mean diameter		14.6	15.1	17.2	19.3	20.1	
	Range		11.8-16.9	13.4-16.7	15.1-18.9	17.8-20.7	19.2-20.8	
	Mean height		5.7	6.1	7.2	8.1	8.8	
	Range		4.8-6.7	5.5-6.5	6.3-8.7	7.3-9.1	8.4-9.3	
	Mean height/diameter ratio		0.40	0.41	0.42	0.42	0.44	
	Range		0.37-0.45	0.36-0.46	0.37-0.47	0.39-0.47	0.40-0.46	
Population IV	Number of snails in group	0	0	0	2	1	7	4
	Mean diameter				15.9	17.5	18.2	18.5
	Range				13.8-18.0	-	16.8-19.6	17.3-19.5
	Mean height				7.0	8.5	8.8	9.2
	Range				6.9-7.1	-	8.1-10.5	8.6-9.7
	Mean height/diameter ratio				0.45	0.49	0.49	0.49
	Range				0.39-0.50	-	0.46-0.59	0.47-0.51

All measurements are to the nearest 0.1 mm

TABLE 9

Results of the Analysis of Variance Tests On Groups of Snails Which Had 4.5 Whorls

	Populations compared	Probability	F value	Degrees of freedom
Height/diameter ratio	I,II,III	<0.01	17.70	2 & 17
Diameter	I,II,III	0.02	4.70	2 & 17
Height	I,II,III	<0.01	13.83	2 & 17

TABLE 10

Results of the Modified Duncan Multiple Range Tests Using Data From the Analysis of Variance Tests in Table 9. Any Means Not Underscored by the Same Line Are Significantly Different At the 0.05 Level, and Any Means That Are Underscored by the Same Line Are Not Significantly Different At the 0.05 Level

Population	III	II	I
Mean height/diameter	0.40	0.51	0.53
Population	II	III	I
Mean diameter	11.1	14.6	14.9
Population	II	III	I
Mean height	5.6	5.7	7.9

whorls. All three analysis of variance tests indicated a highly significant difference between the samples tested ($P \leq 0.02$). The modified Duncan Multiple Range Tests showed that sample III was significantly different from other samples in the tests, in terms of the height/diameter ratio but not the height nor the diameter. Sample II was significantly different from samples I and III in terms of diameter but not in terms of the height nor the height/diameter ratio. Sample I was significantly different from samples II and III in terms of height, but not in terms of the diameter nor the height/diameter ratio. All of the results of the modified Duncan Multiple Range Tests were significant at the .05 level.

In Tables 11 and 12 are shown the results of the analysis of variance tests and modified Duncan Multiple Range Tests for groups of snails that had 4.8 whorls. Again, all of the analysis of variance tests showed a highly significant difference between the samples tested ($P < 0.01$). The modified Duncan Multiple Range Tests showed that sample III was significantly different from the other samples in the tests in terms of the height/diameter ratio and diameter, but not in terms of the height. Sample II was significantly different from the other samples only in terms of the height. Sample I was significantly different from the other samples tested on the basis of diameter and height, but not on the basis of the height/diameter ratio. The significance level for these modified Duncan Multiple Range tests was .05.

The results of the analysis of variance tests and the modified Duncan Multiple Range tests for groups of snails that had 5.0 whorls are shown in tables 13 and 14. On the basis of height, the analysis

TABLE 11

Results of the Analysis of Variance Tests On Groups of Snails Which Had 4.8 Whorls

	Populations compared	Probability	F value	Degrees of freedom
Height/diameter ratio	I,II,III	<0.01	30.29	2 & 24
Diameter	I,II,III	<0.01	29.36	2 & 24
Height	I,II,III	<0.01	9.76	2 & 24

TABLE 12

Results of the Modified Duncan Multiple Range Tests Using Data From the Analysis of Variance Tests in Table 11. Any Means Not Underscored by the Same Line Are Significantly Different At the 0.05 Level, and Any Means That Are Underscored by the Same Line Are Not Significantly Different At the 0.05 Level

Population	III	II	I
Mean height/diameter ratio	0.41	0.49	0.52
Population	II	I	III
Mean diameter	12.2	13.7	15.1
Population	II	III	I
Mean height	5.9	6.1	7.2

TABLE 13

Results of the Analysis of Variance Tests On Groups of Snails Which Had 5.0 Whorls

	Populations compared	Probability	F value	Degrees of freedom
Height/diameter ratio	I,II,III,IV	<0.01	11.87	3 & 58
Diameter	I,III,IV	0.06	3.01	2 & 51
Height	II,III,IV	0.80	0.23	2 & 39

TABLE 14

Results of the Modified Duncan Multiple Range Tests Using Data From the Analysis of Variance Tests in Table 13. Any Means Not Underscored by the Same Line Are Significantly Different At the 0.05 Level, and Any Means That Are Underscored by the Same Line Are Not Significantly Different At the 0.05 Level

Population	III	IV	II	I
Mean height/diameter	0.42	0.45	0.51	0.51
Population	IV	I	III	
Mean diameter	15.9	16.6	17.2	
Population	II	IV	III	
Mean height	6.9	7.0	7.2	

of variance tests indicated that samples II, III, and IV had a very high probability that they came from the same population (P=.80). Analysis of variance indicated no significant difference between samples I, III, and IV on the basis of diameter, however, the probability was low (P=.06). The analysis of variance test on the basis of the height/diameter ratio indicated a highly significant difference among samples I, II, III, and IV ($P<.01$). The modified Duncan Multiple Range tests confirmed the analysis of variance tests in table 13. There was no significant difference between samples tested in terms of height or diameter. However, sample III was significantly different from all of the other samples except sample IV in terms of height/diameter ratio. There was no significant difference between samples I, II, and IV on the basis of the height/diameter ratio. Again, the significance level for the Duncan Multiple Range tests was .05.

Tables 15 and 16 show the results of the analysis of variance tests and the modified Duncan Multiple Range tests for groups of snails that had 5.5 whorls. The analysis of variance tests for diameter and for the height/diameter ratio show a significant difference between the samples tested ($P\leq.03$). In terms of the height of the shell, the analysis of variance test indicated no significant difference between the samples compared, but at a low probability (P=.08). The modified Duncan Multiple Range test for height of shell indicated no significant difference between the samples that were tested. In terms of diameter, only sample IV was significantly different from the other samples tested. On the basis of the height/diameter ratio, sample III was significantly different from sample I but not from sample IV. As in previous modified Duncan Multiple Range tests, the

TABLE 15

Results of the Analysis of Variance Tests On Groups of Snails Which Had 5.5 Whorls

	Populations compared	Probability	F value	Degrees of freedom
Height/diameter ratio	I,III,IV	0.03	4.85	2 & 11
Diameter	I,III,IV	0.02	5.66	2 & 11
Height	I,III,IV	0.08	3.14	2 & 11

TABLE 16

Results of the Modified Duncan Multiple Range Tests Using Data From the Analysis of Variance Tests in Table 15. Any Means Not Underscored by the Same Line Are Significantly Different At the 0.05 Level, and Any Means That Are Underscored by the Same Line Are Not Significantly Different At the 0.05 Level

Population	III	IV	I
Mean height/diameter	0.44	0.49	0.52
Population	IV	I	III
Mean diameter	18.2	19.0	20.1
Population	IV	III	I
Mean height	8.8	8.8	9.9

significance level was .05.

Measurements of the Reproductive Tract

The results of the measurements of the reproductive tracts are shown in table 17. All of these measurements were made on preserved specimens, and therefore in adults were much shorter than expected from the measurements of freshly killed specimens. However, the relative size of the different parts of the reproductive tract that were measured fit the key given by Pilsbry (1939). That is, the internally plicate part of the penis is "decidedly less than half the entire length of the penis," and "the epiphallus is much shorter than the penis." Measurements of the diameter of embryos found in the uterus were not affected by the preservative.

In sample I snails, the length of the penis ranged from 5.0 to 7.5mm with a mean length of 5.9mm. The measurements of the internally plicated part of the penis, in this group of snails, ranged from 1.0 to 2.3mm and had a mean of 1.6mm. The mean of the epiphallus measurements was 2.8mm from a range of 2.0 to 3.9mm.

The range of the length of penes for sample II was 3.5 to 5.3mm with a mean of 4.5mm. The length of the internally plicate part of the penis ranged from 1.2 to 2.0mm with a mean of 1.6mm. Epiphallus measurements ranged from 1.7 to 2.6mm with a mean of 2.1mm.

The length of the penes in sample III had a mean value of 5.7mm from a range of 5.0 to 6.5mm. The mean length of the internally plicate part of the penis in this group was 2.1mm on a range of 1.9 to 2.5mm. The epiphallus measurements had a range of 2.1 to 2.6mm for a mean of 2.4mm.

TABLE 17

Reproductive Tract Measurements

Population	Snail number	Length of penis	Length of internally plicate part of penis	Length of epiphallus	Length of penial retractor muscle	Origin of penial retractor muscle	Number of young	Size of young
I	B	7.5	1.5	2.4	4.4	JEP	11	1.2,1.5,1.4,1.6,1.4 1.5,1.3,1.7,1.7,1.6 1.8
I	E	5.5	2.3	2.6	2.6	"	0	----------
I	4	5.8	2.0	3.0	1.8	"	7	1.0,1.0,1.3,1.0,1.3 1.1,0.9
I	8	5.0	1.5	2.8	3.8	"	5	1.2,0.8,1.2,1.1,1.3
I	23	6.5	1.0	3.9	3.3	"	10	1.4,1.3,1.3,1.4,1.2 1.3,1.7,1.6,1.0,1.4
I	37	5.0	1.5	2.0	1.7	"	3	1.2,0.9,0.9
II	108	3.5	1.2	1.7	2.7	"	0	----------
II	111	5.3	2.0	1.9	1.1	"	4	1.7,1.3,1.6,1.8
II	114	5.0	1.5	2.3	2.1	"	1	1.7
II	121	5.0	1.8	2.6	*	"	5	0.5,2.0,1.8,1.5,1.9
II	122	4.7	**	2.5	1.1	"	4	1.1,1.5,1.3,1.3
II	126	3.6	1.7	1.8	0.8	"	0	----------
III	203	5.0	**	2.6	3.1	"	5	2.0,1.6,1.6,1.8,1.7
III	213	reproductive system lost						
III	215	5.2	2.5	2.4	1.6	"	2	1.9,2.1
III	226	6.2	1.9	2.5	2.0	"	3	0.9,0.7,0.7
III	233	6.5	2.1	2.5	3.9	"	6	1.6,1.7,1.9,1.8,1.8 1.8
III	249	5.7	1.9	2.1	3.6	"	2	1.4,0.8
IV	308	5.3	1.0	2.1	1.5	"	4	3.1,2.7,2.8,1.7
IV	309	6.3	2.2	2.8	1.5	"	3	3.6,3.6,3.7
IV	310	6.6	2.0	2.8	1.4	"	0	----------
IV	312	3.9	1.3	1.7	0.6	"	3	3.5,3.0,3.1
IV	313	6.4	1.9	3.8	1.5	"	3	3.3,3.1,3.2
IV	314	6.5	1.8	2.3	1.8	"	5	3.5,3.3,3.5,3.4,3.2

JEP = junction of the epiphallus and the penis
* retractor muscle lost during dissection
** anterior end of penis retracted into the posterior end of the penis

Sample IV snails had a mean penis length of 5.8mm with a range of 3.9 to 6.6mm. The range of the lengths of the internally plicate part of the penis was 1.0 to 2.2mm with a mean of 1.7mm. The range of measurements for the length of the epiphallus was 1.7 to 3.8mm with a mean of 2.6mm.

One of the taxonomic characteristics of the subgenus Oreohelix is that the penial retractor muscle must have its origin at the junction of the epiphallus and the penis (Pilsbry, 1939). This was found to be true in all of the snails examined (see table 17).

Appendix B contains drawings of the reproductive tracts of the snails used to obtain the above measurements. The camera lucida drawings were made before the measurements were taken. The reproductive tracts were positioned to show as many of the different parts as possible (see fig. 4).

Radular Tooth Formulae

Table 18 shows the results of counting the teeth per row on the radulae of six snails from each sample. In sample I snails the teeth per row ranged from 50 to 57 with a mean value of 53. The range of teeth per row for sample II was 44 to 53 with a mean of 50. The mean number of teeth per row in sample III was 55 with a range of 51 to 58. In sample IV snails counted the mean was 59 teeth per row with a range of 55 to 62 teeth per row.

The radular tooth formulae for sample I ranged from 25-1-24 to 28-1-28. This type of formula represents the number of teeth on either side of the central tooth, and the central tooth itself. The average tooth formula for sample I was 26-1-26. The radular

Figure 4. Genitalia of snail 249 from population III. Abbreviations: al- albumin gland, e- epiphallus, hd- hermaphorditic duct, p- penis, pr- penial retractor, pg- prostrate gland, sp- spermatheca, spd- spermathecal duct, t- talon, u- uterus, v- vagina, vd- vas deferens.

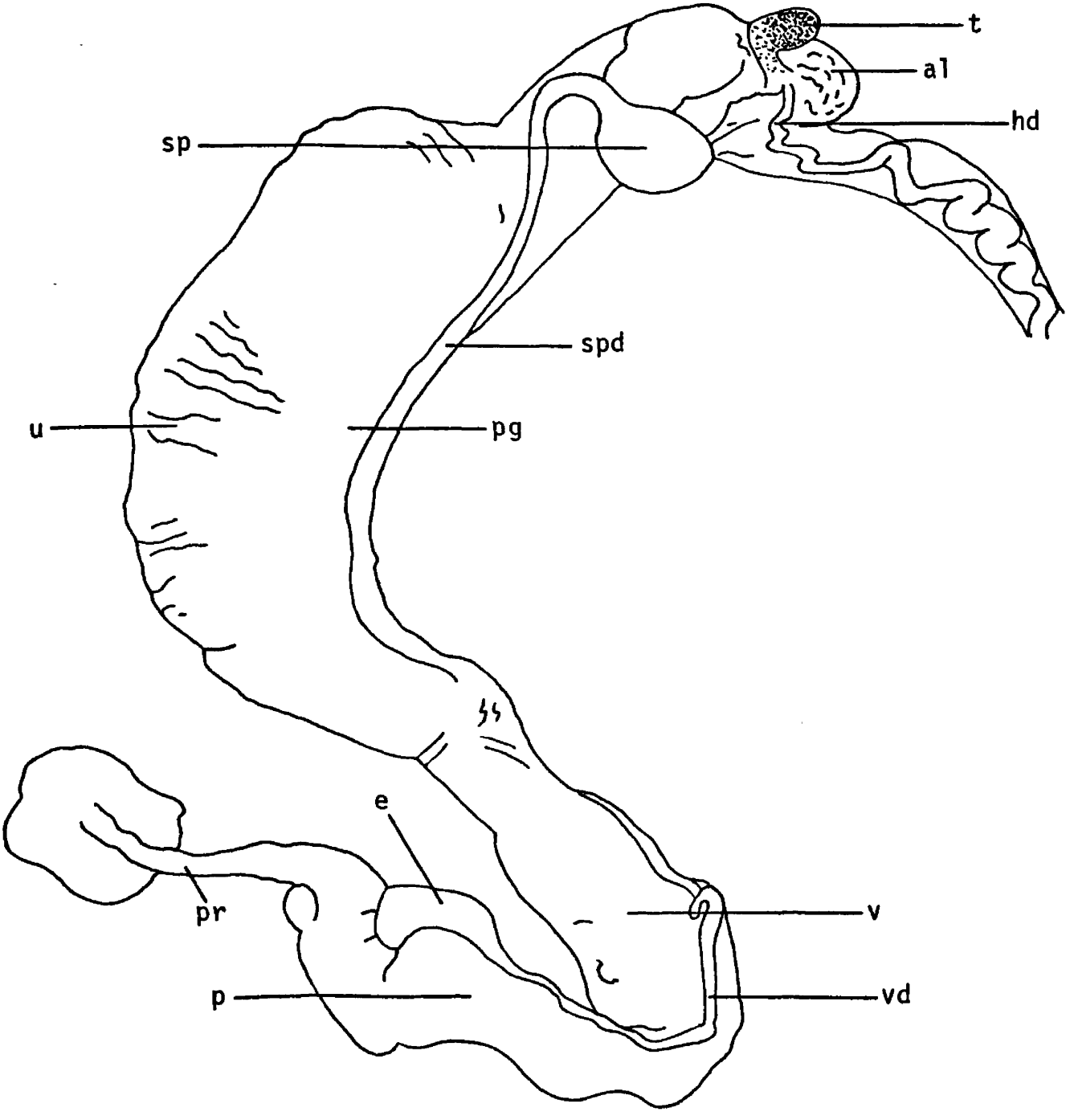


TABLE 18

Radular Tooth Formulae

Population	Snail number	Number of teeth per row	Tooth formula
I	B	51	25-1-25
I	E	57	28-1-28
I	4	53	26-1-26
I	8	55	27-1-27
I	23	54	27-1-26
I	37	50	25-1-24
II	108	49	24-1-24
II	111	53	26-1-26
II	114	53	26-1-26
II	121	44	22-1-21
II	122	*	*
II	126	49	24-1-24
III	203	51	30-1-30
III	213	58	29-1-28
III	215	57	28-1-28
III	226	53	26-1-26
III	233	55	27-1-27
III	249	53	26-1-26
IV	308	61	30-1-30
IV	309	59	29-1-29
IV	310	62	30-1-31
IV	312	55	27-1-27
IV	313	62	31-1-30
IV	314	56	28-1-27

* radula was badly folded in the slide and could not be counted

tooth formulae for sample II ranged from 22-1-21 to 26-1-26. The average tooth formula for sample II was 25-1-24. For sample III the range of the radular formulae was 26-1-26 to 30-1-30, with the average formula 27-1-27. The average tooth formula for sample IV was 29-1-29. This was from a range in formulae of 27-1-27 to 30-1-31.

In appendix C are the results of drawings of the teeth in one row of the radula of each snail numbered in table 18. The teeth shown are those that represent the different shapes seen in one row of teeth from the central tooth to one edge of the radula. Teeth near the posterior end of the radula were drawn to ensure that the drawings would show unworn shape and denticulation.

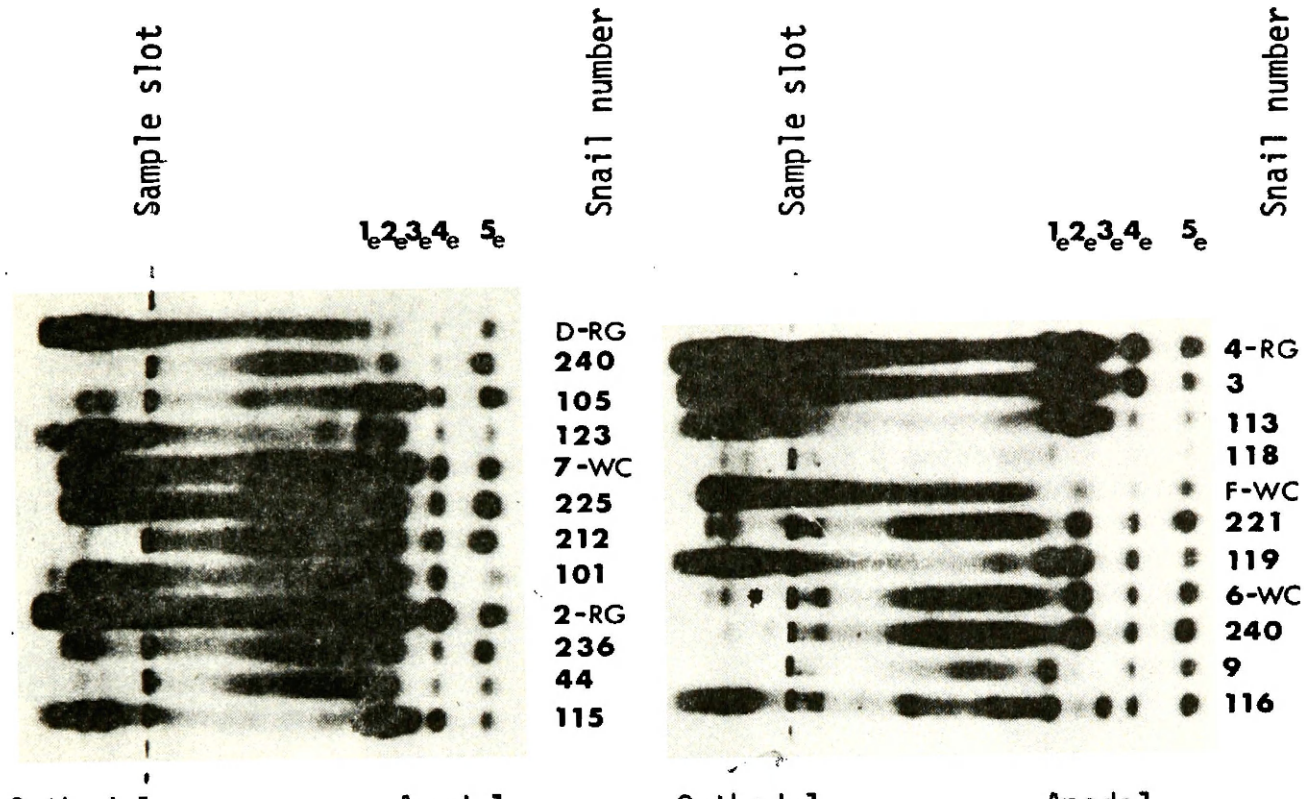
Electrophoresis

A total of fifteen different esterases were easily identifiable from seven different gels (see figs. 5 and 6). A maximum of five esterases were shown to have migrated cathodally. This indicated a net positive charge for each of these groups of enzymes. The other ten esterases which were identified, migrated toward the anode. This indicated a net negative charge.

After careful examination of the gels, it was decided that the last five cross bands that were visible anodally would be used in comparing populations. The reason for this was that these five cross-bands were the only ones that appeared in the same place in every gel, and were clear enough to be usable for comparisons between populations.

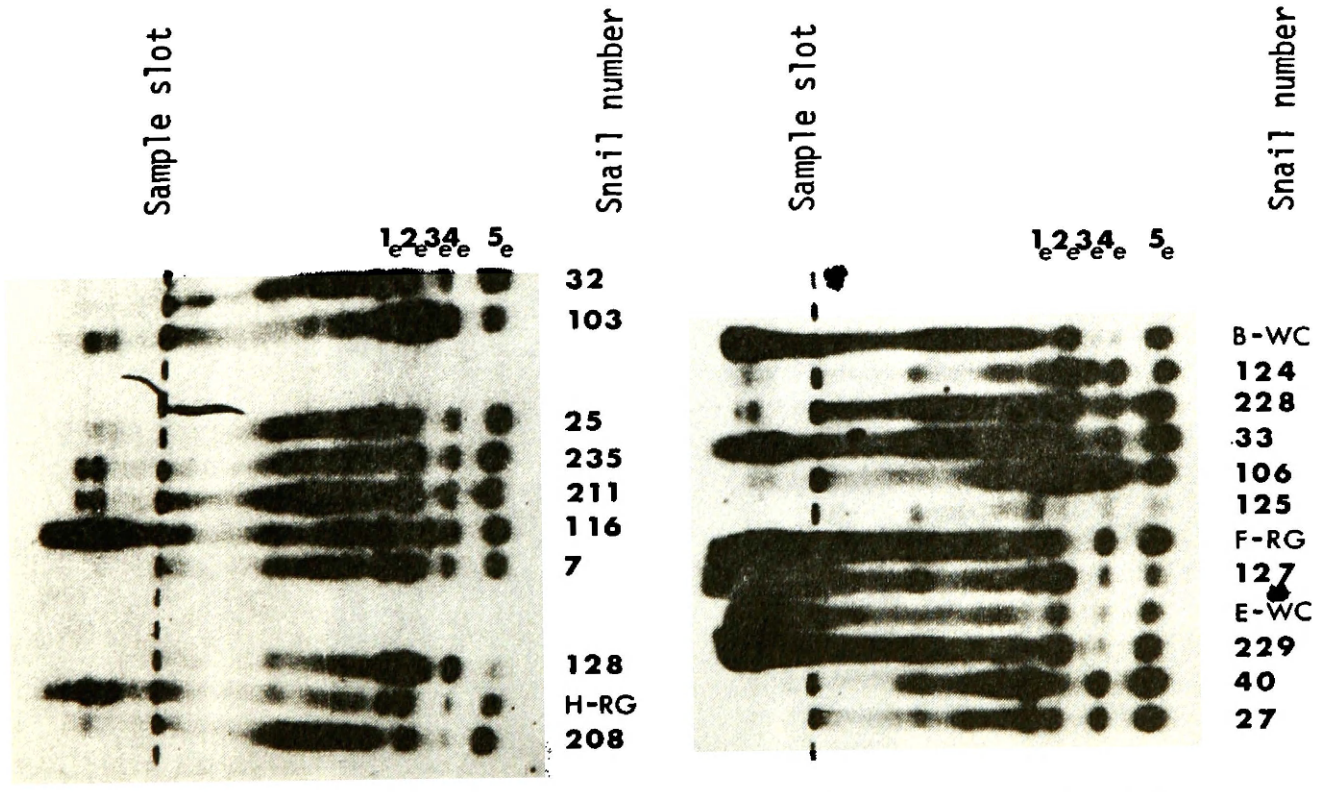
The band nearest the anode was designated 5_e, the next nearest band was designated 4_e, and so forth to 1_e (see fig. 7).

Figure 5. Stained electrophoretic gels



a. Gel of 6-15-74 chamber 2

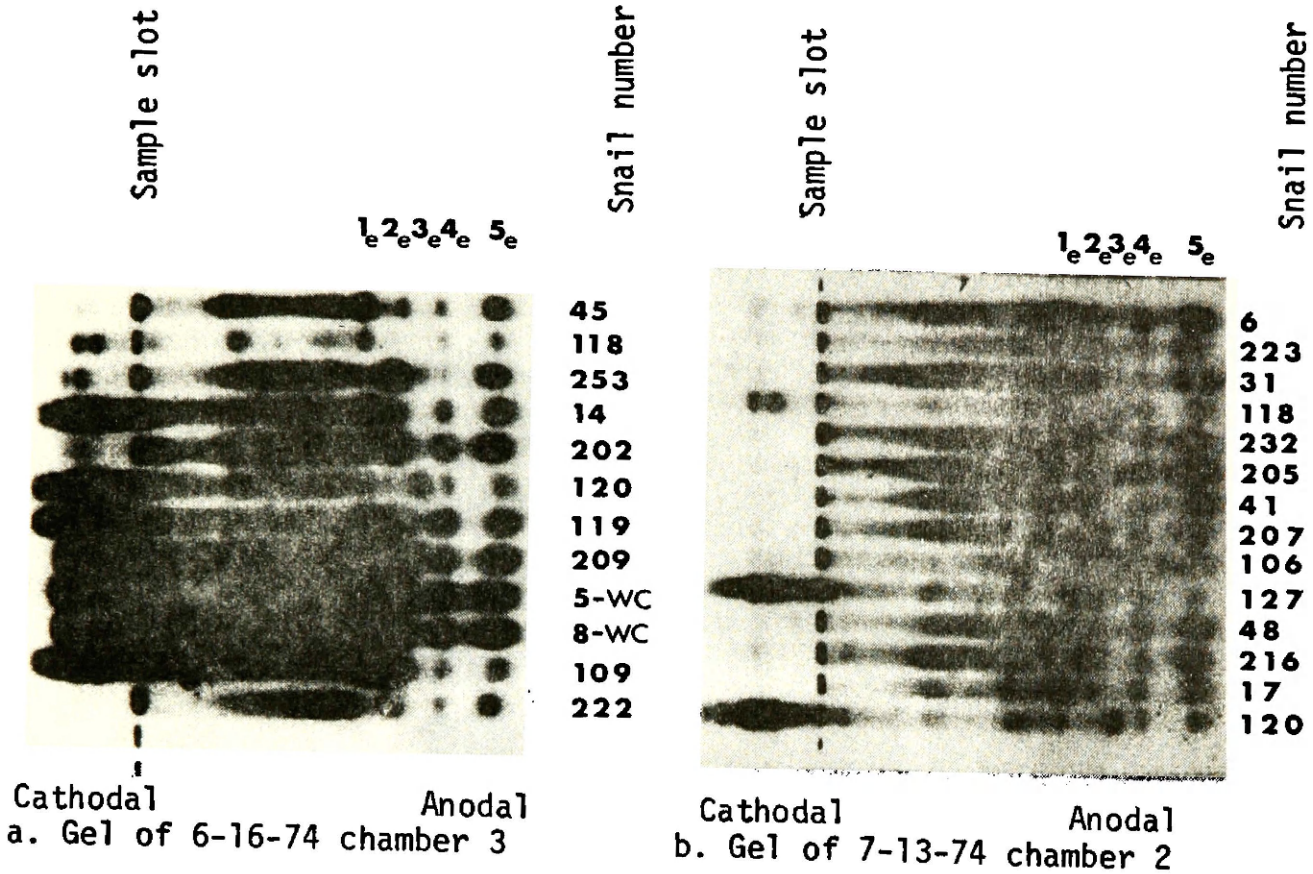
b. Gel of 6-15-74 chamber 3



c. Gel of 6-16-74 chamber 1

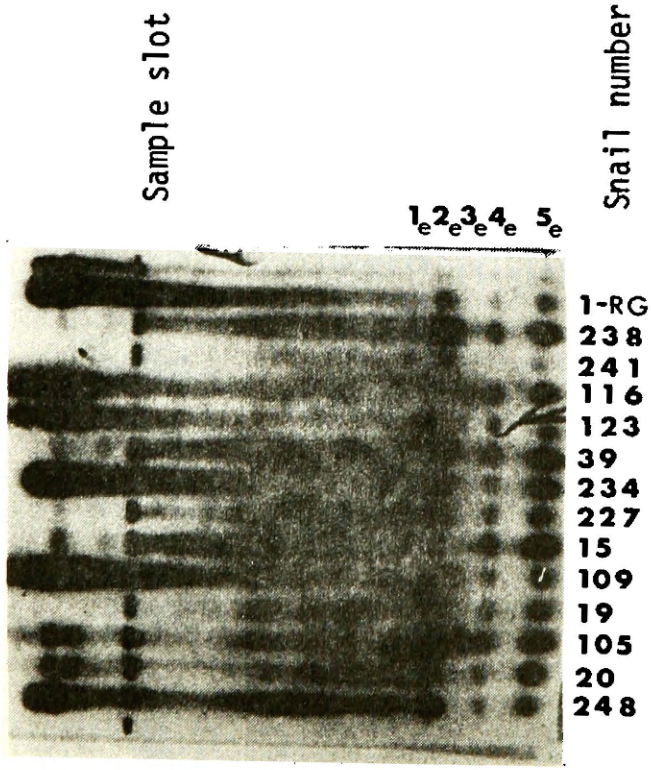
d. Gel of 6-16-74 chamber 2

Figure 6. Stained electrophoretic gels

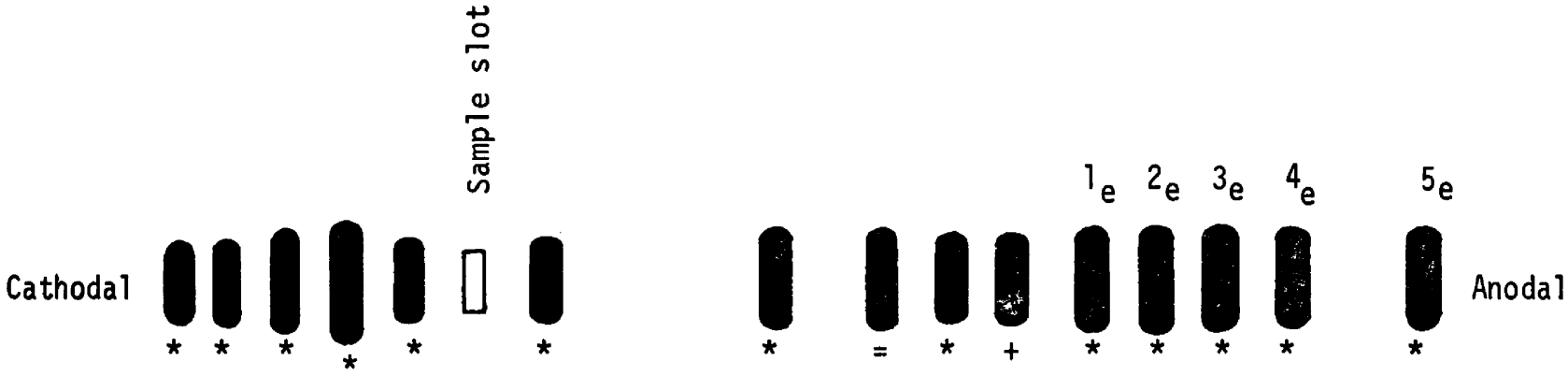


a. Gel of 6-16-74 chamber 3

b. Gel of 7-13-74 chamber 2



c. Gel of 7-13-74 chamber 3



* In sample 120 fig. 6
\+ In sample 017 fig. 6
= In sample 031 fig. 6

Figure 7. Relative positions of the 15 different esterase bands identifiable from the 7 gels (figs. 5 and 6).

Examination of the gels gave the results shown in table 19. All twenty-six specimens of sample I had esterase bands 1_e, 2_e, 4_e, and 5_e, and none had esterase band 3_e. In sample II ten specimens had esterase bands 1_e, 2_e, 3_e, 4_e, and 5_e, however, 6 specimens had only 1_e, 2_e, 4_e, and 5_e. Sample III had esterase bands 2_e, 3_e, 4_e, and 5_e. As in sample II, two patterns were shown: 2_e, 3_e, 4_e, 5_e or 2_e, 4_e, 5_e. Six specimens had the 2_e, 3_e, 4_e, 5_e pattern and 24 had the 2_e, 4_e, 5_e pattern. However, neither of these patterns were found in samples I and II.

TABLE 19

Esterase Patterns of Cathodal Migrating Enzymes $1_e 2_e 3_e 4_e 5_e$

Population I		Population II		Population III	
Bands	$1_e 2_e 3_e 4_e 5_e$	Bands	$1_e 2_e 3_e 4_e 5_e$	Bands	$1_e 2_e 3_e 4_e 5_e$
Snail		Snail		Snail	
D	* * - * *	101	* * * * *	B1	- * * * *
F	* * - * *	103	* * * * *	E1	- * - * *
H	* * - * *	105	* * * * *	F1	- * - * *
I	* * - * *	106	* * * * *	5WC	- * - * *
1	* * - * *	109	* * * * *	8WC	- * - * *
2	* * - * *	113	* * * * *	202	- * - * *
03	* * - * *	115	* * * * *	205	- * - * *
4	* * - * *	116	* * * * *	207	- * - * *
06	* * - * *	118	* * - * *	208	- * - * *
07	* * - * *	119	* * - * *	209	- * * * *
09	* * - * *	120	* * * * *	211	- * - * *
014	* * - * *	123	* * - * *	212	- * - * *
015	* * - * *	124	* * * * *	216	- * - * *
017	* * - * *	125	* * - * *	221	- * - * *
019	* * - * *	127	* * - * *	222	- * - * *
020	* * - * *	128	* * - * *	223	- * - * *
025	* * - * *			225	- * - * *
027	* * - * *			227	- * - * *
031	* * - * *			228	- * - * *
032	* * - * *			229	- * * * *
033	* * - * *			232	- * * * *
039	* * - * *			234	- * - * *
040	* * - * *			235	- * - * *
041	* * - * *			236	- * - * *
044	* * - * *			238	- * - * *
045	* * - * *			240	- * - * *
048	* * - * *			241	- * - * *
				248	- * - * *
				253	- * * * *
				7WC	- * * * *
				6WC	- * - * *

* band present in the pattern of the designated snail
- band not present in the pattern of the designated snail

CHAPTER IV

DISCUSSION

"Explorations in Colorado, Utah, Montana, and Idaho brought to light numerous local forms of Oreohelix haydeni which have been named subspecies. Most of them are highly variable. Their territory has been carefully worked only in widely separated places. It is to be anticipated that some subspecific distinctions now drawn with difficulty may in the future prove subject to change; yet the distribution of the haydeni group of forms will probably remain conspicuously discontinuous, and our present knowledge of the subject seems most easily expressed by recognition of the numerous named races" (Pilsbry, 1939).

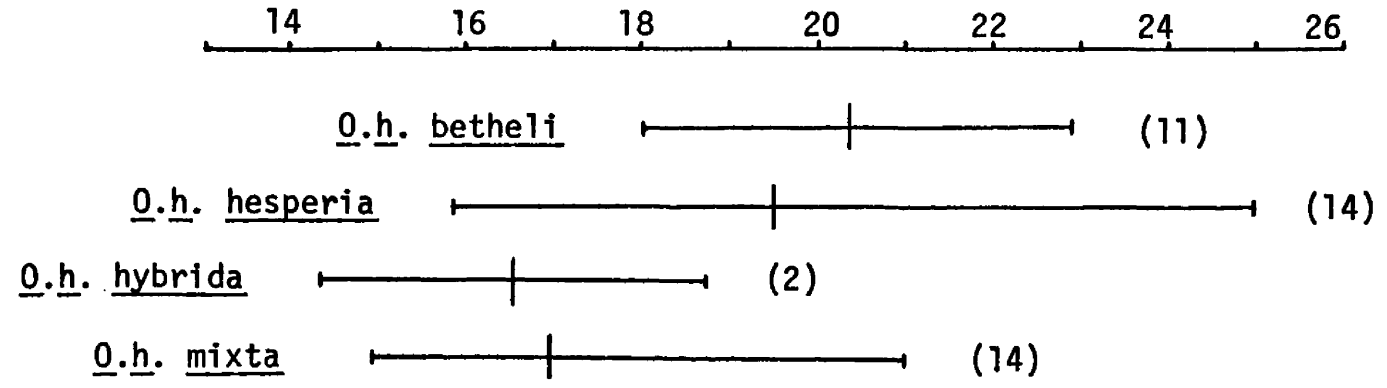
All of the subspecies of Oreohelix haydeni were described before 1940. Most of them were described between 1886 and 1916. All of their descriptions (Pilsbry, 1939) address several characteristics which are discussed in comparative terms, ie.: more depressed, not as depressed as, of greater caliber than, umbilicus narrower than, and so forth. Nearly all of the descriptions have several characteristics in common, ie, descriptive terms indicating width and height of a carination (strong or weak); descriptive terms to describe the height/diameter ratio (depressed spire, spire elevated; flattened spire); description of the whorl shape (whorls convex; whorls flattened above, rounded beneath; globose whorls); the number of times the umbilicus

diameter can be divided into the shell diameter; number of color bands (Pilsbry, 1939). Apparently, establishing a new subspecies was a "matter of judgement rather than of demonstration" (Mayr, 1969). In view of the fact that the high degree of variation of shell characteristics within the species of this genus was known, it seems surprising that so many subspecies were named. The amount of inconclusive subjectivity involved in some of these designations is evidenced in the following two examples.

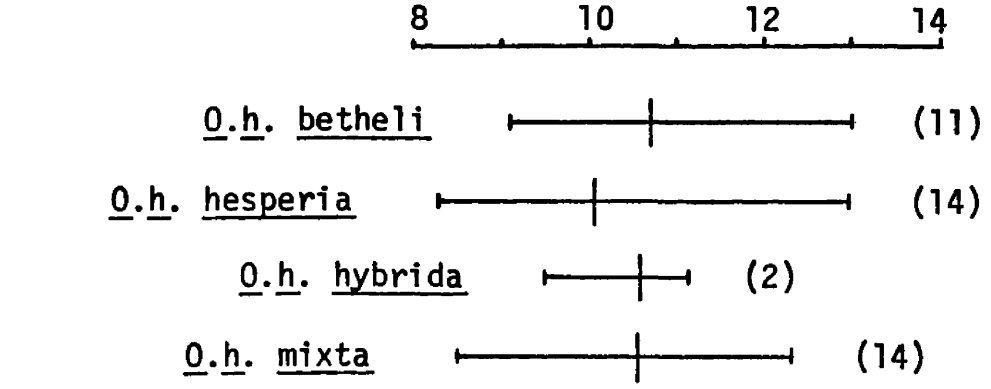
Oreohelix haydeni betheli Pilsbry and Cockerell was described in 1913 and O. h. hesperia Pilsbry was described in 1939. Both subspecies have "strong ribs," two color bands, one, or none, both of these subspecies appear more depressed than O. haydeni, and the division of the umbilicus diameter into the shell diameter results in similar values (4-5 vs 3 2/3 - 4 1/3). O. h. betheli was described as having "great variation in size, umbilicus, and sculpture," (Pilsbry, 1939, p. 473). The description of O. h. hesperia included the statement "many specimens of this race do not appear distinguishable from the most strongly sculptured shells of O. h. betheli" (Pilsbry, 1939, p. 475). Figure 8 shows a comparison of the diameters, heights, and height/diameter ratios of specimens from these two subspecies. Although this comparison was based on only a few specimens (11 and 14), the similarity of the two is apparent.

Oreohelix haydeni hybrida Hemphill was described in 1890, and O. h. mixta Pilsbry was described in 1916. Both of these subspecies had "weak ribs" two color bands to none, the umbilicus diameter divided into the shell diameter resulted in similar values (5½-6 vs 6 and less),

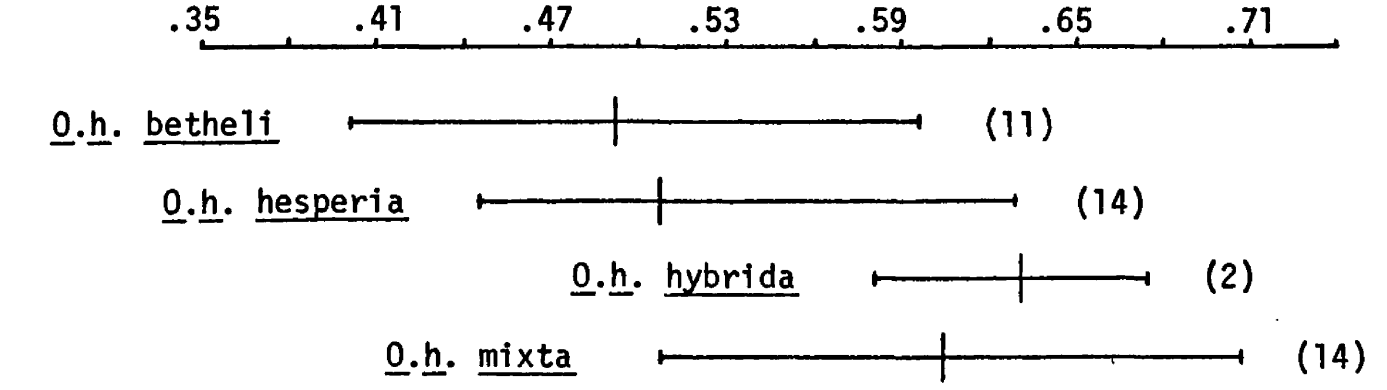
Figure 8. Comparisons of four subspecies of Oreohelix haydeni in terms of their shell diameter, shell height, and height/diameter ratio. Numbers in parentheses are the number of snails compared in each group. Numbers on the scales in a. and b. represent millimeters.



a. Shell diameter



b. Shell height



c. Height/diameter ratio

and the whorls were similarly shaped. In addition, O. h. mixta was said to resemble O. h. hybrida in shape. Figure 8 shows a comparison of these two subspecies, again in terms of diameter, height, and height/diameter ratio. The Oreohelix haydeni hybrida values are based on measurements from only two specimens. For that reason no definite conclusions can be drawn. It appears, however, that O. h. hybrida and O. h. mixta are very similar. The only major point of difference between these two subspecies is location. Taxonomists agree, however, that geographic information alone is not a sufficient basis on which to describe a subspecies (Doyen and Slobodchikoff, 1974; Edward, 1954; Mayr, 1969).

In this study, four populations of O. haydeni are compared. All of these populations are located in Western Montana, and none of them has been described previously. The nearest previously described subspecies of O. haydeni, O. h. oquirrhensis is located at the old Byrne Resort at Bearmouth hotsprings. This is approximately ten miles west of the locations of populations I and II.

Eight meristic characteristics, three morphological characteristics, and one biochemical characteristic were compared in this investigation. The biochemical characteristic compared was esterase pattern as shown by starch-gel electrophoresis of serum prepared from head and foot tissue. The morphological characteristics compared were genitalia morphology, radular morphology, and color bands. The meristic characteristics compared were shell-size measurements and sculpture.

On the basis of certain meristic characters, two population samples could be statistically separated from the other two samples

and from each other. Sample II was clearly distinguishable from the other samples on the basis of smaller shell diameter and height (table 7). The height/diameter ratio and height of population sample III made it possible to separate this sample from the others (table 7). Comparisons among these population samples involving carinations show varying degrees of difference (table 7), however, the amount of overlap in number for these characteristics was very large and therefore their value as distinctive characteristics was reduced. Difference in whorl size among all of the samples appeared not to be significant (table 5). The aperture size reflected the shape of the shell, ie; depressed in sample III and proportionately smaller in sample II as compared to samples I and IV.

Population samples I and IV were difficult to separate. Their diameters, heights, and height/diameter ratios were quite similar (table 7). Whorl size and number of carinations (table 5) were also closely comparable.

When the four samples were divided into groups based on whorl size, the statistical comparisons appeared to be about the same. Sample II appeared separable on the basis of diameter at all whorl sizes tested (tables 10 and 12). Sample III appeared separable on the basis of height/diameter ratio at most whorl sizes. Samples I and IV were separable at some whorl sizes but not at others. It appeared that the conclusions drawn on the basis of the entire sample were true for groups of the same whorl size. The number of carinations based on whorl size were not compared because the majority of carinations could be followed back several whorls on nearly every shell.

The use of the reproductive tract for classification purposes

appeared to have limited application in Oreohelix. Pilsbry (1939) studied the then 27 species and 47 subspecies of the subgenus Oreohelix. A close examination of most of these taxa show that they could be placed in only three different groups on the basis of the genitalia.

The group in which Oreohelix haydeni is placed is named the strigosa group. The characteristics of this group, in terms of the genitalia, are: epiphallus much shorter than the penis; internally plicate part of the long penis decidedly less than half of the entire length; viviparous (Pilsbry, 1939). This study examined 24 of the 155 specimens that were collected. In all cases, the genitalia were as described by Pilsbry, and they appeared qualitatively similar.

Appendix B shows camera lucida drawings made of the genitalia of six snails from each population that was studied. The drawings show the variation both in a population and between populations in regard to overall size, shape, and origin of the penial retractor muscle. Other differences can be ascribed to the orientation of the different parts of the genitalia at the time the drawings were made. Sample II snail genitalia were, in general, smaller than those of the other samples. This is because of the small size of the snails in this population. The genitalia from population sample IV appeared, in general, larger than the other populations. This is probably because the young that were found in the uterus of sample IV snails were nearly two times as large as those from snails in the other samples.

On the basis of this study, it appeared that there are no morphological differences in the genitalia of these four populations. This does not imply that the genitalia can not be used in classification,

it means that the genitalia can not, by themselves, be used as the distinctive characteristic, at the level of population or subspecies, in the species Oreohelix haydeni.

F. C. Baker (1928) proposed a classification of the genera of the family Lymnaeidae based largely upon the form and denticulation of the teeth of the radula.

In a study of Lymnaeidae in Western Montana, Russell (1967, p.2) stated that, "Radular formulae are never as consistant as Baker's descriptions would lead one to believe." After investigation of the Stagnicola emarginata group of the Lymnaeidae Russell (1967, p. 32) stated, "On the basis of the morphology of the genitalia and radula, I have seen no essential differences between the various members of this group which I have studied."

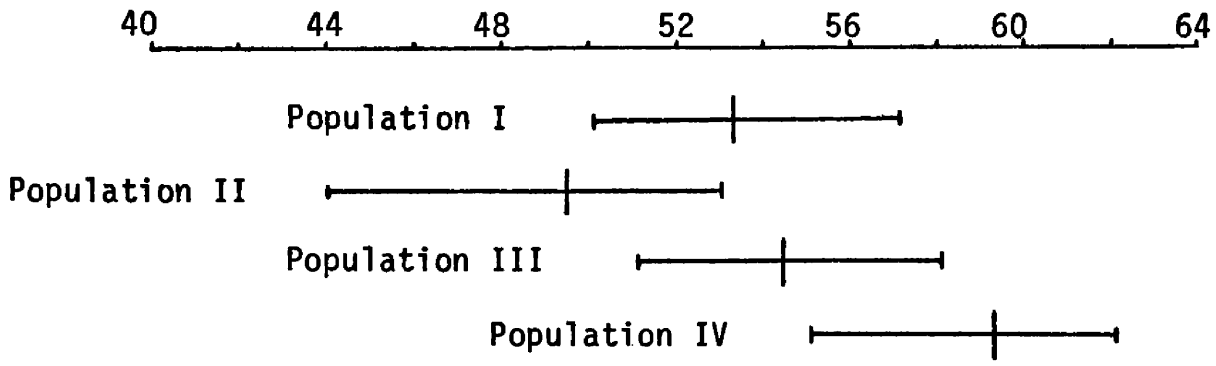
The form and denticulation of teeth from the four study populations are shown in appendix C. The considerable variation both within and between samples is readily apparent. The number of cusps on individual teeth and the position and shape of the cusps varies significantly both within populations and between populations. It is apparent that there is a general constancy of form within each sample, but certainly not to the degree that would serve as a characteristic capable of separating the populations.

Baker (1928) also noted that the radular tooth formulae are taxonomically significant at the generic level. However, Walter (1969) said, "It is probable that each population tends to have a different radular tooth formula since published knowledge indicates that the radula may grow and show an increasing tooth-count as long as the snail

continues to grow."

Of the 24 radulae mounted for this study, a comparison of the mean number of teeth per row for each population sample gave 53.3 for sample I, 49.6 for sample II, 54.5 for sample III, and 59.2 for sample IV. Two samples appeared to differ significantly from the others. However, there was a considerable degree of overlap between these samples (fig. 9). The overlap was so large that the radular tooth formula did not appear useful in discriminating one population from another.

Figure 9. The range in the number of teeth per row in the radulae of six specimens from each of the four study populations.



Because none of the specimens collected for this study displayed color banding, attempts to separate populations on this basis were useless. The lack of banding, however, may be taxonomically important.

The electrophoretic tests performed during this study showed that population sample I, in every specimen tested, had four easily identifiable esterase bands, labeled for this study 1_e, 2_e, 4_e, 5_e. Sample I did not have band 3_e in any specimen (table 19). Population sample II had two patterns. Six of sixteen (37.5%) had the same

pattern as sample I. The other ten specimens had the 3_e band. Thus, unless sample I specimens display the $1_e2_e3_e4_e5_e$ pattern, samples I and II can not be separated solely by the esterase pattern. Population III also had two esterase patterns. However, because none of the specimens from this sample had esterase band 1_e and all specimens from samples I and II did have the 1_e band, sample III could always be separated from samples I and II.

Populations I and II are located approximately one mile apart, and they are separated by the Clarks Fork River. These two populations, at some time in the past probably were able to interbreed. Since the isolation of these two populations, either a mutation causing the creation of the 3_e band in population II has occured or because of random variation the 3_e band has been lost from population I. However, because population III also has the 3_e band it seems more likely that all populations had the 3_e band and that population I has lost it via random variation.

Thus, these populations could be separated on the basis of their electrophoretic patterns. It also seemed probable that all geographically isolated populations would show a distinctive pattern (Davis and Lindsay, 1967). The ability of the electrophoretic pattern to identify a particular specimen, providing a catalogue of patterns is available, seems certain. However, it seems clear that the electrophoretic pattern can not be used as the only criterion in classification because then every geographically isolated population would be a different species or subspecies.

Although 13 characters were compared during this study, most of

them, by themselves were incapable of separating the populations. The most appropriate method for comparison of these four populations was to compare all of the parameters simultaneously. A 4X4 graph of the populations (fig. 10) tabulates the characteristics that appear significantly different.

Population II differed from the other three populations by six of seven characters, the constant ones being height and diameter of shell, width of aperture, and location. Population II is a dwarf population. Pilsbry (1910) said, "The size of snails...is almost wholly a function of the exposure. Snails living on northern or northwestern exposure are invariably larger than those from southern or eastern exposures, regardless of elevation..." Population II is located on a northern exposure and yet is the smallest population.

Population III differed from the other three populations by five of seven characters. Five characters were consistantly different: height of shell, height/diameter ratio, carinations above the periphery, electrophoretic pattern, and location. The distinctive characteristics in population III were the extremely depressed spire and the distinctive electrophoretic pattern.

Population IV differed from the other three populations by four of six characteristics. Carinations above the periphery and location were the only consistently different characteristics.

Population I differed from populations II, III, and IV by four of seven characters: carinations below the periphery and location were the only two appearing consistantly. If it can be assumed, as Davis and Lindsay (1967) propose, that all geographically isolated

Figure 10. A summary of characteristics compared between the four study populations. If a number is present in a square, it indicates that there is a difference between those two populations for that parameter.

Key:
1. Diameter of shell
2. Height of shell
3. Height/diameter ratio
4. Number of whorls
5. Width of aperture
6. Height of aperture
7. Carinations above the periphery
8. Carinations below the periphery
9. Color bands
10. Reproductive tract
11. Radular formula
12. Electrophoretic pattern (population IV not included)
13. Location

		Population			
		I	II	III	IV
Population	I		1,2,5,6,8 12*,13	2,3,6,7,8 12,13	3,7,8,13
	II			1,2,3,5,7 12,13	1,2,5,6,7 13
	III				2,3,6,7,13
	IV				

* Separation was not possible in every case

populations would show a distinctive electrophoretic pattern, then, a distinctive electrophoretic pattern for each population would increase the consistently different characteristics by one for populations I, II, and IV.

Population IV was located late in the study and the limited number of specimens were collected in the Autumn. A larger number of specimens might show more similarity between populations I and IV in terms of height/diameter ratio. In addition, the number of carinations both above and below the periphery was extremely variable (see table 5). This leaves only location and electrophoretic pattern as distinctive differences between these two populations. Thus, on the basis of this study populations II and III were distinctly different from each other and from populations I and IV. Populations I and IV did not appear separable.

Despite the differences shown between the four populations which were used in this study, the problem of whether or not to designate one, some, all, or none of these four populations as new subspecies of Oreohelix haydeni still remains. The first step is to compare each of these four populations to each of the established subspecies. Figures 11, 12, and 13 show these four populations compared with the established subspecies of O. haydeni in terms of diameter of shell, height of shell, and height/diameter ratio of the shell. Only population sample II was significantly different than the described subspecies in terms of diameter of shell. In terms of height of shell, population samples II and III appeared significantly different from the established subspecies. Population III was the only population that appeared signif-

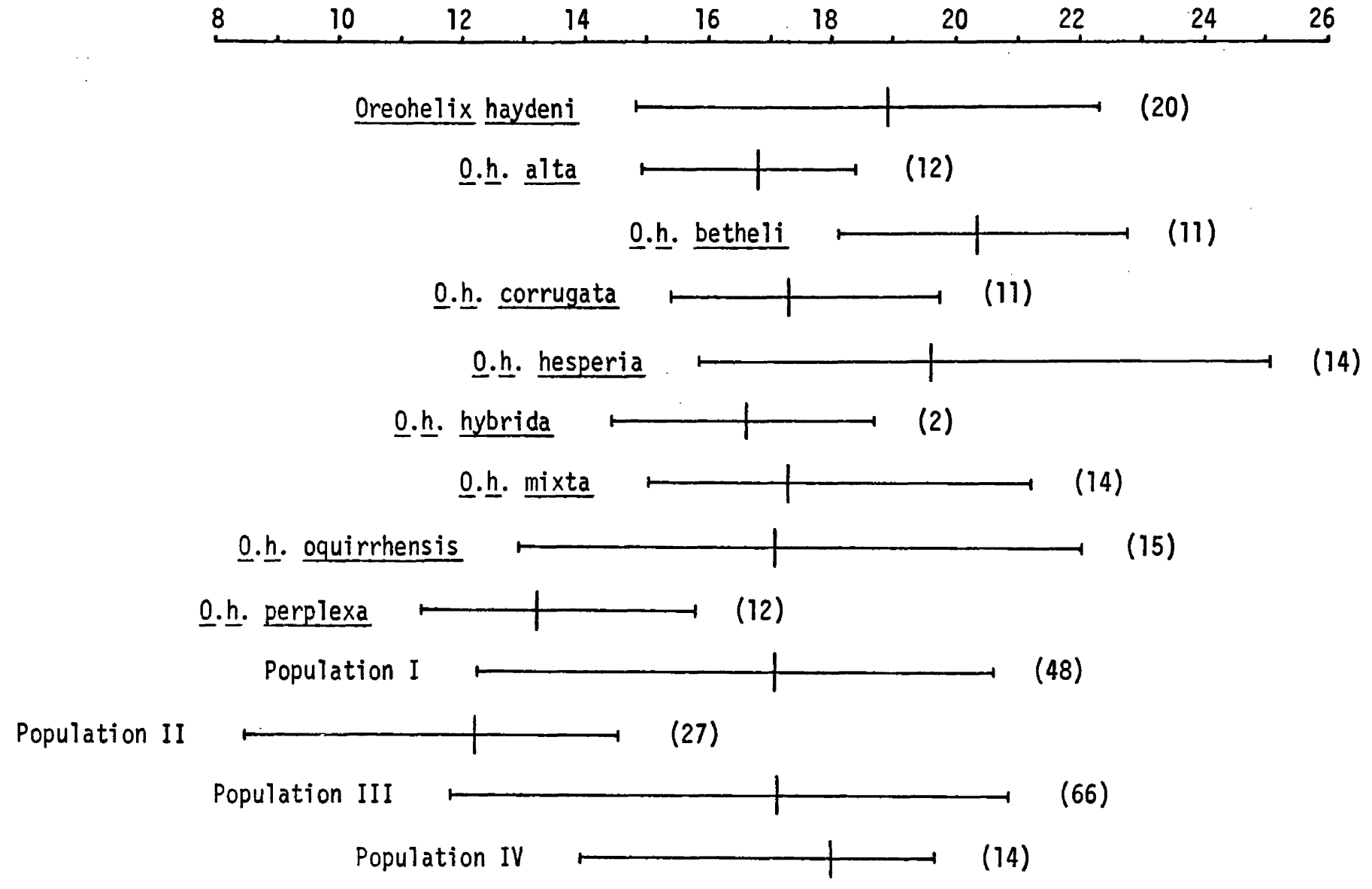


Figure 11. A comparison of the established subspecies of Oreohelix haydeni and the four study populations, in terms of shell diameter. Numbers in parentheses are the number of snails compared in each group. Numbers on the scale represent millimeters.

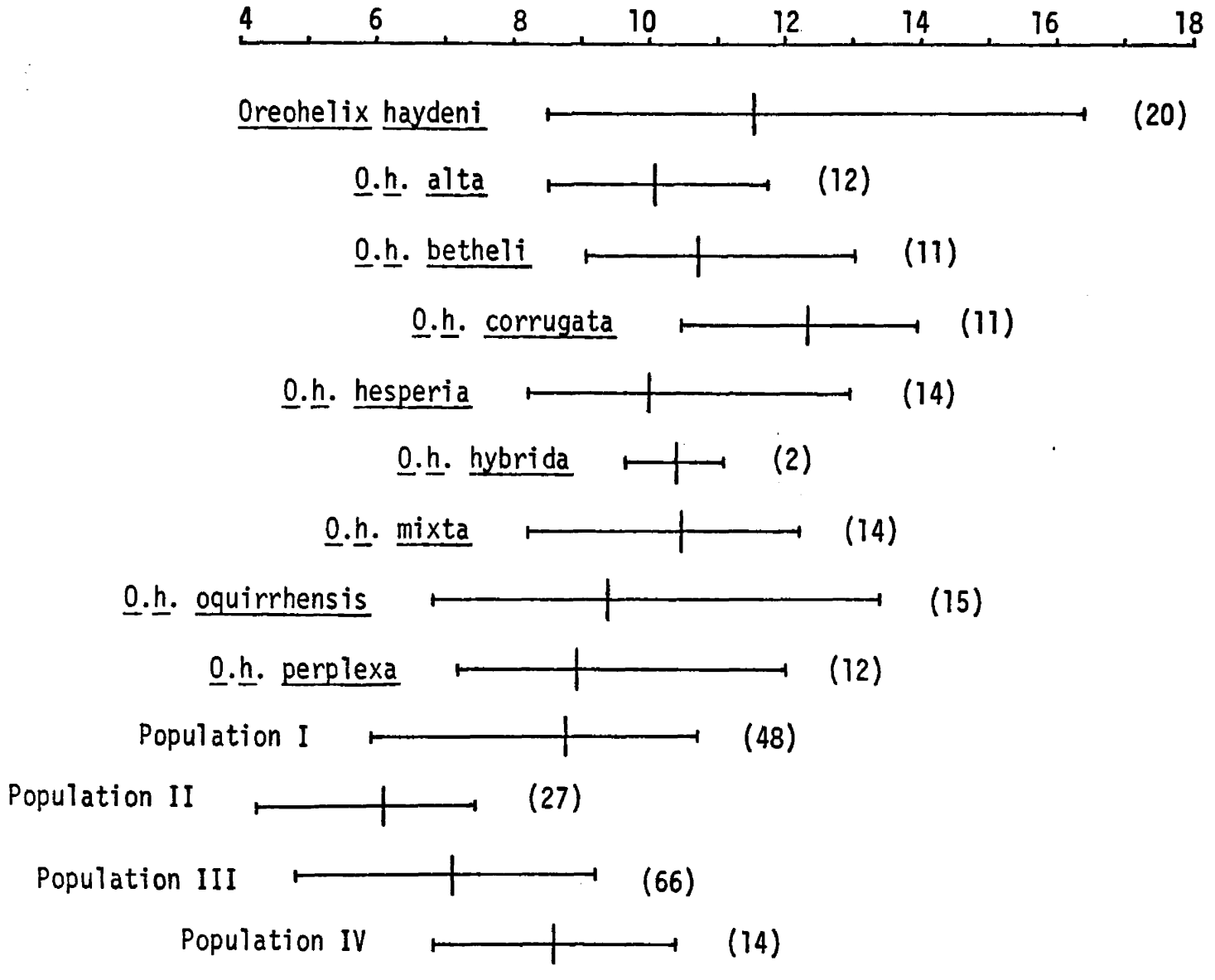


Figure 12. A comparison of the established subspecies of Oreohelix haydeni and the four study Populations, in terms of shell height. Numbers in parentheses are the number of snails compared in each group. Numbers on the scale represent millimeters.

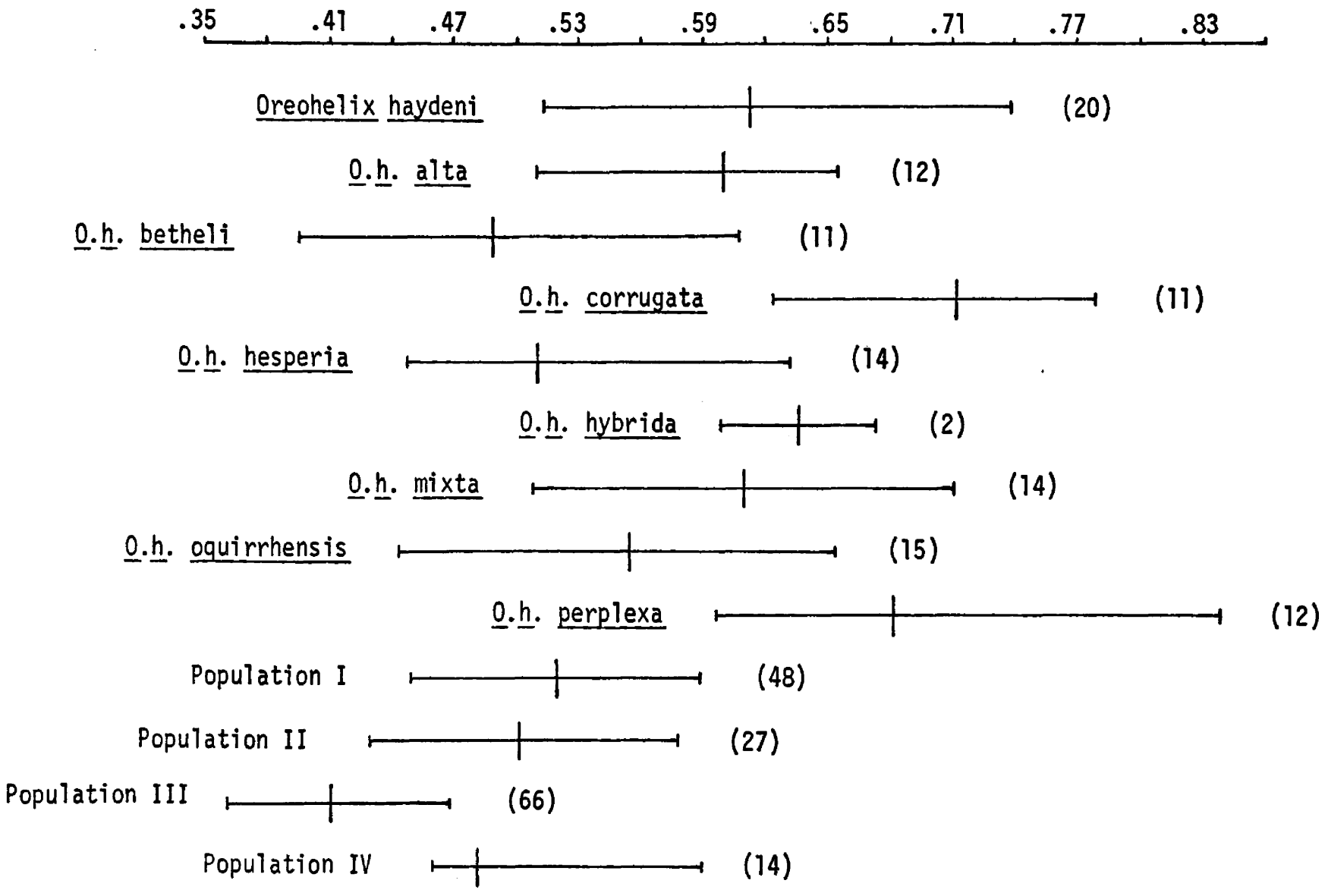


Figure 13. A comparison of the established subspecies of Oreohelix haydeni and the four study populations, in terms of the height/diameter ratio. Numbers in parentheses are the number of snails measured in each group.

icantly different than the established subspecies, in terms of height/diameter ratio. Only Oreohelix haydeni and O. h. corrugata lack color banding within the population. All of the other established subspecies display from two to zero color bands. None of the 151 specimens collected for this study displayed color banding.

When geographical location was included, there were five characters (height, diameter, height/diameter ratio, color, location) that could be compared between the established subspecies and the four new populations used in this study. If, as proposed, electrophoretic patterns are distinctive, then the electrophoretic pattern would make a sixth character. Under these conditions, population I differed from the ten established populations in only two characters: location and electrophoretic pattern. Population II differed in four characters: diameter of shell, height of shell, location, and electrophoretic pattern. Population III differed in four characteristics, also: height of shell, height/diameter ratio, location, and electrophoretic pattern. Population IV differed in two characters: location and electrophoretic pattern.

Less than a decade ago taxonomists probably would not have hesitated to designate populations II and III as new subspecies of Oreohelix haydeni. However, more contempory taxonomists might hesitate, questioning the usefulness of subspecies and the degree of difference necessary to separate two populations into subspecies.

"The subspecies designation has induced many authors to compare carefully material from every newly established locality with specimens from the type locality of a previously described subspecies. Whenever

a thorough biometric-morphological analysis established a mean difference between these samples, this was considered sufficient justification by the authors to describe a new subspecies" (Mayr, 1963, p. 347). It would seem that the larger the number of subspecies in a particular species becomes, the more difficult it becomes to designate a new subspecies. The only alternative is to use more and more arbitrary differences (Mayr, 1963).

Edwards (1954) said that taxonomists should "recognize only truly distinctive allopatric populations as subspecies." Lidicher (1962) defined a subspecies as "...a relatively homogeneous and genetically distinct portion of a species which represents a separately evolving, or recently evolved lineage with its own evolutionary tendencies, inhabits a definite geographical area, is usually at least partially isolated, and may intergrade gradually, although over a fairly narrow zone, with adjacent subspecies."

Some taxonomists consider the subspecies concept worthwhile. Durrant (1955) said, "It is the most important tool that students of evolution have from the viewpoint of geographical variation and distribution, in attempting to contribute to the understanding of what happens to animals at the level of the infraspecific categories." Fox (1955) stated, "I do not find the system of trinomial nomenclature inefficient and superfluous for reference purposes. I have found it exceedingly useful in the analysis of geographical variation as well as in the analysis of evolutionary level."

Others disagree: Wilson and Brown (1953) and Inger (1961) pointed out four aspects of the subspecies which reduce its usefulness:

1. The tendency of different characters to show independent trends of geographical variation.
2. The independent reoccurrence of similar or phenotypically indistinguishable populations in geographically separated areas.
3. The reoccurrence of microgeographic races within formally recognized subspecies.
4. The arbitrariness of the degree of distinction considered by different specialists as justifying subspecies separation of slightly differentiated local populations.

In the controversy over the subspecific designation, it appears that few if any converts were won by the arguments of either side. The subspecific designation is still being used, although the criteria are more stringent, and authors are still critical of its use.

Based on this study, two of the four populations appeared to be distinctly different from all established subspecies of Oreohelix haydeni. Thus, it is possible that populations II and III could be new subspecies of O. haydeni. The disposition of populations I and IV is less clear. However, without a series of specimens from each of the described subspecies for comparative purposes, no definite conclusions could be drawn.

The methods used in this study have not entirely solved the problem of finding a more objective method of designating subspecies of O. haydeni. Particular characters are still weighted more than others in order to differentiate populations. Further research could apply numerical taxonomic techniques which probably are the most objective methods available to solve this problem.

CHAPTER V

SUMMARY

Subordinate taxa within the genus Oreohelix are difficult to differentiate. There appears to be a great multiplicity of forms and parallelisms of shell characters among species or races not directly related (Pilsbry, 1939). Apparently, the genitalia are not useful in systematics, for some 26 species are named, but only three morphological variations in the genitalia have been recorded. The result has led to dependency on shell characters alone for the description of both species and subspecies. This has resulted in much subjectivity in regard to the degree of difference in characteristics needed to designate species and subspecies.

Paper chromatography and chromosome number have been investigated in an attempt to establish a more stable classification (Burch, 1961a; 1961b).

Electrophoresis also had been investigated and it has been shown that it has potential use in the taxonomy of Gastropoda (Pace and Lindsay, 1967; Davis and Lindsay, 1967).

This study compared four geographically isolated populations of Oreohelix haydeni and various methods were studied in an attempt to develop a more consistent and objective method of designating subspecies.

The four study populations were located in Western Montana. One hundred and fifty-five specimens were collected from these four popula-

tions. Height and diameter of shell, height/diameter ratio, width and height of aperture, whorl number, and number of carinations above and below the periphery were determined for each snail. Each shell was checked for color banding. Six snails from each sample were dissected, and their radulae and genitalia were removed. A total of 73 snails were dissected so that the head and foot tissues could be reduced to a serum which was used in the electrophoretic part of the study. The analysis of variance test and a modification of the Duncan Multiple Range Test were used to make comparisons between the samples for diameter and height of shell, height/diameter ratio, and carinations above and below the periphery. All of the other characters were compared on a visual basis.

The results showed that some characters varied significantly, but that others did not. Mean number of whorls did not differ significantly among samples. Between samples, there were significant differences in the mean number of carinations but the overlap in total number either above or below the periphery was considerable. The apertural dimensions reflected the shape and size of the shell. That is, if the shell was smaller than another the aperture was also smaller, and if the shell was depressed the aperture was also depressed. In terms of the mean diameter of the shell, sample II was significantly smaller than the other three samples (12.1mm vs 17.0, 17.1, and 17.9mm for samples I, III, and IV respectively). Both samples II and III were smaller, in terms of mean height, than samples I and IV. Samples II and III were 6.1 and 7.1mm respectively vs 8.7 and 8.8mm in samples I and IV respectively. Sample III was the only sample that varied

significantly from the others in terms of mean height/diameter ratio, 0.41 for sample III vs 0.48, 0.50, and 0.52 for samples IV, II, and I respectively. Each sample was divided into groups based on the number of whorls. Statistical tests conducted on these groups gave results similar to the previous tests.

Measurements and comparisons of the genitalia were made on preserved specimens. All measurements were considerably less than similar measurements of live dissected specimens noted by Pilsbry (1939). However, proportions were similar to proportions measured by Pilsbry. Sample II measurements were smaller than the others, but this was a result of the smaller shells.

Radular tooth formulae means varied among the samples: 26-1-26, 25-1-24, 27-1-27, and 29-1-29 for samples I, II, III, and IV respectively. Overlap in number of teeth was considerable.

Electrophoretic studies of the populations indicated a total of 15 identifiable esterase bands. Five bands were singled out for comparison of the samples. These five bands were labeled $1_e2_e3_e4_e5_e$. All specimens of sample I had a $1_e2_e4_e5_e$ pattern. Sample II had two patterns: $1_e2_e3_e4_e5_e$ and $1_e2_e4_e5_e$. Sample III also had two patterns: $2_e3_e4_e5_e$ and $2_e4_e5_e$. Population IV was not included in this part of the study.

On the basis of shell characters, samples II and III were separable from samples I and IV. Population II apparently is a dwarf population and is significantly smaller than the other three populations. Population III has as extremely depressed spire. This was confirmed by the height/diameter ratio differences between sample III and the other samples.

The reproductive tracts were similar in appearance and were, therefore, not useful in differentiation. All morphological characters were as described by Pilsbry (1939).

Radular tooth formulae were highly variable both within and among samples. Only sample II and sample IV did not overlap in the ranges of their values. Overlap with other samples negated the usefulness of these formulae for identification.

Electrophoretic tests showed that specimens from sample III could be separated from specimens from samples I and II because of the lack of the l_e band in specimens from sample III. Separation of samples I and II was not always possible.

When all 13 characters were compared simultaneously, sample I consistently differed from samples II, III, and IV by location and carinations below the periphery. Sample IV consistently differed from the others by location and carinations above the periphery. Sample III had five consistently differing characters: height, height/diameter ratio, carinations above the periphery, electrophoretic pattern, and location. Sample II had four consistently differing characters: height, diameter, width of aperture, and location.

Populations I and IV were quite similar and should probably be grouped together. Populations II and III were distinctive, and could be separated from each other and from populations I and IV.

In comparisons with small samples of each of the established subspecies of Oreohelix haydeni, both population II and III still appeared distinctive. It was difficult to determine where populations

I and IV should be placed.

The problem of finding a more objective method of designating subspecies has not been entirely solved, because the weighting of particular characteristics is still used.

APPENDIX A

In this appendix are listed the individual measurements of the parameters consolidated in table 5. Maximum diameter, maximum height, maximum width of aperture, and maximum height of aperture were measured to the nearest 0.1mm.

SHELL MEASUREMENTS OF POPULATION I

Snail number	Maximum diameter	Maximum height	Height/diameter ratio	Number of carinations above the periphery	Number of carinations below the periphery	Number of whorls	Maximum width of aperture	Maximum height of aperture
17	14.6	6.9	0.4726	3	7	4.5	5.1	4.4
44	18.0	8.3	0.4611	3	7	5.0	6.7	5.0
2	14.9	7.6	0.5101	3	8	5.0	5.4	4.7
39	18.3	9.6	0.5246	3	5	5.5	6.6	5.2
9	16.4	8.8	0.5366	4	4	4.5	5.8	4.9
28	20.6	10.0	0.4854	2	7	4.5	7.2	6.0
14	13.3	6.2	0.4662	2	6	4.5	4.6	3.9
36	17.0	9.3	0.5412	4	7	5.0	5.9	5.0
3	17.1	9.6	0.5614	2	6	5.0	5.7	5.1
10	16.1	8.9	0.5528	3	7	5.0	5.8	5.3
7	16.4	9.5	0.5793	4	7	4.5	5.7	5.1
38	18.4	9.8	0.5326	3	8	5.0	6.1	5.9
27	18.9	10.1	0.5344	4	8	5.0	6.5	5.6
37	14.6	7.9	0.5411	3	5	4.8	4.9	4.5
11	19.8	10.4	0.5253	3	7	5.5	6.9	5.8
16	18.0	9.4	0.5222	2	5	5.0	6.3	5.8
19	12.1	5.9	0.4876	4	7	4.8	4.1	3.4
18	15.4	7.0	0.4545	5	8	5.0	5.3	4.4
23	17.8	10.0	0.5618	2	7	5.3	6.4	6.0
15	15.7	7.3	0.4650	3	9	5.0	5.6	4.8
40	17.1	8.1	0.4737	3	7	5.0	5.8	4.9
42	16.3	7.9	0.4847	3	9	5.0	5.4	4.6
6	18.2	9.3	0.5110	3	6	5.5	6.4	5.8
32	18.9	9.6	0.5079	2	9	5.3	6.8	5.9
21	18.1	9.7	0.5359	4	7	5.3	6.0	5.8
31	19.4	9.7	0.5000	3	8	5.3	7.1	5.9
45	18.9	9.6	0.5079	4	6	5.3	7.0	6.0
48	16.2	8.3	0.5123	2	5	5.0	5.9	4.8
26	19.0	9.8	0.5158	3	6	5.3	6.8	6.0
41	17.5	9.7	0.5543	2	7	5.0	6.3	5.6
25	18.8	10.0	0.5319	4	10	5.3	6.9	6.5
33	17.0	9.1	0.5353	1	8	5.0	6.1	5.7
47	13.6	8.0	0.5882	3	8	4.5	4.9	4.8
8	18.6	10.8	0.5806	4	7	5.3	7.3	6.2
20	14.1	7.2	0.5106	4	9	5.0	4.9	4.4
13	13.9	7.2	0.5180	2	7	4.8	4.9	4.2

SHELL MEASUREMENTS OF POPULATION I (Continued)

Snail number	Maximum diameter	Maximum height	Height/diameter ratio	Number of carinations above the periphery	Number of carinations below the periphery	Number of whorls	Maximum width of aperture	Maximum height of aperture
12	14.9	7.3	0.4899	2	7	5.0	5.3	4.4
35	18.0	9.0	0.5000	4	8	5.3	5.9	5.7
4	16.2	8.2	0.5062	3	7	5.0	5.9	5.0
5	14.3	7.6	0.5315	4	11	4.8	5.3	4.4
A	19.5	9.2	0.4718	4	8	5.3	6.8	6.2
B	19.8	9.6	0.4848	2	8	5.3	7.0	5.9
C	18.8	10.3	0.5479	3	7	5.3	6.7	5.6
D	16.7	7.8	0.4671	2	8	5.0	5.8	4.7
E	16.9	8.5	0.5030	2	6	5.3	6.0	5.2
F	16.9	8.9	0.5266	3	8	5.3	5.8	5.0
G	18.3	10.3	0.5628	2	9	5.0	6.6	5.8
H	15.8	7.8	0.4937	5	9	5.0	5.3	4.8

SHELL MEASUREMENTS OF POPULATION II

103	13.5	6.6	0.4889	4	7	5.0	4.5	3.5
101	11.9	5.5	0.4622	3	5	4.8	4.1	2.9
102	13.5	6.8	0.5037	3	8	5.0	4.6	3.3
106	12.5	6.3	0.5040	3	7	4.8	4.1	3.2
121	12.0	6.4	0.5333	3	6	4.5	4.4	3.3
105	14.5	7.5	0.5172	4	6	5.0	4.9	3.8
126	10.3	5.7	0.5534	3	6	4.8	3.7	2.6
114	13.3	7.3	0.5489	3	5	5.0	4.6	3.6
117	13.3	5.8	0.4361	3	7	4.8	4.5	3.5
127	9.1	4.8	0.5275	2	6	4.3	3.2	2.6
115	11.7	5.8	0.4957	3	4	4.8	4.0	3.3
104	11.9	6.2	0.5210	4	4	4.8	4.2	3.5
125	9.3	5.0	0.5376	2	6	4.3	3.1	2.4
111	13.3	6.6	0.4962	2	5	5.0	4.5	3.8
116	13.8	6.9	0.5000	3	7	5.0	5.0	3.7
110	12.9	6.5	0.5039	*	*	4.8	4.9	3.5
120	11.9	5.9	0.4958	4	8	4.8	4.4	3.2

* shell accidently crushed before carinations were counted

SHELL MEASUREMENTS OF POPULATION II (Continued)

Snail number	Maximum diameter	Maximum height	Height/diameter ratio	Number of carinations above the periphery	Number of carinations below the periphery	Number of whorls	Maximum width of aperture	Maximum height of aperture
108	12.4	6.0	0.4839	3	7	4.5	4.7	3.6
128	8.3	4.3	0.5181	0	4	4.5	3.0	2.3
109	12.6	6.6	0.5238	3	5	4.8	4.2	3.3
113	11.9	6.0	0.5042	3	3	4.5	3.9	3.1
112	12.6	6.8	0.5397	2	5	5.0	4.5	3.7
124	11.9	5.4	0.4538	3	6	4.5	4.3	3.2
123	10.3	6.0	0.5825	3	5	4.5	3.4	2.4
122	12.0	5.1	0.4250	3	7	4.8	3.9	3.5
107	10.9	5.1	0.4679	4	5	4.5	3.7	3.0
118	12.9	6.0	0.4651	2	6	4.8	4.8	3.8
119	13.3	6.6	0.4962	2	7	5.0	4.4	3.5

SHELL MEASUREMENTS OF POPULATION III

223	19.5	7.9	0.4051	2	5	5.3	6.8	5.1
219	20.7	8.7	0.4203	1	6	5.3	7.0	5.6
202	17.8	7.3	0.4101	2	6	5.0	6.2	4.9
236	16.9	7.5	0.4438	1	6	5.0	5.8	4.8
207	15.2	5.8	0.3816	2	3	4.5	5.2	4.3
206	17.6	7.8	0.4432	1	5	5.0	5.9	4.6
259	20.8	8.4	0.4038	2	5	5.5	7.2	5.0
209	12.5	4.8	0.3840	2	5	4.5	4.8	3.2
240	19.6	7.9	0.4031	2	7	5.3	6.5	4.8
249	18.2	7.4	0.4066	3	6	5.3	6.2	4.6
229	11.8	4.8	0.4068	1	5	4.5	4.3	3.4
224	15.4	6.5	0.4221	3	4	4.8	5.5	4.4
251	17.5	7.4	0.4229	2	4	5.0	6.2	5.2
247	17.1	6.4	0.3743	3	5	5.0	5.6	4.1
230	16.2	6.5	0.4012	3	6	4.8	5.3	4.1
226	14.4	5.6	0.3889	2	7	4.5	4.9	3.7
214	16.9	7.0	0.4142	2	5	5.0	5.8	4.7
228	15.6	6.3	0.4038	2	5	4.8	5.7	4.3
238	20.3	9.3	0.4581	1	7	5.5	7.0	5.7
217	16.8	6.8	0.4048	1	5	5.0	6.0	4.2

SHELL MEASUREMENTS OF POPULATION III (Continued)

Snail number	Maximum diameter	Maximum height	Height/diameter ratio	Number of carinations above the periphery	Number of carinations below the periphery	Number of whorls	Maximum width of aperture	Maximum height of aperture
222	16.4	7.0	0.4269	2	7	5.0	6.0	4.8
258	16.2	7.3	0.4506	1	5	5.0	5.6	4.0
216	16.4	6.4	0.3902	2	7	5.0	5.6	4.2
213	19.9	8.1	0.4070	1	7	5.3	6.8	5.8
233	18.8	7.8	0.4149	1	5	5.0	6.5	5.2
241	14.5	6.0	0.4138	1	5	4.8	4.9	3.9
242	15.1	6.3	0.4172	2	7	5.0	5.0	3.8
248	14.7	5.9	0.4014	1	5	4.8	5.3	3.8
210	17.1	6.6	0.3860	1	5	5.0	5.5	4.8
257	14.4	5.8	0.4028	2	4	4.8	4.6	3.7
227	15.9	6.5	0.4088	1	6	4.8	5.2	4.1
220	18.8	8.2	0.4362	2	5	5.3	6.3	5.4
243	14.1	6.0	0.4255	2	6	4.8	4.5	3.5
221	18.1	7.2	0.3978	2	5	5.0	6.3	4.5
218	19.4	9.1	0.4691	2	6	5.3	6.9	5.2
A1	18.3	7.5	0.4098	*	*	5.0	6.1	5.0
B1	18.9	7.8	0.4127	*	*	5.0	6.5	4.9
C1	19.2	8.8	0.4582	2	7	5.5	6.4	5.2
D1	18.7	7.7	0.4118	*	*	5.0	6.3	5.1
E1	16.6	7.4	0.4458	*	*	5.0	5.9	4.7
F1	17.5	6.7	0.3829	*	*	5.0	6.2	4.3
208	16.5	6.5	0.3939	1	8	5.0	5.7	4.4
253	18.4	8.7	0.4728	2	6	5.0	6.4	5.0
239	17.3	7.0	0.4046	2	4	5.0	6.7	4.7
211	14.9	5.5	0.3691	2	6	4.8	4.9	4.0
234	17.2	7.7	0.4477	2	7	5.0	5.7	4.9
205	17.4	7.3	0.4195	2	6	5.0	5.7	4.9
246	13.4	6.1	0.4552	2	7	4.8	4.8	4.0
254	18.3	7.4	0.4044	1	6	5.3	6.4	5.2
201	16.3	7.1	0.4356	1	5	5.0	5.8	4.6
215	15.9	6.4	0.4025	1	5	4.8	5.1	4.0
256	16.9	6.4	0.3787	2	6	5.0	5.5	4.4
203	18.6	7.3	0.3925	2	7	5.3	6.1	4.6
244	16.1	6.7	0.4161	1	6	5.0	5.2	4.0
225	17.8	7.5	0.4213	1	7	5.3	6.1	4.9
260	15.5	6.1	0.3935	1	6	4.5	5.4	4.4

* shell was broken before carinations were counted

SHELL MEASUREMENTS OF POPULATION III (Continued)

Snail number	Maximum diameter	Maximum height	Height/diameter ratio	Number of carinations above the periphery	Number of carinations below the periphery	Number of whorls	Maximum width of aperture	Maximum height of aperture
231	16.9	6.2	0.3669	2	6	4.5	5.8	4.6
255	16.7	6.0	0.3593	1	6	4.8	5.8	4.3
204	19.9	8.5	0.4271	2	5	5.3	6.9	5.3
235	17.7	7.1	0.4011	1	6	5.0	6.2	5.2
237	19.6	9.0	0.4592	2	7	5.3	7.0	5.5
232	14.9	6.7	0.4497	2	4	4.5	5.3	4.1
252	17.2	7.0	0.4070	1	5	5.0	6.2	5.0
245	17.7	7.5	0.4237	1	8	5.0	6.0	4.8
250	17.8	7.2	0.4045	2	6	5.0	5.9	4.8
212	16.7	7.5	0.4491	1	5	5.0	5.5	4.5
261	20.4	8.2	0.4020	2	5	5.3	6.8	5.5

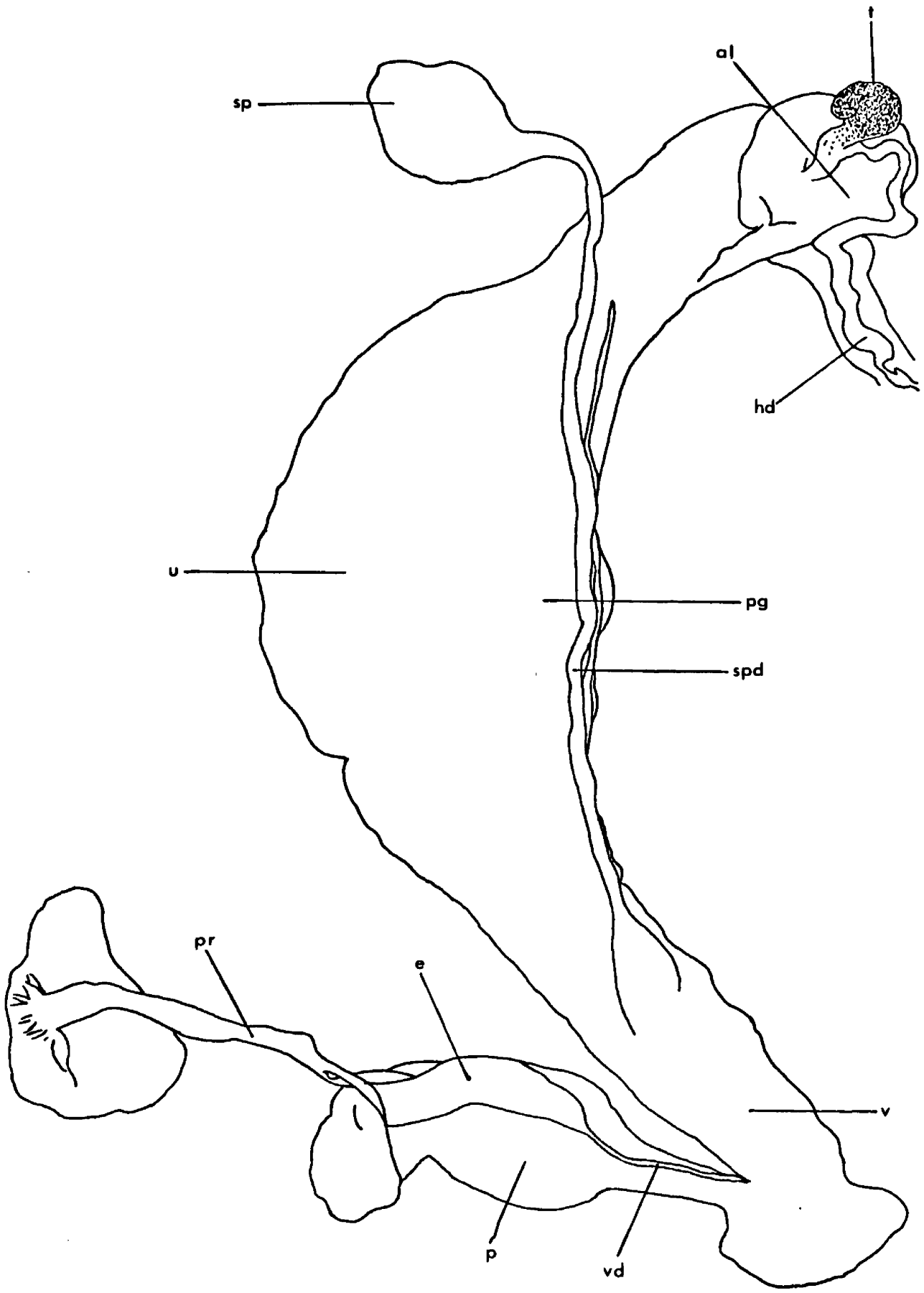
SHELL MEASUREMENTS OF POPULATION IV

300	18.0	9.1	0.5056	1	4	5.8	6.4	5.4
301	16.8	8.2	0.4881	3	5	5.5	6.4	5.2
302	17.3	8.6	0.4971	2	4	5.8	6.6	5.6
303	18.6	8.8	0.4731	2	5	5.5	6.6	5.5
304	17.9	10.5	0.5866	3	5	5.5	6.6	5.5
305	19.5	9.2	0.4718	2	5	5.8	6.4	5.1
307	13.8	6.9	0.5000	2	7	5.0	5.0	4.2
308	17.7	8.1	0.4576	2	7	5.5	6.1	5.3
309	18.1	8.3	0.4586	2	6	5.5	6.1	5.3
310	18.5	9.2	0.4973	3	4	5.5	7.0	5.6
311	17.5	8.5	0.4857	3	5	5.3	6.1	5.1
312	19.6	9.0	0.4592	2	6	5.5	7.0	5.5
313	18.0	7.1	0.3944	2	7	5.0	6.2	5.2
314	19.2	9.7	0.5052	3	6	5.8	7.0	5.7

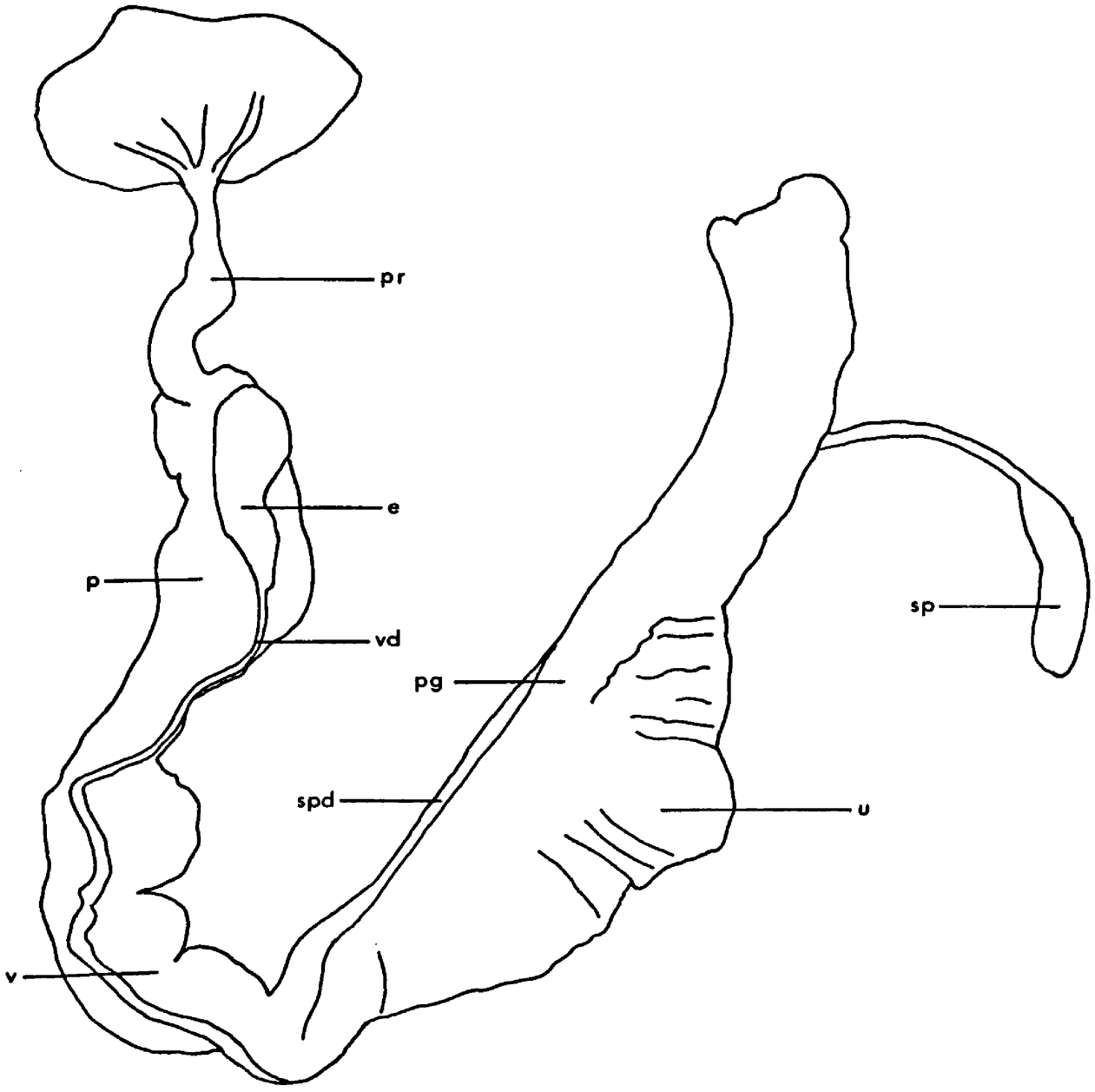
APPENDIX B

This appendix contains drawings of the reproductive tracts of certain snails from each population. The number or letter at the top center of each page indicates the snail from which the reproductive tract was taken. Numbers 1 to 99 are snails from population I, numbers 100 to 199 are population II snails, numbers 200 to 299 are snails from population III, and numbers 300 to 399 are population IV snails. B-RG and E-RG indicate snails from population I. Not all of the drawings are oriented the same. Key: al=albumin gland, e=epiphallus, hd=hermaphroditic duct, p=penis, pr=penial retractor muscle, pg= prostrate gland, sp=spermatheca, spd=spermathecal duct, t=talon, u=uterus, v=vagina, vd=vas deferens.

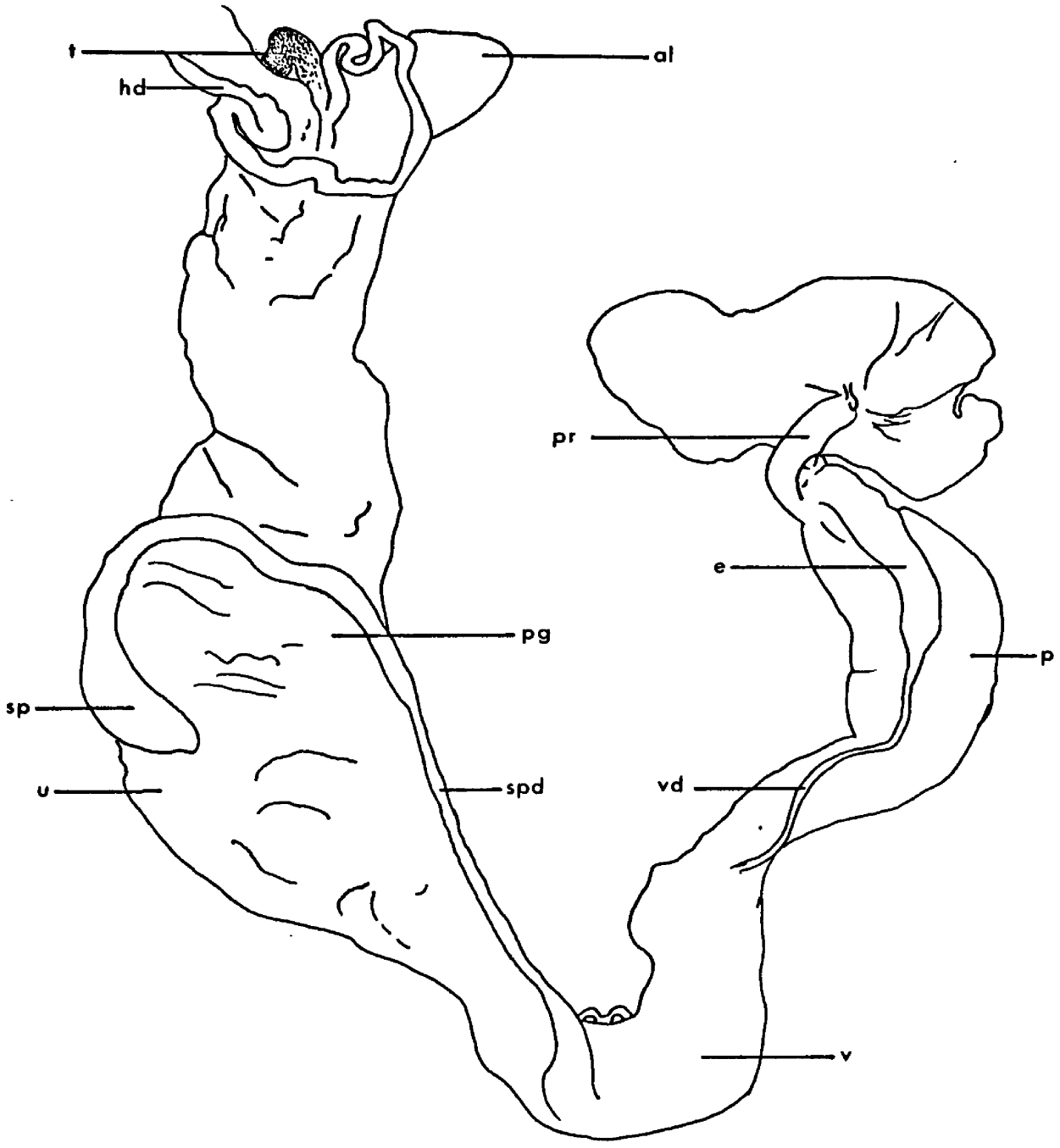
B-RG



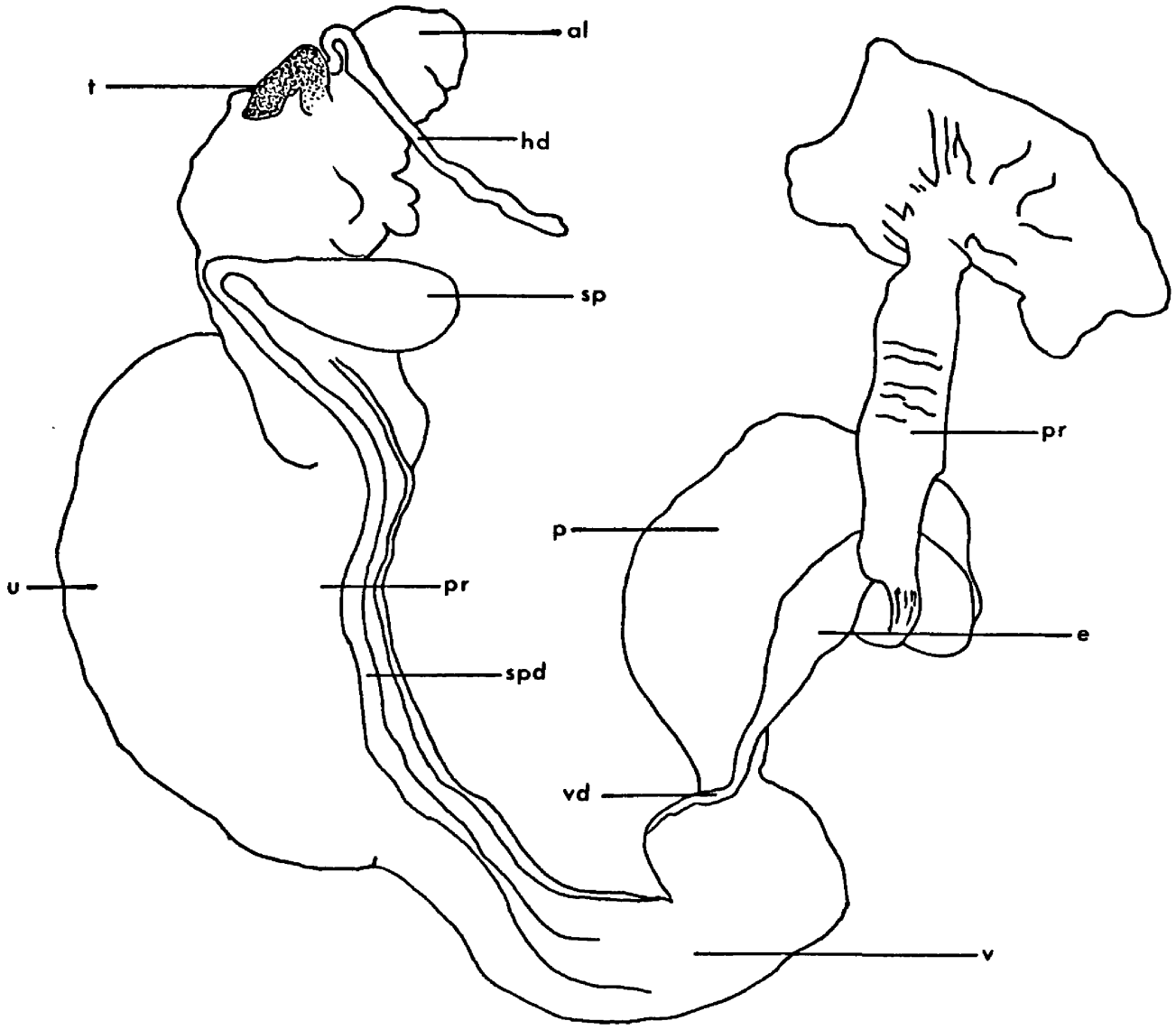
E-RG



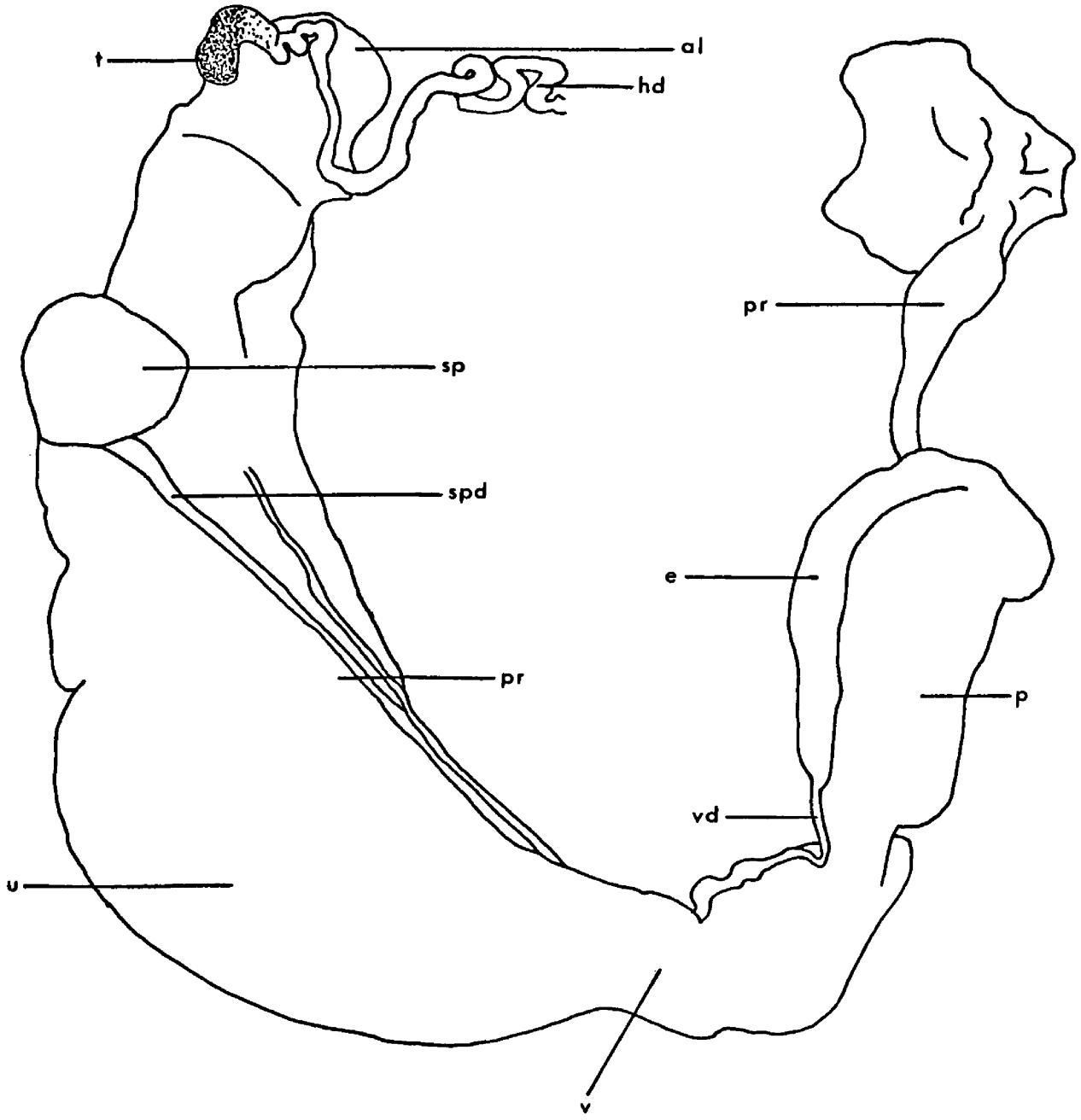
4



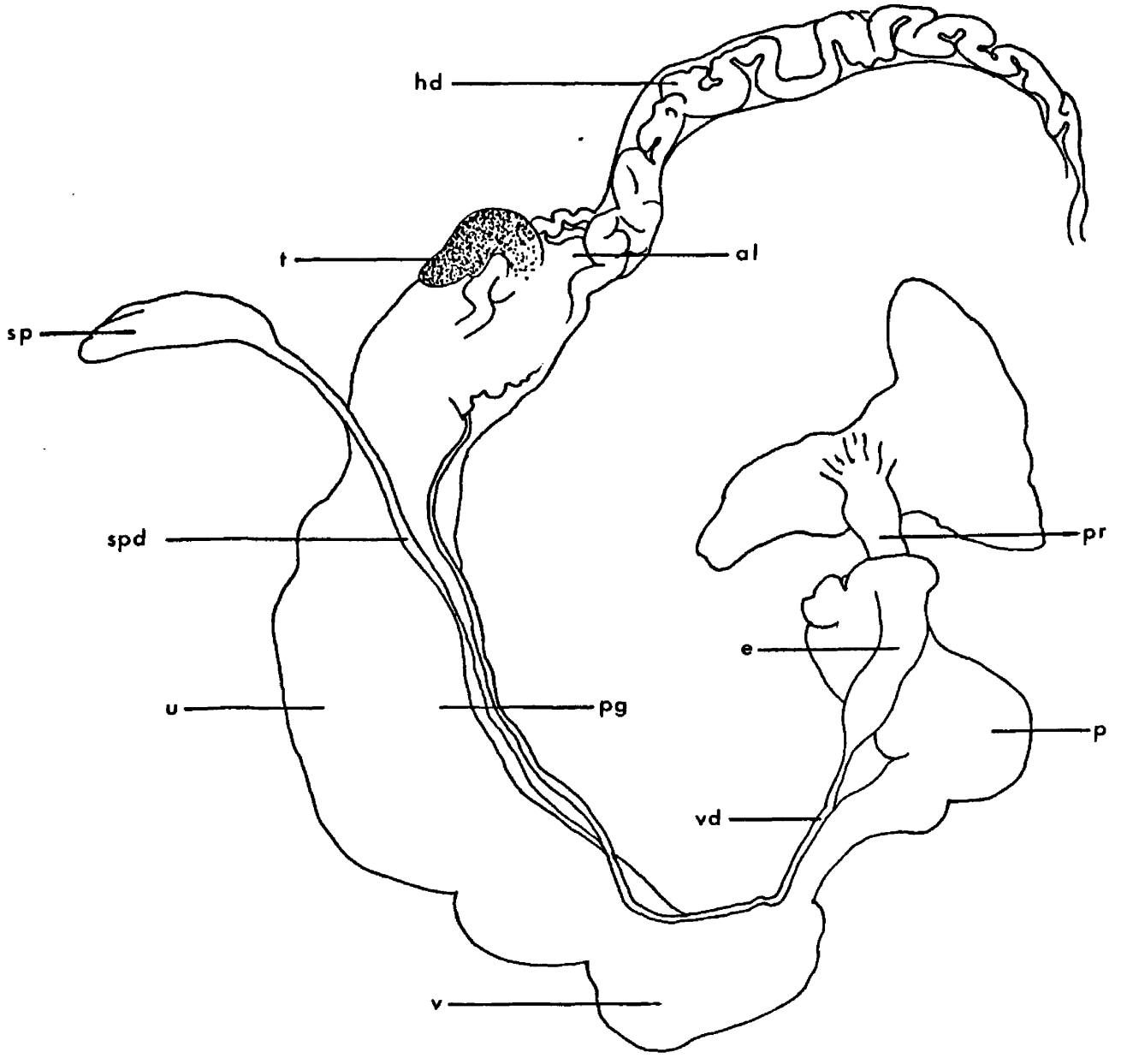
8



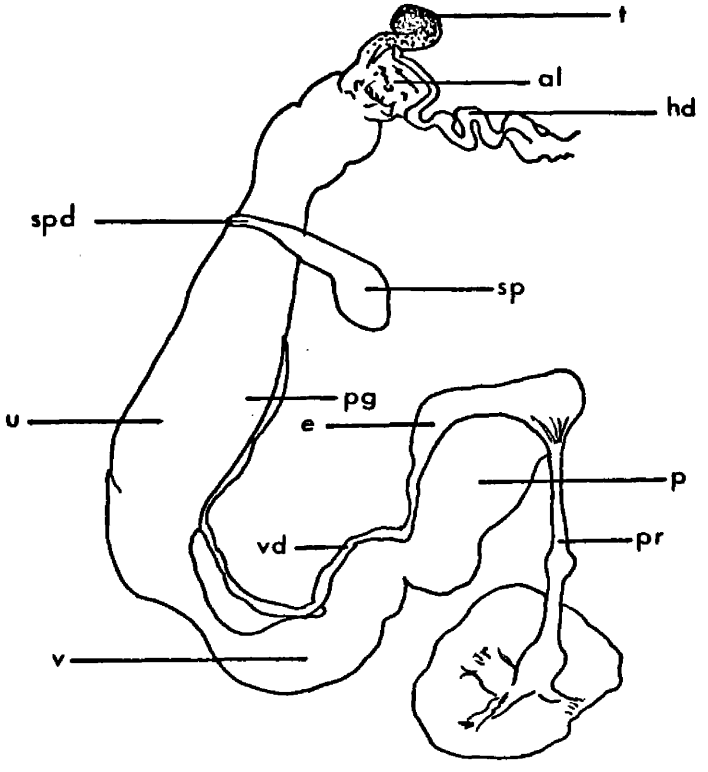
23



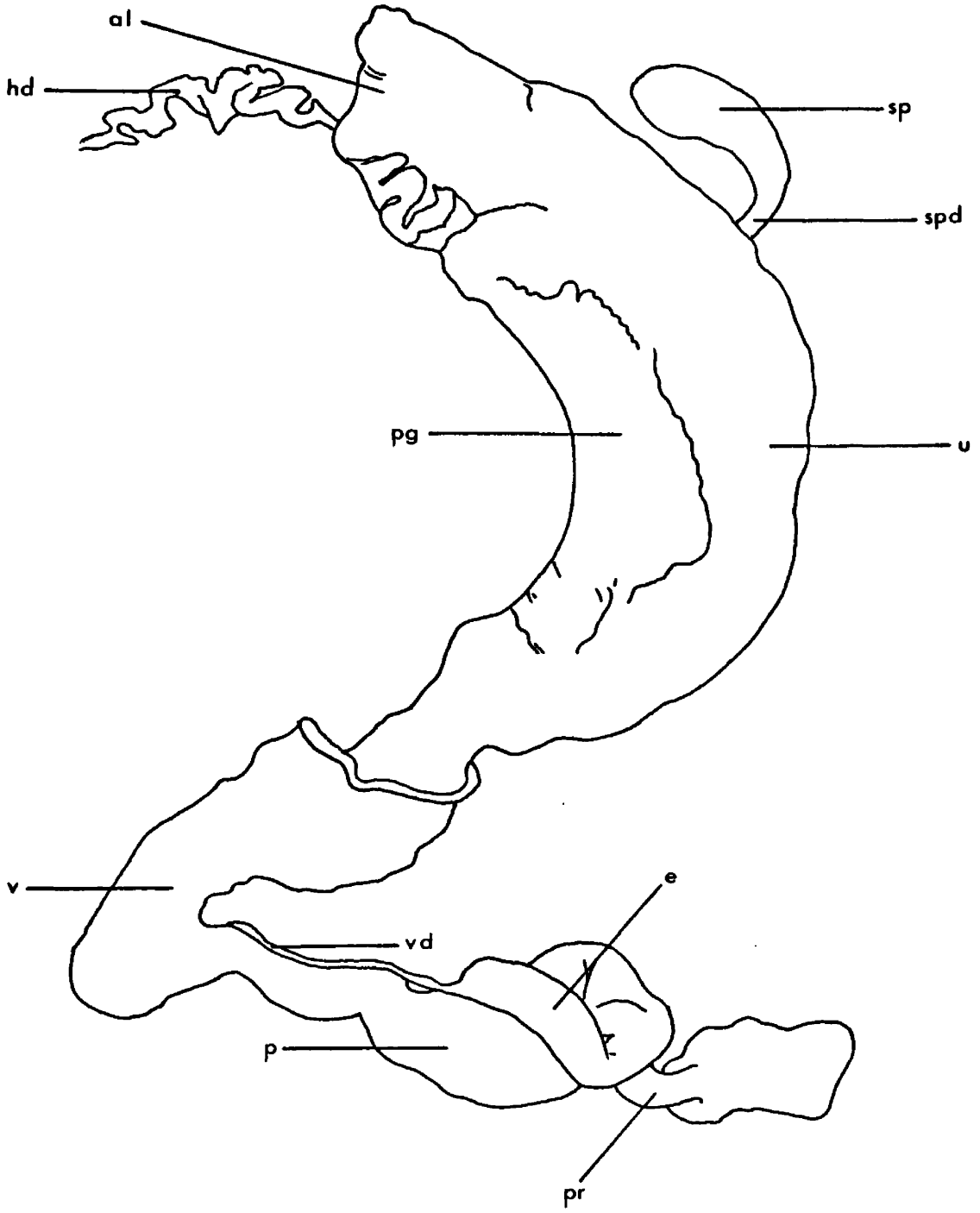
37



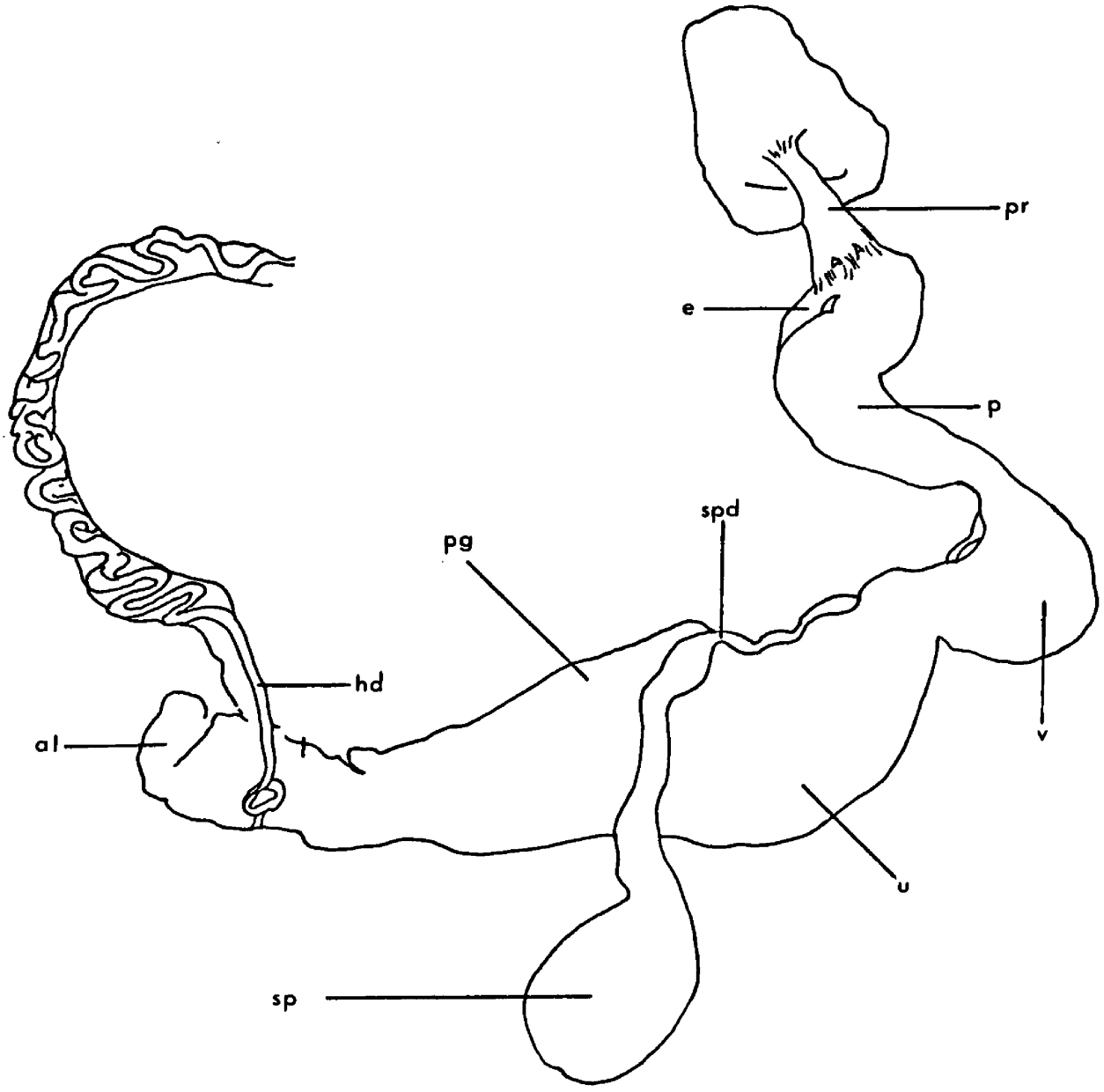
108



111



114



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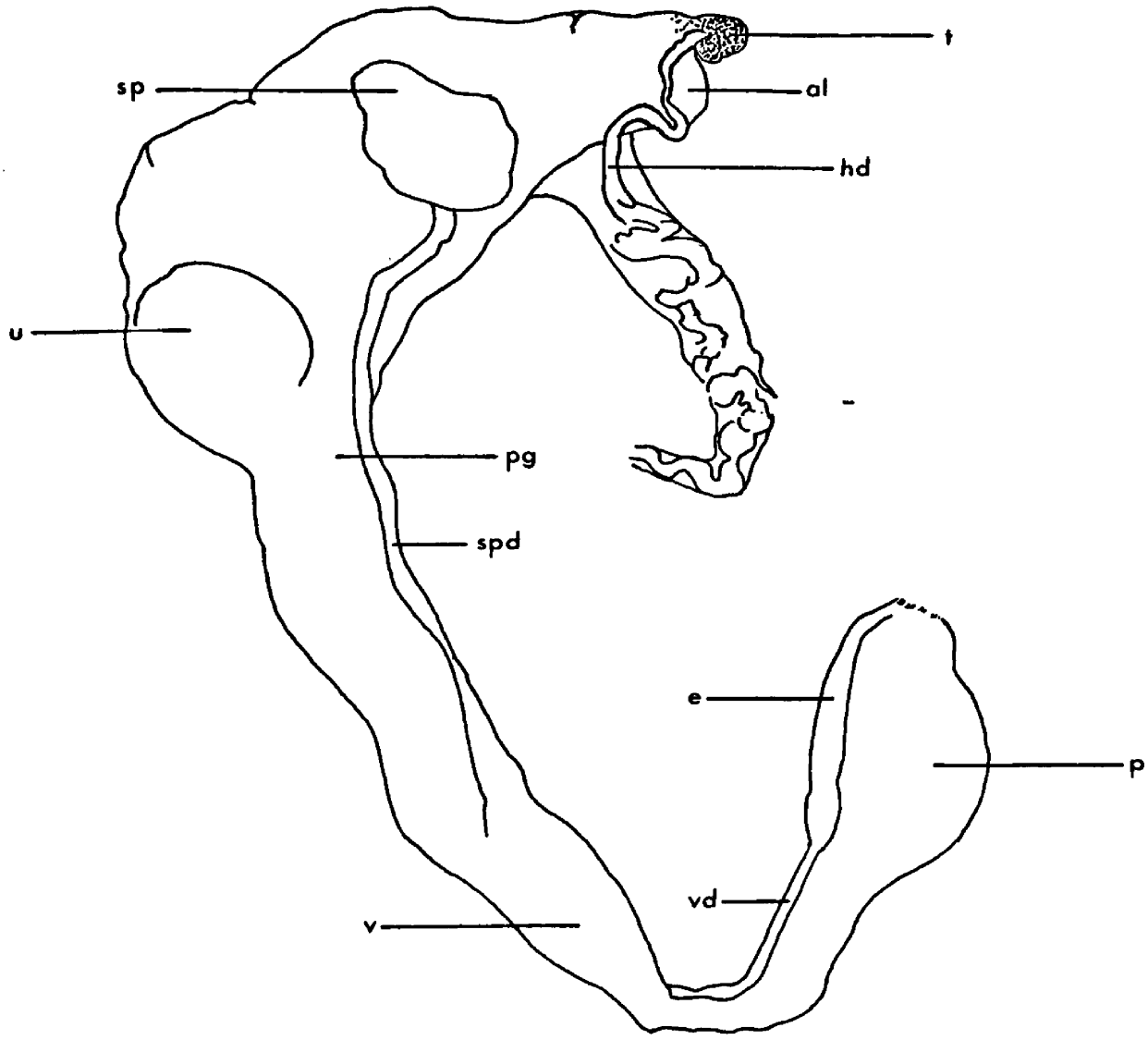

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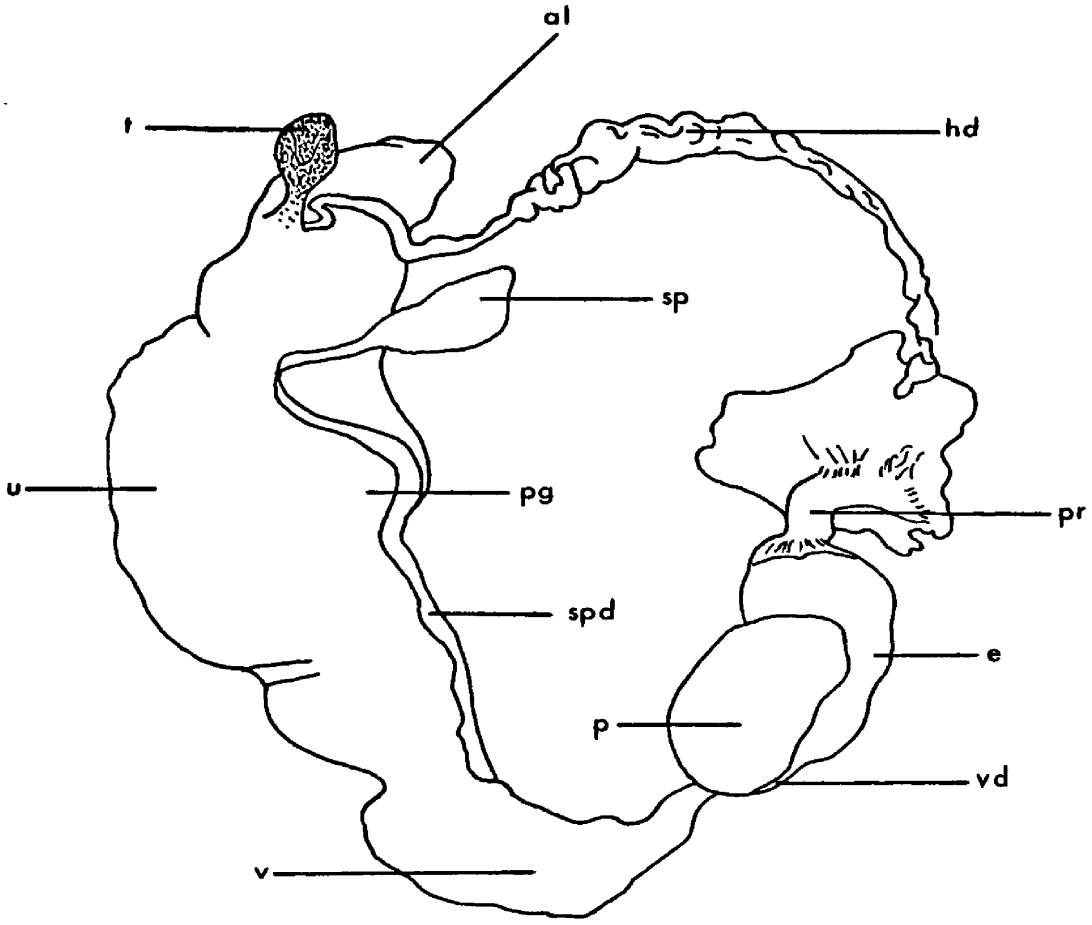

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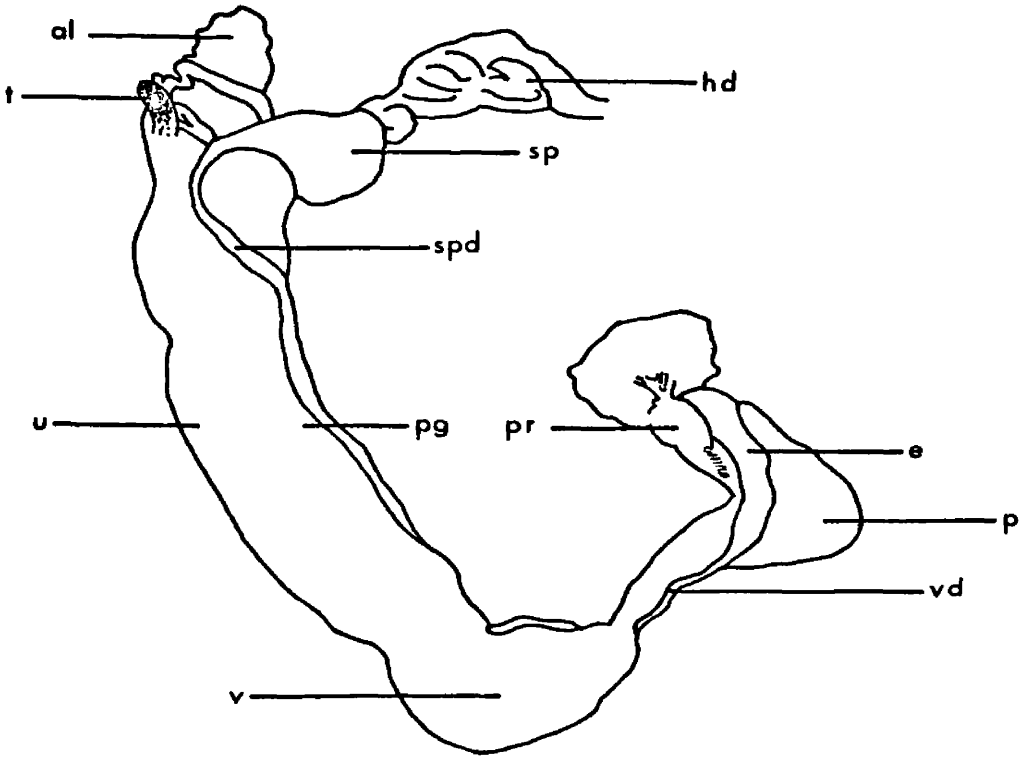

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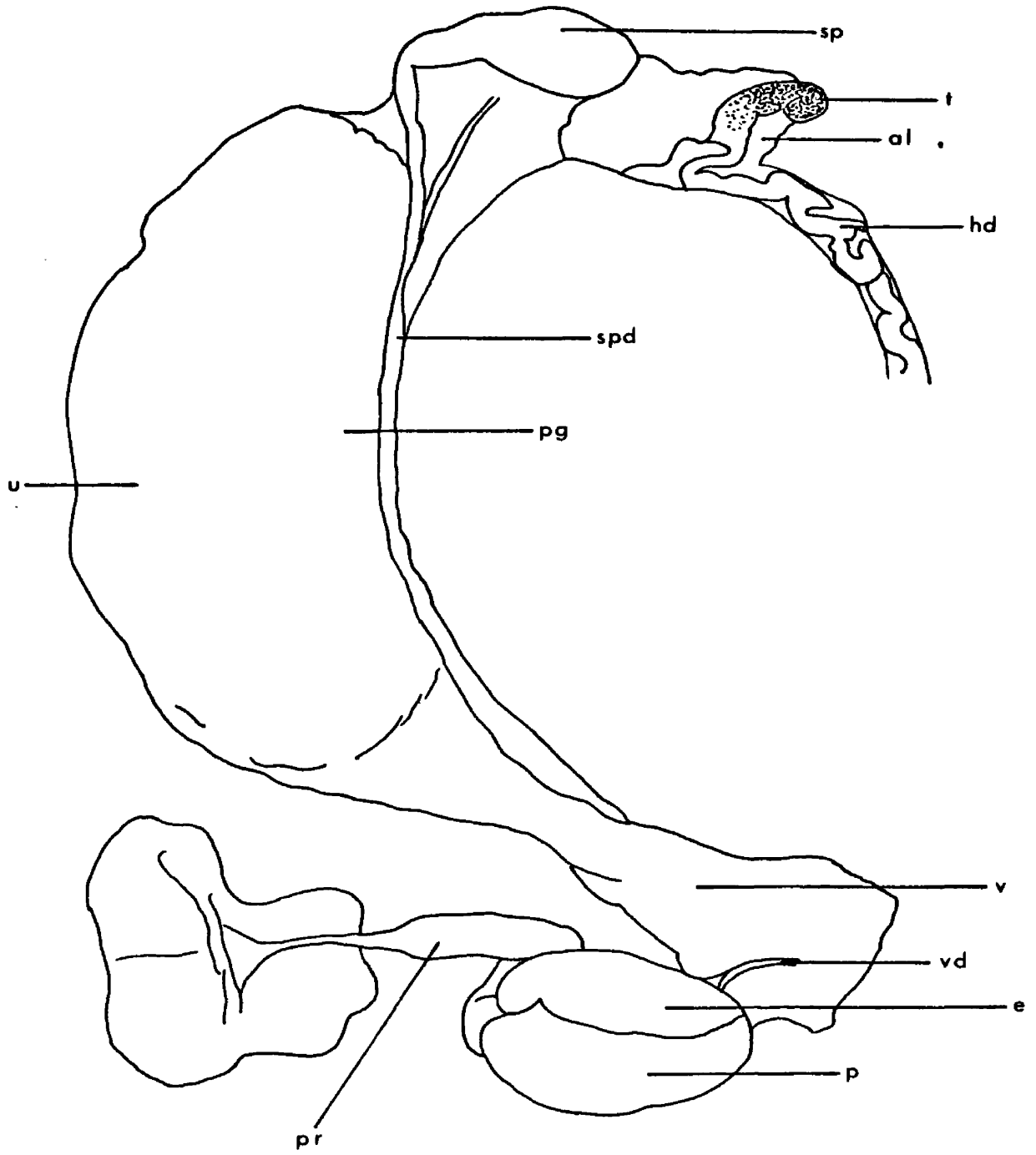

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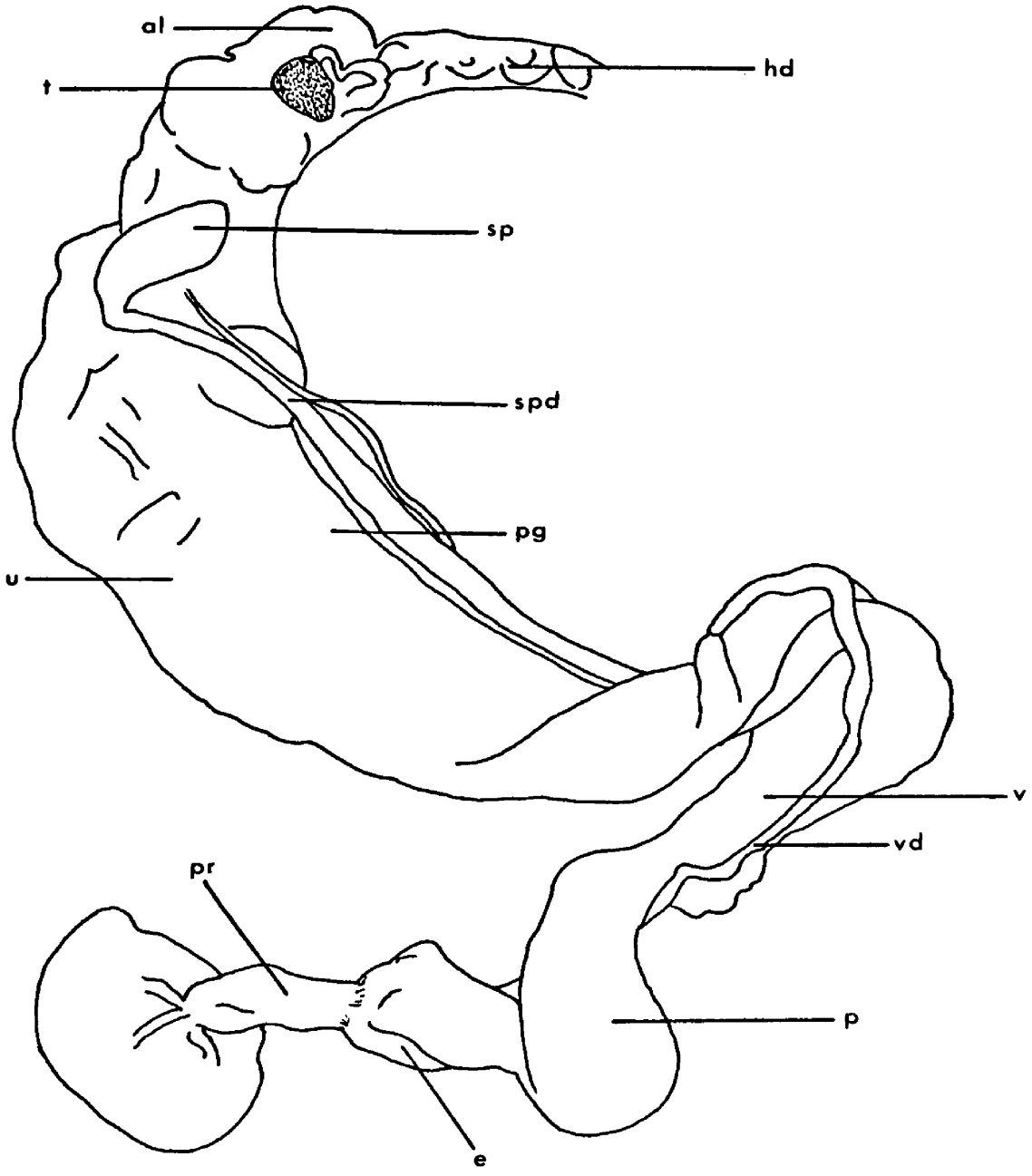

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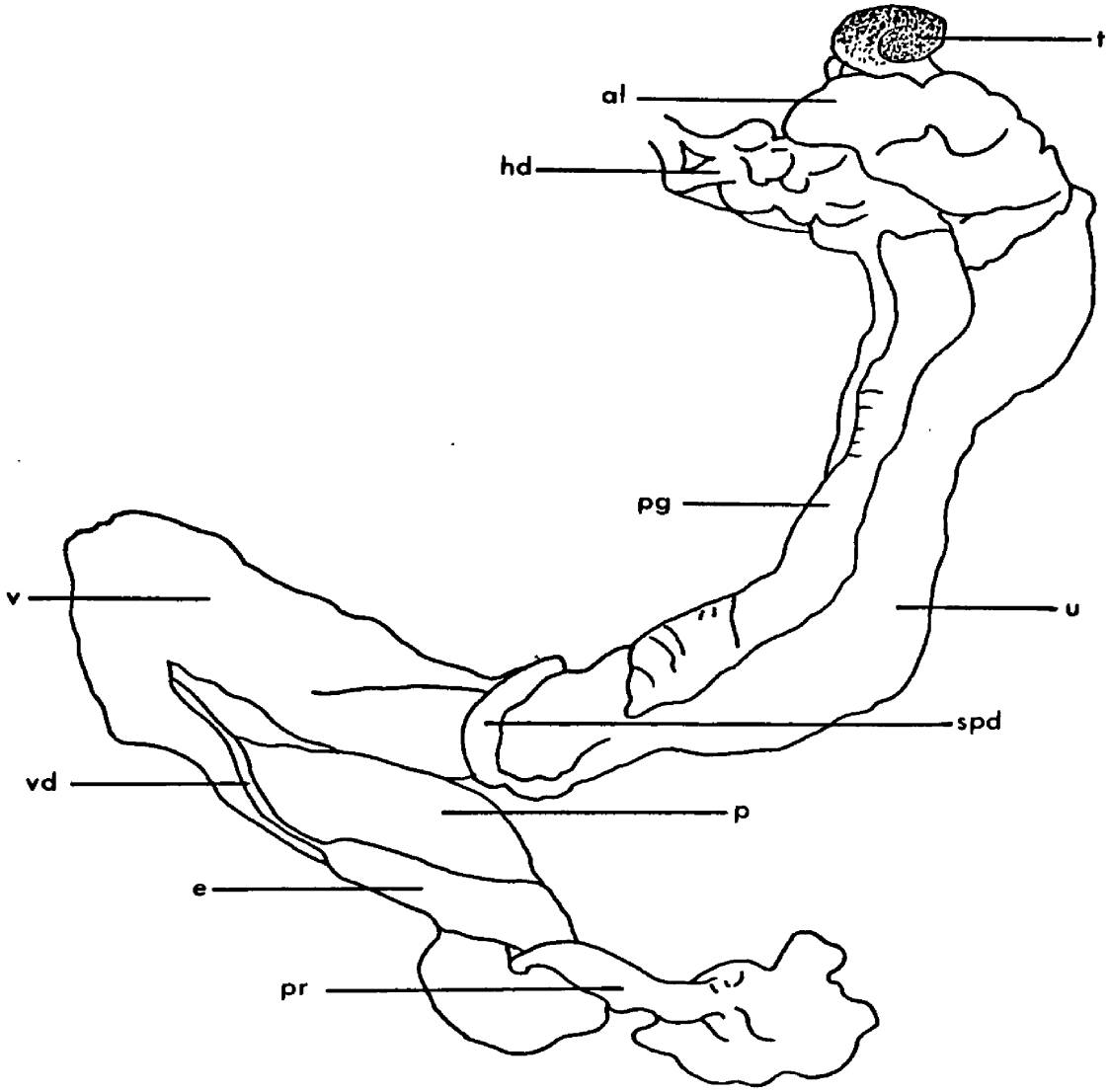

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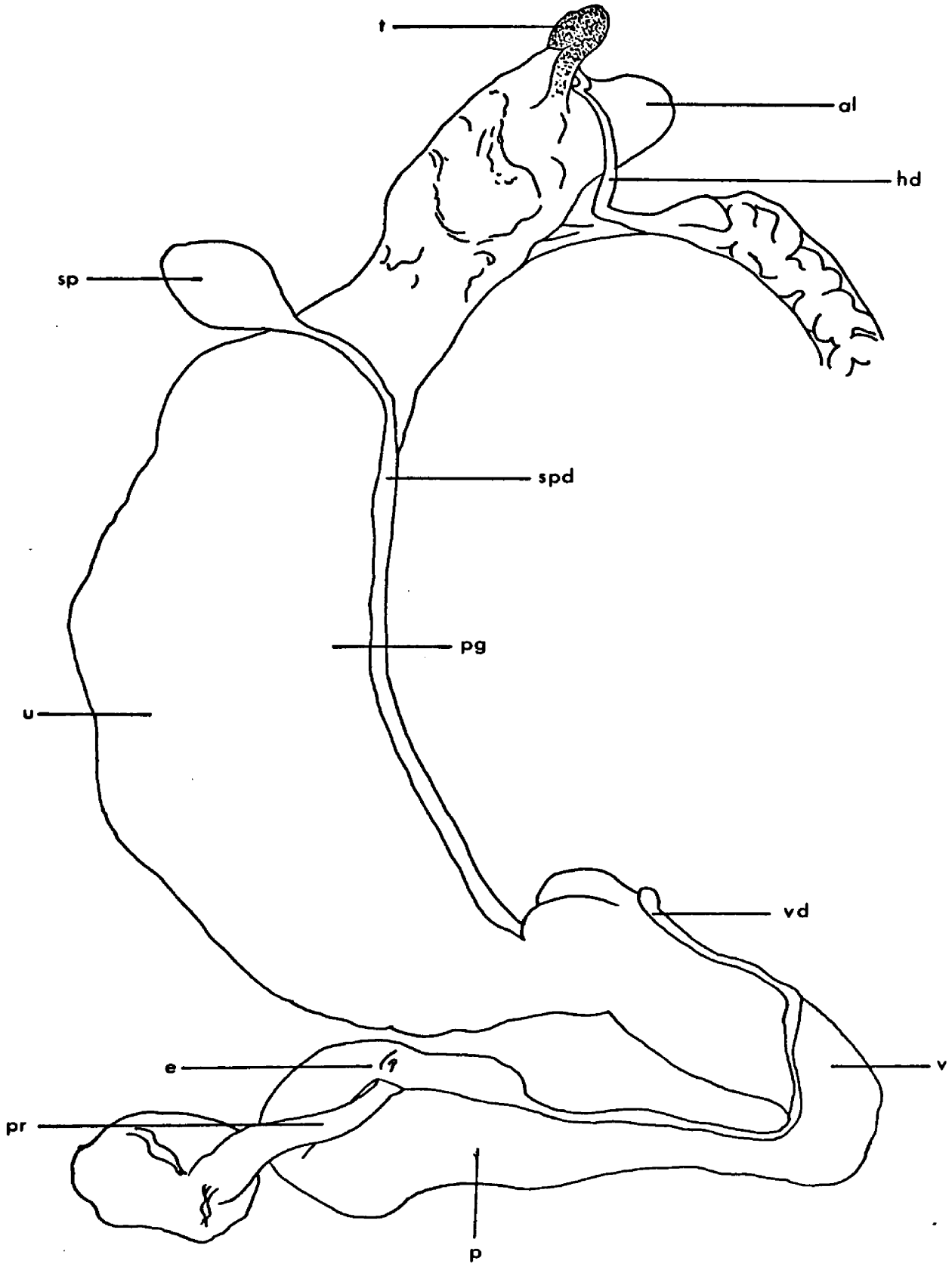

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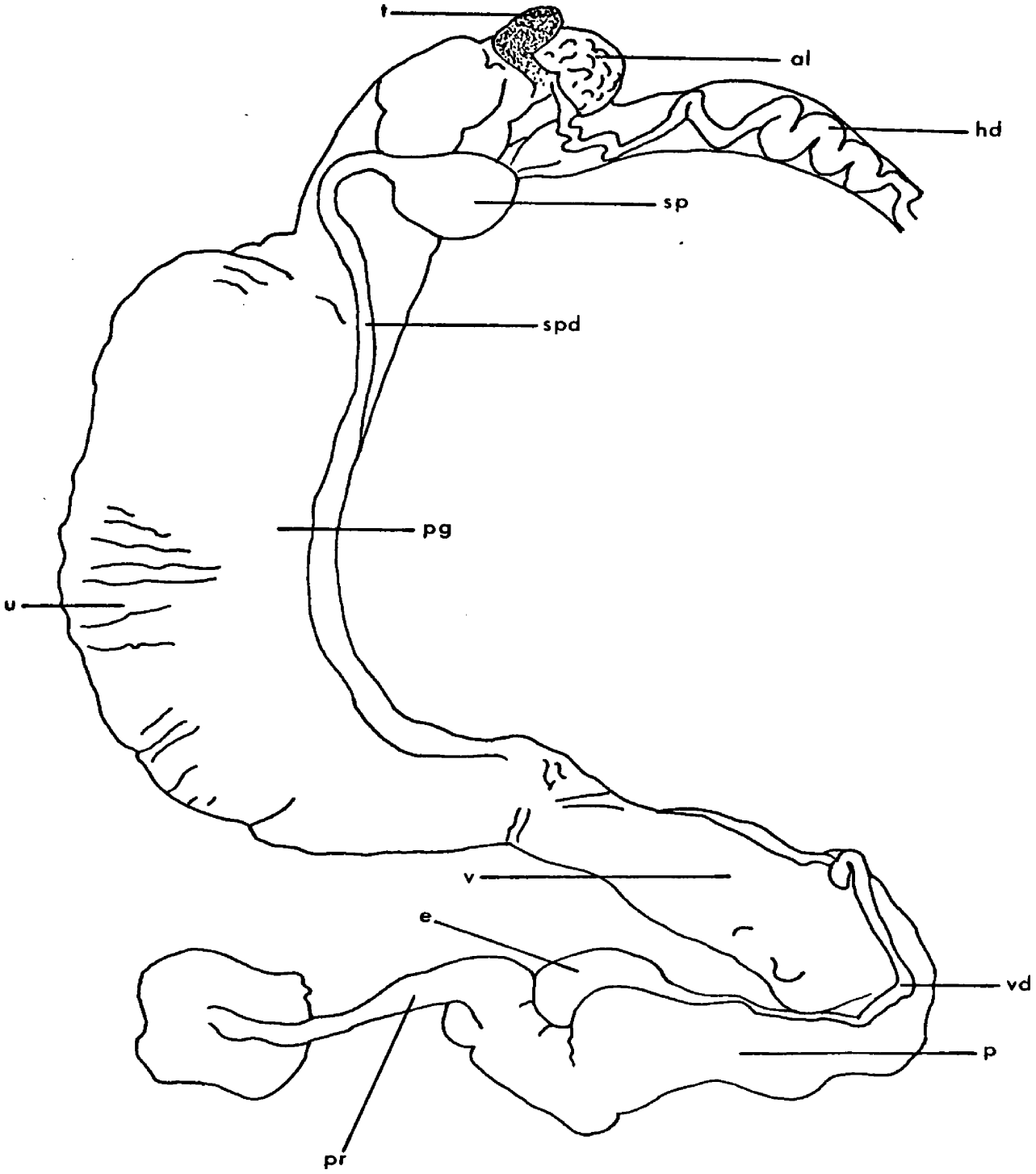

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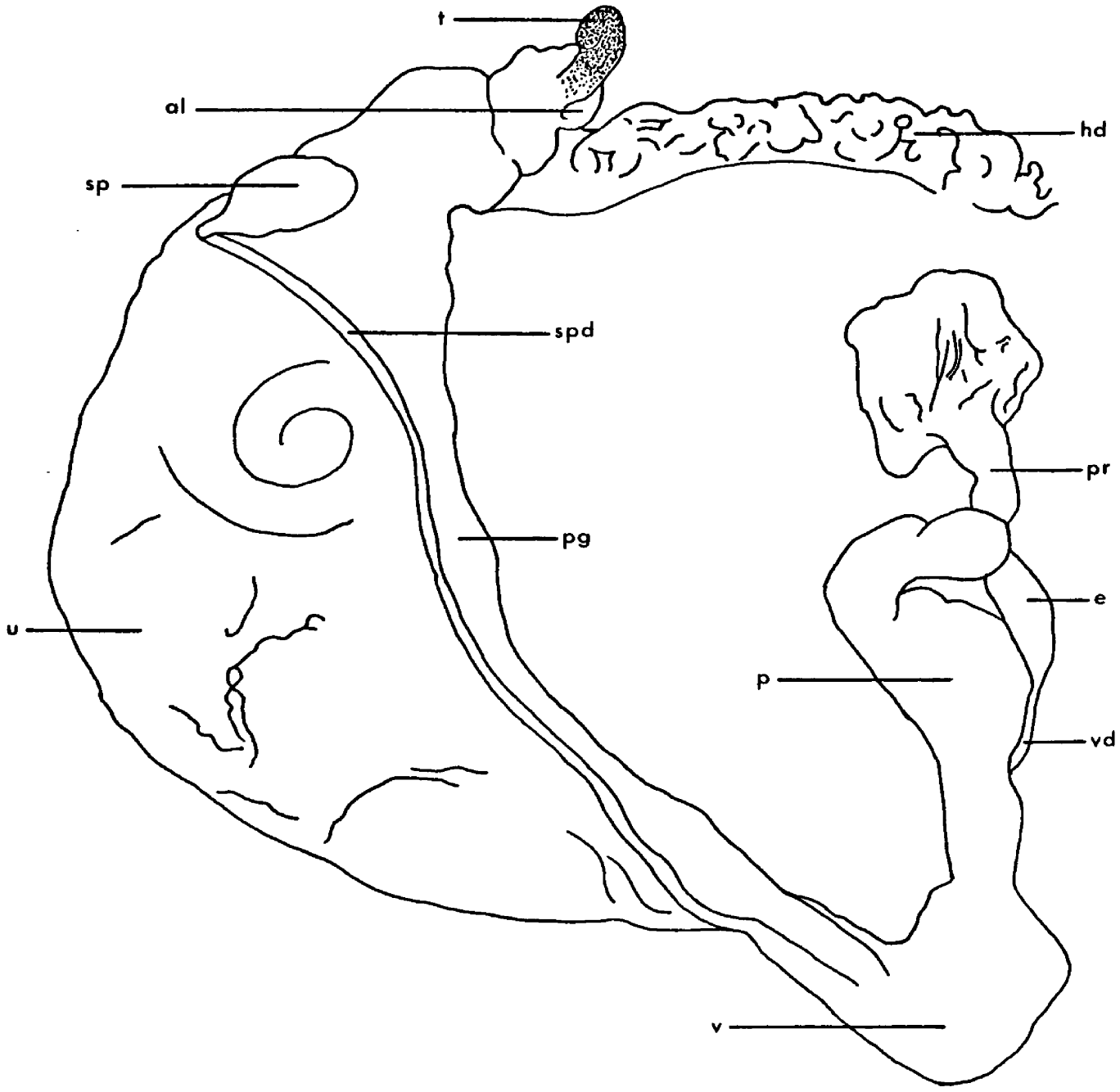

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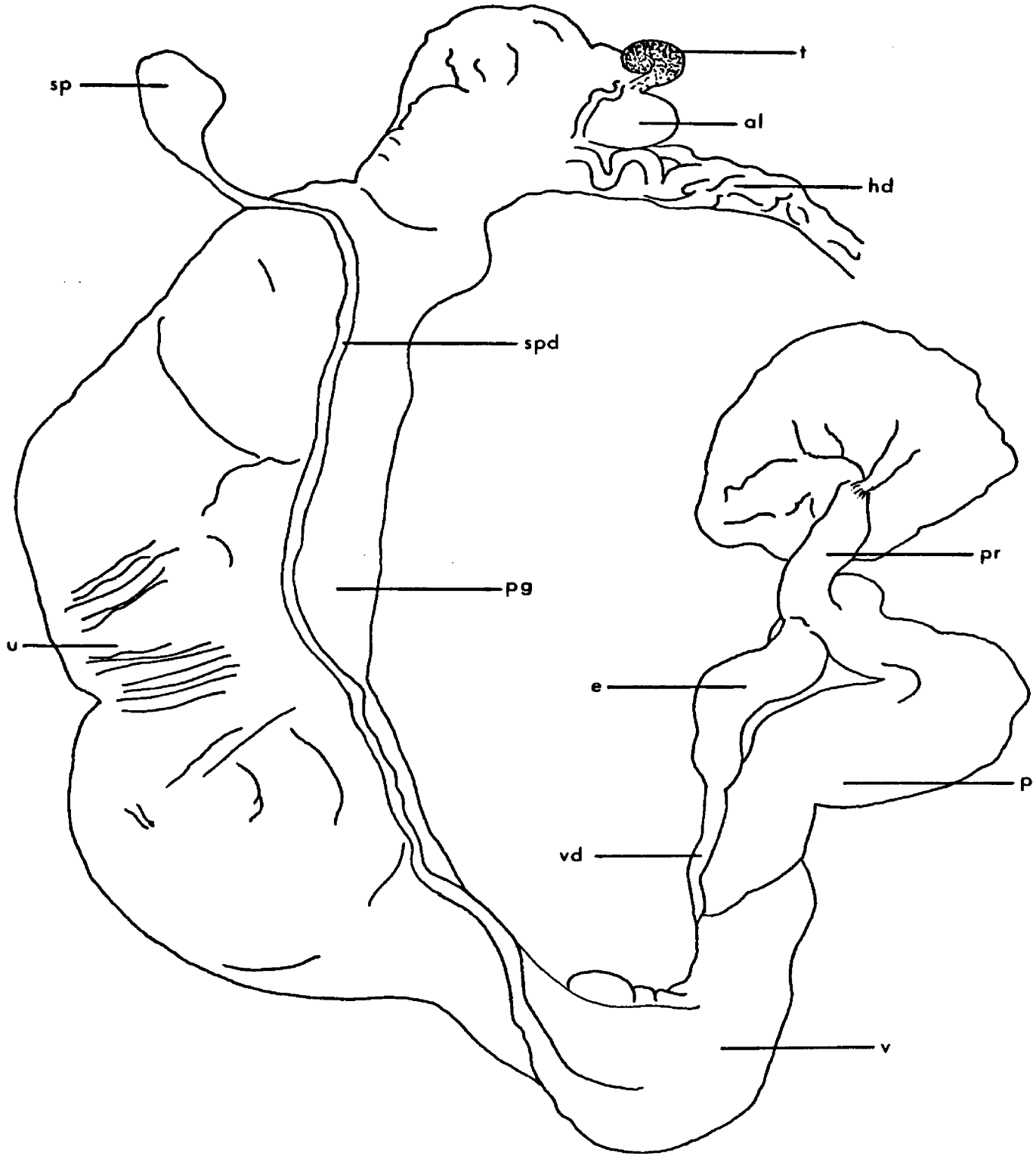

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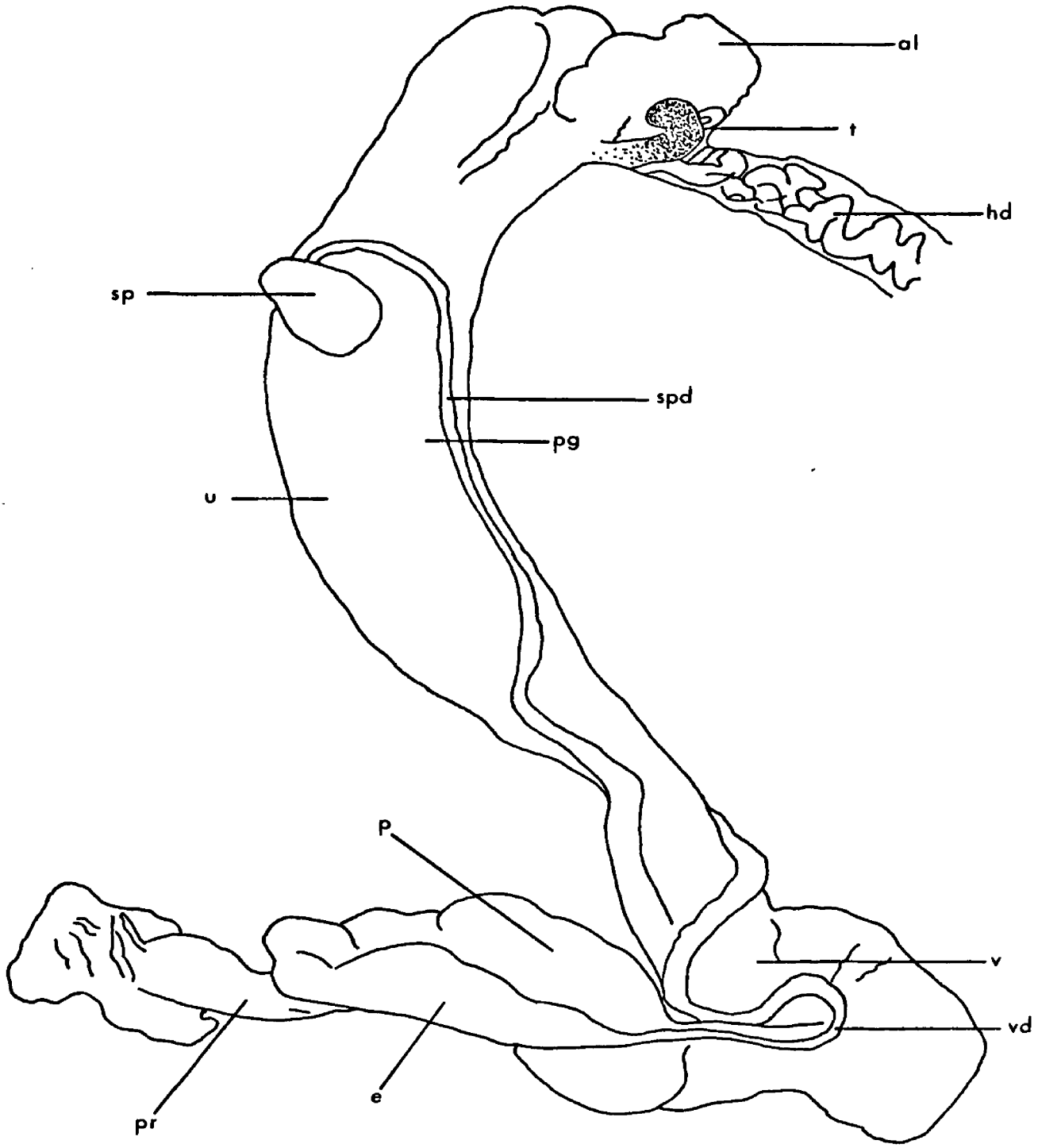

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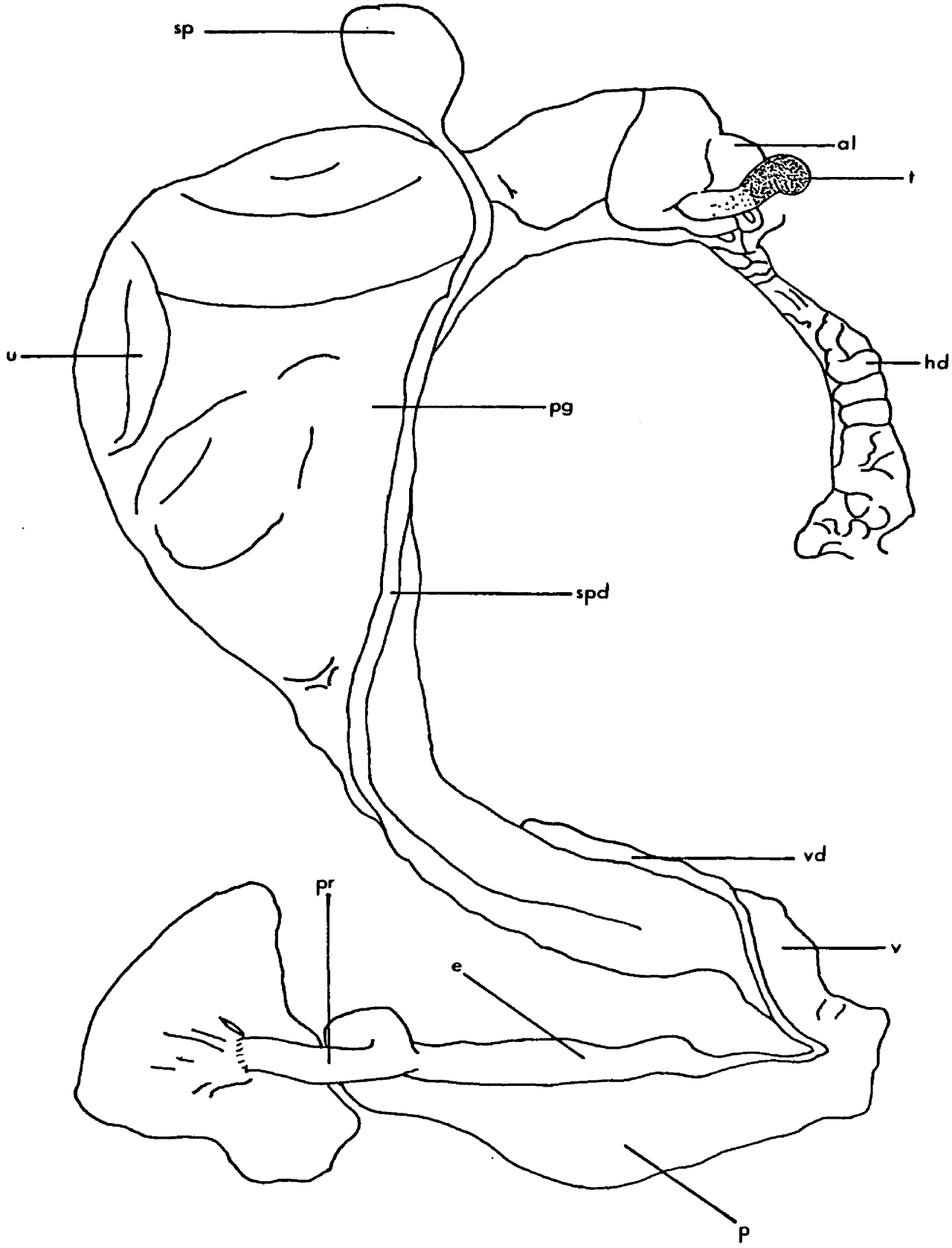

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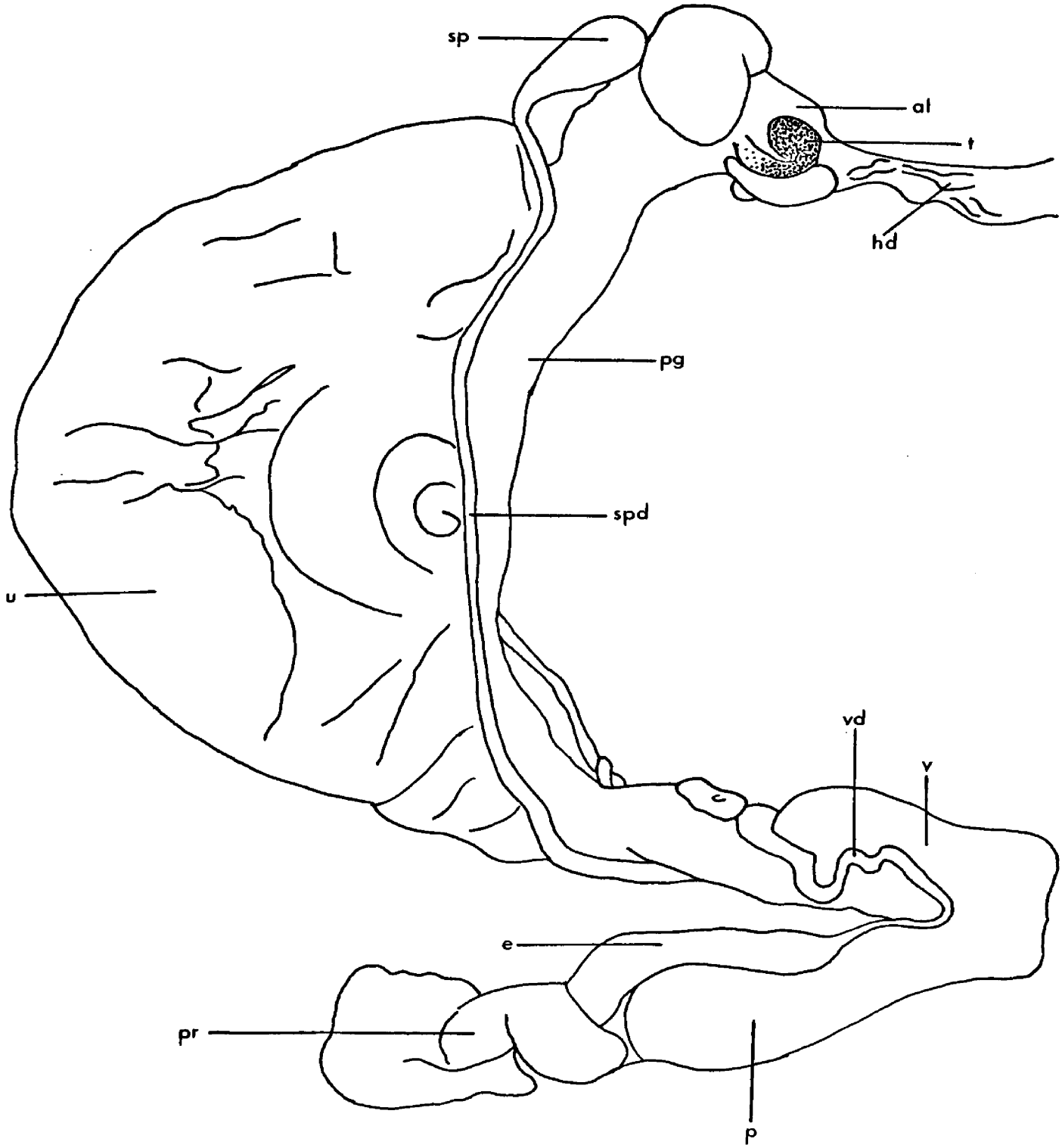
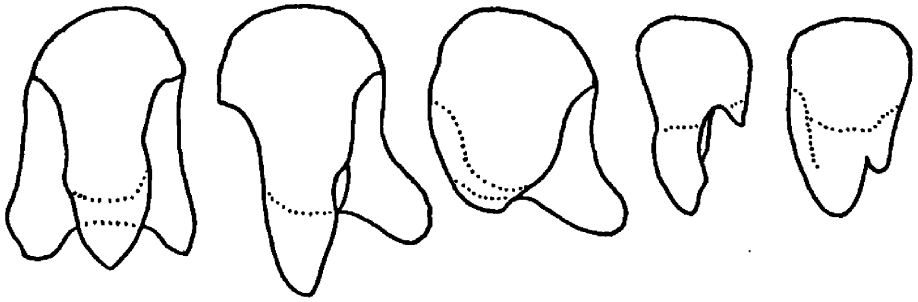


APPENDIX C

In this appendix is shown the variation of the teeth of the radulae of different snails. The letter or number to left of each row of teeth indicates the snail from which the radula was taken. "C" represents the central tooth, the other teeth show the variation in shape and denticulation from the central tooth to the margin of the radula. The orientation is not the same for all of the drawings. As in Appendix B, E-RG-B-RG and 1 to 99 are population I snails, 100 to 199 are snails from population II, 200 to 299 are snails from population III, and 300 to 399 are population IV snails.

C

E-RG



C

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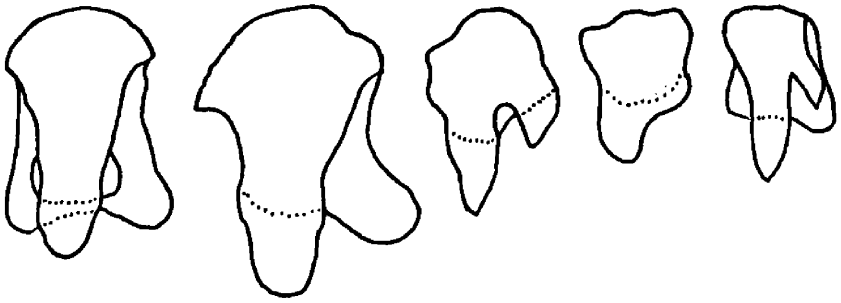

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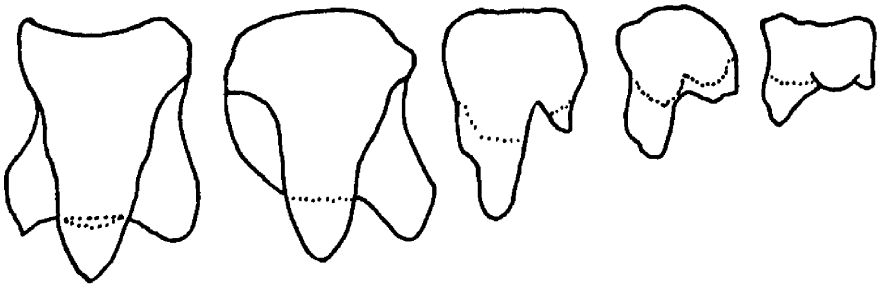
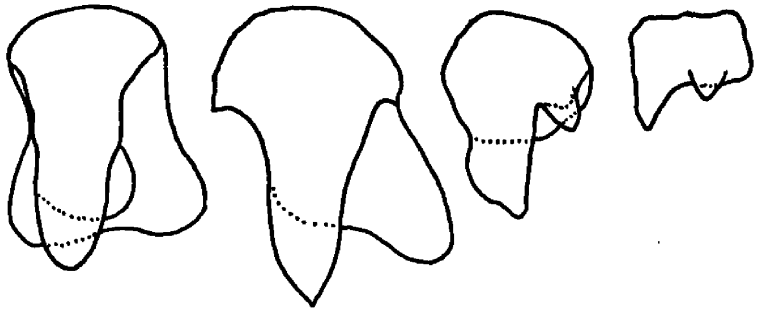


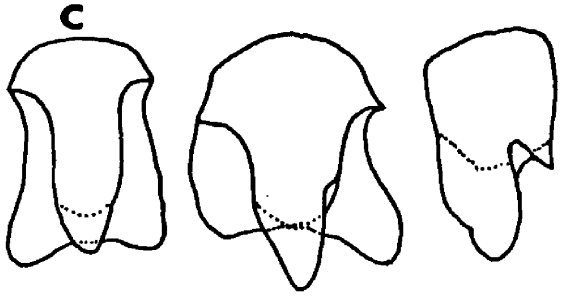
C

23



C

B-RG



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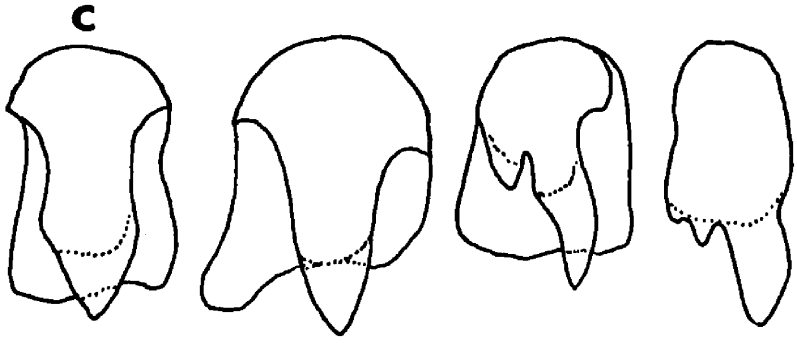

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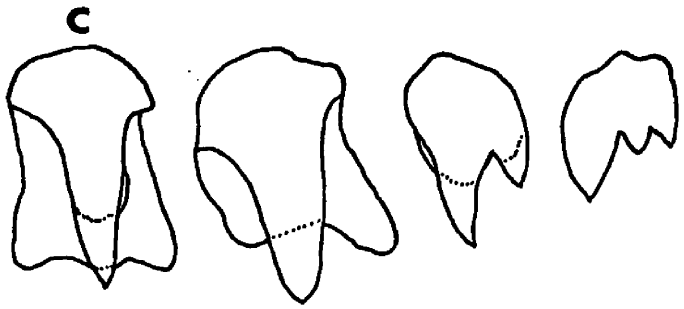

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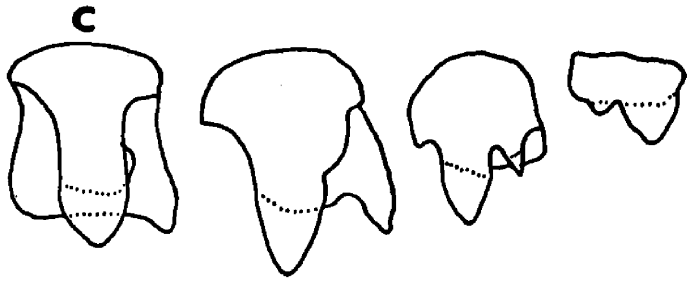

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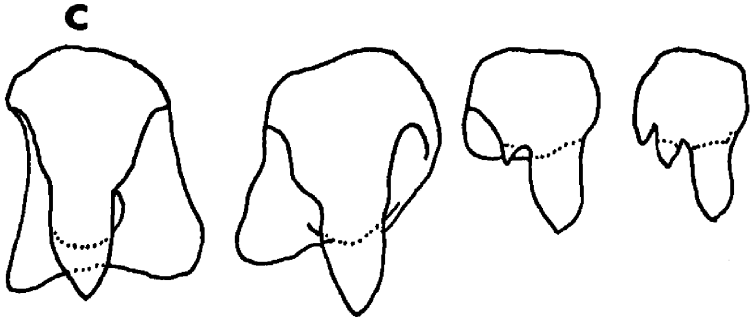

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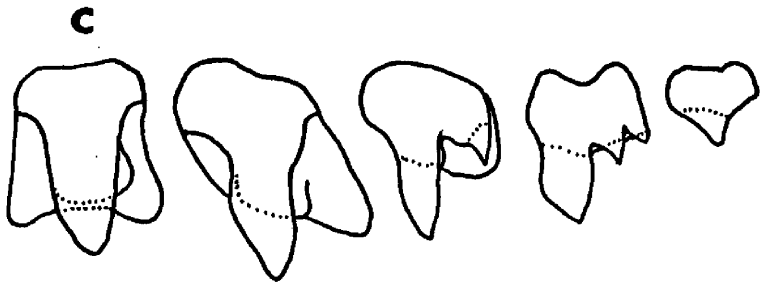

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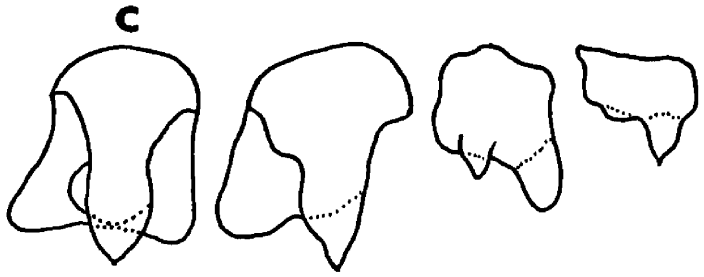

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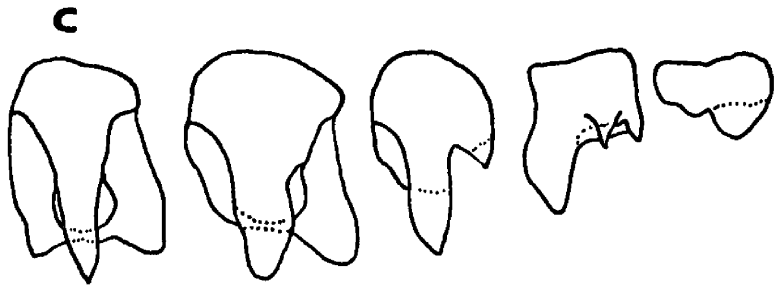

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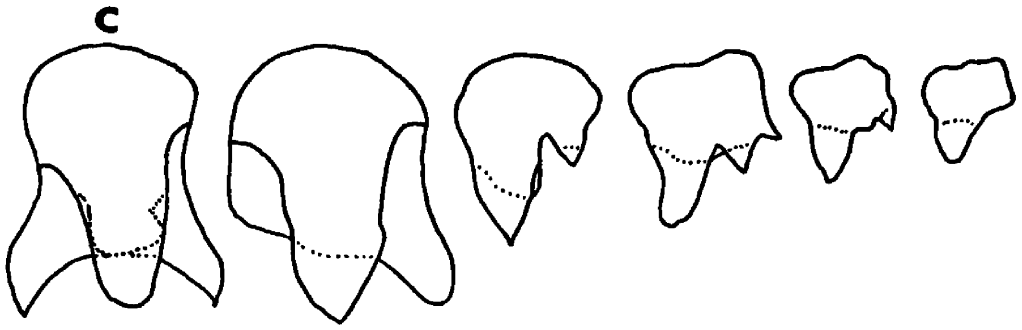

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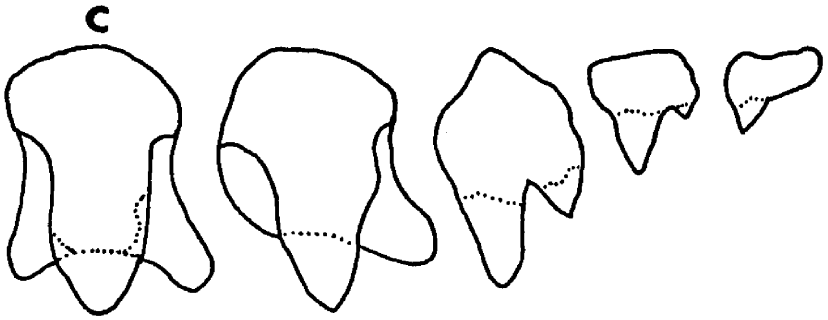

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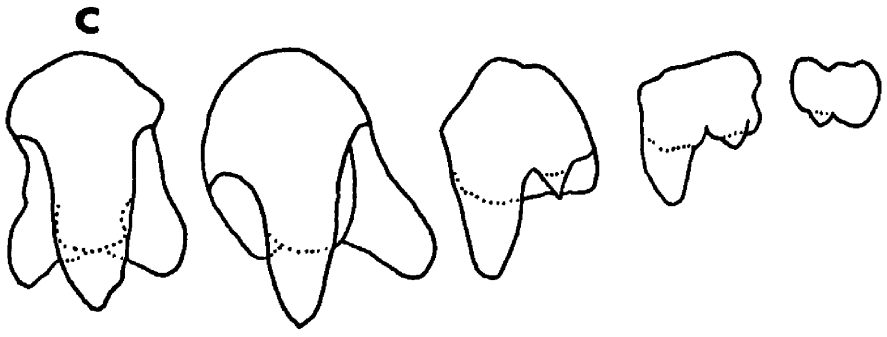

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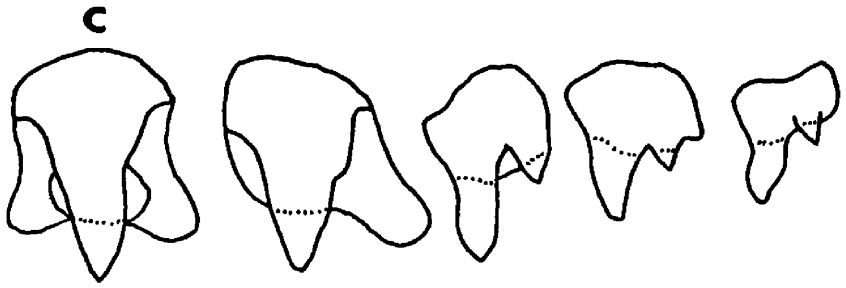

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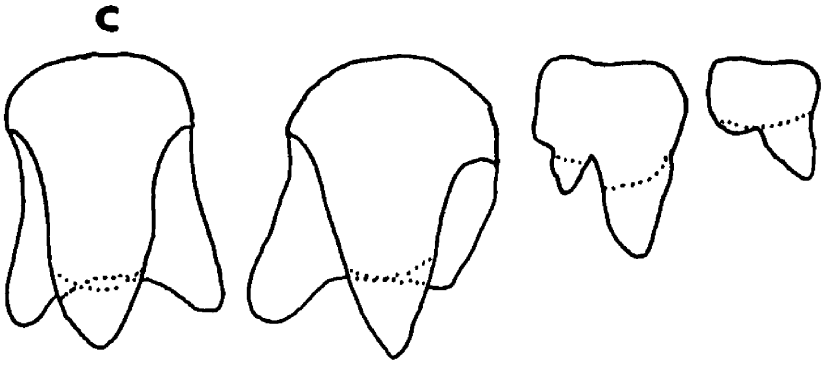

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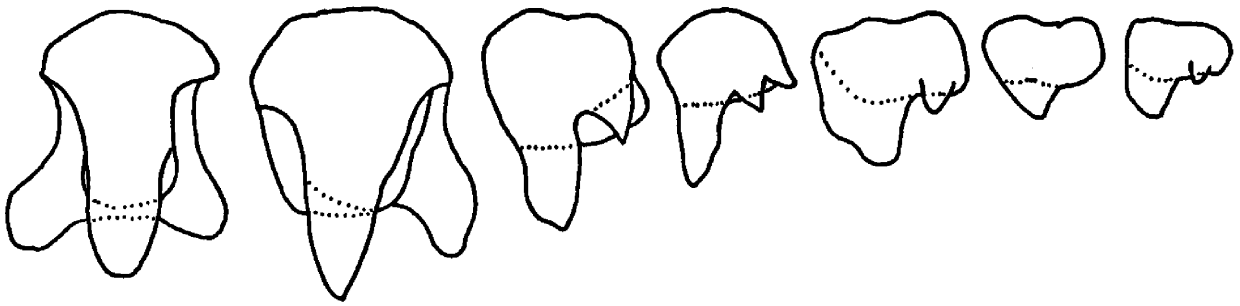

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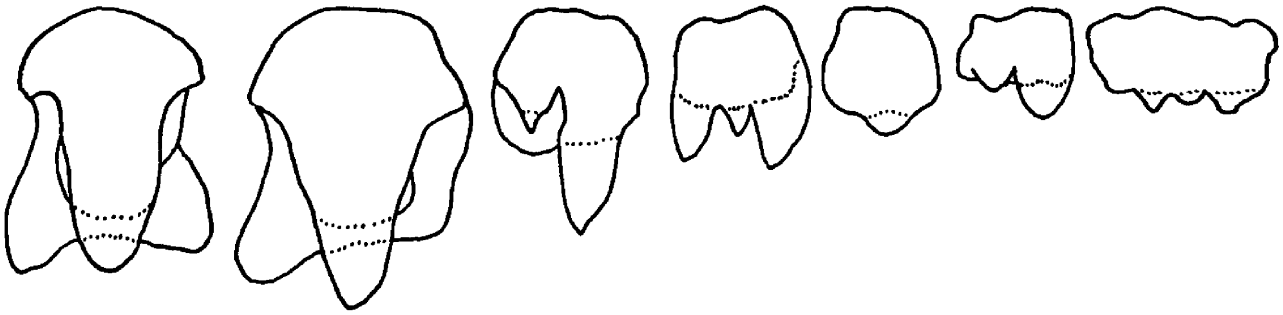

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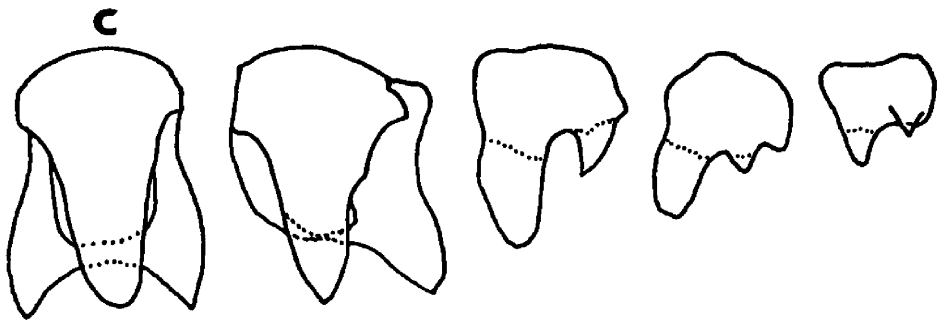

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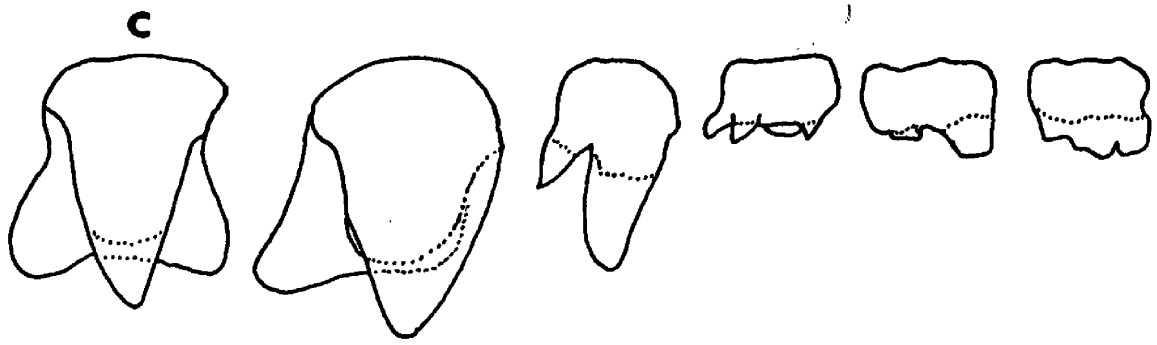

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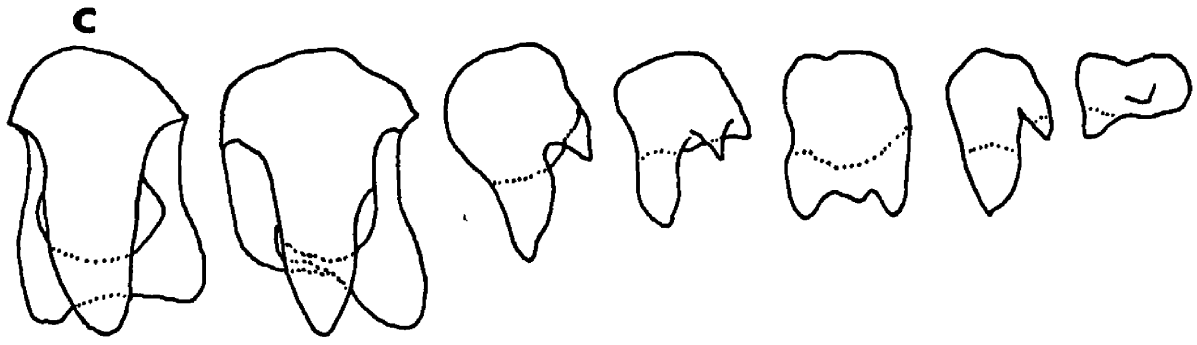

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