STERKIANA

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CONTENTS

ANNOUNCEMENT

STERKIANA is named after Dr. Victor Sterki (1846-1933) of New Philadelphia, Ohio, famed for his work on the Sphaeriidae, Pupillidae, and Valloniidae. It is fitting that this serial should bear his name both because of his association with the Midwest and his lifelong interest in non-marine Mollusca.

The purpose of STERKIANA is to serve malacologists and paleontologists interested in the living and fossil non-marine Mollusca of North and South America by disseminating information in that special field. Since its resources are modest, STERKIANA is not printed by conventional means. Costs are kept at a minimum by utilizing various talents and services available to the Editor. Subscription and reprint prices are based on cost of paper and mailing charges.

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STERKIANA es una coleccion de trabajos sobre los Moluscos extra-marinos viventes y fosiles de las dos Americas, editada por un grupo de malacólogos de los Estados Unidos centrales. Contenirá en el porvenir trabajos en inglés, francés, y español que serán acceptados por la mesa directiva. La correspondencia deberá ser dirigida al Editor.

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FLUORESCENCE CAUSED BY *PSEUDOMONAS* IN THE MUCUS OF *ANGUISPIRA KOCHI* (PFEIFFER)

JOHN M. BAUM AND HUGH C. RAWLS *

INTRODUCTION

Fluorescence under ultraviolet light has been reported to occur in the mucus of *Helix aspersa* (Müller (Turchini, 1926; Fischer and Saddy, 1949) and in the epiphragms of certain other snails (Fischer, 1956). The mucus from living specimens of certain endodontid species and the alcohol used to preserve these and other species of the family have been found to emit a blue fluorescence when exposed to ultraviolet light (Rawls and Yates, 1971). The same kind of fluorescence has been found to occur in one polygyrid species (Rawls and Baum, 1971).

The mechanism responsible for such fluorescence apparently has been unknown, although Derrien and Turchini (1925) noted the possible bacterial origins of the phenomenon. Investigation by the senior author has shown that *Pseudomonas*, a genus of bacteria capable of producing fluorescent pigments (Breed, Murray, and Smith, 1957), is present in the mucus of *Anguispira kochi* and certain non-fluorescent land snails. The object of the study on which this paper is based was to compare the fluorescence of mucus from specimens of *A. kochi* with the fluorescence of pigments produced by pseudomonads isolated from the mucus of these snails, and thereby to determine whether the bacteria are the agents of the fluorescence.

Michejda (1958), Michejda and Urbanski (1958) and Wright (1959, 1964) described chromatographic studies using the ninhydrin and ultraviolet patterns of snail mucus for taxonomic purposes. Michejda (1958) found the chromatographic patterns of mucus similar to those of the foot. Wright (1959) discovered that the substances producing the fluorescent pattern are not derived from the snail tissues proper but from body surface secretions. McGee (1964) extracted from the eggs of *Tegula funebralis* a crude green pigment which partitioned into zeaxanthin, lutein, alpha-carotene, and an unknown green pigment with an attached protein; the last pigment had absorption maxima at 640 and 273 nm in the oxidized state and 273 nm in the reduced state, with 273 nm being near the peak exhibited by Coenzyme Q.

From the foot of *Monodonta turbinata*, Bannister, Bannister, and Micallef, in 1968 isolated a green pigment which had absorption maxima at 680 and 370 nm, and inflections at 440, 330, and 270 nm in methanolic hydrochloric acid. Rawls and Yates (1971) reported that, when the mucus of *Anguispira alternata*, *A. kochi* and *Discus patulus* is exposed to ultra-violet light, a blue fluorescence is emitted; and they suggested that this characteristic might be common to all the Endodontidae. Shortly thereafter, Rawls and Baum (1971) reported a blue-green fluorescence in live

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and preserved specimens of *Mesodon clausus*, the only polygyrid species known to exhibit the phenomenon.

The biochemistry of mucoid substances in snails has not been studied sufficiently, according to Wright (1959), who indicated the presence of an amino sugar in the mucus of *Lymnaea palustris;* he reported that others have extracted glucosamine, mannose and galacturonic acid from the mucin of snail mucus, and galactogen from the albumen glands and egg capsules of *Helix pomatia*. Using the mucus of *Ariolimax columbianus*, Taylor (1963) found protein, polysaccharide and inorganic ions to be present, with glucosamine and fucose comprising 40 per cent of the macromolecular substance.

Pigment production by *Pseudomonas* bacteria has been investigated. Breed *et al.* (1957) described the bacteria as straight, soil or water-inhabiting rods which may produce diffusible pigments varying from fluorescent green through blue, violet, lilac, rose, and yellow. Elliott (1958) demonstrated that riboflavin was absent as a free component of the bacterial pigment. Chodat and Gouda (1961) found several genetically linked pigmented substances, possessing a common chemical skeleton, in culture filtrates of *Pseudomonas fluorescens*. De Ley, in 1964, reported that *Pseudomonas* produces phenazine pigments including pyocyanin, chlororaphin, and oxychlororaphin, phenazine alpha-carboxylic acid, iodinin, indigoidine, brown pigments, and fluorescent pigments the biological function of which is not clearly known but which appear to play a role in electron transfer. De Ley (1964) also reported that a pterine and a flavine were believed to be responsible for blue and yellow fluorescence, respectively, and that phenazine-1-ol had been suggested as the fluorescent compound. Newkirk and Hulcher (1969) showed serine, threonine, glutamic acid and lysine to comprise 26 per cent of the fluorescent molecular mass of the pigment.

The fluorescent, water-soluble pigment produced by *Pseudomonas* has been called by a variety of names, and its physical and chemical nature as well as its fractions only recently have begun to be elucidated. Jordan (1899) and Sullivan (1905) referred to the substance simply as 'the fluorescent pigment.' Burton, Campbell, and Eagles (1948), King, Campbell, and Eagles (1948), Totter and Moseley (1953), King, Ward, and Raney (1954), and Lenhoff (1963) all used the term 'fluorescin.' Elliot (1958) felt that 'pyoverdine' should be used because it specifically identified the bacterial pigment complex. Osawa *et al.* (1963) identified the substances, 'Fluorescence I and Fluorescence II,' in the bacterial pigment. Fluorescence I was the main constituent of the fluorescent pigment produced by the strains studied (Osawa, *et al.*, 1964). Later, Osawa *et al.* (1965) crystallized a non-fluorescent 'C-substance' from Fluorescence II. Newkirk and Hulcher (1969) isolated 'Compound I' from the pigment produced by *Pseudomonas mildenbergii*.

There is a variance of opinion concerning the substances necessary for fluorescent pigment production by *Pseudomonas* species. Using strains of *P. fluorescens*, Jordan (1899) devised a medium containing asparagine with sulfur and phosphorus. Sullivan (1905) found that *P. aeruginosa* grown on a medium of asparagine, magnesium sulfate, dipotassium hydrogen phosphate and glycerine proved to produce pyocyanin and a blue-green fluorescent pigment; he discovered that the carbon contained in sugars and glycerine aided pigment formation, and that sulfates, a high phosphate content, and alkalinity were necessary for the development of blue-green fluorescent pigments. Georgia and Poe (1931) found that magnesium ions and sulfate and phosphate ions are necessary constituents for fluorescent pigment production by *P. fluorescens;* later (1932), comparing peptones as suitable agents of support for the production of fluorescent pigments, they found considerable variation in composition and discovered that some peptones lacked the necessary constituents required for pigment formation.

Burton *et al.* (1948) noted that at high concentrations of phosphate there was a decrease in the formation of pyocyanin and a proportional increase of pyoverdine. Using *P. aeruginosa*, King *et al.* (1948) concluded that magnesium ion was essential for pyoverdine production; that the formation of pyoverdine depended upon the concentrations of dipotassium hydrogen phosphate and iron; that although the sulfate ion was not essential for growth, its presence was required for pyoverdine production; and that the production of pyoverdine could be maintained at a high level over a wide range of magnesium, phosphate, and sulfate concentrations. In their development of Medium A for pyocyanin enhancement, King *et al.* (1954) found asparagine to be unnecessary and a greater production of pyoverdine to occur when the phosphate concentration was increased. Pigment production was increased by the addition of glucose to the medium, and influenced by the type of peptone used (Kluyver, 1956). A glutamic acid medium com-

posed of sodium glutamate, potassium dihydrogen phosphate, magnesium sulfate, and sodium chloride was developed by Osawa's (1963) coworkers, who felt that this medium gave more distinct color tones than did the A and B media developed by King *et al.* (1954).

Pigment production appears to be influenced by the concentration of the medium. Simpler modifications of media promote pigment production (Jordan, 1899) and even if the necessary constituents are present fluorescent pigment production will not occur if the organisms are grown on a highly concentrated medium (Georgia and Poe, 1932).

The presence of acid appears to inhibit the production of pigment by affecting the metabolic activities of the bacteria, rather than interfering with the pigment itself, for the pigment is not destroyed by the acid once it is formed (Jordan, 1899). Slight alkaline or acidic changes have little effect on pigment production, but great variations in either direction result in a colorless growth (Sullivan, 1905). The optimum pH for fluorescent pigment formation appears to be in the neutral range. Georgia and Poe (1931) used a pH range of 6.8 to 7.2, and later (1932) found that a pH between 6.9 and 7.1 permitted optimum fluorescent pigment production. King *et al.* (1954) found a pH of 7.0 to 8.0 to be satisfactory for the determination of pyoverdine on Medium B. Osawa *et al.* (1963) successfully used a pH of 7.2.

Iron appears to be of great importance in the formation of pyoverdine. In 1948, Burton *et al.* noted maximum bacterial growth and an appreciable production of pyoverdine in media free of ferrous sulfate. King *et al.* (1948) stressed that the amount of iron added to media must be minimal. Iron and pyoverdine combine to form part of a respiratory cytochrome; in the absence of iron, the pigment is useless and is excreted into the medium (Knight, 1951). Totter and Moseley (1953) found an inverse relationship to exist between available iron and fluorescent pigment production, and thought pyoverdine to be a substitute for iron-containing cytochromes when a forerunner of the cytochromes is absent. Garibaldi and Neilands (1956) found that *Pseudomonas* exhibits no visual evidence of the formation of iron-binding agents. Lenhoff (1963) observed that when *P. fluorescens* was grown at high oxygen tension (constant aeration) and in the absence of iron, large amounts of pyoverdine were produced, and less than 0.1 per cent protein was attributed to cytochrome c; at low oxygen tension (no aeration) with iron present, cytochrome c comprised 3.5 per cent of the soluble cellular protein and minimal pyoverdine production occurred. Kraft and Ayres (1964) counted over one million cells per milliliter before the pigment became apparent, and they concluded that all of the iron in the medium has to be utilized by the organisms before pyoverdine would be produced. Wasserman (1964) found the ferrous ion to be utilized in pigment formation and believed that the bacteria reduce iron from the ferric to the ferrous state when it serves as the chromogen for compounds formed in metabolism. Garibaldi (1967) discovered that an iron-free medium will not support pseudomonad growth, for it is an absolute requirement of enzyme synthesis; and that conalbumen, an iron-binding egg-white protein, was responsible for an increase in pigment production.

The fluorescent color of pyoverdine appears to vary slightly with the species of *Pseudomonas* producing it, and with the conditions under which the organisms are grown. When *P. ovalis* was grown aerobically in asparagine broth and examined under a black light, Elliot (1958) observed that the top few milliliters become yellow and later diffuse throughout the entire medium; when grown anaerobically, small quantities of blue fluorescing pyoverdine are produced; and that the yellow-green fluorescence changes to blue when the pigment is diluted. Paton (1959) found that the presence of the fluorescent pigment was not dependent upon being visible to the naked eye. Kraft and Ayres (1961) found *P. aeruginosa* to fluoresce a bright, blue-green when grown in asparagine broth. Fluorescence I, isolated by Osawa *et al.* (1963), appeared as a brilliant bluish-white under ultraviolet light and a yellow-green under ordinary light. Two parts of Fluorescence I - fluorescent blue and yellow-green substances, separated by gel filtration - were shown to be the same compound (Osawa *et al.*, (1964). The Fluorescence II fraction was yellow-brown in visible light and a fluorescent yolk-yellow under ultraviolet light (Osawa *et al.*, 1965). Compound I, elucidated by Newkirk and Hulcher in 1969, emitted an intense blue-green fluorescence when exposed to ultra-violet light.

Color changes by pyoverdine because of pH alterations have been found to be reversible (Jordan, 1899; Sullivan, 1905; Elliot, 1958; Wasserman, 1965; Newkirk and Hulcher, 1969). The fluorescent pigment, in a study by Young (1947), was orange when acidified and blue-green when neutra-

lized. Elliot (1958) made a study of visible and fluorescent color changes when the pH of the pigment was altered. Changes in the tone of Fluorescence I as well as the ultra-violet absorption, fluorescent character, and electrophoretic pattern due to changes in pH were noted by Osawa *et al.* (1964), who observed that the fluorescent yellow-green portion of Fluorescence I was very bright at a pH of 8.6 and changed to a blue fluorescence at a pH of 7.5. Wasserman (1965) noted that at a pH of 9.8 the fluorescent color was yellow-green to blue-green; and, when acidified, ranged from blue at neutrality to blue-white, white, whitish-orange, or orange at a pH of 2.0. Newkirk and Hulcher (1969) observed that the blue-green fluorescence from *P. mildenbergii* was most strongly colored at a pH between 7.0 and 9.0.

Hugo and Turner (1957) isolated a soil pseudomonad possessing a green fluorescent pigment which had absorption maxima (405-415 nm in alkaline solution shifting to 360-380 nm when acidified) that were identical to those of the pigment produced by *P. aeruginosa, P. eisenbergii,* and *P. fluorescens* and that corresponded to those of phenazine-1-ol. Crude pyoverdine, dissolved in water, gave absorption maxima at 400 nm and 275 nm (Elliot, 1958). The absorption maxima of pigments produced by *P. aeruginosa* grown in asparagine broth gave peaks at 410 and 270 nm; and at 405 nm for *P. fragi* (Kraft and Ayres, 1961). The peak for Fluorescence II was at 360 nm (Osawa *et al.*, (1963). Both the blue and the yellow-green portions of Fluorescence I had absorption maxima at 400, 260, and 230 nm at a pH of 7.0 (Osawa *et al.*, 1964). The absorption maxima of the C-substance were at 346, 256, and 210 nm (Osawa *et al.*, 1965). Wasserman (1965) found that the absence of an absorption maximum at 405 nm was related to the disappearance of visible color, and he attributed the peak at 260 nm to nucleic acids liberated into the culture medium by the degradation of non-viable cells. Compound I, isolated by Newkirk and Hulcher (1969), had maxima at 402 and 278 nm when the pH was 7.0; in 0.1 M sodium hydroxide, maxima of 401, 311-312, and 278 nm were obtained; and in 0.1 M hydrochloric acid the maxima shifted to 370-376 and 277 nm.

The maximal emission wavelength of Fluorescence I was shown to be 460 nm in two strains of *P. aeruginosa* and 470 nm in another (Osawa *et al., 1964).* Study of the C-substance gave a fluorescence emission maximum at 490 nm with an excitation wavelength of 360 nm (Osawa *et al.*, 1965). The wavelength of emission for Compound I was at 462 nm with an excitation wavelength of 402 nm (Newkirk and Hulcher 1969).

Discoveries concerning the relationships between other bacteria and the pigment produced by *Pseudomonas* have been reported. Bacteriostatic substances have been noted in the fluorescent pigment of *Pseudomonas* (Young, 1947) and Chmura and Pelczar (1959) observed that when *P. aeruginosa* was grown along with *Serratia marcescens,* enhancement of the pseudomonad pigment occurred.

Species of *Pseudomonas,* some of them pigment-producing, appear to be well established as inhabitants of a variety of marine molluscs. Colwell and Liston (1960) found that the Pseudomonas/Vibrio group was the largest single bacterial group inhabiting Pacific oysters, *Crassostrea gigas.* Pigment-producing strains of *P. fluorescens* have been isolated for *Cyclostoma elegans* and *C. sulcatum* by Mahdihassen (1961), who found that the bacterial growth of these strains was kept under control in the molluscan host. Colwell and Liston (1962), in an extensive bacterial study of marine vertebrates and invertebrates, discovered that 48 percent of the bacteria found in the invertebrate group belonged to the genus *Pseudomonas;* this genus and *Achromobacter* were found in the snail, *Lambis lambis,* and in the squid, *Loligo opalescens,* with 89 percent of the pseudomonads from the squid producing a green fluorescent pigment in culture; and in *C. gigas, Pseudomonas* was one of the predominant genera isolated from the body tissue and fluids. Surianinova (1962) isolated pseudomonads from the surface and various body parts of *Mytilus galloprovincialis.* Beeson and Johnson (1967) found that clams of the genus *Donax* contained *Pseudomonas* and *Vibrio* as their bacterial symbionts.

MATERIALS AND METHODS

Specimens of *Anguispira kochi* were collected from the southeastern and southwestern borders of Foley's Woods, a nature preserve near Paris, Illinois, and from the northeastern and southeastern borders of Fox Ridge State Park, eight miles south of Charleston, Illinois. *A. alternata* and *M. thyroidus* from the same localities, and specimens of *Liguus* supplied by Mr. E. C. Winte, were used for comparative purposes.

A bacterial transfer loop was used to take mucus samples from individuals of each species. A loopful of mucus was streaked on Petri plates containing Medium

B (King *et al.*, 1954) to obtain isolated colonies (Pelczar and Reid 1965). This procedure was repeated the following day using the same snails and yielded 48 inoculated plates. Medium B was made in the following manner: Difco Proteose Peptone, 20 gm; Glycerine, 10 ml; K_2HPO_4, 1.5 gm; $MgSO_4$, 1.5 gm; Agar, 15 gm; Distilled water, 1,000 ml; and pH adjusted to 7.0-7.2. For liquid media, the agar was omitted.

After 48 hours, the colonies that fluoresced under a UVSL 13 combination ultra-violet light (Ultra-violet Products, Inc.) were isolated in pure culture by re-streaking isolated colonies at least three consecutive times, during which no foreign bacterial growth was detected. A gram stain of each pure culture was made as a primary test for purity and for gross identification. Bacto-Differentiation Discs, Oxidase (Difco Laboratories, 1966) were employed as a means for determining the bacteria as members of the genus, *Pseudomonas*. The pure isolates were streaked on nutrient agar (Difco) slants and refrigerated for storage.

All of the cultures used in the investigation were grown at room temperature without constant mechanical aeration. Capall or Kaput (Bellco) caps enclosed the 16 mm culture tubes. Before inoculating any media for experimental purposes, each of the *Pseudomonas* isolates used in the investigation was transferred from the slant and grown in 3 ml of nutrient broth (Difco) for 48 hours. To obtain simultaneous growth of all the cultures isolated from a particular species of snail, a loopful of each of the 48-hour pseudomonad suspensions was inoculated into a common culture tube containing 3 ml of nutrient broth. The bacteria in this suspension were then inoculated on the experimental medium. Because Jordan (1899) and Sullivan (1905) reported that daylight will decompose pyoverdine, all fluorescent pigments produced by the *Pseudomonas* isolates were stored in a dark place.

To compare solubility properties of the bacterial pigment and the fluorescent pigment present in the mucus of *A. kochi*, pseudomonad cultures were grown simultaneously for several days in culture tubes containing 5 ml of liquid Medium B. Pigment production in these tubes was checked visually and under ultra-violet light. Mucus from several specimens of *A. kochi* was dissolved in 10 ml distilled water and its fluorescence checked under ultra-violet light. The bacterial suspension and the mucus solution both then were centrifuged in a Sorvall superspeed centrifuge at 13,500 RPM for 30 minutes. Several drops of each fluorescent supernatant were added to test tubes containing a number of solvents. The tubes were shaken and then examined under ultra-violet light to determine whether the fluorescent substances were soluble in the given solvents.

As a second experimental method, mucus was extracted from snails by means of a medicine dropper calibrated with one ml water. A total of one ml of mucus was taken from seven specimens of *A. kochi* and dissolved in 5 ml of 0.01 M phosphate buffer, pH 7.0. The isolates were grown in 3 ml of Medium B for several days and then added to 2 ml of the buffer. Both solutions were centrifuged at 13,500 RPM for 30 minutes. More buffer was added to each supernatant, bringing the total volume of each to 10 ml. The fluorescent solutions then were put into dialysis bags and suspended in 40 ml of the phosphate buffer contained in a sealed 250 ml flask. The dialytic property of the solutions was observed under ultra-violet light.

Bacteria isolated from *A. kochi* were all inoculated into a common tube of nutrient broth and then streaked on three plates of Medium B to obtain solid masses of growth. After 72 hours, the bacteria were scraped from the plates with a clean glass slide and placed in a tube containing 10 ml distilled water. The tube was covered with Parafilm (American Can Co.) and agitated until a homogeneous suspension was obtained. The suspension was centrifuged and 0.1 N HCl and 0.1 N NaOH were added to 3 ml portions of the supernatant to obtain pH readings of 4.0, 7.0, and 10.0 on a Beckman Zeromatic pH-meter. The same procedure was followed for a 1:10 dilution of the supernatant and for one ml of mucus dissolved in 10 ml distilled water. This solution also was centrifuged. The pH of the mucus was recorded before pH changes were effected by addition of HCl and NaOH. Observations then were made of the fluorescent color changes produced by dilution and alteration of pH.

Pseudomonad isolates obtained from *A. kochi* were grown simultaneously in culture tubes of Medium B for several days until pigment production was evident. Additional mucus samples obtained from several specimens of *A. kochi* were dissolved in distilled water and poured into a culture tube. All tubes then were autoclaved at 250° C at 15 lbs for 15 minutes. The fluorescent substances then were observed under ultra-violet light.

In an effort to determine any visible difference in pigment production between the bacteria isolated from *A. kochi* and from *M. thyroidus*, cultures isolated from

the two species were grown independently on plates of Plate Count Agar (Difco), 1 percent Tryptone (Difco) Agar, and 0.5 per cent Peptone (Difco) Agar. After 48 hours, the cultures were examined visually and under ultra-violet light to determine whether any extraordinary pigment formation had occurred.

For the spectrophotometric study, pigments produced by the *Pseudomonas* isolates and the mucus solutions from *A. kochi*, *M. thyroidus*, and specimens of *Liguus* species were compared. Mucus solutions from *M. thyroidus* and *Liguus* specimens were studied spectrophotometrically to provide records for comparison of non-fluorescent mucus with the bacterial pigment and the mucus of *A. kochi*. A total of one ml of mucus was extracted from six specimens of *A. kochi* and diluted in 10 ml of 0.01 M phosphate buffer. Several loops of mucus from other individuals of *A. kochi* were diluted in 4 ml quantities of standard buffer (Sargent-Welch) at pH 4.0 and pH 10.0. A total of one ml of mucus from seven specimens of *M. thyroidus* and a total of one ml from two specimens of *Liguus* were each added to 10 ml phosphate buffer. Individual Petri plates of Medium B were inoculated to obtain a solid mass of growth with each isolate. A suspension of nutrient broth containing all of the 48-hour isolates also was inoculated in the same manner to obtain a simultaneous growth of the organisms. After 72 hours, clean glass slides were used to scrape the bacteria from the plates and to place the scrapings in tubes containing 10 ml of the three buffer solutions (pH 4.0 7.0 and 10.0). The tubes were covered with Parafilm and shaken to obtain homogeneous suspensions. All of the mucus and bacterial suspensions were centrifuged at 13,500 RPM for 30 minutes. In most cases, repeated centrifugation was needed. Phillips (1964) stated that aliphatic ethers are transparent, except for possible end absorption, so ether was added to prevent bacterial growth in those solutions containing phosphate buffer. In solutions containing the other two buffers, the addition of ether was not necessary because spectrophotometric recordings were made immediately following centrifugation. In addition to these solutions, the supernatant containing the phosphate buffer and pigments produced by all of the pseudomonad isolates from *A. kochi* was added to a solution of mucus from *Mesodon thyroidus* in proportions of 1:1, 1:3, 3:1. Absorption spectra were recorded on a Beckman DB-G spectrophotometer. The phosphate buffer with ether was used as a reference in recording all absorption spectra at a pH of 7.0, and the other two buffers were used when spectra were run at their corresponding pH values.

Haynes (1951) stated that most pseudomonads will not grow at 37° C, and Schneierson, Amsterdam and Perlman (1960) used varied amounts of chloramphenicol to prevent pigment production by *P. aeruginosa* in culture. In this study, efforts were made to prevent bacterial growth and/or pigment production in live specimens of *A. kochi* by controlling temperature and by using chloromycetin (chloramphenicol). Mucus was extracted from the snails in order to diminish the amounts of bacteria and pigment already present. Three specimens of *A. kochi* so treated were placed in an oven at 37° C; two additional specimens were treated respectively with solutions containing 30 mg chloromycetin/ml distilled water and 15 mg/ml, the solutions administered by pipette into the shell aperture; the shells were turned so that their apertures faced upward to prevent the chloromycetin from draining out. The use of temperature control and chloromycetin was continued for 18 hours, at which time the snails were observed under ultra-violet light to determine the effect, if any, of fluorescent pigment production.

RESULTS

The mucus from *A. kochi* and *A. alternata* was viscous and contained a visible amber pigment. A brilliant blue fluorescence was emitted when mucus from specimens collected during the summer months was exposed to ultra-violet light. Recent investigation has shown the mucus from specimens of *A. alternata* collected during the spring months to fluoresce blue-green. When most of the mucus had been extracted from any individual of these two species, the amber color disappeared and the newly-produced mucus became less viscous but the color of its fluorescence did not seem to be affected. The mucus from *M. thyroidus* was not viscous, nor was it pigmented, and no fluorescence could be detected under ultra-violet light.

Bacteria which produce fluorescing pigments were isolated from the mucus of *A. kochi* and *A. alternata*, as well as from the non-fluorescent mucus of *Mesodon thyroidus*. All of the bacteria that produced fluorescent pigments on Medium B were gram-negative rods and oxidase positive; these characteristics are indicative of the genus, *Pseudomonas*. Sixty-three fluorescent isolates were obtained from the mucus of the three snail species; twenty-two isolates from *A. kochi*, 20 from *A. alternata*, and 21 from *M. thyroidus*. Differences in ha-

bitat did not appear to affect the fluorescence in the snails, or the snail-bacteria relationship.

When grown on solid or in liquid Medium B, most of the isolates produced a blue-green fluorescent pigment, but some colonies produced fluorescent blue or yellow-green pigments when grown on agar plates of Medium B. It was impossible to group the colonies by their fluorescent pigments because distinct color demarcations could not be made. The pigment produced by all of the isolates was yellow-green in daylight and diffused evenly throughout the agar plates of Medium B. In culture tubes of Medium B, the pigment had the same visible color but was formed near the surface of the medium.

Both the fluorescent pigment present in the mucus of *A. kochi* and the pigment produced by the bacteria isolated from this species have the same solubility properties. These properties and the solvents used are shown in Table 1.

TABLE 1. Solubility properties of the fluorescent bacterial and mucus pigments[a]

Solvent	BP[b]	MP[c]
Water	s	s
Methyl alcohol (absolute)	s	s
Ethyl alcohol (85%)	s	s
Isopropyl alcohol (absolute)	s	s
Formaline (10%)	s	s
Glacial acetic acid (conc.)	s	s
Hydrochloric acid (conc.)	s	s
Glycerine	s	s
Acetone	s	s
Dioxane	s	s
Chloroform	i	i
Ether	i	i
Amyl acetate	i	i
Aniline	i	i
Benzene	i	i
Essence of Euparal	i	i
Wintergreen oil	i	i
Xylol	i	i

[a] s : soluble; i : insoluble

[b] Bacterial pigment

[c] Mucus pigment

The fluorescent pigment from the mucus of *A. kochi* and the pigment formed by the *Pseudomonas* isolates both exhibited the same dialytic property. After a period of 24 hours, fluorescence was apparent on both sides of the dialysing membrane.

Autoclaving at 250° C for 15 minutes at 15 p.s.i. did not appear to affect either the pigment present in *A. kochi* mucus or the pigment produced by isolates from the mucus.

The pigment produced by the bacterial isolates fluoresced blue-green in liquid Medium B. At a pH of 7.0, the pigment fluoresced blue, much like that of *A. kochi* mucus, but was not as brilliant; and changed to fluorescent whitish-blue at a pH of 4.0, then to blue-green at a pH of 10.0. All color changes were reversible. When the original solution was diluted 1:10 with distilled water, the blue-green fluorescence changed to a blue fluorescence of the same hue as found in the mucus of *A. kochi*. The diluted bacterial pigment did not show any color changes when pH alterations were made. The pH of the mucus from *A. kochi* was found to be 7.0, and the blue fluorescent color did not change when the pH was altered to acid or basic values. Mucus from the recently collected specimens of *A. alternata* emitted a blue-green fluorescence, but when the mucus was diluted with distilled water a blue fluorescence was observed.

There were no apparent differences in pigment production between those pseudomonads isolated from *A. kochi* and those isolated from *M. thyroidus*. Each culture isolated from the two species produced fluorescent pigments that diffused evenly throughout the Medium B agar but did not diffuse throughout Plate Count agar, Tryptone agar and Peptone agar. Fluorescence, when present, was confined to the colonies themselves. Pigment production by the cultures isolated from both snail species was variable on Plate Count and Peptone agar; approximately 50 per cent of these isolates produced fluorescent pigments in the two media. The Tryptone agar supported small white colonies when streaked with isolates from *A. kochi* and *M. thyroidus*, but fluorescence was difficult to determine because of the reflection of light by the colonies.

The absorption peaks of those pigments formed by each culture isolated from *A. kochi* ranged from 258 to 270 nm and from 315 to 410 nm. Two peaks, one at 265 nm and another at 400 nm, were found to be characteristic of the isolates. The spectra of the mucus solutions from the non-fluorescent snails, *M. thyroidus* and *Liguus*, were similar, each with a peak at about 270 nm (Fig. 1-A). When the supernatant of the fluorescent pigments from simultaneously grown *A. kochi* isolates was added to *M. thyroidus* mucus, absorption peaks similar to those of the supernatant alone

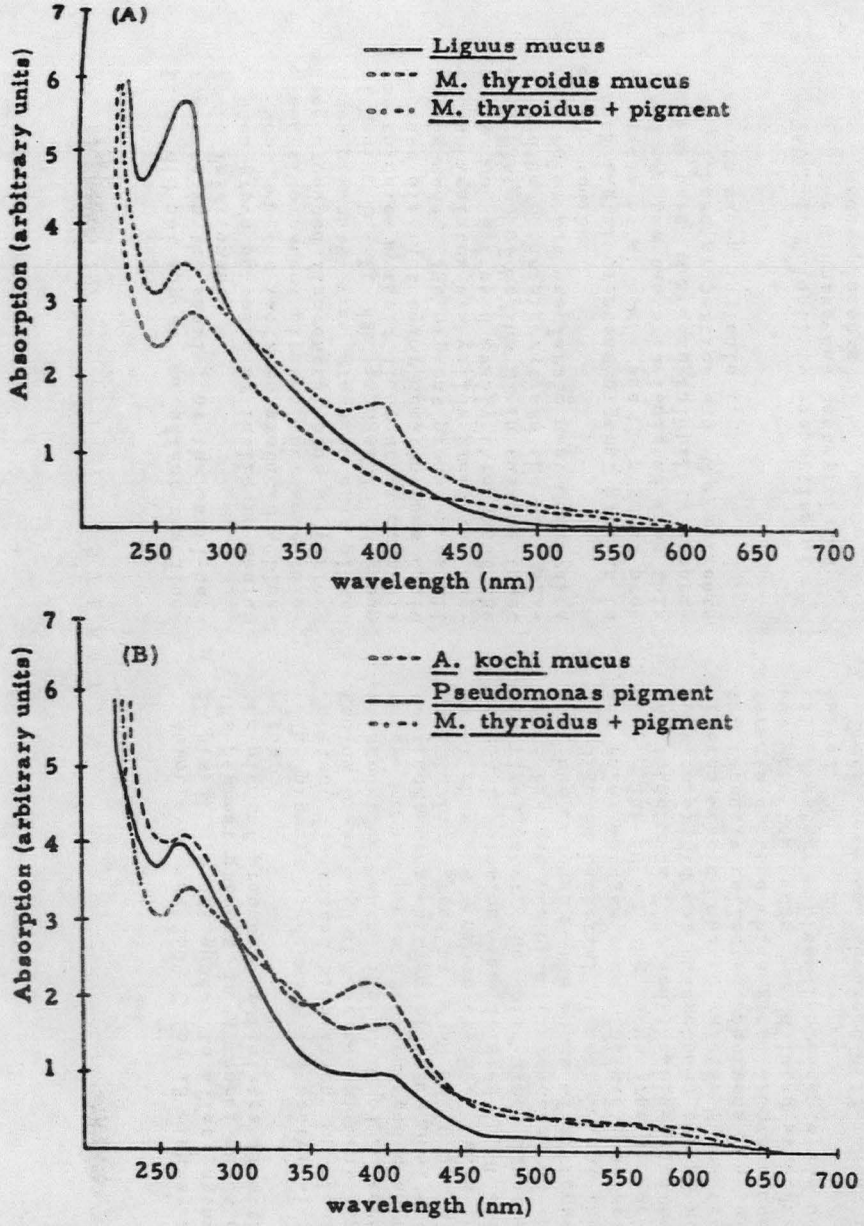

FIG. 1. Absorption spectra (pH 7.0) of snail mucus solutions, mucus solutions with bacterial pigment, and bacterial pigment.

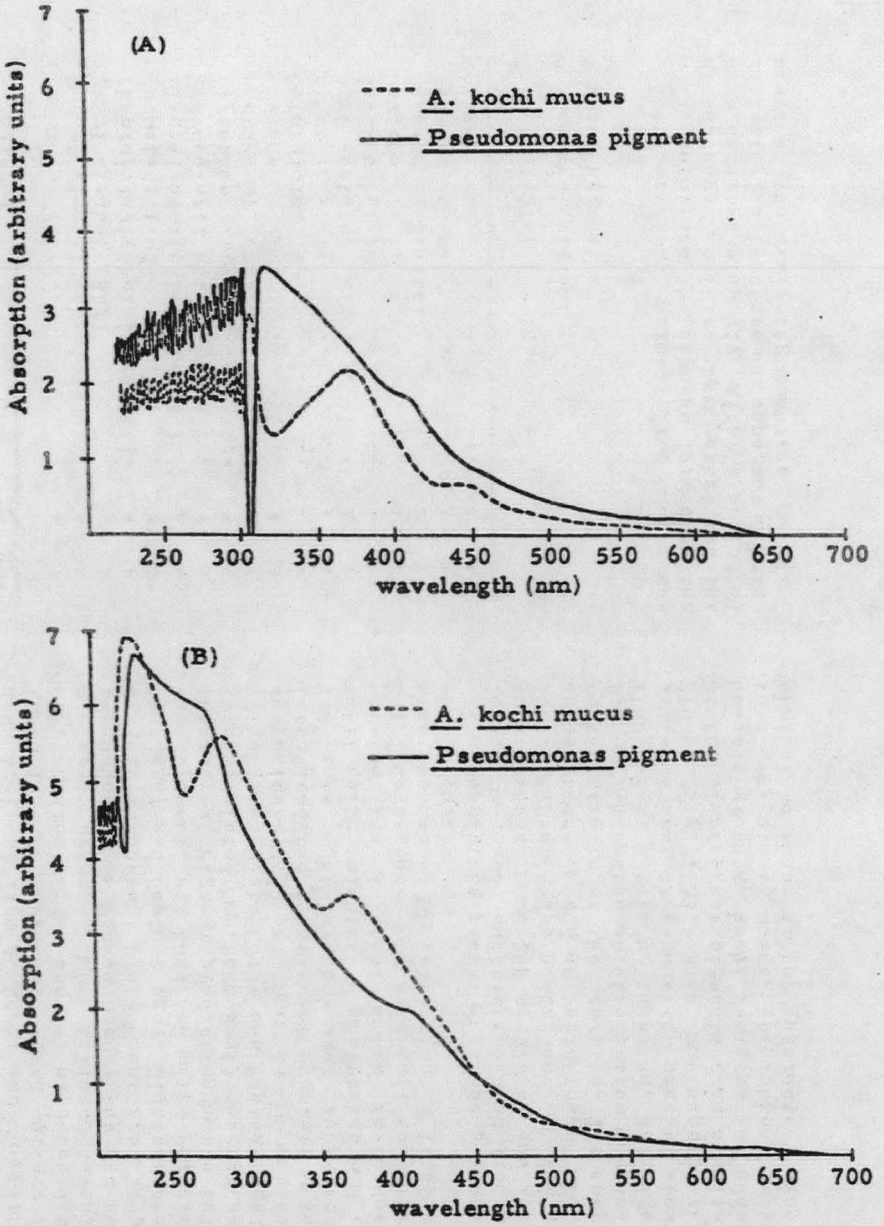

FIG. 2. Absorption spectra of *A. kochi* mucus solution and bacterial pigment at (A) pH 4.0 and (B) pH 10.0.

were obtained (Figs. 1-A and 1-B). The absorption spectra of the supernatant *M. thyroidus* mucus solution were the same for the 1:1, 1:3 and 3:1 proportions. Figure 1-B depicts the absorption spectra of the bacterial pigment compared to the mucus solution from *A. kochi*. The spectra are similar, with the peaks of the bacterial pigment at about 265 and 400 nm and those of the mucus solution at about 265 and 385 nm. Changes in pH to 4.0 and 10.0 appeared to have similar effects on the absorption spectra of both the bacterial pigment and the pigment from the mucus of *A. kochi* (Figs. 2-A and 2-B). At a pH of 4.0, the bacterial pigment had a peak at about 320 nm with an inflection at about 405 nm. The mucus solution showed absorption peaks at about 310, 370, and 450 nm. At a pH of 10.0, the bacterial pigment showed peaks at about 235, 270, and 400 nm. Absorption peaks at about 225, 290, and 365 nm appeared when the mucus was altered to the same pH. Oscillations occurred in all spectra recorded in the acidic and basic ranges. When the pH was 4.0, oscillation began at about 305 nm for both the bacterial pigment and the mucus solution. At a pH of 10.0, the recordings of both the bacterial pigment and the mucus solution showed oscillations beginning at 215 nm. The wavelength of emitted fluorescence in all cases was 462 nm.

Efforts to prevent fluorescence in living specimens of *A. kochi* were based on work by Haynes (1951) and Schneierson *et al.* (1960), but in this study they were failures. Within 18 hours after controlled temperature and chloramphenicol experiments were started, the subject snails were dead.

DISCUSSION

King *et al.* (1954) reported that, if only one medium could be used for general purpose, Medium B would be suitable for detecting *Pseudomonas*. They observed that a slight yellowing of either Medium A or B was the most frequently encountered reaction with bacteria other than pseudomonads, and that upon examination with ultra-violet light the foreign substance would not fluoresce. According to the Difco Supplementary Literature (1966), oxidase differentiation discs may be used to differentiate strains of *Pseudomonas* from other organisms, a positive reaction being indicative of the genus. Osawa *et al.* *(1963)* used the production of fluorescent pigments and the oxidase reaction to confirm their *Pseudomonas* identifications. Because the colonies used in this study produced fluorescent pigments on Medium B and were oxidase positive, gram negative rods, their identification as pseudomonads appears to be justified. Colwell and Liston (1961) discussed the difficulty that they encountered when using Bergey's Manual (Breed *et al.*, 1957) to identify their strains of *Pseudomonas* at the species level. Consequently, this study made no attempt to identify any of the isolates beyond the generic level.

The literature indicates that *Pseudomonas* is a predominant genus of bacteria found in many marine molluscs. The results from this study indicate that these bacteria are present in the mucus of *A. kochi*, *A. alternata*, and *M. thyroidus*, and they suggest that pseudomonads will be found in the mucus of other species of land snails, fluorescent and non-fluorescent alike. Because pseudomonads have been isolated from fluorescent and non-fluorescent mucus, a presence-absence aspect surely is not responsible for the fluorescence which has been observed in certain species. However, the presence of these bacteria establishes a potential for the production of diffusible pigments. If *Pseudomonas* bacteria are responsible for the phenomenon, the physiology of fluorescent snails must be responsible for the production of substances like or unlike those found in Medium B which enable the bacteria to produce the fluorescent pigment. Conversely, there may be substances, such as iron, present in the non-fluorescent mucus that inhibit the liberation of the pigment. In describing the media for the support of pigment production, King *et al.* (1954) stated that the usefulness of a culture medium is determined not only by the presence of certain necessary constituents, but also by the absence of minimal concentrations of substances which may have adverse effects. This principle is applicable to the constituents of snail mucus with regard to its role as a medium for the enhancement of fluorescent pigments produced by pseudomonads.

The results of previous work in developing ideal media for fluorescent pigment production are useful for speculating on the nature of substances in mucus which would permit the production of fluorescent pigments. It would seem that sulfates (Sullivan, 1905; Georgia and Poe, 1931; Burton *et al.*, 1948; King *et al.*, 1948, 1954), are necessary inorganic constituents for the production of such pigments. Burton *et al.* (1948), King *et al.* (1948), Knight (1951), Totter and Moseley (1953), and Kraft and Ayres (1964) all indicated that the amount of free iron in such media should be minimal. It is possible that the mucus

from non-fluorescent snails may lack sulfates phosphates, and magnesium; or have a high concentration of iron; or both. Conversely, the mucus from *A. kochi* and other fluorescent snails may possess the needed sulfate phosphate and magnesium ions; or have a low iron content; or both. The media used by Georgia and Poe (1931, 1932), King *et al.* (1954), and Osawa *et al.* (1963) all had a pH near or at neutrality. The mucus from *A. kochi* was found to have a pH of 7.0, indicating another factor which may be of some importance.

Paton (1959), Osawa *et al.* (1963), and Wasserman (1965) found that the occurrence of fluorescence was not dependent upon the bacterial pigment being visible to the naked eye. Likewise, the mucus from *A. kochi* exhibited the phenomenon of fluorescence even when the amber pigment was not visible. Both fluorescent substances dialyzed, suggesting a small molecular size; and withstood autoclaving, an indication that they are not composed of protein. These results and the solubility properties described in Table 1 indicate that the fluorescent substance in the mucus of *A. kochi* and the fluorescent substance produced by bacterial isolates from these snails are similar, if not identical.

The blue-green fluorescence exhibited by pseudomonad isolates changed from that color to blue when the pH was brought to 7.0; when the bacterial pigment was diluted, however, a blue fluorescence was emitted which did not change color when the pH was altered. Although the mucus from *A. kochi* fluoresced blue, not the blue-green shown by the bacterial pigment, the pH of the mucus was found to be 7.0, indicating that if the bacterial pigment were present in the mucus it would fluoresce blue. The blue fluorescence of the mucus did not change color when the pH was altered, and the diluted bacterial pigment reacted similarly, suggesting that if the bacterial pigment is present in the mucus, its quantity is minute. The pH of mucus and the small quantity of the bacterial pigment both may be responsible for the fluorescent color of the mucus.

It is reasonable to assume that a low oxygen tension exists in the mucus of land snails such as *A. kochi*. Lenhoff (1963) found that when *P. fluorescens* was grown at low oxygen tension (non-aerated) on a medium containing less than 8 X 10^{-6} M ferric chloride, a blue fluorescence appeared with only a trace of pyoverdine being excreted into the medium. This further explains the blue color of the fluorescence of mucus as being a function of small quantities of pigment, and it also suggests that oxygen tension and iron concentration affect the color of the fluorescence exhibited.

Although this study was concerned with *A. kochi*, investigation indicates that the blue fluorescence in the mucus of *A. alternata* is identical to that of *A. kochi*. Having isolated pseudomonads from members of both species, it seems probable that whatever is causing fluorescence in one is responsible for that phenomenon in the other. The blue-green fluorescent pigment in recently collected specimens of *A. alternata* resembles the bacterial pigment both in original color and in the fact that when the mucus is diluted with water the fluorescence changes to blue. The presence of blue and blue-green fluorescence in the mucus of *A. alternata* is compatible with the hypothesis that pigment formation by *Pseudomonas* may be a function of the physiology of the snail.

When grown on Medium B, Plate Count Agar, Tryptone agar, and Peptone agar, there did not appear to be any significant difference in pigment production among the cultures isolated from *A. kochi* and those isolated from *M. thyroidus*. It is thus apparent that *A. kochi* has not selected for strains of *Pseudomonas* having nutritional requirements which are more easily satisfied than those of pseudomonads found in *M. thyroidus*. Once again, it appears that the fluorescence of the mucus is dependent on the physiology of the snails rather than on metabolism of the bacteria.

Mucus samples from *A. kochi*, *M. thyroidus*, and *Liguus* species all had an absorption peak at about 265 nm (pH 7.0). The bacterial pigment and the mucus from *A. kochi* had a second peak at about 385 and 400 nm, respectively. It appears that these second peaks are related to the fluorescent nature of the substances. Wasserman (1965) suggested that an absorption peak at 260 nm may be due to nucleic acids liberated into the medium by the degradation of non-viable cells by other bacteria, a suggestion which is substantiated by our determination that *Pseudomonas* is present in the mucus of *A. kochi*, *A. alternata*, *M. thyroidus*, and *Liguus*.

The absorption maxima at about 265 and 400 nm appear to be indicative of the fluorescent pigments produced by the pseudomonad isolates. Variable results obtained by others (Hugo and Turner, 1957; Elliot, 1958; Kraft and Ayres, 1961; Osawa *et al.*, 1964; Wasserman, 1965; Newkirk and Hulcher, 1969) regarding the absorption maxima of *Pseudomonas* pigments is in accordance with the variations observed in this study. The absorption curves of *A. kochi* mucus and

bacterial pigment are similar, although the peak at about 400 nm for the bacterial pigment does not match the peak at about 385 nm for mucus. Neither of the fluorescing substances in this study has been isolated and purified, and it is possible that during the production of the pigment by the bacteria in the snail mucus something in the mucus might be metabolized by the bacteria and shift the peak to a lower wavelength. This is borne out by the fact that the absorption maxima of the bacterial pigment to which *M. thyroidus* mucus had been added exhibited peaks which resemble those of the bacterial pigment alone. This indicates that the peak at about 385 nm might not be merely a function of the physical mixing of the mucus and the bacterial pigment, but might be a function of bacterial metabolism.

The effects of pH on the absorption spectra of the two pigments do not correlate with the spectra of pseudomonad pigments reported by Osawa *et al.* (1964) and Newkirk and Hulcher (1969). The oscillations and absorption maxima found in this study are unique. At first, the oscillations appeared to be artifacts; however, they disappeared when water blanks were recorded, then reappeared when the pigments were recorded a second time, and we conclude that they are valid characteristics. Although the corresponding buffer was used as a reference at each pH, it is possible that the buffer might have reacted with the pigments and so altered the absorption curves. If this is true, the oscillations show that the buffer probably reacted with both pigments in a similar manner, for the oscillations begin at the same wavelength for each pigment at each pH. The two substances also have several peaks in common. At a pH of 4.0, the bacterial pigment had a peak at about 320 nm, which is near that of 310 nm for the mucus; and at a pH of 10.0, the bacterial pigment had peaks at about 235 and 270 nm, which are near the peaks at about 225 and 290 nm for the mucus. Osawa *et al.* (1964) believed that a change occurs in the structure of the bacterial pigment between a pH of 4.0 and 5.0, and such a change might account for the change in absorption spectra recorded in the acidic range. Perhaps a similar change occurs when the substances are made alkaline. Mucus and bacterial pigment samples both exhibited a fluorescence-emission peak at about 462 nm, a value reported by Newkirk and Hulcher (1969) for pigments produced by *Pseudomonas mildenbergii*, which substantiates the contention that fluorescence of the mucus is caused by pigments produced by pseudomonads.

It has been established that pyocyanin is soluble in chloroform (Sullivan, 1905; Véron, 1961; Azuma and Witter, 1964) and that the fluorescent pigments produced by pseudomonads are not (Sullivan, 1905; Verōn, 1961; Wasserman, 1964). Because Medium B is selective for pyoverdine production (King *et al.*, 1954) and the pigment produced on this medium was insoluble in chloroform, and because the fluorescent color and the absorption and emission values of the bacterial and mucus samples are similar, it is apparent that fluorescence in *A. kochi* is a function of the pigment, pyoverdine, produced by pseudomonads present in the mucus of snails of this species.

This study sought to determine whether bacteria of the genus *Pseudomonas* are responsible for fluorescence observed in the mucus of *A. kochi*. The presence of the bacteria in the mucus has been demonstrated, along with the fact that isolates from *A. kochi* mucus produce fluorescent pigments. The pigment present in the mucus of snails of this species has characteristics similar to those of the pigment produced by bacterial isolates from the mucus of these snails. It follows that pseudomonads are indeed the causative agents of fluorescence in *A. kochi* mucus. The burden of the evidence presented in this paper suggests, moreover, that bacteria of the genus *Pseudomonas* probably will be found to be responsible for similar fluorescence in certain other species of land snails. We know that several related species of endodontid snails fluoresce under ultra-violet light (Rawls and Yates, 1971), and that at least one species of polygyrid exhibits fluorescence (Rawls and Baum, 1971); and this study has established pseudomonads as the agents of the phenomenon in one species of endodontid (and by inference, in a second). Among the questions yet to be answered are those relating to the role of pseudomonads in fluorescence by other endodontids and by representatives of other families; and those concerning the absence of fluorescence by snails in which pseudomonads are present. We hope to report some progress toward answers in subsequent papers.

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THE ANATOMICAL DISTRIBUTION OF FLUORESCENCE CAUSED BY PSEUDOMONADS IN *ANGUISPIRA KOCHI* (PFEIFFER)

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INTRODUCTION

During a study of chromatograms of tissues of various species of land snails, certain chromatograms were observed to exhibit a vivid, blue fluorescence when subjected to ultraviolet illumination. Further investigation indicated that this fluorescence was characteristic of certain endodontid species and suggested that it would be found to occur in all members of the family (Rawls and Yates, 1971). A subsequent study reported the same kind of fluorescence in the mucus of *Mesodon clausus*, a polygyrid (Rawls and Baum, 1971). Fluorescence of a different kind has been reported in the mucus of *Helix aspersa* Müller, an helicid (Turchini, 1926; Fischer and Saddy, 1949), and in the epiphragms of certain other snails (Fischer, 1956).

A related study by Baum and Rawls (1972) has established the fact that bacteria of the genus *Pseudomonas* are the causative agents of fluorescence in the mucus of *Anguispira kochi*. The role of pseudomonads in fluorescence by *A. kochi* having been determined, further investigation was indicated by the observation that the phenomenon did not occur uniformly over the body of the snail. The purpose of this paper, therefore, is to demonstrate the anatomical distribution of bacterial fluorescence in *A. kochi*, and to attempt to establish a basis for understanding why fluorescence occurs in certain body parts and not in others.

Earlier researchers were concerned with the fluorescent colors observed in different animals (Stübel, 1911; De Kowalski, 1911; von Prowazek, 1914; Arloing *et al.*, 1925; Derrien and Turchini, 1925; Turchini, 1926). Later work included the study of fluorescence in specific tissues and organs (Bommer, 1929, 1933; Exner and Klemperer, 1930; Exner, 1932, 1933, 1934; Erös, 1932; Böck, 1934; Von Querner, 1933; Hamperl, 1934; Sutro, 1936). Turchini (1926) specifically called attention to a yellow fluorescence in the mucus of *Helix aspersa* Müller, apparently the first mention of fluorescence of any kind in land snails; a footnote indicates that Derrien communicated orally to Turchini regarding solubility of the fluorescent substance. More recently, Fischer and Saddy (1948, 1949), Fischer and Brunel-Capelle (1953), and Fischer (1953, 1954, 1955, 1956, 1959, 1967, and 1970) have described fluorescence in the shells and soft parts of a number of molluscs. Most of the studies to date have concentrated on the colors observed, but Prowazek (1914) discussed the possible causes of their formation, Bommer (1929, 1933) attempted the extraction and identification of fluorescent compounds, and Derrien and Turchini (1925) commented on the possible bacterial origins of fluorescent substances.

Baum and Rawls (1972) have demonstrated that pigments produced by bacteria of the genus *Pseudomonas* fluoresce in the mucus of specimens of *A. kochi* exposed to ultraviolet light, and have shown that pseudomonads are present in the mucus of other

land snails but fail to fluoresce except in culture. Pseudomonads of various species previously have been isolated from several kinds of organisms. Meyer (1925) found that pseudomonads are present in the nephridia of certain land molluscs, and Mahdihassen (1960) demonstrated that pseudomonads, as symbionts in *Cyclostoma elegans* did not grow at random but were kept under some sort of physiological control in that host. The presence of pseudomonads in certain marine vertebrates was noted by Colwell and Liston (1961), who compiled a list of taxonomic relationships among the species of *Pseudomonas*. Beeson and Johnson (1967), studying bacterial isolates from the digestive gland and the gut of the bean clam, *Donax gouldi*, showed *Pseudomonas* to be present. Specific pigments of pseudomonad origin have been reported; Osawa (1964) noted the production of a brilliant, yellow-green fluorescent pigment in a broth culture of *Pseudomonas aeruginosa;* and Newkirk and Hulcher (1969) isolated pigments produced by *Pseudomonas mildenbergii*, pigments which exhibited absorption maxima at 402 nm and 278 nm, and an emission maximum at 462 nm. Pigments of non-bacterial origin have been reported from certain molluscs: McGee (1964) extracted green and yellow pigments from eggs of *Tegula funebralis* and *T. brunnea*, and noted that in the oxidized form the pigments exhibited a green fluorescence which showed a maximum absorption at 273 nm; and Bannister, Bannister, and Micallef (1968) demonstrated the presence of a green fluorescent pigment in homogenates of the foot of trochids of the genus *Monodonta*.

Chromatography has been used in attempts to identify fluorescent compounds present in various body tissues, and in attempts to determine relationships within taxonomic groups. Hadorn and Mitchell (1951), investigating the patterns produced by *Drosophila* squashes, found that the contents of the digestive tract did not influence the fluorescent patterns. Later, Buzzati-Traverso and Rechnitzer (1953), using paper chromatography of untreated squashes of muscle tissue of certain marine fish, suggested that geographic races of some species could be distinguished. Subsequent study by Buzzati-Traverso (1953) revealed that, in snails, contamination of tissue by intestinal contents usually produced additional bands on the chromatograms. Kirk, Main, and Beyer (1954), describing methods of identifying snails by the use of paper chromatography, concluded that fluorescent patterns in snails were uninfluenced by variations in age or in the environment. Similar results were reported by Varty (1956) in solving taxonomic problems in insects of the genus *Adelges*. Homogenates of body tissues and mucus were made from specimens of several species of freshwater snails by Michejda (1958), who observed that fluorescent patterns from hepatopancreas were extremely variable but that the pattern produced by mucus was similar to that obtained from tissues of the foot, implying that all fluorescent compounds present in tissue smashes were also present in the body surface mucus. Michejda and Urbanski (1958) using ultraviolet analysis of chromatograms, compared the foot and the whole organism of representatives of several families, including one endodontid species, *Discus rotundatus*. Later, Michejda and Turbanska (1958) qualitatively compared the ultraviolet patterns of various tissues and concluded that homogenates of foot muscle were much less fluorescent than those of skin and suggested a lack of mucus-secreting glands in the muscular tissue. Wright (1959) remarked that the most reasonable explanation for variations in chromatograms seemed to lie in seasonal metabolic changes in the snails.

MATERIALS AND METHODS

Specimens of *Anguispira kochi* were collected during 1970 and 1971 from several locations in Foley's Woods, a nature conservatory near Paris, Illinois. All of the snails were placed in a terrarium partially filled with humus, soil, and leaf litter taken from the collection sites. Snails which were collected in the fall months were retained in the terrarium until early February, when the anatomical studies were begun. Work on those collected in early spring and midsummer was carried out immediately following collection.

To become familiar with the histological structure of *A. kochi* and to determine sites of fluorescence a number of microscope slides were prepared. Standard histological techniques were employed to prepare cross-sections of the entire organism. The sections were placed serially on slides and stained with Harris' haematoxylin and eosin. Coverslips were placed on the sections to make permanent slides to be used for reference.

Attempts at gross dissection and histological fixation for microscopic study under ultraviolet light were abandoned because of the water solubility of the mucus and fluorescent material. An alternate method employing a freezing (CO_2) microtome was adopted. A stereoscope with its

base reversed was positioned over the stage of the microtome to permit the observation of tissues as sections were removed. Ultraviolet illumination was provided by a UVSL-13 Mineralite. For observation otherwise, an incandescent illuminator (Bausch and Lomb) was fixed in its mount in the stereoscope. An ultraviolet filter was attached below the stereoscope objective head to absorb stray ultraviolet light and keep it from entering the stereoscope; the filter absorbed 89 percent of the ultraviolet between 200 nm and 320 nm, and 84 percent in the range of 320 nm to 378 nm, as determined with a manual spectrophotometer (Hitachi-Perkin-Elmer). All wavelengths above 378 nm passed through the filter uninhibited.

Preparation of the snails for sectioning was accomplished by two methods, the first of which utilized no tissue fixation. The specimen was placed in a tightly sealed container of water to drown the snail and relax its body. After about 24 hours it was removed from the container and the shell was broken away. The body of the snail was placed in a perforated plastic container and allowed to remain in a stream of tap water for 20-30 minutes to rinse away surface mucus from the tissues. Rinsing completed the preparation and the snail was ready for immediate sectioning.

The second method of preparation was a variation of the technique described by Miller (1967) for preparation for dissection. The snail was drowned by immersion in water for about 24 hours and then heated gradually (approximately 1° per minute) to a temperature of 56° C, which is the temperature at which collagen is denatured, at which time the dead animal could be pulled easily from the shell. The animal was then placed in the water rinse to remove the surface mucus. Both techniques were used interchangeably; the second is the better method, from the standpoint of preserving the shell for further use.

After the excess mucus was removed from the body by rinsing, the snail was placed directly on the freezing head of the microtome. Standard embedding media exhibit fluorescence under ultraviolet light, so distilled water was used as the embedding medium. The snail was positioned on its left side and several drops of water were placed around the body and frozen to form a base, after which more water was added and quickly frozen to form a block containing the snail.

The angle of the blade was maintained at about 20-30 degrees, and to obtain the best possible sections without smearing the tissues or the fluorescent material, the knife blade was cooled with ice at all times during use. After the block was frozen and initial cuts were made, the surface of the block and the exposed tissues were kept at the freezing point to prevent the fluorescent material from moving in solution. Sections were made at 50 microns, thickness of the sections being unimportant because tissues removed by sectioning were not saved, and the exposed tissues were observed directly on the frozen block.

Three series of observations were made utilizing the modified frozen section technique described. The first was concerned with those specimens taken in the fall of 1970, and the animals were not examined until February of 1971. The second utilized a collection of specimens taken early in April, 1971, when the activity of the snails was first becoming evident; the animals were sectioned in the first three weeks after collection. The third series employed snails which had been collected in mid-summer, and sectioning was carried out within a week following collection. In each case, the frozen block was observed immediately following removal of each section and the location and relative intensity of fluorescence was noted. Previous study (Baum and Rawls, 1972) has shown that the fluorescence of a solution of mucus from *A. kochi* could be quenched by lowering the pH significantly; this phenomenon could be detected visually. As one means of confirming that the fluorescence seen in the tissues of the snail was indeed that caused by pseudomonad pigment, 01 N HCl was added dropwise directly onto the body as a section was removed by the microtome blade. Another method of confirming the presence of pseudomonad pigment in tissues utilized a filter which eliminated all but the desired wavelength, that characteristic of pseudomonad pigments. To record fluorescence emission values, a standard mucus solution was used; the standard contained one ml of mucus diluted to 10 ml with 0.1 Molar KH_2PO_4 buffer solution, pH 6.9 and was centrifuged at 13,500 rpm for 30 minutes to remove any particulate matter. An Aminco-Bowman spectrofluorimeter was utilized to obtain emission values. Four separate scans were made at 265, 300, 350, and 400 nm, and emission values were read through the range of 200 – 700 nm. Adipose tissue fluoresces blue, according to Popper (1941), and to avoid confusing such fluorescence with that of pseudomonad pigment we used an alcoholic solution of chlorophyll which, according to Haitinger (1938), yields a red color by reaction with any fatty tissue present. This last confirmatory test seems to us to be unnecessary; quenching visible fluorescence by lowering the pH of the surface of the tis-

sue and interposing a filter to eliminate all wavelengths but that emitted by pseudomonad pigment would seem to be sufficient confirmation of the presence of the pigment.

RESULTS

Examination of a fresh specimen of *Anguispira kochi* under ultraviolet light reveals fluorescence over all of the surface of the foot, with the exception of the sole, and on the surface of the mantle edge. The fluorescence is most vivid around the genital and anal apertures, and least apparent over the antennae and most of the surface of the head. The edge of the mantle fluoresces brightly. No value is placed on gross dissection under ultraviolet light, even when carried out under chloroform or ether, because all internal structures are contaminated by the fluorescent material in suspension in the body surface mucus.

Cross-sections of the body, stained with haematoxylin and eosin, were studied before sectioning of frozen tissues was undertaken. The edge of the foot is covered with a layer of epithelial cells of the columnar type, the rest of the body being covered with a stratified epithelial layer interrupted irregularly by intercellular spaces and the necks of gland cells. Inward from the epithelium, most of the foot is composed of a layer of connective tissue cells and fibers forming a reticulum in which are situated a number of mucus cells and small amoeboid cells. The mucus cells were found as individuals in the connective tissue above the sole of the foot, but in aggregations in the fringes of the foot; some of these cells were deeply placed in the connective tissue layer and had very long necks reaching through the connective tissue to open at the surface epithelium of the body. Internal to the connective tissue layer is a thick layer of non-striated muscle which, in turn, is covered on its inner surface by a simple squamous epithelium enclosing a hemocoelic cavity that extends throughout much of the length of the foot.

Some tissues and organs of certain kinds of animals contain naturally fluorescent pigments (Metcalf and Patton, 1944). In *Anguispira kochi*, for instance, the digestive gland emits a faint blue-green fluorescence when excited by ultraviolet light; portions of the reproductive tract fluoresce green; and the pericardial tissues at times emit an orange fluorescence. None of these is the result of excitation of *Pseudomonas* pigment, because acidification of the tissues with 0.1 N HCl quenches the blue fluorescence emitted by the bacterial pigment; fluorescence of other than bacterial origin is not affected by lowering the pH, nor do such fluorescent materials exhibit solubilities characteristic of pseudomonad pigments (Baum and Rawls, 1972). The fluorescence of pseudomonad pigment is also characteristic; spectrofluorimetry reveals a single band at an emission value of 462 nm.

Frozen sections of the foot viewed under ultraviolet light exhibited fluorescence in mucus gland cells, deep beneath the epithelium; the epithelial cells themselves did not fluoresce. No fluorescence was observed in the layer of connective tissue, with the exception of that seen in the necks of gland cells which opened to the surface of the body. The muscular layer beneath the connective tissue layer failed to exhibit any fluorescence, as did the simple squamous layer next inward. The hemocoelic space enclosed by the epithelium fluoresced brilliantly.

Sectioning the viscera revealed little fluorescence. The digestive gland, the nephridium and the pericardial structures all failed to show any fluorescence of pseudomonad origin. Observable fluorescence in the reproductive system varied with the dates of collection. Specimens taken in the fall months and sectioned in the winter exhibited fluorescence in major portions of the reproductive system; in the penis, vagina, ovotestis, seminal vesicles, and the genital atrium. Spring and summer collections revealed fluorescence only within the genital atrium. Other than the reproductive system, only the hemocoelic space exhibited any variation in fluorescence during the year; no fluorescence was found in the fall-winter group, but a vivid fluorescence was seen in specimens collected in the spring and summer months. Brilliant fluorescence was observed in the gland cells in the subepithelial connective tissue layer of the edge of the mantle, and in the mantle cavity. Less vivid fluorescence could be seen throughout the rest of the mantle, but bright spots occurred in scattered locations; on the right, along the posterior arc of the mantle edge, immediately above the anal opening, the greatest concentration of fluorescence was observed. In several cases, the area where the mantle overlapped the foot contained a substantial amount of the fluorescent material, but it was extracellular and appeared to be mucoid material which had not been effectively rinsed away during preparation for sectioning. Lastly, fluorescence was observed in the cavity of the anterior portion of the

gut, but never any deeper than in the pharyngeal bulb.

DISCUSSION

Fluorescence observed in *Anguispira kochi* is produced by pseudomonads, which are motile bacteria commonly found in soil and water (Baum and Rawls, 1972), and it is reasonable to expect that, because soil and leaf litter constitute its habitat, *A. kochi* easily could become infected by pseudomonads. Bacteria may be selective and show preference for particular tissues in certain host organisms (Colwell and Liston, 1961), however, and this study was carried out to determine whether fluorescence of pseudomonad origin is preferentially distributed in the body of *A. kochi*.

No fluorescence was seen in the muscular layer of the foot. Michejda and Turbanska (1958) reported that homogenates of foot muscle of *Lymnaea* failed to fluoresce on chromatograms while those of the skin fluoresced vividly, and they suggested that the reason for this was the lack of mucus-secreting glands in the muscular tissue. Mucus gland cells were never observed in the muscle layer of the foot of *A. kochi*, nor did the necks of any of the extramuscular glands pass through the muscle tissue.

Observation of the visceral structures revealed that the internal organs generally did not exhibit fluorescence of pseudomonad origin; the nephridium and the digestive gland failed to fluoresce, although Meyer (1925) and Mahdihassen (1960) indicated that *Pseudomonas* was invariably present in the nephridia of *Cyclostoma*. Baum and Rawls (1972) established that *Pseudomonas* occurs in non-fluorescent as well as fluorescent snails, and suggested that the physiology of the snail may be the factor which determines fluorescent pigment production by such bacteria. If *Pseudomonas* occurs in the nephridium of any snail, the conditions therein may not permit pigment production, or may in some way alter the pigment molecule so that fluorescence is blocked; previous study (Baum and Rawls, 1972) has shown that sulfates and magnesium are required by bacteria of the genus *Pseudomonas* to enhance pigment production, and it is reasonable to assume that these might be lacking in certain organs but present in others.

If we assume that certain portions of the organism are uninhabitable by pseudomonads, we could explain the occurrence of fluorescence in selected parts of the body. However, we have no evidence that pseudomonads do not exist in all portions of the body of *A. kochi;* the gut, for example, fluoresces only at the oral and anal openings but it is possible—even probable—that these bacteria could be found elsewhere in the digestive tract and that their pigment production is simply blocked or modified by pH or some other factor.

Since all mucus gland cells exhibited fluorescence it seems apparent that something in the physiology of these structures is conducive to pigment formation by pseudomonads. The margin of the mantle fluoresces vividly because it is rich in mucus cells; fluorescence found in the mantle cavity could be a product of pigment diffusion or bacterial motility.

Seasonal variation in the occurrence of fluorescent material was noted in two portions of the body; the reproductive tract and the hemocoel. There was no fluorescence observed in the reproductive organs of specimens collected in the spring and summer months, but in those snails collected during the fall these structures did exhibit the phenomenon. Wright (1959) suggested that seasonal variations in chromatograms derived from snail tissues were the result of changes in metabolic activity; preparation for hibernation in the fall and increased sexual activity in the spring. Increased sexual activity might be coupled with a change in the concentration of some factor which inhibits fluorescent pigment formation in the reproductive organs. Conversely, a similar alteration might occur in the fall and winter months and result in the enhancement of pigment development. Fluorescence seen in the hemocoelic fluid of the spring and summer specimens but not in those collected in the fall, would indicate a change in metabolic activity might be related to the presence or absence of the pigment. The fluorescence observed in this cavity was never as vivid as that of external structures, suggesting a somewhat lower concentration of the pigment, and there is the possibility that the pigment, which is diffusible, passes directly into the hemocoel via the vascularity of the mantle; it is also possible that some pseudomonads are present at all times in the hemocoel.

Kirk, Main, and Beyer (1954) established that no significant differences between young, intermediate and mature land snails could be detected in the characteristic ultraviolet patterns of chromatograms derived from tissues taken from those snails. We have determined that no differences are apparent with respect to the sites of fluorescence in different age groups of *A. kochi*, which suggests the possibility of

a genetic factor, related to the metabolism of the specific tissues that fluoresce because of available pseudomonad pigment.

SUMMARY AND CONCLUSIONS

The anatomical distribution of fluorescence caused by pigments produced by pseudomonad bacteria in *Anguispira kochi* was investigated by the use of a modified frozen section technique, the purpose being to determine whether this fluorescence is selectively located in the body of the snail.

As a result of this study, it is apparent that the fluorescence caused by ultraviolet stimulation of pseudomonad pigments is confined to the body surface mucus and the subepithelial mucus glands, to the body orifices, to the fluid of the hemocoel, and to the reproductive structures; the variable fluorescence in the hemocoel and the reproductive tract seems to be correlated with seasons of the year, but no satisfactory explanation for this apparent relationship can be given at this time. We believe that some specific factor or group of factors directly related to the physiology of the snail will be found to be responsible for the selective distribution of pseudomonad-induced fluorescence in *A. kochi;* and that research along this line may provide answers to questions concerning fluorescence in other snails which exhibit the phenomenon, and to the problem of the absence of fluorescence in snails known to harbor pseudomonad bacteria.

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FIELD JOURNALS OF HENRY A. PILSBRY PERTAINING TO ARIZONA

ANNOTATIONS BY JOSEPH C. BEQUAERT AND WALTER B. MILLER

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The notes compiled here have been extracted from the field journals of Henry A. Pilsbry. The journals are on file in the Department of Mollusks, Academy of Natural Sciences of Philadelphia. We thank Dr. Robert Robertson and Mrs. Nancy Rulon for making the journals available and for other assistance.

The journals pertain to two expeditions, one in 1906 and one in 1910. Other information concerning the expedition was published by Pilsbry and Ferriss (1910, 1911, and 1915). Journal accounts of parts of these expeditions occurring outside Arizona were compiled by Metcalf (1970).

Annotations by J. C. Bequaert and W. B. Miller are inserted in the text in parentheses and in different type. Although we have tried to conform to the original notes, a few punctuation marks have been added for clarity and scientific names have been italicized (seldom done in the original notes).

In 1906, Pilsbry and James H. Ferriss left Joliet, Illinois, the home of Ferriss, on Oct. 8 and arrived in Albuquerque on Oct. 12 and proceeded by train by way of Grants, New Mexico, to Williams, Arizona.

Sat. Oct. 13/06. Arrived at Williams after Canyon train had left. Friday went up Bill Williams Mountain. Just below it in aspens, found *Vitrina (pellucida) alaskana* under damp stones, somewhat numerous. Higher up, & up to within ½ mile of summit found *Vallonia, Thys. (Microphysula) ingersolli?, Pupilla blandi* (referred to *P. hebes* by Pilsbry and Ferriss, 1911:197), and *Pyr. striatella (Discus cronkhitei)* etc. No trace of *Oreohelix* or larger shells. Rock is a sort of hard conglomerate, not stratified, & probably volcanic or Cambrian. At top there seems to be some stratified rock. *Vitrina (p. alaskana)* is much less voluminous than *V. limpida*. The only shell lobe is a small one at suture. Tail does not project behind the shell. Many composites still in bloom, but flowers mostly frost-killed. Thin ice formed Sunday morning, but soon warms up in the sun.

Arrived at Bass Camp, at rim of Canyon Oct. 16. Found *Sonorella* under low white sandstone rocks at Bass Sta. At Camp we coll. *Pupilla* etc, *Sonorella (coloradensis) & Oreohelix (yavapai extremitatis* at its type locality, Pilsbry and Ferriss, 1911: 184) on talus ca 200 ft below rim.

Oct 17 went down around 1st amphitheatre of trail, & at S.E. side of its head, under the great white cross-bed lime and *sandstone* cliff, the talus runs over the sandstone, covering it rather thickly. It is covered with humus & shrubs (deciduous), one like currant bush, others very thorny, like osage orange. Here among moss & grass, & under & around stones found *Oreohelix y. (yavapai) profundorum* very common. Very few *Sonorella*, but *Cochlicopa*,

Pupilla etc. Got a lot of dirt. This place is 800-1000 ft below rim. Then crossed over the Le Conte Plateau to Huethawalee Butte (this feature could not be located), which is whitish sandstone over red sandstone of the Plateau. Found it very dry. No shells whatever.

Oct 18. Cleaned up in morning. pm, went to small amphitheatre W of Bass Camp, & about ½ to ¾ mile W of top of trail. Here found *Succinea, Euconulus (fulvus)*, a few dead *Pupilla (syngenes)*, *Sonorella (coloradoensis)* & *Oreo(helix)* all on upper talus, 1-300 ft. below rim.

Oct. 19. To springs about 2 miles W of Bass Trail. They seep out at base of cross bedded sandstones. On talus below, found *Sonorella, Vitrina, Cochlicopa* etc.

Oct. 20. Went down trail to River. At about 5000 ft found *Sonorella(coloradoensis)* bones under slabs of red sandstone. (top of red wall limestone 4364 ft base/ little below of do. 3824) found *Sonorella (coloradoensis)* again, few bones below redwall limestone talus of do. about 3000 ft.

River here 200 ft across at waters edge --a sullen turbid yellow stream, in a canyon of dull purplish rock, perhaps 200 ft deep & 3-400 ft wide. There is a little sand in places & some much-worn rounded drift-wood lodged in crevices.

Bench-mark US Geo Sur. low water--2000 ft
Bench-mark US Geo Sur. high water-2230 ft
Bench-mark to bottom of river ... 2147 ft

Vegetation here, except for a few cacti, the mesquite & large Opuntias, is about same as on upper taluses. but sparser.

Oct 21. Crossed to camp on Shinumo Creek.

Oct 22. Went up Shinumo Cr. Found banded *Sonorella (coloradoensis)* in talus of angular granite stones on S. side of Cr., not far above camp. Further up got stone-colored frog & tadpoles. Frogs rather stupid and easily caught. do. tadpoles. Followed left tributary to Creek--a small stream --about 1 mile up. It zigzags in granite walls with here & there a talus. Found bandless *Sonorella (coloradoensis)* in taluses on S. side--flatter & larger apert. than banded one from the large creek. All day we found *Thys(anophora) horni* & a few *Succinea*. Very cold here last night--could not have been much above freezing but warmer today. Canyon so deep the sun does not get in except a few hours a day.

Oct. 23. Went down creek to mouth, & Ferriss fished without success. Creek enters the granite iron-colored like river gorge. Camp is about one mile from the river. No snails but *Succinea* & a few *Thys. horni*. Found cattails & rushes near creek, also Fireweed. The erect, large-leaved *Opuntia* is particularly fine. Returned to camp to lunch, & in pm. went up to junction of left branch with Creek. This is fully a mile above camp. Here we took minnows from a pool near the creek. Also got 1 (one) small red-warted frog or toad near the creek & several of the gray frog. The latter varies in tint & distinctness of markings. It is bright yellow along sides where belly joins the sides. They cling close to vertical rocks & are easily caught thereon, but take to water freely if alarmed.

All rocks we have found snails in along Creek here are the steeply tilted granitic with veins of quartz & often porphyretic (sic) or conglomerate with strata of pebbles. Taluses are of *particularly angular* hard rock, sharp to handle. Camp is probably 2-300 ft above river level. Left branch of creek may be 100 ft higher. It is very narrow, with iron-like, subvertical sides & few steep taluses. This morning it was 44° F. noon in tent 54° 7 pm. 62°

Oct 24. Went up left branch (=White Creek) to amphitheatre. It passes out of granite into level bedded sandstone, then opens into a grand amphitheatre bounded by the Red Wall. We spent night on talus of latter, & coll(ected) thin, hardly lipped *Sonorella (coloradoensis)* there. Also *V. (Retinella) indentata*. The veg. is quite diff.--oaks & manzanita etc found maidenhairs (ferns) in canyon of cr. returned evg. of Oct. 25. Caught mice & skinned 6. Plenty of frogs on White Creek.

Got letters from Adeline (Mrs. Pilsbry). (On Oct. 26?)

Oct. 26. Returned to Bass Camp.

Oct. 27. Collected in cove to the right of trail below crossbed sandstone head of Starvation Tank Wash. Talus is overgrown with shrubbery, but no pinions, though below it is covered with them. There is an oak of the white oak group here, mostly very scrubby. Found *Sonorella (coloradoensis)* in abundance, not deep.

Returned to trail cove. I went up on talus about 4-500 yds. W. of head of cove, where pinions abund. Found *Sonorella (coloradoensis)* where pinion thin out, in abundance. *Oreohelix (yavapai* subsp.) was found about 200 yds W. of head of canyon, only one or two. This is near mouth of the bay, thus (sketch). The dead ones increase in quantity eastward, but I could find no live ones until last segment of talus, which is ca 30-40' high & about 100 long, with a great rock in middle. This is nearly opposite the zigzag stairs by which trail descends the crossbed sandstone. These taluses of Crossbed overlie red sandstone & cover it where we worked, thus (sketch).

In Mojave Canyon, --amphitheatre above head of White Creek--there is much greasewood & holly-leaved oak. No white oak. This is at base of the Red Wall on its talus.

Oct 28. Feathery shrub is called buckbrush or Forget your troubles. Said to have properties of cinchona. Went from Camp to Bass Station & later to Grand Canyon.

Oct. 29. Monday. Worked on cherty limestone talus, from about 75 to 350 ft below rim, which is here 6866 ft above sea level. Above found sinistral *Pupilla (syngenes)* & *Oreohelix, V. (Retinella) indentata* & *Euconulus (fulvus)* at 300 ft the *Oreo.* are scarce but *Sonorella* sets in, & *V. indentata* is common, *Pupilla* rare. The talus is very steep but well covered with vegetation. Our work was in area covered by zigzags of the B A (Bright Angel) Trail thus (sketch). To the right of trail, along the crossbed sandstone the *Oreo.* have a tendency to *(a)* descending last whorl. This is near base of the X bed (=Crossbed) sandstone. Along cliff talus to the right there are dead *Oreo.* & *Sonor.*, etc former not (with) descending (last whorl), but could find no live ones. Trail runs down a rift in X-bed, filled with talus of limestone & at sides, esp. below, some sandstone.

Oct 30. Tuesday. Returned to Williams. Collected above large dam (left) this pm. found *Conulus, V.* (Retinella) *indentata, Z(onitoides) arborea* & one minute *Pupa (Gastrocopta pilsbryana,* Pilsbry and Ferriss, 1911: 197) at low altitude, under & around malpai rock, chiefly among aspens. Enroute now for Albuquerque.

Pilsbry and Ferriss returned to Albuquerque, made a short trip into the Sandia Mountains, and then proceeded southwest to Deming, Luna County, N.M., where they visited the Florida Mountains. From Deming they travelled by rail west to Bowie, Arizona.

Nov. 6. Sta., S. P. RR. Started from Bowie at 11:40 am. Later loaded & went to Buckeye canyon, which leads up from the east towards Dos Cabezas. Camped at Mill of Buckeye Mining Co.

Nov. 7. Went up canyon. Wide, stony bed, with stream sometimes above, sometimes hidden. About 1 mile up, Ferriss rolled a rock of several cu ft over his leg. It carried him 12-15 ft down the heavy rock. Calf was badly bruised, & he returned to camp. I ascended left (south) side to some low crags near top of ridge & deep under heavy rocks found large *Sonorella (bicipitis).* The earth was dry & dusty. Went up canyon past Buckeye Gold Mine (now not working). About ½ mile above mine the canyon branches. Took right branch (west branch) & about ½ miles up found small *Sonorella (bicipitis* at its type locality) on left (south) side, 20-40 ft above bed of canyon, in same situations as the large ones found below. *Vitrina* & *V. indentata* were found sparingly, in both places. Returned to camp about dark.

Nov. 8. Went up to Buckeye Mine & crossed ridge to left. On this ridge sketched map of Dos Cabezas. Saw fine view of Graham range just north of Dos C. Skirted along head of left br. of Buckeye Canyon, high up, finding small *Sonorella (bicipitis).* Crossed over ridge to Happy Camp Canyon, where same small *Sonorella (bicipitis)* occurred. Went down canyon to mouth. About a mile up it united with Tarbox Canyon, -- a large one--& the common course of the two is about a mile wide. The mouth is half closed by a reef of nearly white rocks of coarse, light gray granite. We climbed over rocks to the south & travelling a couple of miles reached 9-mile water-hole (cited by Pilsbry and Ferriss, 1910: 59), where Mortimer Wien had camp & supper. Quail & rabbit.

Nov. 9. Spent a couple of hrs. hunting large *Sonorella (bicipitis)* in rocks of 9-mile (water-hole). The rock is coarse grained light gray granite, size of a small house, cavities beneath. Found a few large *Sonorella (bicipitis).* Left about 11 o'clock, arrived at Dixon's place (=Knape Ranch of Topo. Map) at Ft Bowie about 2 o'clock. Dixon's place is about a mile from the fort. We went up Bull Hill (cherty limestone). Found *Holospira (cionella)* in a little wash facing the Fort. The fort is 5500 ft elevation & this (station of *Holospira)* is about 6000-6200. We looked in vain on this hill for *Sonorella.* On the next & higher quartzite hill, back of Dixon's I found *Sonorella bowiensis* in a long-leaved oak thicket below low bluff, about half or 2/3 way up. Also a *Ashmunella* like *proxima* (nominate *A. proxima* of Pilsbry and Ferriss, 1910: 100). Lower down we found *Holospira (cionella).* Got photos of both hills & fort.

Nov. 10. Went to Big Emigrant Canyon (=Emigrant Cn. of Topo. Map) & camped at Riggs cabin. 2 of the Riggs were there with a cowboy.

Nov. 11. Went to head of W. branch. Among pinions there found *Holospira (arizonensis emigrans* at its type locality), limestone. Also large *Sonorella (optata* at its type locality). Ferriss also took this in a ravine in hill where canyon ... (The word "Camp" starts a new page. It is not clear whether this page contains notes

of Nov. 12 or whether this is the continuation of the notes for Nov. 11). Camp. All through the valley which opens N. & looks out on Bowie & Graham Mts., at about 2000 ft elev. (above camp) there is *Sonorella*. Got some live ones. The ridge towards Mesa is coarse, rotten granitic rock. *Sonorella* also on & to the outside of this ridge. The valley is elsewhere limestone becoming cherty above, & summit seems to be angular, friable quartzite, among which *Oreohelix* occurs. Cleaned up catch to date this morning.

Nov. 13. Travelled all day from Big E. (Emigrant) to White Tail Canyon. About 10 miles straight across. Omitted Wood Canyon.

Nov. 14 Wednesday. White Tail. Ferriss staid in camp & cleaned up. I went down to Reider's Mine & went up canyon. It is heavily pinion wooded below on N. slopes; above is white oak (leaves all fallen) & a few longleaf pines. Just at & around mine, along the canyon found *Oreohelix*, *Sonorella (virilis leucura)*, *Ashmunella* & *Holo(spira)*. They continued to summit, above 2000 ft. above (above the mine, presumably) (Ridge here said to be 7000-7500 ft.). Found *Oreo(helix)* & *Holo(spira)* on the very top, the others just below. The ridge lowers & becomes knife-like towards the cliffs at top of Mackay Canyon. Not much below these cliffs: White oaks and a few pines. Down this canyon (Mackay) there are live oaks, but no pinions where I went. Snails were scarce, but got four genera about halfway down. The mouth of this canyon is just opposite a conspicuous crag on N. E. rim of main canyon, composed of several turrets & points. Points collected at (Mackay Cn. stations 12 and 14 of Pilsbry and Ferriss, 1910: Fig. 6) are marked a, b, c, on map (sketched in journal). These branches are limestone.

Nov. 15. Went up draw back of Gardners Mine (where we camped). (The following is inserted separately on page: "*Holo* & bluntkeeled *Oreo* on summit of narrow ridge, under stones. This is just above a mine on W. side, & just where ridge drops a couple of hundred ft.") Found *Holo*, *Ash*, *Son*, & *Oreo.* from lower slopes to summit. Ferriss got dirt with *Pupa (Gastrocopta)* at summit. On the Pinery side of the summit, near where it drops a couple of hundred ft., & above the hill-top mine (Hilltop of Topo. Map) I took a bluntly angular *Oreohelix* & *Holo(spira)* under hot stones in the sun. Down northward found same snails as near Gardners.

Nov. 16. Up White Tail. Found a new scaly *Ashmunella* & small *Sonorella (micra* at its type locality) in igneous rock slide near road on N.E. side, ½ mile above Gardners. Then Ferriss went up left fork of canyon & I up Indian Creek. Climbed to cliffs finding a few live *Sonorella*, some *Ash(munella)* & *Holo(spira)*. Also got to summit.

17th Nov. Visited same rock slide (talus). F. (Ferriss) went up Mountain & got *Sonorella* (small) and *Ash(munella)*. I went around N.E. side of same Mt & got both in a talus (igneous) ("Sta. 16" inserted here, seemingly the same designation as on the map of Pilsbry and Ferriss, 1910: Fig. 6) ca. 12-1500 ft higher than talus at foot of Mt. There is a smaller rock & a higher coarser-rock talus here. Only *Sonorella* found in latter, but I opened but one mine (=digging). Later found *Sonorella* in a large rock talus ca. 200 ft higher than camp & near it, on right side of canyon (Sta. 15). Kept a small spec(imen) of the rock from top of mt. talus. It is the same all over this ridge but ridge to S.W. is limestone, cherty on top of ridge.

Nov 18, 1906. Snowed. Stayed in & cleaned up.

Nov 19 Monday. Moved over to Paradise Canyon & camped about 2 miles below Paradise. Thermometer down to 20° in morning.

Nov 20 Tuesday. temp. 17° in morning. Mort. (Mortimer Wien mentioned above and by Pilsbry and Ferriss, 1910:118 as "our guide and driver") & I went over to the cienega (San Simon cienega, 1 mi. E of New Mexico border) near Steen's Peak range (Steins, in Peloncillo Mts.) to look for blind fish. Found a few dead *Physa* along ditch but no chance to seine. Ice. Ferriss went up on left side of canyon towards Paradise & got an *Oreohelix* hairy below & *Holospira*. Also *Pupa* dirt, with *Bif.* (=*Gastrocopta*) *perversa* etc. Snow melting slowly, but still much on the Mts.

Nov. 21 Wednesday. Moved over to Cave Creek & camped above Rieds (=Reed's Ranch, now part of Southwestern Research Station of the American Museum). Visited cliffs (Reed's Mountain) opposite Ried's (=Reed's) in afternoon & found *Oreo(helix) clappi*.

Nov. 22 Thursday. F. (Ferriss) & I went down creek. Worked on a talus at foot of (Reed's) Mt., back of Rieds (Reed's Ranch) (below) & got fine lot of *Ash. chiricahuana*, *A. ferrissii* & some *Sonorella (virilis circumstriata* at its type locality). Then went up opposite side to cliffs & got a few *Ash(munella) proxima (?)* etc. Then up S. fork of canyon about 2 miles. *Ash(munella) angulata* is everywhere, on both sides in small numbers. Also on a rocky heap in middle of canyon. Also found *Sonorella* here. Cave Cr. is guarded by hills crowned with cliffs 500-800 ft high up s

far as Rieds (=Reed's). Then it branches out. One branch runs toward Paradise & has cut down to comparatively low level the divide betw. Paradise Canyon & Cave Cr. On the S. side it opens up a great amphitheatre, with a ridge & hill in centre. Cave (Crystal Cave of Topo. Map) is on a branch to right, above branch leading to Paradise.

Nov. 23. Friday. Visited (Crystal) Cave & found *Oreo(helix) chiricahuana* & *Holospira (chiricahuana* at its type locality). Colony is about 1/8 mile long & perhaps 100 yds wide at the widest. Live ones are under dead agave, sotol, & bear grass, with *Thysanophora, Succinea* & *Vit. (=Retinella) indentata*. The very steep sides are of steeply dipping shale, more or less calcareous, & the soft earth formed by its decomposition. In P.M. went up middle of amphitheatre on left, up a ravine leading to central Mt. On neck connecting this Mt. with the border of amphitheatre found a slender *Holospira (chiricahuana gracilis* at its type locality). All thro. this amphitheatre, E. of this hill & ridge, there is *Oreo(helix) clappi, Ash(munella) chiricahuana* & a few *Sonorella (virilis). O. clappi* is always dirty & not deep, often at roots of grass. Rieds (Reed's) Mt. has cliffs of green & red, like the Red Wall limestone in Grand Canyon.

Nov. 24. Saturday. up to N.E. border of Cave Cr. amphitheatre, N.E. of the Creek. A limestone & shale ridge projects betw. Cave Cr. & the wash the cave is on. On N. side of this ridge at 2 places we found *Holo(spira)* and *Oreo(helix) chiricahuana* with a few other things. This cave wash heads up in a little bay in the rim, in which at its mouth there is a little grove of aspens among the oak scrub.—the only aspens I have seen in the canyon. Opp. these aspens on the south side, in a talus 2-300 ft above bed of wash, & 100 below cliffs, we found *Oreo(helix) barbata* in plenty, also *Ash(munella) chiric(ahuana)* & *angulata*, etc. Going down we found *Oreo-(helix) chiricahuana* on S. side of the limestone ridge, near its crest, for about half its length. The shells here are about typical size, & live perhaps 1000 or 1500 ft or more higher than the cave.

Nov. 25. Sunday. Went up to Sawmill. It is in Barfoot Park, at the head of Pine Canyon (as now on Topo. Map) (sometimes called Riggs Canyon or by some, Sawmill Canyon.) Sawmill is to be moved in the spring. Stayed with Mr. Boyer & Mr. Sanders. On S. (?) side of Barfoot there is a Mt. with cliffs above & very extensive talus of Trachyte below. In this we got a hairy *Oreohelix, Ash(munella) duplic(idens), Cochlicopa* & a few dead *Sonorellas*. In a park of yellow pine, about a mile beyond from ridge crossed by latter, we got *Ash(munella) esuritor*. Type loc is a small conical rock & earth pile about 10 ft to left of road. We found a few dead ones about a mile below, near the road. This is where a few yellow pine first appear going up, among oak scrub. The *Ash(munella) esuritor* park is the first yellow pine park on the road up.

Nov 26 Monday. Went down the Pine Canyon about a mile. Found a few bones of *Ash(munella) metamorphosa* (later synonymized with *A. esuritor* by Pilsbry) but little else. Left for Cave Cr. camp about noon. All the Barfoot & Rustlers park country in 6 inches of snow. Looks like Labrador. It is all covered with yellow pine & spruce. The rock is Trachyte, as it is also at head of N. Fork of Cave Cr. The *esuritor* park drains into Turkey Cr. (of the east slope of the Chiricahuas; there is another Turkey Creek on the west slope), the stream in Paradise Canyon.

Nov. 27. (Date entered but nothing more.)

On the expedition of 1910, Pilsbry and Lorenzo E. Daniels travelled by train to El Paso, Texas, and then to the Big Hatchet Mts. of New Mexico, where they made several collections. From the Big Hatchets they continued westward from Hachita via rail to Bisbee, Arizona. Later, in Tucson, Arizona, they were joined by James H. Ferriss.

Aug. 28. Sunday. About 7 am. started for town. Reached Hachita about 10, & left for west at 11:10 am., reaching Bisbee about 4 pm. The southern end of the Chiricahuas look very good. Several large & fine canyons & no doubt much country not yet explored, easily reached from Rodeo, by taking a team there. All this country & west to these Mts. is covered with luxuriant grass & looks fine. Lots of cattle. Bisbee is built in and on steep sides of a gulch. Hills mostly red, very arid and barren, but westward there seems to be limestone.

Aug. 29, 1910. Went up Tombstone rd. to the first draw on left, which we ascended. On the slopes below low limestone cliffs on the hills on left we found abundant bones of *Holospira (arizonensis mularis* at its type locality) & *Sonorella (bartschi)*. The brush was here all dead and charred. Could find no living snails. Going west along ridge we crossed a saddle, then a low limestone hill & another saddle, coming to the high limestone mt. at head of the draw. Here, on the ledge halfway up cliff we found a few living *Sonorella (bartschi* at its type locality, Mt. Ballard, two

miles west of Bisbee). There were no *Holospiras* here, whatever, but on slope below the cliff, several hundred ft. lower, there were some bones. On these Mts., there are 3 agaves, one smooth, one the common big one, & another with hook-serrate leaves; but they are all rather rare. Some Sotol & beargrass, but not much. No prickly pear, but a few candle cactus, some of them huge, & one or two small cereus, very rare. Absence of prickly plants quite noticeable. A very small scrub oak (specimen kept) many flowers, mostly different from those of Hachitas (the Big Hatchet Mts. of New Mexico). The range of limestone hills seems to border the mts. S & SW. Mines are chiefly in them. Mts. N. & E. are malpai, which seems quite barren of snails.

Aug. 30/1910. Walked about 4 miles up Tombstone road—about a mile beyond the watershed & then up a draw on the left about a mile to some cliffs. Found no snails whatever. The Mts are all igneous rock on the left (N. W. slope) covered with trees about 8-12 ft high, Oak, etc., etc. No cliffs. On the other side they are more barren & the reddish rock crops out in low cliffs in places at the hill tops. Very beautiful goldenrod is abundant. Took photos of hill where we coll. *Sonorellas*, & of Bisbee.

Aug 31. 1910. Took Bisbee & Warren Trolley to Warren, where we went east about 2 miles to a rather high limestone peak. Found a few *Sonorellas (bartschi)* & multitudes of *Holospiras (ferrissi fossor* at its type locality) under stones on N. & NW slopes, low cliffs near summit. *Holo(spira)* lives in mellow earth under stones, in nests of 6-20. Often stands apex up in earth, the rest buried. Got a lot of plants.

Sept. 1. On train for Benson. The San Pedro Mts (in Mexico), opp. the Mule range SW. looks good for a *Holo(spira)* & a *Sonorella*. Reached from Naco Sta., where conveyance can probably be had to Mts, about 5 miles distant. As we go along the Huachucas are seen westward—a fine range. They are visible from the top of Bisbee Mts.

Arr. Benson about noon. Walked down to San Pedro R., about a mile east to S.P. RR bridge. The stream is 20-30 ft wide, turbid carrying a full load of silt. It flows between vertical earth banks about 8-9 ft high, in a floodplain covered with mesquite. Along the borders, where bank is concave, there is more or less drift. Gathered a sack. It contains *Holospira, Bifidaria (=Gastrocopta)*, Zonitidae, Planorbis etc. (listed by Pilsbry and Ferriss, 1915:389-390). Took train for Tucson at 4:30 & arrived there 6:30 Pacific time.

Sept. 2. Changed dryers (of plants in presses), & in pm. went up to the Desert Laboratory, on a volcanic hill about 2 miles W. of town (Tumamoc Hill). Met Dr. (B.E.) Livingston of Johns Hopkins who is working there. In evening went out to see Dr. (F.) Shreve, who is in charge during Dr. McDougall's (reference is to D.T. MacDougal) absence in Colo.

Sept. 3, 1910. Tried out the volcanic hills west of town, at W. end of Congress St. Got a few *Bif. (Chaenaxis) tuba* under stones. Also some *Thys(anophora) hornii*. More were in drift along the fence at right (north) side of road, near the foot of the hill. Walked up the Santa Cruz river. It is an insignificant stream,—one can jump across dryshod, nearly anywhere. Meanders in a sandy bed, between vertical (or broken down) drab earth banks which rise perhaps 10 ft from bed. These banks may be 100 ft apart. Willows etc grow in about half of the river bed, the rest being shifting sand. Collected some drift. Probably all the drift shells came out of the earth banks, which were perhaps deposited in a cienega. They contain *Planorbis, Lymnaea, Physa, Pisidium, Paludestrina* & *Anodonta* in quantities, & some *Zonitoides* also (listed by Pilsbry and Ferriss, 1915: 399-400).

Sept. 4/10. Sunday Went over plants & tied them up for shipment. Mailed 2 boxes to Vanatta mainly Mule Mt. shells. Went down to river & got 2 bottles of minnows. Sifted down the drift.

Sept 5/10. Ferriss arrived. Went to Tempe by morning train. Worked in Salt River drift. The Mts. in sight all the way there are eruptive, dark rock with little vegetation. The river bed is perhaps 200 yds wide, but only about 25 to 50 ft is occupied by the stream. It contains a good many fish up to a foot long. Took sleeper for Tucson where we arrived this morning (6th).

Sept 8. Train (from Tucson) to Siding #4 on Nogales branch S.P., then by wagon (Sr. Grijalva) to Agua Caliente, a fine spring of tepid water at western base of Santa Ritas, at mouth of A.C. Canyon. Found *Sonorella (walkeri aguacalientensis* at its type locality) abundant in banks of stream flowing from the canyon, immediately S. of spring (Sta #1). It occurs also on bluff above (Sta #2) and south of the draw.

Saturday, Sept. 10, 1910. Went up (the word "Soldier" has been written in quotes and then crossed out. Soldier Canyon is identified in Pilsbry, 1939:288) cn., opening between two high granite peaks, north of Agua Caliente. Rock all granite, mostly coarse, and much like 9-mile water hole in the Dos Cabezas. About halfway up (Sta 3) found *Sonorella (walkeri*, at one station

cited by Pilsbry and Ferriss, 1915: 395), like those of Agua Caliente but larger, etc. Got about 12 good live ones. Saw one giant cactus, the only one in this Mt. so far. Returned down Agua Caliente.

The 'apron' or approach to the Santa Ritas has no big yuccas. There are many Fouquierias, barrel cacti, Opuntias 2 spp. & cylindric Opuntias 2 species one very spiny called Choya, the other called ____ (left blank) Also mimosas. Scarcely any Agave.

(Insert at top of page: "Bert Yost at Agua Caliente.")

Sunday Sept 11. Ferriss & I went up to Mr. Jenkins upper camp, in A(gua) Cal(iente) Canyon at about 5500 to 6000 ft. He has a cabin & a tent & is working a prospect for Mr. Eliot of El Paso. Found a large *Sonorella (walkeri)* in the main canyon & back to the left above cabin & also in Walnut Cn. *(S. walkeri* at Station 5, its type locality, Pilsbry and Ferriss, 1915:395) which branches off a short distance—200 yds—below cabin.

Monday Sept. 12. Went up to aspens which are a few hundred ft. below summit of Mt. Hopkins, at head of 'Pine Canyon' a branch of Walnut Cn. Thence down to 'Walnut Basin,' where big walnuts (one 4 ft diam) & bracken grows luxuriantly. Found no shells, but many flowers—yellow columbine, gentian, etc. A heavy succession of falls over huge granite rocks lies between basin & fork of canyon. In Walnut Cn. above & below mine we got *Sonorellas*.

Tuesday Sept. 13. Hunted large & small *Sonorellas* near mine in Walnut Cn. (Sta. 5). (Insert at top of page: "Sta. 4 is the pool at Agua Caliente"). They live in piles of granite, often 50 to 1000 lbs in weight, from 100 yds below to same distance above the mine. In some piles there are only large *Sonorellas*, in others small, while in some both occur. Got most of the small *Sonorellas* above mine about 50 yds or more. Did not find any of the small ones sticking fast to rock, but it had been raining hard on the 12th. Their mucus (of *S. clappi)* is very tenacious & sticky, white, while that of the big *Sonorellas (S. walkeri)* is orange tinted. Got a rattler out of the rock here. In afternoon moved camp up to saddle at head of Agua Caliente Cn.

Sept. 14. Cleaned up shells. Caught a bat & lizard on cabin. After dinner Daniels & I went over saddle & directly down slope to the bed of the W. branch (of) Madera Canyon (spelling of Topo. Map), just below Johns old camp. (Inserts on back of page: "Sta. 6—at canyon S of & near camp, where we get water. Small *Sonorellas* coll. by Ferriss.' and 'Madera Canyon (spelled Madeira on some maps) is also called White House (Casablanca) Canyon.'). We found a few large Sonorellas & *Euconulus (fulvus)* about half way down (Sta. 7). About 100 yds above Old John's Camp there is an extensive rock pile in bed of canyon, perhaps 10 ft above the stream. Here we took many small reddish *Sonorellas* & a few large ones (Sta 8) *(S. santaritana, S. walkeri,* and *S. clappi* are sympatric).

Sept. 15. All of us ascended Mt. Hopkins, & went down the other side of the saddle between heads of Madera & Josephine Canyons. At perhaps 6000 ft or so we found medium sized *Sonorella (santaritana* is the only species recorded from Sta. 9 in Josephine Cn.) in friable angular rock in banks of Canyon (Sta 9), Also found the same a few hundred yds up the branch leading to saddle next to Baldy (Sta. 10). (Old Baldy of the Santa Rita Mts. is Mt. Wrightson).

Sept. 16. Ferriss & Daniels made the following stations. (Sketch entitled "Stations near the head of Agua Caliente Canyon" indicates location of stations 11-14). I went down to Jenkins Cabin but found nobody at home. Changed dryers (on plant press). Collected at Sta 5, Walnut, finding big-mouth & small-mouth *Sonorellas* in the same rock pile in two cases.

(Insertions on back of page: "Big Santa Rita *Sonorella* is gray-blue above with a slight trace of brown, becoming light brown at border of foot, sole brown." and "Kent Cn. is on the White House side. We got into the head of Camperel Canyon on the Greaterville side.")

Sept 17. Collected more at stations on preceding page (Stations 10-13). Cleaned up catch.

Sept 18 Sunday. Daniels & I went down Madera Canyon to the fork & up fork about 1 mile ("Sta 15" in margin). Found large *Sonorella (walkeri* at Sta. 15,) chiefly close to & just below fork in the eastern branch. Saw no small *Sonorella*. Collected a brilliant red & black snake.

Sept. 19 Monday. Went over to Mr. Brandts camp near head of E. branch of Madera.

Sept. 20. Trekked over saddle N. of Old Baldy (= Mt. Wrightson of Topo. Map) into Kent (?) ("Camperel" inserted later) Canyon (=present Gardner Cn. of Topo. Map), which flows east. Extremely rough & rocky sides full of cliffs. Found a few small *Sonorellas (granulatissima occidentalis* at its type locality, Station 17) on the way down & at about 500 ft. (The saddle must be about 8000 ft.) ("Sta 17" written in alongside of page). The big pines on saddle make a park-like open forest. They

extend down the canyon east as far as we went. There are also huge spruce & hemlock trees in & on S. side of the canyon. We got down to walnuts, thro a zone or group of small aspens & then small-leafed maples but did not reach sycamores. Got down to 2d branch from the north. Went all the way back to camp, arriving at 10 p.m.

(Insertions on back of page: "Sta 16. East side Mt. Hopkins about 1 mile S. of Sta. 7, Msdera Canyon." and "Baboquivari Mts. ca. 60 miles due west from Santa Ritas, 45 from RR.").

Sept. 21. I went down to A. E. Jenkins camp after breakfast. In afternoon he packed us down to lower camp (about 5000 ft.) where we spent the night. I got little speckled toad & a blue snake here.

Sept 22. Jenkins took us over past the Santa Cruz river, where at Amado's ranch (Sta 18) we collected a quantity of drift with Pupae etc. (species listed by Pilsbry and Ferriss, 1915:400).

Sept 23. Started west about 7 o'clock. Stopped at 11 at a small water course with dirty water in some holes. The country rises slowly, rolling, with much mesquite at first, then grassy, parklike, with scattered large mesquites, chiefly in the swales, finally confined to them. Along this stream collected many plants and grass. Catsclaw & mesquite on banks of the rocky bed. Took photo of outfit. This was N. of the Cerro Colorado, a rocky craggy Mt. ("Sta. 20" written in margin). There is scattered barrel cacti around here. The same beautiful, park-like grass country continues west of the Cerro Colorado, nearly to the Guijea (=Guija, but also written "Gija" in Pilsbry and Ferriss, 1915) Mts. which lie south. We camped here. There is a fine stream & a mine—the Mts are coarse granite with ledges of white quartz. Got paper package of drift, rather poor. (Species from drift mentioned by Pilsbry and Ferriss, 1915:408).

Sept 24/10. Continued journey to Baboquivari arriving about 3:30 in Oro Fino Cn. (Jupiter Canyon of Topo. Map) where the Gold Bullion Mine is situated. There is a Mexican ranch & windmill in mouth of the canyon. Daniels ascended butte on left. F(erriss) & I went across north to collect. All got *Sonorellas*.

(Insert on back of page: "Tumacacori Mts. E. of Cerro Colo. & S. of siding No. 4, 6 miles to base look good for a *Sonorella*.")

Sept. 25. Sunday. Baboquivaris have desert vegetation. Lower canyons & their zigzag continuations on plain green with mesquite & catclaw. Lower hills & slopes have many barrel cacti, 2 or 3 giant cacti, *Agave treleasei* & a larger one, some sotol & many fine flat leaved nopal, one blue, other green. The larger cacti absent on higher Mts. leaving rainbow & a flat mamillaria (sic). There is some scattered oak, size of a peach tree on W. & N. slopes above, but grass covers the whole Mts. What few herbaceous flowers there are seem all or nearly all to be same as S(anta) Ritas. Some thistle. There are a very few stunted pinyons around the crags of high peaks. Mint, ferns numerous but few species

Sept. Sunday. Sta. 23 is on S. side of amphitheatre of N. branch (of Thomas Cn). Sta. 24 is on west side of ridge leading N. to the peak. Sta. 25 is on E. side of ridge, nearer the peak. Both 24 & 25 are under oak trees with the shells not deep, under leaves, on or under stones. (Stations in the Baboquivari Mts. are indicated by Pilsbry and Ferriss, 1915, on Fig. 8). The rings are extremely numerous & large. (White rings produced on stones by aestivating *Sonorellas*. Small red-bellied snake & green scorpions small but malevolent, at Sta. 22 (in Oro Fino Cn. = Jupiter Cn.) Baboquivari Peak a huge obelisk of dull red stone, in places covered with yellow-green lichens; on ledge oak trees grow. If accessible at all, it is on the corner facing canyon, where the angle slopes a little & is somewhat splintered. Oaks on top. A deep & very craggy canyon makes in from west & heads up against canyon where peak is. The Mts. are on simple ridge with long lateral spurs. Passed obelisk. & ascended ridge N., then descended a branch of head of Thomas Cn. Collected *Sonorellas (baboquivariensis)* at various places, the first perhaps 600 ft below summit of ridge. Brown Cn. (of Topo. Map = "Sycamore Cn." of Pilsbry and Ferriss, 1915: Fig. 8; both names are indicated in Pilsbry and Ferriss, 1923: Fig. 8) is much richer than the others. There are sycamores, hackberry, walnut, a beautiful dark oak, & more flowers but about all are Santa Rita sorts. After a long tramp found camp, about 8 o'clock. (Insert at top of page: "(found water in head of middle cn. about ½ mile below Bab. peak)").

Sept 26 Monday Cleaned catch in forenoon. After lunch F(erriss) & I went up about 3 miles, then up a branch (of Sycamore = Brown Cn.) to the south (or S.W.) about ¼ mile. Got large mouth *Sonorella* & one *Succinea* (Sta 31).

(Insert on back of page: "Mr. G. N. Sayre, of Tucson, has special knowledge of Baboquivari Mts. Also Prof. Forbes of the U. of Ariz. at Tucson, said to have climbed the peak twice.").

Tuesday Sept 27/10. Packed up & started for the Gija (=Guija) Wash, where we arrived about 1:30. (The "side-trip" to the Baboquivaris was made by wagon, Pilsbry and Ferriss, 1915:412). After dinner Ferriss & I went up the hill above mine (N.W. end of range). It is covered with coarse-grained granite, weathering to gravel. (On) rounded boulder or angular rocks when (we) attacked (the site) near top above mine (we) found *Sonorella (sitiens* at its type locality). ('Sta. 32" appears in margin of page near here). Very rare & hard to get suitable rock being scarce. Most loose rock too massive. Ascended rounded top of hill, & worked along ridge south ½ mile where there is a low rock dyke, where we took a few more *Sonorella (sitiens)* & got a bull snake. These are large hills rather than Mts. rounded, mostly grass-covered, with much crown-of-thorns & sotol on top, few cacti but there is a pygmy agave *(parviflora)*. Worked back to head of canyon which makes in from north, same rock at head.

Sept. 28. Wednesday Small toad Gija (=Guija). Soles & inner side of thighs yellow, belly & throat white, back pink gray with some black maculations & red warts. Ferriss & I left wagon & went across mesa to the nearest crag over granite of the Cerro Colorado, back of the Cerro Colorado Mine on the Arivaca road. Found very few bones of *Sonorella (sitiens arida* at its type locality) on both N. & S. sides of the crag. Saved sample of rock. Northern slope of Cerro Colorado rounded & grassy. S. side worn into fantastic pinnacles, very craggy, dull red. N. side Sta. 33. S. side Sta 34.

Sept. 29 Thursday. Returned to Tucson.

Sept 30 Friday. Fixed up plants.

Oct 1. Ferriss & I went to Desert Laboratory (on Tumamoc Hill). Met Dr. MacDougal. Continued up hill finding *Sonorella (tumamocensis* at its type locality) from a short distance above Laboratory to top of hill in sharp, coarse tufa. Sta 35. Found more on east slope going down.

(Insert in margin of page½ "Mineral Hill Group is W. of S. from Tucson.").

Oct. 2. At 1:20 Daniels & I started south with Mexican driver. Went past E. end Tucson range, through Papago Indian reservation, to a group of low hills about 20 miles from Tucson. The hills stand on a high mesa, but are isolated from each other & from other ranges. About 7 miles to Sharitas (=Sierrita Mts.) & 9 to Black Mt. at the Mission. Stayed at L.D. Chilson's camp. Address: 207 Pennington St., Tucson. The east end of San Xavier Mine Hill is white limestone; the ~~west end is white~~ or whitish quartz. It has a spur to the north, & the end is bifid by reason of an insignificant ravine between rocky ridges, the S. ridge a low cliff. Halfway up the ravine this cliff has fallen in a tumble of huge quartz blocks. They are too heavy to move, but in the crevices there is some small stone. The tumble is about 200 ft long 40 wide, (not continuous), rocks partly piled, partly scattered, lower end of tumble is about 200 ft above mesa. Sparse bushy vegetation all over hills & mesa-mesquite, catclaw, Palo Verde, & some Fouquieria. On southern slopes tree cacti. On mesa also many Opuntia, flat & cylindric, & some barrel cacti. Also a few yuccas. On Mts. there is some sotol. San Xavier hill is white limestone W. end quartz. The spur northward is coarse pinkish-gray granite. No agave in these hills. (Here there is a sketch entitled "San Xavier Hill from west, about 2½ miles distant." An arrow points to a locality *ca.* halfway up the hill that is designated "*Sonorella* Sta 36." This would be the type locality of *S. eremita.* The sketch is similar to that in Pilsbry and Ferriss, 1915: Fig. 7).

Oct 4/10. Tuesday. Went west to low granitic hills about 3½ miles from camp. N & NEE sides covered with big boulders like 9-mile (Water Hole of Dos Cabezas Mts.) or Guijas, but found no shells. In pm explored Mineral Hill with same result. Collected mistleto (sic) on palo verde, etc. Coral snake.

Oct 5/10 Wednesday. Returned to Tucson. Stopped at San Xavier Mission to see the buildings. Also collected a *Sonorella (papagorum* at its type locality) on Black Mt., N. slope of the east end close under the summit. The hill is vesicular tufa exactly like Laboratory (Tumamoc) Hill at Tucson. Very steep, & *almost covered* with rock-slides. There is some ocotillo, mesquite, catclaw & paloverde & some giant cactus, but the latter is almost all on southern slope. Also the edible red berry grows here. No agave or sotol seen.

Oct. 6. Thursday. Worked on Sentinel Peak (now called locally "A" Mountain) for *Sonorella* but without success. Got a few more on Laboratory Hill.

Oct. 7/10. Friday. Walked over to El Gato (Cat Mountain) the most eastern of the high peaks of the Tucson Range. No suitable rock piles & no *Sonorella* found.

Oct. 8/10. Saturday. Overhauled & packed up baggage. Had phone from Ferriss at Oracle saying he would return Sunday night.

Oct. 10/10. Monday. Left Tucson at 1:30, arriving at Dragoon about 5:30 pm.

Oct. 11.10. Tuesday. Got Mr. Peake, dry ~~farmer~~ & hotel keeper to drive us to Co-

chise Stronghold in the Dragoons. It is about 10 miles down the west side.

(The preceding two dates are repeated here).

Monday, 10 Oct. at noon took S.P. train for Dragoon. Arrived 5:30 & stayed with Mr. Peake.

Tuesday 11th Oct. Drove out to Cochise Stronghold (Tweed Cn. of Pilsbry and Ferriss, 1915: Fig. 1 = Stronghold Cn. West of Topo. Map) on west side of Dragoons, & up into (Tweed) canyon to near its apparent head. On the south the ridge is very craggy & irregular. On N. side the Mts. slope with few cliffs & are mainly limestone, white & hard, often crystalline. At E. end there is a fine pile of coarse granite, with spines & towers above, huge boulders below. Another tower like mass stands on S. side near E. end. E. of it a canyon runs up about 1½ miles, ending in a limestone ridge (Sta 7) which divides it from the next canyon south. This next canyon has rough craggy ridges on both sides, & a limestone ridge (Sta 8) across the apex. Sta 1 is a slide of heavy, angular stone on the E. side of the crag not far up the canyon. In it we found *Sonorella (ferrissi)* mainly bones, 1 (one) live one only. They make no tracks on the rock, & do not seem to seal up to rock. Sta 9 is on the E. side of the rocky bed of the canyon, near the foot of the 'falls.' Many live *Sonorella (apache)* taken there by Ferriss. Sta 7 is the limestone ridge at head of Canyon, & 8 is higher on same ridge eastward. Sta 6 is in a crag about ½ way up the Mt. on the east side of Cataract Canyon ("Cataract Branch" of Pilsbry and Ferriss, 1915: Fig. 1) & overlooking a big canyon on the side towards Pearce. Only got *Pupa* dirt here. Sta 6½ is W of this on about same level.

Saturday. *Holo(spira)* Sta. 16, on mesa in mouth of Cochise (Stronghold Cn. West= Tweed Cn). On sloping stony sides of wash ca 15 ft deep which meanders over plains, under dead sotol, *rarely* dead Spanish bayonet trunks or stones. Where apron of Mts begins to slope steeply the arroyo deepens--30-40 ft sides become steep & *Holo(spira)* seen up to where granite begins at base of Mt. Mesa between washes is grassy with some bear grass clumps. Some small oaks, juniper, catclaw etc in washes. Above granite ledge at base of Mt (Sta 18) there is limestone with coarse *Holo(spira)*. Above this another granite dyke. This is directly on the course of the wash, which becomes a ravine or canyon at head of apron where granite begins.

Small flat *Sonorella (ferrissi)* from the limestone ridge makes small rings on stones rarely. Found living ones laid aperture up under stones & in earth, with a white epiphragm like eastern Helices. The larger *Sonorella (apache* at its type locality, Station 8 of Pilsbry and Ferriss, 1915: Fig. 1) from Cataract branch (of Stronghold Cn. West = Tweed Cn.) makes no marks on rocks & was not found sealed to them.

Sta. 19, on slope S. of draw 18, 50 ft above foot of mt. SE side of the granite.

Sta. 20. Mouth of 2d cn. from spur.

Sta 21 beside ✠ (sketch map) of E slope of same cn. Sta 21 all over hillside where there is lime from bed of ravine to ridge. Hill is partly lime, partly andesite.

Oct 16/10. Ferriss & I hiked out of Dragoon (Mountains), carrying my dunnage to Dragoon. Packed up in 2 trunks & left same day.

As related by Pilsbry and Ferriss (1915:365), Ferriss and Daniels remained in the Dragoon Mountains for a time after the departure of Pilsbry. The journal contains a list of stations (22a to 42) from which Ferriss and Daniels collected. The listing is similar to that published by Pilsbry and Ferriss (1915: 367-368.

Inserted in the journal of 1910 are three receipts from "J. Ivancovich, Wholesale Grocer, 31 East Congress Street, Tucson. A. T., Telephone Main 221, P.O. Box 125." These receipts give an indication of what provender was taken along on a malacological field expedition in 1910. Two of the receipts are dated Sept. 9, 1910, which would have been the day before the party left for the Santa Rita and Baboquivari Mts. The third receipt is dated Oct. 1, the day before Pilsbry and Daniels went on a shorter trip to the southwest of Tucson.

RECEIPTS OF SEPT. 9 (COMBINED)

2 doz. Lemons	.40	2 Sl(iced) Peaches	.30
1 qt. Scudders Syrup	.50	2 B(lack) Berry Jam	.40
1 Sx Pioneer 8	.95	4 K.O. Sard(ines)	.50
5 # Lard (Pure)	.90	4 E(vaporated) Milk	.70
¾ doz. Uneedas	.60	6 # Cheese (very mell)	1.50
3 pkg. G(raham) Crax	.40	3 # ----- Rice	.30
1 # B(aking) Powd.	.45	Onions (3#)	.15
3 Cans Nesco Cof(fee)	1.05	2 S(alt) P(ork) Bacon	4.10
2 Oats (Can).	.35	5 # Swt. Pot(atoes)	.40
1 Bx D(omino) Sugar	.55	5 # Spuds	.10
¼ # Best G.P. Tea	.20	2 Pts G(rape) Juice	.50
8 Cans Q(uarter) Peaches	1.20	1 Bottle Olives Q (Lg)	.45
4 Cans Cots	.60		
		PAID:	$17.55

RECEIPT OF OCT. 1

3 # Rice	.25
1 # Nesco Coffee gd(ground	.35
1 qt Scud(ders) Syrup	.50
3 B(lack) B(erry) Jam	.60
1 S(alt) P(ork) Bacon 3½	1.10
1 Doz. Lemons	.20
2 Gr(aham) Crax	.25
PAID	$3.25

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LEO GEORGE HERTLEIN

(1898-1972)

The October 1970 number of the Nautilus (vol. 84, no. 2) was subtitled the LEO GEORGE HERTLEIN HONOR ISSUE by his friends who contributed a biographic sketch, a list of taxa proposed in his honor, a bibliography of his works, a list of names proposed by him, and papers on Mollusca, particularly the marine forms of the West Coast of the Americas which were the main field of his scientific endeavors.

The sad news of his death on January 15 of this year came as a shock to all his friends who had recently congratulated him on his retirement and his nomination as Curator Emeritus of Geology at the Academy of Sciences. Our friend Allyn G. Smith, his colleague of many years, sent along the sad news but as yet there are no details except that the end came very suddenly.

Little can be added to the biography given by Addicott (*Nautilus* vol. 84, no. 2, p. 37-41) except to add a few personal reminiscences from a friendship of some 35 years during which we met only three times but maintained an exchange of reprints and information which I take this opportunity to record. I remember particularly a visit to the Academy in 1966 which was about equally divided between examination of specimens and renewing acquaintance with old friends. On one of the numerous occasions when I went in to see Leo, I was surprised to find that he had callers, namely Carroll Lane Fenton and his wife Mildred, the paleontologists and authors of the *Fossil Book* which was then being revised for a projected new edition. G. Dallas Hanna soon joined the group and the conversation was lively and stimulating. I wish I had taken a picture of that group but I was too busy listening and putting in my oar now and then to think of it. It is sad to think that such a meeting can never again take place for only Mrs. Fenton and the writer now survive.

Leo Hertlein was president of the AMU in 1966-67. The meetings of 1967 were held in Ottawa and with rare tact and thoughtfulness, Leo asked me to give the banquet address, knowing that Ottawa was my home town and that I had begun my studies of Mollusca with those of the immediate vicinity, later extending my interest to all of Canada and later still to other parts of North America. The privilege of giving that address, which he modestly represented as a favor to *him*, was an experience which one can never forget and it is forever linked with the memory of the unselfish friend who made it possible.

Others have recorded the early years of his life, the hardships and accomplishments that marked his long life of service to science and to his colleagues and friends, so there is no need to repeat them here. Much as we shall miss him, we shall treasure the memory of his friendship and kindness as long as we live.

A. L.

ENDANGERED LAND SNAILS OF THE EASTERN UNITED STATES

LESLIE HUBRICHT

Listed below are the land snails of the eastern United States which because of restricted ranges or other factors could readily become extinct should adverse conditions arise. Most of the species are known only from a single locality. Others are found in areas which are undergoing rapid development with destruction of their habitats, such as the Florida Keys.

In recent years there have been a series of severe Spring droughts in the southeastern United States which have greatly reduced the land snail population and apparently caused the extinction of many local colonies. Because a species is living in a park or other location where the habitat will be protected from fire or timber cutting will not protect it from some natural catastrophe. The restricted ranges of some of the species are probably due to the destruction of their habitats by climatic changes.

CEPOLIS VARIANS (Menke).

POLYGYRA UVULIFERA MARGUERITAE Pilsbry. May be extinct as the type locality is now built up.

POLYGYRA POSTELLIANA PENINSULAE Pilsbry.

STENOTREMA PILSBRYI (Ferriss).

STENOTREMA GLASSI Branson. I have visited the type locality of this species several times without finding it.

STENOTREMA HUBRICHTI Pilsbry.

MESODON CLAUSUS TROSSULUS Hubricht.

MESODON CLARKI NANTAHALA (Clench & Banks).

MESODON ARCHERI Pilsbry. I have visited the type locality for this species several times without finding it.

MESODON CLENCHI (Rehder).

MESODON LEATHERWOODI Pratt.

MESODON JONESIANUS (Archer).

TRIODOPSIS PLATYSAYOIDES (Brooks).

TRIODOPSIS OCCIDENTALIS (Pilsbry & Ferriss).

HOJEDA INAGUENSIS (Weinland).

LIGUUS FASCIATUS (Say). This species is now practically extinct on most of the Keys. In part due to over collecting and to cutting of the hammocks, but also to spraying with insecticides for mosquito control which has been disastrous for the arboreal snails. The colonies within the Park are not too secure as I have been informed that there is considerable poaching.

ORTHALICUS RESES (Say).

ORTHALICUS FLORIDENSIS Pilsbry.

GUPPYA MIAMIENSIS Pilsbry. I did not find this species on two trips to southern Florida. It may be only a form of *Guppya gundlachi* (Pfeiffer).

GLYPHYALINIA APPROXIMA (Walker & Pilsbry).

GLYPHYALINIA PECKI Hubricht.

PARAVITREA CLAPPI (Pilsbry). I have tried to find this species several times but have so far been unsuccessful. Whether I have not looked in the right places or whether it has met with some disaster is not known.

PARAVITREA SMITHI (Walker).

PARAVITREA AULACOGYRA (Pilsbry & Ferriss). I have visited the type locality of this species several times without finding it.

PILSBRYNA AUREA H. B. Baker.

ANGUISPIRA PAUCICOSTATA Kutchka.

ANGUISPIRA PICTA (Clapp).

ANGUISPIRA CLARKI Vanatta. I was not able to find this species at the type locality. Mr. J.B. Clark bought forty acres on the north side of the Beachy Farm, cut off the timber and then sold the land. His cutting of the timber may have caused the extinction of the type colony. A few dead shells have been found in West Virginia, but it has yet to be collected alive.

DISCUS MACCLINTOCKI (F. C. Baker). The known range of this species as a living snail is an area about ten feet long and a foot wide at the mouth of a cave in Bixby State Park, Clayton Co., Iowa. When I was there there were many dead shells with their spires opened, probably by cychrine beetles. An increase in this predation could result in its extinction.

POLYGYRISCUS VIRGINIANUS (Burch).

HELICODISCUS SALUDENSIS (Morrison).

HELICODISCUS HEXODON Hubricht.

HELICODISCUS NOTIUS SPECUS Hubricht.

SUCCINEA CHITTENANGOENSIS Pilsbry.

VERTIGO ALABAMENSIS ALABAMENSIS Clapp.

VERTIGO ARTHURI von Martens.

VERTIGO WHEELERI Pilsbry. This species is probably only an aberrant *V. rugosula* Sterki.

VERTIGO HEBARDI Vanatta.

BOTHRIOPUPA VARIOLOSA (Gould).

STERKIA EYRIESI RHOADSI (Pilsbry).

LUCIDELLA TANTILLA (Pilsbry). I was not able to find any of the last four species during two trips to southern Florida.

LAND SNAIL RECORDS FROM MISSOURI

LESLIE HUBRICHT

This paper lists all of the land snails from Missouri in the collection of the author. Fossil records are not included as these were published elsewhere (Sterkiana 13:7-17, 1964). Much more collecting needs to be done before the fauna of Missouri will be really known. There are very few records for northern Missouri. Only the area around St. Louis can be said to be thoroughly studied.

OTALA LACTEA (Müller). St. Louis.

POLYGYRA LEPORINA (Gould). Franklin, Howell, Jefferson, St. Louis.

POLYGYRA JACKSONI (Bland). Dade, Ozark.

POLYGYRA DORFEUILLIANA Lea. Barry, Camden, Franklin, Howell, Jefferson, Lawrence, McDonald, Miller, Morgan, Ozark, Phelps, St. Charles, Ste. Genevieve, St. François, St. Louis, Taney, Texas, Washington, Warren.

STENOTREMA LABROSUM (Bland). Barry, Taney, Webster, Wright.

STENOTREMA BARBATUM Clapp. Boone, Franklin, Jefferson, St. Louis.

STENOTREMA STENOTREMA STENOTREMA(Pfeiffer). Boone, McDonald, Ozark.

STENOTREMA BLANDIANUM (Pilsbry). Christian, Greene.

STENOTREMA LEAI ALICIAE (Pilsbry). Barry, Boone, Howell, Jefferson, Lincoln, St. Charles, St. Louis, Texas.

STENOTREMA FRATERNUM FRATERNUM (Say). Boone, Franklin, Jefferson, Mississippi, Oregon, St. Louis, Washington.

MESODON THYROIDUS (Say). Boone, Camden, Franklin, Jefferson, Perry, St. Charles, St. Louis, Warren.

MESODON CLAUSUS CLAUSUS (Say). Barry, Boone, Christian, Howell, Jasper, Jefferson, Miller, Ozark, St. Charles, Ste. Genevieve, St. Louis, Webster.

MESODON ZALETUS (Binney). Barry, Camden, Carter, Douglas, Franklin, Jefferson, Laclede, McDonald, Oregon, Pulaski, Ste. Genevieve, St. François, St. Louis, Warren, Washington, Wayne, Webster.

MESODON PENNSYLVANICUS (Green). Boone, Washington.

MESODON ELEVATUS (Say). Barry, Boone, Camden, Carter, Douglas, Franklin, Jasper, Laclede, Pulaski, St. Louis, Warren, Webster, Wright.

MESODON INFLECTUS (Say). Barry, Boone, Christian, Franklin, Howell, Iron, Jasper, Jefferson, Laclede, Lawrence, McDonald, Miller, Morgan, Oregon, Osage, Ozark, Reynolds, St. Charles, Ste. Genevieve, St. Louis, Stone, Taney, Texas, Warren, Washington, Wayne, Webster, Wright.

TRIODOPSIS NEGLECTA (Pilsbry). Barry, Jasper, McDonald, Taney, Webster.

TRIODOPSIS DISCOIDEA Pilsbry. Cape Girardeau, Franklin, Jefferson, St. Louis.

TRIODOPSIS CRAGINI Call. Camden.

TRIODOPSIS DENOTATA (Férussac). Mississippi.

TRIODOPSIS FOSTERI (F.C. Baker). Boone, Camden, Cape Girardeau, Carter, Franklin, Jefferson, St. Charles, Ste. Genevieve, St. Louis, Scott, Warren, Washington, Wayne, Wright.

TRIODOPSIS ALLENI (Wetherby). Barry, Boone, Camden, Franklin, Howell, Jasper, Jefferson, Lawrence, Phelps, St. Charles, St. Louis, Texas, Warren, Washington, Wayne, Webster, Wright.

TRIODOPSIS MULTILINEATA (Say). Mississippi, St. Charles, St. Louis.

ALLOGONA PROFUNDA (Say). Boone, St. Louis. The record from Washington County is erroneous.

RABDOTUS DEALBATUS DEALBATUS (Say). Franklin, Jefferson, McDonald, St. Louis, Wayne.

LAMELLAXIS GRACILIS (Hutton). St. Louis (greenhouse).

LAMELLAXIS MAURITIANUS (Pfeiffer). St. Louis (greenhouse).

LAMELLAXIS CLAVULINUS (Potiez & Michaud). St. Louis (greenhouse).

LAMELLAXIS MICRA (d'Orbigny). St. Louis (greenhouse).

OPEAS PUMILUM (Pfeiffer). St. Louis (greenhouse).

HAPLOTREMA CONCAVUM (Say). Boone, Franklin, Jefferson, McDonald, Miller, Ozark, St. Charles, St. Louis, Webster.

EUCONULUS CHERSINUS CHERSINUS (Say). Christian, Franklin, Jefferson, St. Charles, St. Louis.

EUCONULUS DENTATUS (Sterki). Jefferson, St. Louis.

GUPPYA STERKII (Dall). Franklin, Jefferson, St. Louis.

GLYPHYALINIA WHEATLEYI (Bland). Jefferson, Ozark, St. Louis, Wright.

GLYPHYALINIA INDENTATA (Say). Christian, Jefferson, Lawrence, McDonald, Mississippi, Ozark, St. Charles, Ste. Genevieve, St. Louis, Taney, Washington.

MESOMPHIX FRIABILIS (W.G. Binney). Jefferson, St. Louis, Wayne.

MESOMPHIX CAPNODES (W.G. Binney). Barry.

PARAVITREA SIGNIFICANS (Bland). Boone, Franklin, Jefferson, Lawrence, McDonald, Ozark, St. Louis.

PARAVITREA SIMPSONI (Pilsbry). Ozark.
HAWAIIA MINUSCULA MINUSCULA (Binney). Barry, Christian, Howell, Jefferson, Ozark, St. Charles, St. Louis, Wright.
VENTRIDENS LIGERUS (Say). Franklin, Warren, Mississippi, St. Charles, St. Louis, Warren.
ZONITOIDES ARBOREUS (Say). Barry, Boone, Christian, Crawford, Franklin, Jefferson, Newton, Ozark, St. Charles, St. Louis, Washington, Wright.
ZONITOIDES LIMATULUS (Binney). St. Louis, Washington.
STRIATURA MERIDIONALIS (Pilsbry & Ferriss). Franklin, Jefferson, McDonald, St. Louis.
ANGUISPIRA ALTERNATA (Say). Boone, Franklin, Jasper, Jefferson, Platte, St. Charles, St. Louis, Warren.
ANGUISPIRA STRONGYLODES (Pfeiffer). Franklin, Jefferson, Mississippi, St. Charles, St. Louis, Wwyne.
DISCUS PATULUS PATULUS (Deshayes). Boone, Franklin, Jefferson, St. Charles, St. Louis, Scott, Washington.
DISCUS PATULUS EDENTULUS Hubricht. Barry, Howell, McDonald, Ozark, Texas, Wright.
HELICODISCUS NOTIUS NOTIUS Hubricht. Christian, Jefferson, McDonald St. Louis, Texas, Wright.
HELICODISCUS PARALLELUS (Say). Franklin, Howell, Jefferson, Lawrence, Ozark, St. Charles, Ste. Genevieve, St. Louis, Washington.
PUNCTUM MINUTISSIMUM (Lea). Douglas, Franklin, Jefferson, St. Louis.
PUNCTUM VITREUM H. B. Baker. Franklin, Jefferson, St. Charles, St. Louis Washington.
LIMAX MAXIMUS Linné. Christian, St. Louis.
LIMAX FLAVUS Linné. Christian, St. Louis.
LEHMANNIA POIRIERI (Mabille). St. Louis (greenhouse).
DEROCERAS LAEVE Müller). Boone, Franklin, St. Charles, St. François, St. Louis, Washington.
PHILOMYCUS CAROLINIANUS (Bosc). Boone, Franklin, Jefferson, Phelps, St. Charles, St. Louis.
PALLIFERA MUTABILIS Hubricht. Boone, Franklin, Jefferson, St. Louis, Wright.
PALLIFERA RAGSDALEI (Webb). Camden, Jefferson, St. Louis.
PALLIFERA MARMOREA Pilsbry. Boone, Franklin, Jefferson, Ozark, St. Charles, St. Louis, Texas, Wright.
PALLIFERA FOSTERI F. C. Baker. Camden, Franklin, St. Louis, Washington.
OXYLOMA SALLEANA (Pfeiffer). Franklin, St. Charles, St. Louis.
SUCCINEA OVALIS OVALIS (Say). Mississippi, St. Louis.
SUCCINEA GROSVENORI Lea. St. Louis.
SUCCINEA WITTERI Shimek. Boone, Iron, Jefferson, St. Louis.
CATINELLA VERMETA (Say). Franklin, Jefferson, Mississippi, St. Charles, St. Louis.
STROBILOPS LABYRINTHICA (Say). Boone, Franklin, Gasconade, Jefferson, McDonald, Ozark, St. Charles, St. Louis, Washington.
STROBILOPS AFFINIS Pilsbry. St. Louis, Washington.
STROBILOPS AENEA Pilsbry. Douglas, McDonald, St. Louis, Washington, Wright.
GASTROCOPTA ARMIFERA (Say). Boone, Christian, Franklin, Howell, Jefferson, Ozark, St. Charles, Ste. Genevieve, St. Louis.
GASTROCOPTA ABBREVIATA (Sterki). St. Louis.
GASTROCOPTA CONTRACTA (Say). Boone, Christian, Franklin, Howell, Jefferson, McDonald, Ozark, St. Charles, St. Louis, Washington, Wright.
GASTROCOPTA HOLZINGERI (Sterki). Douglas, St. Louis.
GASTROCOPTA PENTODON (Say). Boone, Jefferson, St. Louis.
GASTROCOPTA TAPPANIANA (C. B. Adams). Boone, Franklin, Jefferson, St. Louis, Washington.
GASTROCOPTA CORTICARIA (Say). Wright.
GASTROCOPTA PROCERA PROCERA (Gould). Boone, Christian, Franklin, Howell, Jefferson, St. Charles, St. Louis, Wright.
PUPOIDES ALBILABRIS (C. B. Adams). Howell, Jefferson, McDonald, Ozark, St. Charles, Ste. Genevieve, St. Louis, Taney, Wright.
VERTIGO MILIUM (Gould). Douglas, St. Louis.
VERTIGO OSCARIANA Sterki. Douglas.
VERTIGO OVATA OVATA (Say). Pulaski, St. Louis.
VERTIGO TRIDENTATA Wolf. Douglas, St. Louis.
VERTIGO GOULDI GOULDI (Binney). Jefferson.
COLUMELLA SIMPLEX (Gould). Douglas, Jefferson, St. Louis, Wright.
VALLONIA PULCHELLA (Müller). Christian, St. Louis, Wright.
VALLONIA EXCENTRICA Sterki. St. Louis.
VALLONIA PARVULA Sterki. Boone, Franklin, Howell, St. Charles, St. Louis.
CIONELLA MORSEANA Doherty. Wayne.
CARYCHIUM EXILE H. C. Lea. Boone, Camden, Douglas, Franklin, Jefferson, Ozark, St. Charles, St. Louis, Washington.
CARYCHIUM EXIGUUM (Say). Franklin, Jefferson.
HELICINA ORBICULATA ORBICULATA (Say). McDonald, Ozark, Taney.
POMATIOPSIS LAPIDARIA (Say). Franklin, Jefferson, Pulaski, St. Louis, Shannon, Stone.

SOME RIVER DRIFT LAND SNAILS FROM OKLAHOMA

LESLIE HUBRICHT

These shells were collected along the South Canadian River at Whitefield, Haskell County, Oklahoma. The collection contains several species not previously reported from that State. Many of the shells had been washed from Pleistocene deposits. The numbers are the number of specimens collected, the larger numbers are estimates.

POLYGYRA LEPORINA (Gould). 50
POLYGYRA TEXASIANA (Moricand). 50
POLYGYRA DORFEUILLIANA Lea. 9
STENOTREMA LEAI ALICIAE (Pilsbry). 25
MESODON INFLECTUS (Say). 2
TRIODOPSIS CRAGINI Call. 1
EUCONULUS CHERSINUS CHERSINUS (Say). 25
GLYPHYALINIA INDENTATA (Say). 35
HAWAIIA MINUSCULA MINUSCULA (Binney). 500
ZONITOIDES ARBOREUS (Say). 25
STRIATURA MERIDIONALIS (Pilsbry & Ferriss). 6
STRIATURA MILIUM (Morse). 9
HELICODISCUS NOTIUS NOTIUS Hubricht. 200
HELICODISCUS PARALLELUS (Say). 200
HELICODISCUS SHIMEKI Hubricht. 27
HELICODISCUS ROUNDYI (Morrison). 12
HELICODISCUS TRIDENS (Morrison). 500
HELICODISCUS SINGLEYANUS (Pilsbry). 200
HELICODISCUS JACKSONI Hubricht. 100
HELICODISCUS NUMMUS (Vanatta). 20
PUNCTUM MINUTISSIMUM (Lea). 75
PUNCTUM VITREUM H. B. Baker. 25
CATINELLA VERMETA (Say). 1
STROBILOPS LABYRINTHICA (Say). 6
STROBILOPS AENEA Pilsbry. 9
STROBILOPS TEXASIANA Pilsbry & Ferriss. 200
STROBILOPS LONSDALEI Ho & Leonard. 1
GASTROCOPTA ARMIFERA (Say). 200
GASTROCOPTA ABBREVIATA (Sterki). 200
GASTROCOPTA RUIDOSENSIS (Cockerell). 2
GASTROCOPTA CONTRACTA (Say). 1000
GASTROCOPTA HOLZINGERI (Sterki). 100
GASTROCOPTA TAPPANIANA (C.B. Adams). 400
GASTROCOPTA PENTODON (Say). 150
GASTROCOPTA CORTICARIA (Say). 1
GASTROCOPTA PROCERA (Gould). 1500 (1 sinistral).
GASTROCOPTA CRISTATA (Pilsbry & Vanatta). 1000
GASTROCOPTA PELLUCIDA HORDEACELLA (Pilsbry). 500
PUPOIDES ALBILABRIS (C.B. Adams). 1000
PUPILLA MUSCORUM (Linné). 1
VERTIGO MILIUM (Gould). 200
VERTIGO RUGOSULA Sterki. 35
VERTIGO ORALIS Sterki. 25
VERTIGO OVATA OVATA (Say). 75
VERTIGO TESKEYAE Hubricht. 25
VERTIGO TRIDENTATA Wolf. 1
VALLONIA GRACILICOSTA Reinhardt. 2
VALLONIA PARVULA Sterki. 50
VALLONIA PERSPECTIVA Sterki. 5
CARYCHIUM EXILE H. C. Lea. 500
CARYCHIUM PEREXIGUUM F.C. Baker. 11
HELICINA ORBICULATA (Say). 3
SNAIL EGGS, very small and flattened, probably *Zonitoides arboreus*. 3.
SNAIL EGGS, very small. 4

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MALACOLOGY IN CUBA

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Cuba, like the other islands of the Greater Antilles, is unusually rich in land shells, especially land prosobranchs of the families Pomatiasidae (Chondropomidae + Annulariidae) and Helicinidae, as well as some pulmonate families like the Urocoptidae and Camaenidae (genera *Zachrysia, Polydontes, Pleurodonte* and others. But for some reason there was more professional and amateur interest on the part of Cubans themselves in their molluscan fauna than almost any other country in the New World besides the U.S.A. Almost from the very beginnings Cubans took a prominent part in the investigation of their native mollusks. Together with such names as the German Pfeiffer and the Frenchman d'Orbigny, we find as almost of equal rank names of Cubans like Poey and Arango. This was not true in say, Hispaniola, Puerto Rico, Mexico, and Central America where with few exceptions, the published material on mollusks was written by foreigners—Ihering in Brazil, Strebel, von Martens, Fischer and Crosse in Mexico and Central America, Crosse in Hispaniola, etc.[1] Moreover, Cuba was the first country in the New World besides the U.S.A. where a malacological society was formed and a malacological periodical established. It is true that at present both periodical and society are defunct as a result of political causes, whereas such organizations exist in Uruguay and Brazil. But the Cuban groups antedated them by many years. What caused this upsurge of popular interest in Cuba in snails, what forms it took, and what the status of the subject is in present-day Cuba will be the subject of my discussion.[2]

I plan to divide my talk into three parts: first the history of malacology in Cuba from the beginning to about 1935. This will be far from a complete account; I will be able to touch only on the highlights. Then I shall give a brief account of the highwater mark which lasted roughly from the founding of the Sociedad Malacológica Carlos de la Torre in 1942 to approximately 1954, when the triannual periodical, the Revista de la Sociedad Malacológica Carlos de la Torre, ceased publication after about 9 volumes, and finally came to an end in the early 1960's when the society, together with all private associations, was abolished by the new government. The last part of my talk will deal with the present-day status of which I have only the sketchiest information.

Cuban shells early came to the attention of Europeans, but they were not cited as coming from Cuba. Bonnani in 1684 in his RECREATIO MENTIS ET OCULI (cl. 3, fig. 5) has a recognizable figure of the incredibly beautiful Cuban *Polymita*, but writes that it came from the *'littore ostiensi Italiae'*—or, what seems to say, the Italian coast near Ostia. Müller named *Buccinum fasciatus* (now *Liguus fasciatus) (1774,*

1. Uruguay had at least one prominent earlier malacologist in Formica Corsi (1852-1939) who was born in Catalunya and lived in Uruguay from 1888 to his death. But most of the descriptions of new forms in the nineteenth century were done by Europeans. For malacology in Canada, which also is an exception, see La Rocque, 1962.

2. This talk was the banquet address at the convention of the American Malacological Union in Cocoa Beach, Florida in 1971.

Verm. Terr. Fluv. 2: 145) as coming from the Indies. In his INDEX TESTACEOLOGICUS Wood has no citations from Cuba and reports *Helix picta* (now *Polymita)* as coming from Amboyna (1825:164). Even as late as 1834 Lea (Trans. Amer. Phil. Soc. 5:49, pl. 19, fig. 57) described *Helicina pulcherrima* (now *Emoda)* from Java. But he had a question mark. The first shell described as coming from Cuba seems to be *Helix bonplandi* (now *Eurycampta)* named by Lamarck in 1822 (Hist. Nat. 6(2):72). Among early travelers was Arthur Morelet who visited Cuba and the Isle of Pines and in 1849 and 1851 published his TESTACEA NOVISSIMA INSULAE CUBANAE ET AMERICAE CENTRALIS, in which he described many new Cuban species, among them the enchanting *Helicina regina* (now *Viana)* and the starry *Helicina constellata* (now *Priotrochatella),* two of the loveliest helicinids in the world.

The first studies dealing with purely Cuban mollusks as such were made by Ludwig Georg Karl Pfeiffer who published his BERICHT ÜBER DIE ERGEBNISSE MEINER REISE NACH CUBA IM WINTER 1838–9 (Report on the results of my trip to Cuba in the winter of 1838-9) in Wiegmann's Archiv für Naturgeschichte (1(5): 346-358). A little later I will have more to say about this trip, which proved to be of great importance in the history of malacology in Cuba. Pfeiffer stayed for only one month but in numerous articles in the Proceedings of the Zoological Society of London, Malakozoologische Blätter and elsewhere he described and discussed large numbers of new species on the basis of his own collecta and material sent to him by Gundlach, Wright, Arango, and others. Unfortunately the entire Pfeiffer collection, with all the invaluable Cuban types, was destroyed in World War II.

In 1838 three German biologists, Pfeiffer, Johannes Christoph Gundlach, and Eduard Otto, were invited to spend some time on the finca or ranch 'Fundador' owned by Charles Booth in Matanzas. (See A. Torre, 1952). Pfeiffer and Otto did not stay long, but Johannes Gundlach (called Juan by the Cubans), who was on his way to visit a friend in Surinam, fell so much in love with Cuba that he spent the rest of his long life there (1810-1896). He was a gentle character wholly devoted to collecting and studying. He suffered from a loss of smell the result of a hunting accident in his youth in Germany. He specialized in birds but collected and wrote on other natural history objects as well. Most of his new species were described by Pfeiffer and Poey; Gundlach apparently only proposed the new names. Pfeiffer alone received more than 200 new molluscan species from this indefatigable collector. In Poey's MEMORIAS (see below), Gundlach contributed articles on birds, bats, reptiles, amphibians, crustaceans, and insects. He named the smallest of all birds, the humming bird *Calypte helenae* for Booth's wife. The Cubans call this bird the 'pájaro mosca' or fly bird because of its diminutive size. He spent most of his time in Cuba but also paid a visit to Puerto Rico. In 1892 he sold his natural history collections to the Museum of Madrid for 8,000 gold pesos and promptly gave all this money to his host who found himself in financial straits. In 1892 he also met Carlos de la Torre who got him a job in the Museo del Instituto de la Habana where he spent most of the rest of his life.

Another foreigner attracted to Cuba was the Frenchman Alcide Dessalines d'Orbigny (1802-1857). He was well known for his work on French paleontology and in 1826 he visited the Americas, where he stayed for eight years. As a result of this trip, he wrote the VOYAGE À L'AMÉRIQUE MÉRIDIONALE, but for us his most important work is the section on Mollusca in Sagra's HISTORIA FÍSICA, POLÍTICA, Y NATURAL DE LA ISLA DE CUBA (1841-1847), which was first published in a French version. The atlas with magnificent illustrations appeared in 1842, in many cases before the written descriptions.

The greatest of Cuban naturalists, and deservedly so, as well as one of the great men of the New World, is Felipe Poey y Aloy (1799-1891). He deserves to be much better known in our own country. He was a remarkable character with widespread interests. He was trained first in philosophy and later in law but he never practiced. He wrote for numerous Cuban and Spanish periodicals on all sorts of subjects. He was the author of some quite charming Spanish poems. He founded the Museo de Historia Natural in Havana (1839), the Real Academia de Ciencias Médicas, Físicas y Naturales de la Habana (1861), and the Sociedad Antropológica de la Isla de Cuba (1877). For years he was a professor in the University of Havana where he taught comparative anatomy, zoology, botany, mineralogy, and geology, in several cases introducing the subjects in the curriculum for the first time and writing the standard textbooks. It was he who trained most Cuban naturalists of the period, among them Don Carlos de la Torre. He wrote on numerous fields of natural history, and his best known published work in this respect is his MEMORIAS SOBRE LA HISTORIA NATURAL DE LA ISLA DE CUBA in two volumes

(1851-1861) which he wrote not only in Spanish but also in Latin and French. The Memorias contain his most important contributions in malacology. He also wrote articles in it on fish, bats, etc. His outstanding opus, ICTIOLOGIA CUBANA, has apparently never been published. It consists of six folio manuscript volumes with more than 700 mostly life-sized, colored illustrations. Though many countries offered to buy the manuscript and illustrations, he sold it for $4,000 to the Spanish government. It was displayed at the Amsterdam International Exposition in 1833, where it won its author a knighthood in the Orden de la Cruz del León Neerlandés. Later the Cuban government of President Machado gave $10,000 to have the manuscript copied and prepared for publication. The task was undertaken by de la Torre and Felipe García Cañizares, but publication never took place because, as J. A. Conde writes (1958:232) of 'crisis económicas sucesivas.'

I have time to mention only one more Cuban malacologist of the earlier generation. He is Rafael Arango y Molina (1837-1893) who is particularly worthy of mention since he is the first Cuban scholar who devoted almost all of his time to malacology. He also wrote on the Radiata of Cuba, but this proved to be only of secondary interest to him. He formed the best collection of Cuban mollusks ever assembled which he later donated to the Real Academia de Ciencias Médicas etc. in Havana. I could not find out where it is now, but it probably is in the collection of the Museo Poey either in the University of Havana or in the national capitol building. He described many new species and wrote extensively in the Proceedings of the Academy of Natural Sciences of Philadelphia, the French Journal de Conchyliologie, and elsewhere. His most important work is the CONTRIBUCIÓN A LA FAUNA MALACOLÓGICA CUBANA (1878-1880), a thoroughly annotated catalogue of Cuban mollusks which also contained some new species and many original observations on ecology, nomenclature, and distribution. Hence it is much more than a simple catalogue. Crosse's work on Cuban malacology (1890) is largely a translation of Arango's Contribución, which is a book of meticulous scholarship and thorough erudition, gratifyingly free of errors. It is probably the best single work on Cuban mollusks ever published.

Between this older generation of scholars —I have to omit many— and the younger people who worked in Cuba before the Castro revolution, stands the figure of Don Carlos de la Torre y de la Huerta (1858-1950). He made important contributions to his country as an educator, author, scientist, and politician and produced several studies in various fields of natural history. He assembled an extensive collection of Cuban shells, now divided between the Museo Poey and the Museum of Comparative Zoology in Harvard University. During his lifetime his fame as a knowledgeable shell expert pervaded the entire malacological community of Cuba. This skill was manifested in his ability of identifying shells by touch alone, without looking at them. When he demonstrated this in the British Museum, Bednall, the Curator, according to Conde (1958:279), was so deeply moved that he was forced to exclaim: "'Only Lamarck achieved anything like it--when he was blind--and then only with larger shells.' Then, rising from his seat, he pressed an ardent kiss upon the noble (amplia) brow of the Cuban naturalist," (translated). His most important works--of which more will be said directly—were written in English with the collaboration of Paul Bartsch and Joseph P. E. Morrison. Most of his other work also appeared in English. His younger contemporaries, in the Cuban manner, heaped fulsome praise upon him, but whether this was entirely merited is a matter of dispute today.

The later Cuban and American students of Cuban mollusks are too numerous to mention. They include practically every American malacologist of note such as Pilsbry, Clench, Henderson, Bartsch etc. Among the many younger Cubans we must not omit mention at least of Carlos Guillermo Aguayo, Miguel L. Jaume, Hortensia Sarasúa and Luis Howell Rivero. But there are many more.

Until the 1940's the study of snails remained largely the province of specialists and more or less trained observers. Then an event occurred which made the 'caracolitos' one of the most popular hobbies among the Cubans of leisure. This was the publication in 1938 by the United States National Museum of the handbook on the CUBAN OPERCULATE LAND SHELLS OF THE SUBFAMILY CHONDROPOMINAE by Carlos de la Torre and Paul Bartsch, to be followed in 1941 by the same authors' THE CUBAN OPERCULATE LAND MOLLUSKS OF THE FAMILY ANNULARIIDAE EXCLUSIVE OF THE SUBFAMILY CHONDROPOMINAE, and in 1942 by THE CYCLOPHORID OPERCULATE LAND MOLLUSKS OF AMERICA, in which they were aided by Joseph P. E. Morrison.

It should be noted that a well illustrated well written, and easily available handbook leads almost immediately to a widespread

popular interest in the subject. This happened in the case of GOULD'S INVERTEBRATA OF MASSACHUSETTS (1841 and 1870), and the huge interest in shells today may well be ascribed primarily to the books by R. Tucker Abbott. A similar effect took place in Cuba when the Torre-Bartsch monographs began to appear. These booklets were magnificently illustrated and, having been published in large quantities, were readily available at less than $2.00 each. I found these three works in the hands of all the Cuban shell people I ever visited. In addition to being well illustrated and easily available, the books were provided with precise locality data for every species and subspecies discussed, so that all these forms could readily be collected and determined. The writing is less praiseworthy. The descriptions are lengthy and repetitive, and it is difficult to winnow the diagnostic features from the clutter of repeated adjectives. In many cases there is no statement of the diagnostic differences at all. However, this fault is largely overcome by the truly excellent figures and the admirable locality data. Probably most readers relied on the figures and the locality data alone to name up their hauls. At any rate, it was the appearance of these three monographs which drove many Cubans into a shell collecting frenzy. Other hobbies were given up for the 'caracoles.' Even Bohemia, the popular weekly periodical had to take notice of the rage, especially since it involved so well-known a public figure as Don Carlos de la Torre. Making land shell collections and naming 'novedades' blossomed as readily under the hot subtropical sun as the excellent Cuban tobacco and sugarcane. This was all to the good, because, as a result, every nook and cranny was hunted down for the snails. Cuba became splendidly known conchologically. But there was a fly in the mixture.

Besides those already mentioned, the Torre-Bartsch monographs had other serious faults. And these faults, as far as I could see, were never overcome by the large number of amateurs and even scholars who were attracted to shell collecting by Torre and Bartsch. The traditions set up by these two students remained, even in the work of competent post-Torre-Bartsch scholars. What were these faults? They were beautifully outlined in a spritely review of the second of the three monographs by H. B. Baker in 1941. I quote Baker's summary: 'The authors have completely finished the Cuban members of this family and, in so doing, have fallen little short of the good old-fashioned standards, as represented in the classic works of Reeve and Isaac Lea. Any future studies can only subtract from such imposing creations which include as new, 5 genera 23 subgenera 101 species and 120 subspecies from one of the best-known islands.' It is significant that the other Torre-Bartsch monographs were not reviewed in the pages of Nautilus.

Among other faults noted by Baker, the most important were: 1) the untenable separation of the Neotropical pomatiasids under the family name Annulariidae, which itself was improperly used; 2) the failure to consider the normal variability of minor sculptural shell features upon which many taxa were erected; 3) the neglect of ecological influences on shell morphology; and 4) the complete omission of reference to the secondary sexual dimorphism--Baker commended the authors for their 'demulcent reticence in matters of sex.'[1] This last is a most important point since in many pomatiasids the male is clearly smaller than the female. Nevertheless, in one of the identification keys (1941:231) the shells 13 mm long and those 11 mm long are assigned to different species. Even where the original descriptions by other authors made reference to this sexual difference in size, Torre & Bartsch pointedly omitted such details in their redescriptions and discussions.

The system set up by Torre & Bartsch is a neat, gentlemanly matter of clean, dry, handsome shells with no unpleasant details of soft parts, dissections, distributional and ecological data or life-history studies. Well does Solem (1961: 193) call it 'a neat card-filing system' based upon single 'key characters' with no attention paid to the role of interspecific variation and phenoecological considerations.'[2]

1. I have another instance of this 'demulcent reticence' regarding s*x. Carlos de la Torre & Charles H. Ramsden (1914, Naut. 28:50) named *Annularia mayensis*, diameter given as 15 mm and the statement added: 'The males are about 4 mm smaller in diameter.' i. e. more than ¼ smaller! Same species described elaborately in Torre & Bartsch (1941:379) diameter 12.8 mm and not a word about sexual dimorphism.

2. The use of key characters is misleading in other fields as well. Sprague de Camp (1970:86) writes: '...the cranial index is only one of many characteristics to be considered in classifying people, and used by itself leads to such curious results as lumping the Swedes, Eskimos, and Hottentots together.

We need not pursue this subject any longer. The surprising thing is, as I have indicated, that all these weaknesses were repeated and elaborated on by the entire Cuban post-Torre-Bartsch generation of workers. They never gave up the name Annulariidae in spite of Baker's clear exposition of its incorrectness on bibliographical and anatomical grounds, they made much use of minor, highly variable microsculpture of the shell surfaces in separating new taxa, they omitted ecological and distributional considerations, and they rigidly excluded all reference to sexual differences. Thus can strong traditions be established. These traditions probably still prevail even in the restricted work on malacology being done in Cuba today, because I receive letters from Cuba telling me that large numbers of manuscripts--among them 'many new species and subspecies'--await publication, 'species and subspecies' found in such well-collected areas as the Yumuri Valley in Matanzas Province and elsewhere.

But don't let me go overboard. The picture is far from being a gloomy one. The greater part of the activities pursued in those post-Torre-Bartsch days were of great value and the few weaknesses do not destroy the whole. Large numbers of individuals were led to an interest in shells and large and valuable collections were made. Interest was aroused in halting the commercial exploitation especially of *Polymita* and some attempts at conservation were made as early as 1940. The Revista itself has much valuable material, superb studies by men like Howell Rivero and Carlos Aguayo among others. Cuba was scoured from end to end for shells and it is probably better known malacologically than any area of equal size in the Neotropics.

This hopeful period in the history of Cuban malacology began to come to an end in 1954 when the Revista folded up in the middle of volume 9. Signs that there was trouble appeared earlier because a full year went by between the appearance of number 1 and number 2 of volume 9 whereas previously all the numbers had appeared at regular intervals three times a year. When Castro came to power in 1959 the society was soon thereafter abolished together with all private clubs and associations. The personal collections, as far as I could determine from Cuban refugee scholars, were more or less voluntarily donated to the government and housed in the capitol building in Havana. Indications are that they are being somewhat less than satisfactorily cared for there.

Now, as to the present. From the little I could learn, there still seems to be a good deal of study going on in very restricted circles. Miguel Jaume, Director of the Felipe Poey Museum and head of the Division of Biology in the Academia de Ciencias, writes that he has a 250 page manuscript dealing with one hundred years of study on the malacological fauna of Cuba which has been in the hands of the printers since 1968. Similarly another of his studies on the mollusks of the Sierra del Rosario and a very extensive bibliography for the study of the mollusks of the Antilles await publication. Dr. H. Sarasúa writes that she is preparing a list of the types of *Cylindrella (=Urocoptis)* of Gundlach in the Museo Poey.

As far as actual publications on mollusks are concerned, they can be counted on the fingers: a few brief reports in the mimeographed TRABAJOS DE DIVULGACIÓN and in the new series called POEYANA, and a single paper in the Nautilus. One of the serious difficulties faced by the Cubans is the lack of modern literature in spite of several trips Jaume has made to Europe and South America. The result is that work being done there is frequently out-of-date.

I will conclude by outlining what I think are the tasks for the future which will bring to the world once more a satisfying knowledge of the rich Cuban fauna. These tasks can be divided into two groups, field work and museum studies. In field work the collection of specimens must be ardently pursued. The restricted habitats of many species and forms and the increasing industrialization of the country puts the very existence of many molluscan populations in jeopardy. Intensive military and industrial construction has already caused serious damage in the San Andrés region and in Guanajay in Pinar del Río Province and probably elsewhere. Some richly populated mogotes and cerros near Remedios in Las Villas Province have already been largely destroyed by mining for calcium to manufacture cement.

In the collections, special attention must be paid to the preservation of large series of alcoholic specimens. Solem (1961:192) complained that in his studies of museum material he could not even sex the specimens because most series consisted only of cleaned shells.

Another pressing task is the study of the life history of the mollusks. So little has been done in this area that even the eggs of any pomatiasid from Cuba have yet to be described. In this connection careful studies of the ecological require-

ments are needed to illuminate the bewildering distribution of many forms.

There is also much need of faunal lists of many areas. Only very few such have appeared up to now.

In the area of museum work, attention must be paid above all to the discovery and preservation as well as, hopefully, publication of all type material. As I have indicated this seems to have begun already.

Finally, museum studies with large series of well localized material are needed to determine the limits of the variations of species and genera. Solem (1961:193) suggests that such work might well result in the synonymization of large numbers of published names. As it is, it will take a stout heart indeed on the part of any student to begin nibbling away at the lofty structure of the Pomatiasidae erected by the labors of Torre and Bartsch.

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REPRINTS OF RARE PAPERS ON MOLLUSCA
MARTIN LISTER (1678) HISTORIAE ANIMALIUM ANGLIAE

(Continued from Sterkiana 44:57)

AURÈLE LA ROCQUE

The four plates reprinted here are nos. 6 to 9 of the original publication of 1678. The fossils represented are mainly Mollusca but there are exceptions. For example plate 7 shows many echinoids, there are a few undoubted brachiopods on plate 8 (fig. 46 and perhaps also upper fig. 44) and other brachiopods appear on plate 9 as noted below.

Most of the figures on these plates are original but some were reproduced from Robert Plot's *Natural History of Oxfordshire* ... of 1677. Lister acknowledged his debt to Plot in his text and I have noted a few of those which can be recognized with certainty from comparison of Lister's work with that of Plot.

In the remarks below, I have tried to identify as best I could the nature of the fossils illustrated by Lister. This has been done earlier but at the moment I do not have the reference at hand.

PLATE 6

The first two rows of specimens are undoubtedly ammonites and so is the first specimen (fig. 7) in the third row. Fig. 6 is a reversed copy of Plot's plate 5, no. 11. Fig. 8 may be a small ammonite but it could also be an enlarged specimen of a small gastropod. It may prove to be the same as Plot's plate 5, no. 8.

Figure 9 is probably also a gastropod, sinistral in the figure but probably dextral in the original, judging by the way dextral gastropods in other plates were drawn as sinistral.

Figure 10 (first of 3 with the same number) is probably a group of gastropods in a matrix of undetermined nature but it may possibly also be a group of spirorbid worm tubes.

Figure 10 (last two figures). These could be gastropods, nautiloid cephalopods, or ammonoids. There are globose, tightly coiled forms in all three groups.

PLATE 7

Top row of figures. All of these are gastropods, all drawn as sinistral. They are all in a fairly good state of preservation except Fig. 14 which is an internal mold of part of the spire; no. 17 shows more of the specimen depicted but all external details of the shell are missing.

Middle three rows. These are all echinoids, some regular, others irregular, drawn in various aspects.

Bottom row. Four belemnites of various sizes, all rather fragmentary.

On this plate the equivalence with Plot can be noted as follows:

LISTER	PLOT
Fig. 17	Plate 6, fig. 11.
Fig. 20	Plate 8, fig. 9.
Fig. 22	Plate 5, fig. 4.
Fig. 23	Plate 5, fig. 5.
Fig. 24	Plate 5, fig. 6
Fig. 25	Plate 5, fig. 3
Fig. 27	Plate 2, figs. 9, 10
Fig. 28, left	Plate 2, fig. 11
Fig. 28, right	Plate 7, fig. 9
Fig. 29	Plate 2, fig. 14
Fig. 30 (2 figs.)	Plate 3, figs. 1, 2.

PLATE 8

Fig. 33. Two views of the same pelecypod, left valve.

Fig. 34. Another pelecypod with both valves joined and closed.

Fig. 35. The two smaller mollusks could be limpets on the interior of a broken oyster shell.

Fig. 36. The figure is rather puzzling, but could be a fragmentary pelecypod valve.

Fig. 37. A pelecypod with both valves closed.

Fig. 38. Possibly a fragmentary specimen of *Inoceramus*.

Fig. 39. An elongate mytilid?

Fig. 40. Internal mold of a very globose pelecypod.

Fig. 41. Same as Fig. 40?

Fig. 42. Same pelecypod, but an external view of a specimen with well preserved valves.

Fig. 43. A large ostreid.

Fig. 44. The upper figure might be a brachiopod but the lower one appears to be a young oyster.

Fig. 45. Gryphaea, Exogyra, or an allied genus, showing both valves.

Fig. 46. Two views of the same brachiopod.

Fig. 47. Possibly a pelecypod, perhaps genus *Mya*, but it could also be a brachiopod.

Figures 39, 40, 41, and 42 appear to be from Plot's plates 5 and 7.

PLATE 9

Fig. 54. Very probably a pelecypod perhaps a cardiid, judging by the part of the hinge shown on the left hand figure; the right hand figure is drawn as though the specimen were a brachiopod but again the costae suggest a cardiid.

Fig. 48. Certainly a fossil pectinoid.

Fig. 49. Probably a fossil pectinoid, perhaps related to the monotids of the Triassic.

Fig. 50. This suggests a brachiopod rather than a pelecypod. It resembles a young productid.

Fig. 51. A pectinoid, with ornamentation which should be diagnostic.

Fig. 52. A specimen without hinge or shell margin, which suggests a pustulose brachiopod.

Fig. 53. This looks more like a brachiopod than a pelecypod but nothing clearly diagnostic is visible.

Fig. 55. On the other hand, this specimen looks more like a pelecypod than a brachiopod.

Fig. 56. The sinus in this specimen makes it almost certain that it is a spiriferoid brachiopod.

Fig. 57. The outline is somewhat puzzling, but it does suggest a rhynchonelloid brachiopod. If so, the structure near the tip of the beak was drawn on the wrong side of the valve.

Fig. 53. This figure appears to be another specimen of the same genus as the other figure 53 just above it.

Fig. 57. Clearly a brachiopod because of the symmetry and the arrangement of the costae as well as the outline of the valve.

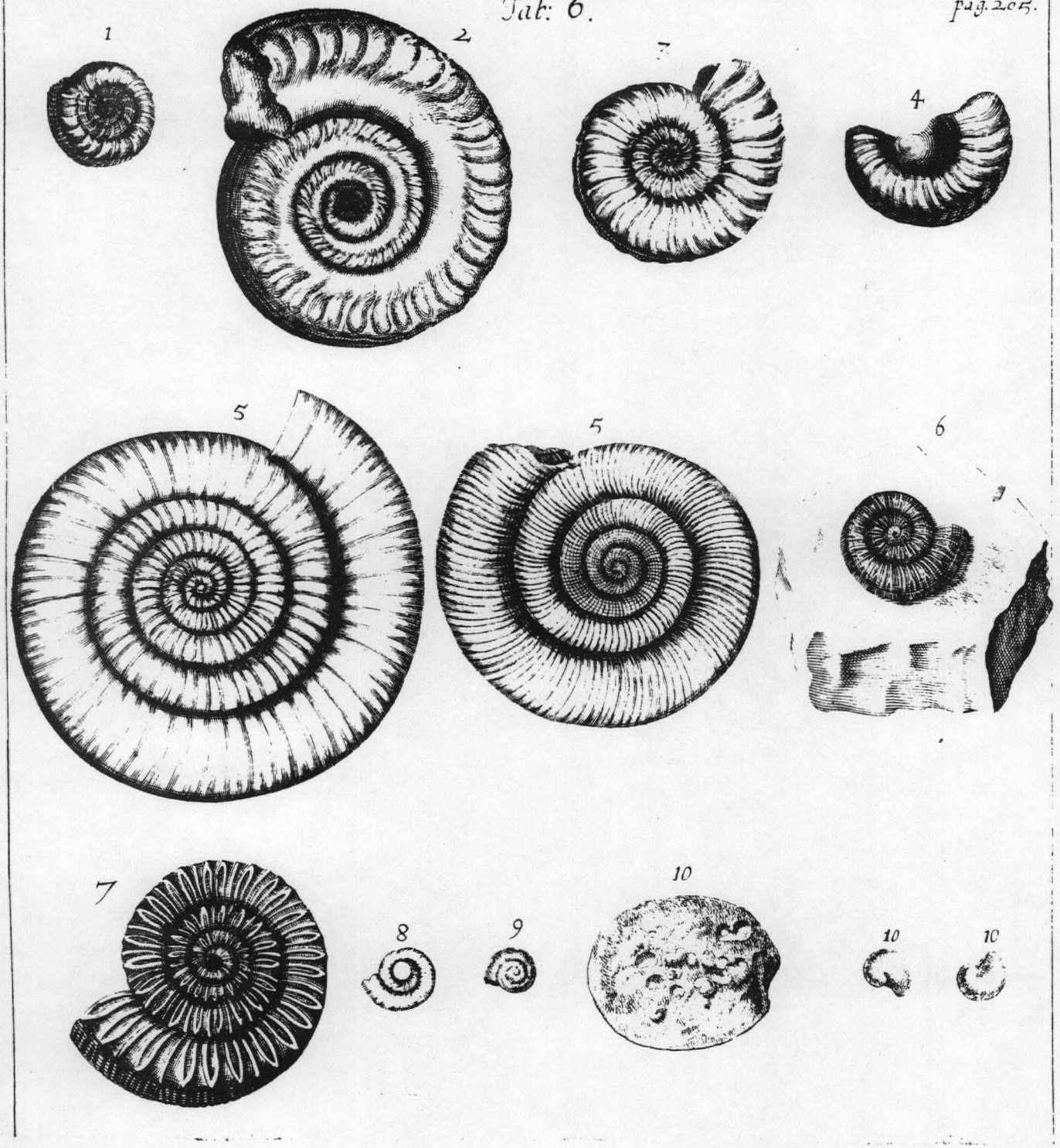
Fig. 58. This could be a brachiopod with a twisted beak. There are quite a few of these in the Paleozoic and Mesozoic.

Fig. 59. This is most probably a very globose brachiopod but the pose of the specimen gives little information as to its taxonomic position save that it is an articulate.

The following equivalences with Plot may be noted.

LISTER	PLOT
Fig. 51	Plate 4, fig. 11.
Fig. 58	Plate 4, fig. 4.
Fig. 59	Plate 4, fig. 5

Tab: 6. pag. 205.



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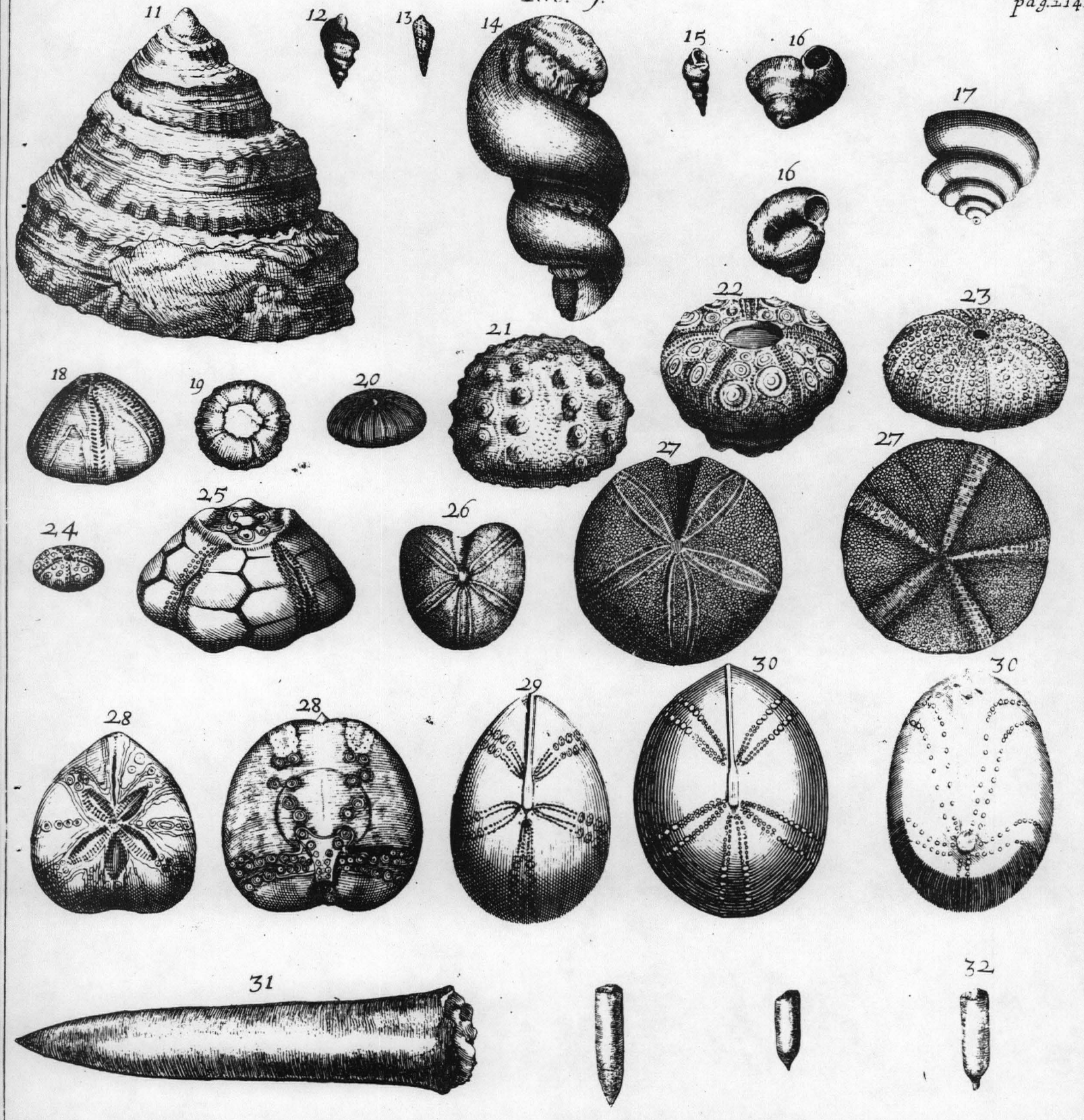
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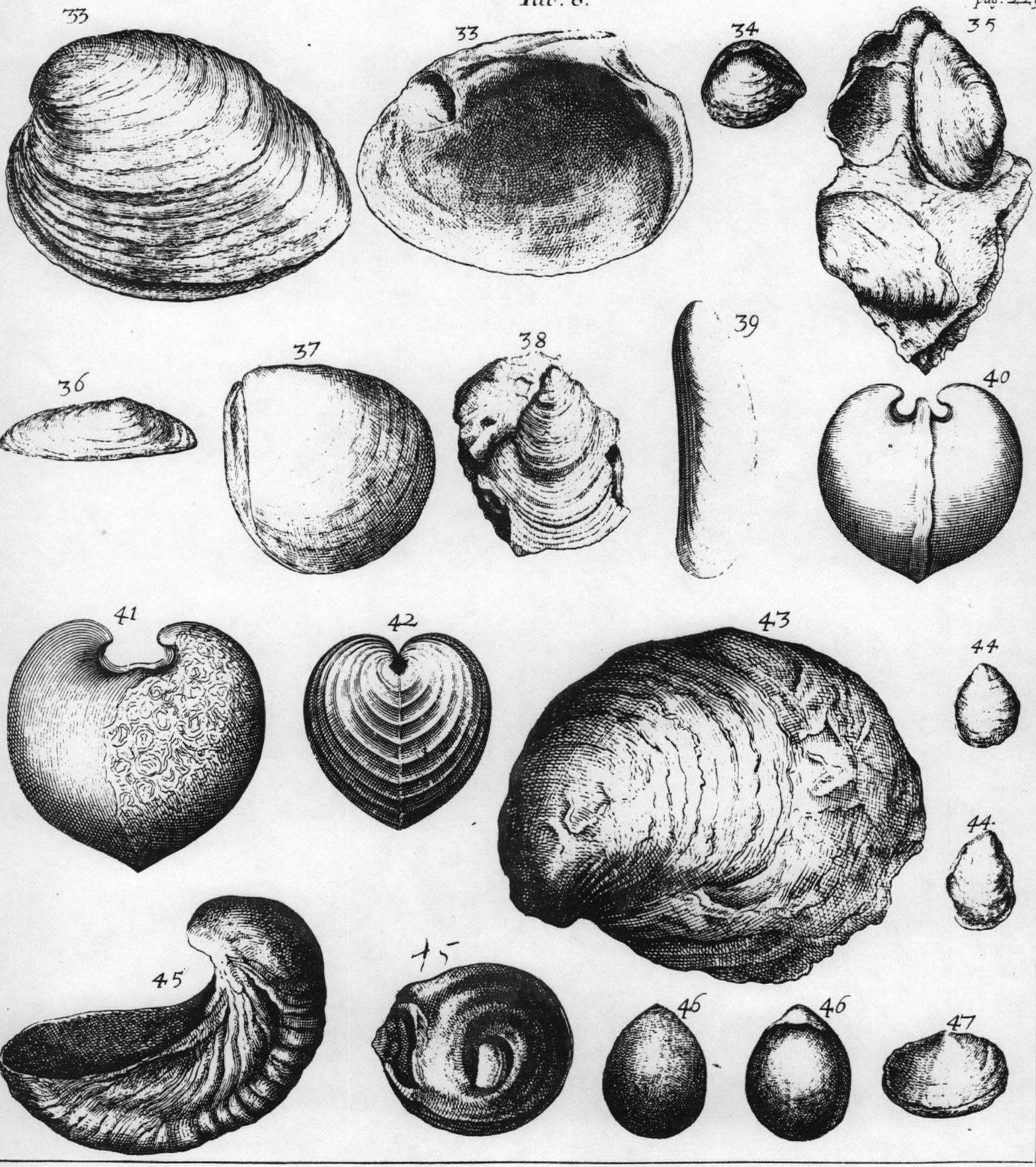

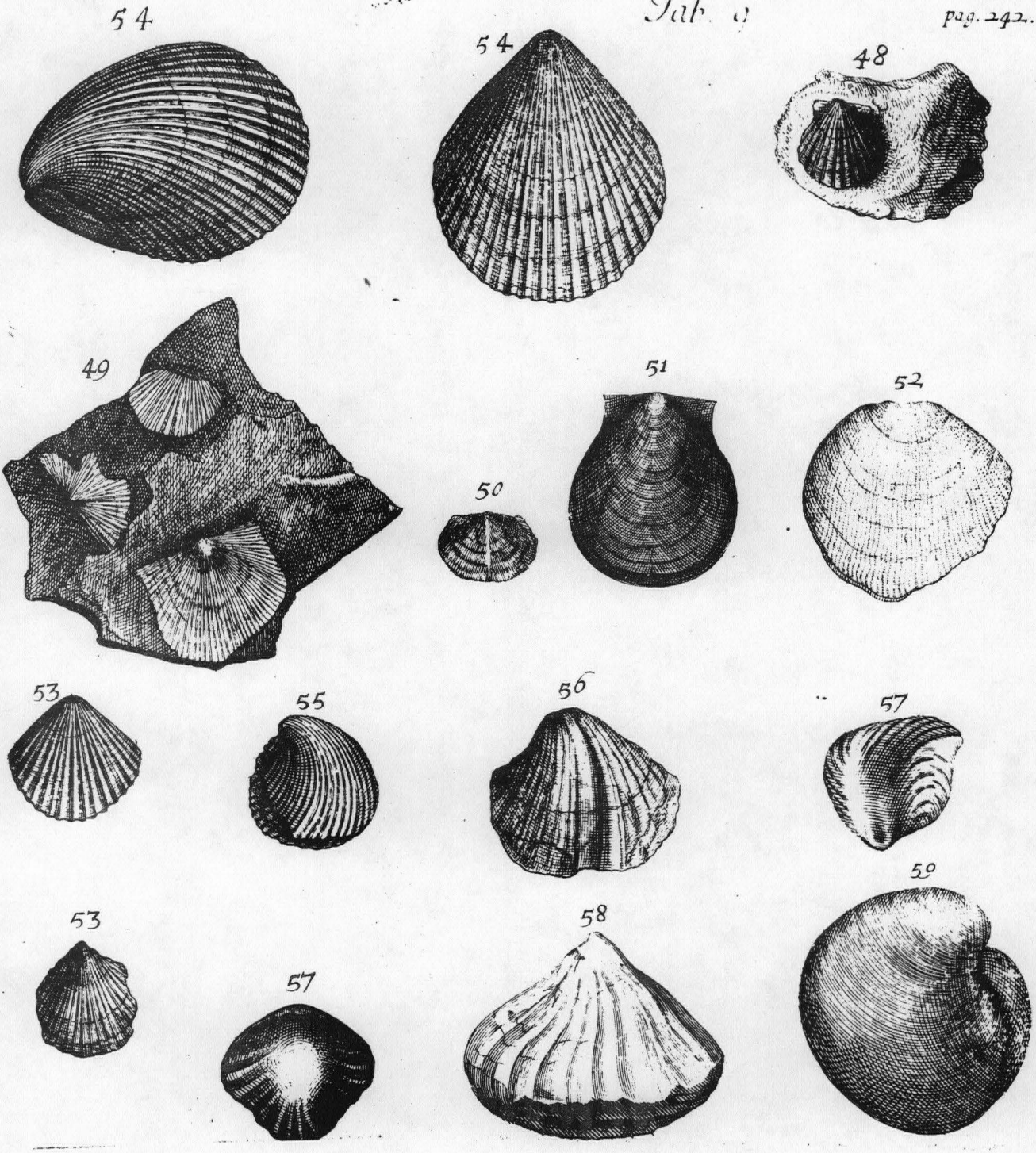
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Class LAMELLIBRANCHIA.

Order EULAMELLIBRANCHIA.

Suborder SUBMYTILACEA.

Family MARGARITANIDÆ.

Ortmann (79, p. 223) has raised the genus *Margaritana* to the rank of a family and (80, p. 13) has proposed a new genus, *Cumberlandia,* for *M. monodonta* (Say), both based upon anatomical peculiarities

For the distribution of the genus in this country, see Walker, Nos. 152 and 153, Ortmann (80, p. 14) and Utterback (135, p. 99).

MARGARITANA MARGARITIFERA (L.).

Unio ocmulgeensis dominus De Gregorio, Moll. di aq. dul. di Amer., 1914, p. 13, pl. 7, fig. a-c.

Family UNIONIDÆ.

Simpson's "Descriptive Catalogue of the Naiades" brings the subject down to January 1, 1913.

For the revised classification so far as it has progressed, see Part I.

In view of the relatively small number of species that have been examined anatomically and the consequent element of uncertainty as to the systematic position of the remainder that must continue until the animals can be critically examined, it has seemed better, for convenience of reference, in this porton of the work to retain the generic names given by Simpson, noting, however, under such species as have been examined anatomically their proper place in the revised classification.

Recent attempts to revive Rafinesque's names for many of the species have created considerable confusion as to the proper nomenclature to be followed.

Vanatta's valuable paper on "Rafinesque's Types of Unio" (140, p. 549), reviewed by Walker (158, p. 43), has given definite information as to what Rafinesque in 1831 understood or claimed to be the species that he had described in 1820.

It has been too hastily assumed by some that these determinations have definitely settled the validity of all of the Rafinesqueian species involved. This is far from correct. It is not claimed, except in one instance, that the so-called types in the Poulson collection are the original types of Rafinesque. And, even if they were, reference to them for the purpose of determining an otherwise unidentifiable description is prohibited by the International Code (Op. Int. Co., I). The requisites for a sufficient description are definitely specified by the Code (Art. 25) and these provisions as defined by the decisions of the International Committee must be applied to each individual case.

Dr. Pilsbry in Vanatta's paper has very aptly stated the situation as follows: "The use of a Rafinesquian name depends upon whether it could be identified by descriptions published prior to any other recognizable name for the same species. That it can be recognized from the types or other specimens from Rafinesque does not entitle his names to acceptance unless the published descriptions are adequate. This question of the adequacy of published diagnoses must be considered for each species separately."

In the same connection, see Walker, (157, p. 74).

Subfamily UNIONINÆ (Swainson, 1840), Ortmann, 1910.

Genus QUADRULA Rafinesque, 1820.

QUADRULA ASKEWI (Marsh).

Frierson (41, p. 136) refers this to *beadleiana* Lea. But Ortmann (81, p. 21) states that it does not group with that species, but is a *Fusconaia* of the *undata* group.

QUADRULA BEADLEIANA (Lea).

Includes *Q. chickasawhensis* (Lea) and *askewi* (Marsh) according to Frierson (41, p. 136). But Ortmann (79, p. 268) says that it is an *Elliptio.*

QUADRULA BURSAPASTORIS (B. H. Wright).

Is a *Fusconaia* according to Ortmann (81, p. 90).

QUADRULA COCCINEA (Conrad).

Is a *Pleurobema* according to Ortmann (77, p. 101) and a variety of *Q. obliqua* (Lam.) (78, p. 117 and 79, p. 263).

Utterback (135, p. 190) quotes *catillus* Con., which Simpson has considered a synonym of *Q. coccinea,* as a variety of *Q. obliqua* (Lam.) and on p. 193 of the same paper considers it identical with *Q. solida* (Lea), having priority and gives it specific rank as such.

QUADRULA COOPERIANA (Lea).

At first referred to *Pleurobema* by Ortmann (78, p. 117), this species is now included in *Plethobasus* by him (79, p. 261).

QUADRULA CYLINDRICA (Say).

Unio cylindricus propetypicus De Gregorio, Moll. di aq. dul. di Amer., 1914, p. 11, pl. 4, fig. 1.

Unio cylindricus acrispatus De Gregorio, Ibid, p. 11, pl. 4, fig. 2.

Vanatta (140, p. 556) states that the *Unio solenoides* Raf. of the Poulson collection is this species.

QUADRULA EBENUS (Lea).

Is *Obovaria obovalis* Raf. of the Poulson collection according to Vanatta (140, p. 558). If identifiable from the original description, *obovalis* would have precedence.

QUADRULA FRIERSONI (B. H. Wright).

Is a *Pleurobema* according to Ortmann (81, p. 30).

QUADRULA HEROS (Say).

This species is the type of *Megalonaias* Utterback.

Frierson (45, p. 61) has identified Barnes' *Unio giganteus* as this species and gives it priority.

QUADRULA INTERMEDIA (Conrad).

Unio tuberosus perlobatus De Gregorio, Moll. di aq. dul. di Amer., 1914, p. 9, pl. I, fig. 3.

QUADRULA KIRTLANDIANA (Lea).

Is a variety of *Q. subrotunda* (Lea) according to Ortmann (78 p. 116).

QUADRULA LACHRYMOSA (Lea).

This species has been identified as the *Obliquaria quadrula* Raf. by Say, Conrad and others and Vanatta (140, p. 556) states that the shell so labelled in the Poulson collection is Lea's *asperrimus*. If identifiable from the original description, it would have priority.

QUADRULA LACHRYMOSA CONTRARYENSIS Utterback.

Quadrula lachrymosa contraryensis Utterback, Amer. Mid. Nat., IV, 1915, p. 138, pl. XVIII, figs. 47a-b.

Type locality: Lake Contrary, St. Joseph, Mo.

PLEUROBEMA MISSOURIENSIS (Marsh).

The type of this species has been figured by Walker (155. p. 140, pl. V, figs. 1-2) and it appears to be a *Quadrula* allied to *Q. subrotunda* (Lea).

QUADRULA OBLIQUA (Lam.).

Includes *pyramidata* Lea, *cocinca* Con., and *plena* Lea according to Ortmann (78, p. 117 and 79, p. 264) and is a *Pleurobema*. Vanatta states (140, p. 557) that the *Obliquaria lateralis* Raf. of the Poulson collection is this species.

QUADRULA PERUVIANA (Lamarck).

Lamarck in his original description refers to the figure in the Encyc. Meth., pl. 248, fig. 7, but the reference was overlooked by Simpson in his Synopsis, but was supplied in the Desc. Catalogue. The species is the form commonly called *plicata* Say by collectors and is characterized by its prominent beaks. It is quite different from *plicata* Say from Lake Erie.

QUADRULA RARIPLICATA (Lamarck).

This species, which has been referred to *plicata* Say by Simpson and authors generally, is neither typical *peruviana* (Lam.) nor typical *plicata* (Say). The type which is still preserved in the Museum at Geneva, Switzerland, is the Ohio River form, which has commonly passed as *plicata* (Say), and is sufficiently distinct to have varietal rank at least. There is some reason to believe that Say's *plicata* is an off-shoot from this race rather than of *undulata* Bar. as has been suggested by Ortmann (79, p. 246). It is also possible that it rather than *undulata* should be considered the *costata* Raf.

QUADRULA PLICATA (Say).

As stated by Ortmann (79, p. 246) the type of this species came from Lake Erie and is undoubtedly the form described by Lea as *Unio hippopæus*. It has been referred to *undulata* Bar. by Ortmann (l. c.), but there is apparently some ground for considering it as more closely allied to *rariplicata* (Lam.). Pending the settlement of this question, it would seem better to keep it separate from either.

QUADRULA PLENA (Lea).

According to Ortmann (78, p. 117) this is probably only a form of *obliqua* (Lam.). Vanatta states (140, p. 558) that the *Obovaria cordata* Raf. of the Poulson collection is this species. If identifiable from the original description, Rafinesque's name would have priority.

QUADRULA PUSTULATA (Lea).

According to Vanatta (140, p. 557) the *Obliquaria nodulata* Raf. of the Poulson collection is this species. If identifiable from the original description, *nodulata* would have precedence.

QUADRULA PUSTULOSA (Lea).

According to Vanatta (140, p. 556) the *Obliquaria retusa* Raf. of the Poulson collection is "probably" this species. The specific name is not preoccupied by *Unio retusa* Lam. and, if identifiable from the original description, Rafinesque's name would have priority.

Utterback (135, p. 131) has suggested that the species should be known as *bullata* Raf., but see next note.

QUADRULA PUSTULOSA PERNODOSA (Lea).

According to Vanatta (140, p. 557) the *Obliquaria bullata* Raf. of the Poulson collection is this form, but the name is preoccupied by *Obliquaria flexuosa bullata* Raf. and Lea's name will stand.

QUADRULA PYRAMIDATA (Lea).

Unio plenus interduos De Gregorio, Moll. di aq. dul. di Amer., 1914, p. 18.

According to Ortmann this is probably only a form of *obliqua* (Lam.). Vanatta states (140, p. 557) that the *Obliquaria rubra* Raf. of the Poulson collection is this species. If identifiable from the original description, *rubra* would have priority.

QUADRULA REFULGENS (Lea).

Includes *sphærica* (Lea) according to Frierson (41 p. 136).

QUADRULA RUBIGINOSA (Lea).

Unio validus continuus De Gregorio, Moll. di aq. di Amer., 1914, p. 21.

This species has been identified as the *Obliquaria flava* Raf. by Say, Conrad and others. According to Vanatta (140, p. 557) the *O. flava* Raf. of the Poulson collection is this species. If identifiable from the original description, Rafinesque's name would have priority.

According to Ortmann (78, p. 116) *rubiginosa* is a variety of *undata* (Bar.).

FUSCONAIA SELECTA Wheeler.

Fusconaia selecta Wheeler, Naut., XXVIII, 1914, p. 76, pl. IV.
Type locality: Cache River, Nemo, Craighead Co., Ark.

QUADRULA SPHÆRICA (Lea).

Is a synonym of *refulgens* (Lea) according to Frierson (41, p. 136).

QUADRULA SUBROTUNDA (Lea.)

According to Vanatta (140, p. 558) the *Obliquaria sintoxia* Raf. of the Poulson collection is this species. If identifiable from the original description, *sintoxia* would have precedence. The species is a *Fusconaia* according to Ortmann (79, p. 244).

FUSCONAIA SUBROTUNDA LEUCOGONE Ortmann.

Fusconaia subrotunda leucogone Ortmann, Naut., XXVII, p. 89.
Type locality: Elk River, Gassaway, Braxton Co., W. Va.

QUADRULA TRAPEZOIDES (Lea).

Bariosta ponderosus Raf. is a synonym and *Bariosta* Raf. is a synonym of *Amblema* Raf., unless the species should prove to be generically distinct according to Frierson (42, p. 7).

FUSCONAIA UNDATA TRIGONOIDES "Frierson" Utterback.

Fusconaia undata trigonoides "Frierson" Utterback, Amer. Mid. Nat., IV, 1915, p. 107, pl. XV, figs. 30A-D.

Type locality: Platte River, Agency Ford, Mo.

QUADRULA UNDULATA (Barnes).

This species has been identified as the *Amblema costata* Raf. by Conrad, Frierson and others. Vanatta states (140, p. 556) that the *Amblema costata* Raf. of the Poulson collection is also this species. If identifiable from the original description, Rafinesque's name would have priority. In considering this question attention should be given to the possibility that *costata* Raf. may be the *rariplicata* Lam.

Costata has been designated by Frierson (42, p. 7) as the type of *Amblema* Raf.

QUADRULA UNDULATA PILSBRYI (Marsh).

According to Utterback (135, p. 119) this is a synonym of *Q. perplicata quintardii* (Cragin).

Genus TRITOGONIA Agassiz, 1852.

The recent discovery of Sterki ('07, p. 48) that in the type species, *T. tuberculata*, all four of the gills are utilized for marsupia, removes the genus from the *Digenæ* of Simpson to the *Tetragenæ*. But in view of the remarkable dimorphism of the species, which is apparently a sexual and not a senile charcter as has been suggested by Ortmann, the subordination of the genus to *Quadrula* as proposed by him would seem to be inexpedient.

TRITOGONIA TUBERCULATA (Barnes).

Quadrula tritogonia Ortmann, Naut., XXII, 1909, p. 101.
Quadrula parkeri Geiser, The Academician I, 1911, p. 15.

The new names proposed by Ortmann and Geiser can not be used, even if the species is referred to *Quadrula*. If *Rotundaria*, with *tuberculata* Raf. as its type, be given generic rank, Barnes' name can still be used in *Quadrula* (Ortmann, 78, p. 116); but if not, the species would take the name of *obesa* Simp. (Vanatta, Naut., XXIII, 1910, p. 102).

Obliquaria verrucosa Raf. is identified as this species by Conrad and the shells so labelled in the Poulson collection are also that species according to Vanatta (140, p. 554). If identifiable from the original description Rafinesque's name has priority.

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1. P: page, C: column, L: line, T: line from top, B: line from bottom, X: space.

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29	2	B 10	motacilla pro motaceila
33	2	B 8	1:39 pro 1:30
38	2	T 2	4:102 pro 4:103
39	2	B 27	submarginata pro subemarginata