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Title DESCRIPTION AND LIFE HISTORY OF CARDICOLA ALSEAE SP. N. (TREMATODA: SANGUINICOLIDAE)

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Cardicola alseae is a blood dwelling trematode found in the fishes Salmo clarki henshawi and Salmo gairdneri gairdneri. Eggs which were ovoid in shape and non-operculate left the adult and passed to the gill capillaries of the secondary lamellae. The miracidium was ovoid, 0.070 mm. long by 0.052 mm. wide, covered with long cilia, and internally had an eyespot composed of 40 to 50 melanin granules. The miracidium was encased in an egg capsule which enlarged prior to eruption and release of the larva. Sporocysts were found in the visceral mass of the snail Oxytrema silicula (Gould). No mother sporocyst generation was identified. Percentage of infection was low, with infected snails having sporocysts of equal size. Usually one to three adult cercariae were present in each sporocyst, along with germinal balls in many stages of development. Cercariae were of the lophocercous, furcocercous, brevifurcate, apharyngeate type with furcae possessing claws on the tips. A delicate dorsal keel

extended at least three-fourths the length of the body and reached its widest point at the dorsal bend of the body. If a cercaria touched the soft part of a potential host, it would attach, drop its tail, and penetrate within 15 to 20 minutes.

The adult fluke was removed from blood vessels of the gills, liver, mesenteries, and kidneys. It was covered with small spines, possessed a highly saccular testis, subterminal mouth, an H-shaped intestine, and lacked a pharynx. The fluke appeared to possess the characteristics of the genus Cardicola. C. alseae differs from the other two blood flukes found in salmonid fish in the morphology of the cercaria, size of the adult worm, number of rows of marginal spines, form of the testis and ovary, and shape of the excretory bladder and intestine.

DESCRIPTION AND LIFE HISTORY OF *CARDICOLA ALSEAE* SP. N. (TREMATODA: SANGUINICOLIDAE)

by

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DESCRIPTION AND LIFE HISTORY OF CARDICOLA ALSEAE SP. N. (TREMATODA: SANGUINICOLIDAE)

INTRODUCTION

The trematode Cardicola alseae sp. n. belongs to the family Sanguinicolidae Graff 1907. All known Sanguinicolidae are trematodes of fish and parasitize the blood circulatory system. Two lines of thought exist as to the proper classification of these fish blood flukes. Hyman (1951) recognized a single genus, Sanguinicola. Yamaguti (1953), following the reasoning of most students of the subject, placed fish blood flukes into seven genera. The systematics used in the following discussion will be those of Yamaguti.

Plehn (1905) described two species of Sanguinicola, S. inermis and S. armata. Both species were removed from the gills of cyprinid fish. The adults were about 1. 0 mm. in length, tapered, and non-spinous. The descriptions and illustrations given by Plehn (1908) are the ones encountered in most present day discussions of the subject. She did not report any studies on the life history of sanguinicolid blood parasites, and at that time, the adult fluke was thought to be a cestode. However, Odhner (1911) concluded that Sanguinicola was a much modified form of a suckerless trematode. Life histories have never been studied in detail, but the fundamental sequence was elucidated by Scheuring (1922) for S. inermis. The adult was located in

the bulbus arteriosus of the heart and in the large branchial vessel. Eggs were produced primarily in the May to November season with only one mature egg found in the ootype at any one time. The mature eggs were normally carried in the bloodstream to the capillaries of the gills, but also lodged in the heart musculature, liver, and kidneys. The eggs were triangular in shape and contained an embryo with a characteristic, deep black pigment spot and a penetration snout. The miracidium broke free from the gills at maturity, penetrated and developed in the water snails, Limnaea stagnalis, Bithynia leachi, Radix ovata, and Valvata piscinalis. The cercariae which developed were furcocercous. These cast aside their tails and penetrated the skin of exposed fish. Massive penetration of cercariae caused death to young fish, although the presence of adult worms generally caused no evident damage to the fish. The greatest damage came from eggs which occurred so numerously that the gill capillaries became plugged.

Woodland (1923) reported the presence of fish blood flukes in the Sudan from slides prepared during a research expedition into the area. His observations and interpretations were made entirely from fixed specimens, resulting in statements which ignited a long term scientific disagreement on morphological details with Odhner. This was the first account of sanguinicolid fish parasites from outside Germany. The first report in the United States came from Van Cleave and Mueller (1932) working with Stizostedion vitreum and Perca

flavescens, wall-eyed pike and yellow perch respectively, in Oneida Lake, New York. Their new species, Sanguinicola occidentalis, was always located in the heart, although the incidence of infection was never very high. Due to the minute size and scarcity of specimens, the description given was a composite of all the worms collected.

Fischthal (1949) made a survey of the parasites of largemouth and smallmouth bass Huro salmoides and Micropterus dolomieu dolomieu collected in lakes throughout Wisconsin. He found a light infection of a sanguinicolid which he called Sanguinicola huronis. It was present in the mesenteric vessels and was the second species described in the United States. As in several other cases, supply of adult trematodes was limited, resulting in a description taken from five specimens. Davis (1953) reported the presence of a blood dwelling fluke in the gills of cutthroat trout in hatcheries in Oregon and California. Most of his notes were fragmentary and did not give descriptions with enough detail to apply to any particular species except perhaps for the blood fluke later described by Wales (1958) as Sanguinicola davisi. It is possible that some of the flukes which Wales (1958) called S. davisi were not that, but other closely related species. Another new species, S. klamathensis Wales (1958), was discovered in the efferent renal vein of rainbow and cutthroat trout. This species was more elongate and spindle-shaped than S. davisi and obviously a new species. No experimental studies on the life history were made.

At that time a lophocercous furcocercous cercaria was likewise found in Flumenicola seminalis Hinds. However, efforts to demonstrate experimentally its association with the adult fluke were unsuccessful. Recently this relationship was established by me during experiments carried out concurrent with the blood fluke study under discussion.

Erickson and Wallace (1959) described a new species, Sanguinicola lophophora, along with experimental studies on the adult, sporocyst, and cercaria. Experimental infection of fish was eventually successful by cercariae which were obtained only from natural infections, and no attempts were made to infect snails experimentally. Sporocysts, but no rediae, were present. This work completed in Minnesota in 1959 was the latest description of a new species of fish blood fluke in the United States. Erickson and Wallace attempted to modify the key (McIntosh 1934) to the genus Sanguinicola to include the two new species. A distinguishing feature, that of folliculiform testes, used by Plehn et al., to separate the genus Sanguinicola from other genera was given considerable attention. This resulted in the inclusion of several genera under Sanguinicola which Odhner (1941) believed would more conveniently have fit into other groups.

The genera in the family Sanguinicolidae are more often parasites of marine fish than of fresh water species. No complete life history studies have been made of any marine species of the family but it is possible that in some instances, the intermediate hosts are

members of the phylum Annelida and not Mollusca. The newer genera of the Sanguinicolidae have been described from marine fish. McIntosh (1934) during routine examinations of Seriola lalandi described the genus Paradeontacylix. Short (1954) described the genus Selachohemecus from the sharp-nosed shark Scoliodon terra-novae and the genus Cardicola (Short 1953) from the white trout, Cynoscion arenarius, and the speckled trout, Cynoscion nebulosus.

Few thorough studies of sanguinicolid worms have been made. This is due perhaps to the scarcity of infected fish, the smallness of the adult worm, and its unusual location in the definitive host.

MATERIALS AND METHODS

The intermediate host, Oxytrema silicula, was collected from the Alsea River above the dam furnishing water to the Alsea Fish Hatchery, Benton County, Oregon. The snails were collected individually and by use of a bottom scraper during the early spring and summer of 1963 and 1964. More than 4000 snails were obtained in this manner. The isolated snails were placed into a cold room (12.8° C) where spontaneous shedding of cercariae occurred. Percentage of infection with the cercariae described below did not exceed one percent of snails collected. No other lophocercous furcocercous cercariae nor any cercariae of Sanguinicola davisi were shed.

When O. silicula was broken from the shell, the sporocysts fell freely from the visceral mass. The sporocysts and cercariae were examined either unstained or by use of neutral red. When cercariae were examined unstained, one drop of sterile human serum was added to one drop of water containing the larvae according to the method of Archibald and Marshall (1931). Presence of serum sometimes stimulated activity of flame cells making determination of a flame cell formula less difficult. Cercariae shed spontaneously were exposed to both young cutthroat trout fingerlings (Salmo clarki henshawi) and steelhead rainbow trout fingerlings (Salmo gairdneri gairdneri) where penetration could be observed to occur on the soft ventral surface.

Penetrations attempted in other areas were not successful. Under high power of the dissection scope and with very bright illumination cercariae could be observed under the outer skin.

Fish used in experimental study were obtained from the Klamath Hatchery as young fingerlings averaging one and one-half inches in length. Extensive collecting failed to show the infection to be present there. Both control fish and those to be used experimentally were taken from the same tanks. Controls were maintained in well aerated aquaria and were killed and thoroughly examined for presence of adult flukes and developing miracidia. During the experiment, 102 controls were examined in the above manner and no evidence of infection was found.

Eggs and miracidia were obtained from naturally infected cut-throat trout fingerlings taken from the Alsea Hatchery. These were examined by placing small pieces of gill into 0. 7 percent saline and observing with the compound microscope. Heavily infected gills were flaccid and possessed a pale color indicating the possible presence of miracidia even before microscopic examination. At 10X magnification all stages from the egg to the matured miracidium could usually be found when a heavy infection existed. Emergence of miracidia was stimulated by placing the gills into warm saline (40° C). The emerged larvae were collected and transferred to dishes containing young Oxytrema silicula taken from Mary's River, Benton County, Oregon.

The adult worm was often difficult to locate. The gills, liver, and other soft organs were placed into physiological saline and carefully teased apart. The worms would usually emerge into the fluid. The adult was small and rather transparent and could be seen with the dissection scope at 30X and with a partially darkened field. Study of internal morphology of the adult is very difficult at best, but live specimens are far superior to stained ones. The majority of observations recorded in this study were made from live specimens. Adults collected were fixed in AFA, stained with Semichon acetocarmine, cleared in oil of clove, and mounted in balsam.

All measurements are in millimeters.

LIFE CYCLE DATA

Fish used in experimental study were placed in small glass aquaria with four or five infected snails. These fish were frequently dead within a few hours presumably from large numbers of attempted penetrations. A total of 40 fish were exposed to infected snails during 1963 and 1964. Of that number 21 were maintained for more than four weeks, six for eight weeks, and three for ten weeks. Fish were killed when they began to appear sluggish. Of the 21 fish examined after four weeks, nine were parasitized. From one to five young flukes were removed from the gills, liver, mesenteric vessels, and kidneys of the infected fish. The largest fluke recovered at that time was 0. 5 in length. The most obvious structure was the testis which varied in shape depending on the contractions of the worm. Two of the three fish maintained for ten weeks were infected; one contained an adult 1. 0 in length. Testis and vitellaria were apparent. No evidence of an ovary or egg production could be seen.

When Oxytrema silicula were exposed to miracidia in a small amount of water, they would swim about erratically for a time and eventually either die or gain entrance to the snail presumably by being eaten rather than by penetration. Freshly introduced miracidia could be found in the mucosa and lumen of the intestinal tract. Two months following exposure sporocysts measuring 0. 2 long by 0. 2 wide

and containing germinal balls were found in the visceral mass. Snails collected but not exposed to miracidia were free from infection.

EGG AND MIRACIDIUM

(Figures 2-3)

The round to ovoid eggs with no apparent operculum averaged 0. 020 long by 0. 015 wide. The cytoplasm was clear with scattered vacuoles throughout. The yolk granules were confined largely to the periphery. In very young eggs the vacuoles were small, when present at all, and as the eggs matured the size of the vacuoles increased. No eggs were found in the main gill capillaries of the trout fingerlings, but were located in the secondary lamellae. Eggs in tandem were common. Eggs were not found in the heart, spleen, liver, and kidney tissue. Various stages of development from the uncleaved egg to the matured miracidium were often present in the same fish.

The miracidium measured 0. 070 long by 0. 052 wide and was encased in a double layered shell of uneven thickness. The larvae which fitted firmly inside the shell possessed long cilia uniformly except on the small terebratorium. A dark, prominent eyespot was present between the midline and lateral surface of the body. This eyespot was composed of 40 to 50 individual melanin granules clustered together to make it the most obvious feature of the miracidium. Conspicuous ridges were present both laterally and dorsally. Emergence of the miracidium was preceded by its increased activity and

by enlargement of the shell. Rupture of the shell resulted in release of the larva which swam free of the gills in a spiral and erratic manner. Larvae did not appear attracted to Oxytrema silicula, but continued to swim in their erratic fashion. Contact with the snail seemed to be by accident. None was seen to bore into the snail. Snails exposed to miracidia for some hours were dissected and miracidia were found in the digestive tract either in the lumen or in the mucosa.

In water the miracidia swam for up to three hours during which period they became less active. They moved toward the source of a beam of light and upon reaching the edge of the container, collected there, but some immediately swam in the opposite direction. Soon, however, most of them swam about, crossing the beam a few times before again moving with it toward the light source to repeat the swimming pattern.

SPOROCYST

(Figure 4)

Sporocysts were located in the stream prosobranch snail, Oxytrema silicula. Usually from 100 to 200 sporocysts were present in the visceral mass of an infected snail. Nearly all those found appeared to be in a similar stage of development. Few small sporocysts were located and those which were present were of a shape similar to mature ones. The average sporocyst measured 0.6 long by 0.45 wide. Germinal balls were scattered throughout the thin-walled sac and cercariae in various stages of development were present. Normally one or two mature cercariae were in each cyst. No birth pore was seen nor were there any indications of a pharynx and gut.

CERCARIA

(Figure 5)

The cercaria was of the lophocercous, furcocercous, brevifurcate, apharyngeate type. The total length was 0. 40; the body was 0. 15 long by 0. 07 wide. An apical papilla with six rows of dark spines was a most obvious structure. The entire body was covered with light colored, delicate spines. The opening of the mouth was subterminal and easily seen. However, other portions of the digestive tract were obscured by the granular penetration glands. In immature cercariae of various stages of development in the sporocysts the esophagus was found to be a very slender tube which terminated in a small sac-like caecum in the anterior one-fourth of the body. Ten large penetration glands filled the anterior of the body back to the region of the excretory bladder. The posterior enlarged portion was often triangular in shape and the entire cell was granular in appearance. No genital primordium could be seen. The excretory bladder was V-shaped, thin-walled, and highly contractile. Due to the dense and massive penetration glands, determination of a flame cell formula was exceedingly difficult. By use of sterile human serum and developing cercariae, a formula, $2[(2 + 2)]$, was derived. The number of flame cells is accurate, but the exact tube connections could

not be shown with absolute certainty. No flame cells were present in the tail.

A very delicate dorsal keel extended at least three-fourths the length of the body from the posterior bend to about the level of the anterior constriction. The keel possessed small folds along its length giving the impression of individual long hairs and resembled the undulating membrane of a trypanosome. Apparently the keel was not attached on the midline, but rather extended somewhat to each side. During embryonic development of the cercaria, this was the last major structure to develop. It began as three individual outpouchings from the dorsal surface of the body. These extended and eventually fused to give the dorsal fold seen in the mature larva.

The tail was 0. 25 long and 0. 03 wide with furcae 0. 05 long. At the tips of the furcae were claw-like projections which resembled small arrow heads. One highly coiled duct extended from the excretory bladder to its point of branching at the base of the furcae. Opening of the ducts was at the furcal tips. The tail was normally contracted resulting in small folds along the tail length. This contraction caused the excretory duct to appear so highly folded that it could easily be mistaken for a double structure. A very delicate membranous fold appeared along both edges of the furcae from the claw-tips to the points of attachment of the forks to the main tail stem.

In the laboratory Oxytrema silicula rarely shed more than 30

cercariae each day. Shedding occurred mainly in the morning at 12.8° C and in darkness. The emerged cercariae seldom lived longer than a few hours at room temperature but could be maintained for as long as two days at 12.8° C. Freshly emerged cercariae were used for direct measurements under light coverslip pressure or were heat killed with boiling 0.5 percent formalin. Measurements taken in both cases were almost identical.

Behavior of the cercariae was distinctive. Freshly emerged larvae tended to fold so the tip of the body was between the furcae of the tail. A sudden stretching of the larvae and rapid lashing of the tail would be followed by the folded position. The cercariae would remain suspended in the water for several hours by swimming, after which they would sink to the bottom of the glass fingerbowls.

ADULT

(Figure 1)

The adult fluke possessed a very delicate body more tapered posteriorly than anteriorly. The apical papilla was small with six rows of short spines. Along the margins to the level of the ootype were five very prominent rows of spines. In very young worms these spines resembled small hairs. Spines tended to be directed posteriorly and probably functioned to hold the adult fluke in place in the circulatory system. With exception of the marginal rows of spines, those over the remainder of the body were not easily seen in fixed specimens.

A small subterminal mouth below the apical papilla led into a very narrow esophagus which extended posteriad to the anterior limits of the testis. An H-shaped gut was present with slender branches extending both anteriorly and posteriorly. The limits of the caeca could not be determined. This structure was much more easily seen in living specimens. Ingested red blood cells or other particulate matter were not seen in any specimens collected.

The ovary was often indistinct and was clearly visible in only two of the specimens collected. The mature ovary lay posterior to the testis and occupied about one-fifth of the total length of the worm.

In some specimens two tubes from the ovary joined to form a thin-walled collapsible tube with a single loop which then passed to the ootype.

One of the most prominent structures in the adult fluke was the ootype which usually contained several ovoid eggs. The ootype lay slightly posterolaterad to the uterus and to the male gonopore. No Mehlis gland was seen. The uterus was relatively short, thin-walled and passed from the ootype to the female gonopore.

A common vitelline duct was present and particularly conspicuous in the area of the ootype. This thin-walled tube passed up the midline of the body dorsal to the vas deferens and branched anterior to the testis. In the live specimen yolk cells could be seen moving back and forth in the structure. These cells were not of a uniform shape and therefore could be distinguished from mature eggs. The yolk cells could be followed from the anterior part of the testis to the ootype. Limits of the vitellaria were not distinct, but were mainly in four areas. Two groups of vitellaria were posterolaterad extending from the gonopores to the ovary and two groups from the ovary anterior to the intestinal caeca on each side.

The testis was very prominent and consisted of a highly saccular structure extending from the intestinal caeca posteriad in the midline of the body to the ovary. It occupied about two-fifths of the total length. The shape of the testis varied in live flukes from a sac with

many folds to one with very few folds. Fixing of the specimens caused the testis to appear more folded and granular than in living forms. The vas deferens could be followed from the testis to the seminal vesicle. This very obvious structure possessed a prominent bend somewhat anterior to the seminal vesicle and male gonopore. The seminal vesicle was a somewhat expanded extension of the vas deferens and appeared to be encased in a very delicate sheath. The male gonopore was posterolaterad to the female opening. The male and female gonopores did not occupy a common atrium.

The excretory vesicle was V-shaped and thin-walled. Two slender tubules were present and each passed anteriad along the lateral portions of the body. The tubules could be followed for only a short distance.

Neither a nerve ring nor lateral nerve cords could be seen even in living specimens because exceptionally heavy vitellaria concentrations obscured them.

Mean values for five adult worms with minima and maxima in parenthesis. Adult, length 1.119(0.919-1.414); width 0.29(0.24-0.40). Ootype, length 0.035(0.024-0.044); width 0.031(0.024-0.036). Testis, length 0.38(0.30-0.46); width 0.17(0.15-0.20). Ovary, length 0.17 (0.09-0.27); width 0.18(0.15-0.25).

DISCUSSION

Blood dwelling trematodes of the type described are quite different from those trematodes which inhabit areas such as the gastrointestinal tract. Normally the cercariae of trematodes are very delicate and fragile larvae which succumb easily. Following penetration into the second intermediate host or as the case may be, direct entry into the definitive host, these larvae mature into the next stage while undergoing significant changes. In the species that penetrate into a second intermediate host and form a metacercaria, an entire period of reorganization and change takes place. The larval features are lost. The excretory system becomes modified and often a large bladder filled with cells and excretory wastes is the most prominent feature of a metacercaria. However, in sanguinicolid blood parasites a different and less noticeable change occurs. The delicate, typical, fork-tailed cercaria penetrates the definitive host directly. During this penetration the tail is dropped but the cercarial body does not become greatly modified. No heavy cuticle is laid down and encystment does not occur. A certain amount of elongation and widening takes place. The small, delicate spines on the cercarial body enlarge, but the rows of spines on the apical papilla change very little. The cercarial penetration glands atrophy and the genital primordium develops further producing the reproductive systems. In the adult,

the typical larval fragility is retained. This is evident in many ways. For example, handling of mature adults required precautions not normally needed with other adult trematodes. The adult body would usually become damaged beyond use if transfer was made with a brush with bristles as soft as sable hair. Since the massive yolk glands take up stain rapidly and tend to mask the other structures, most of the studies required living specimens. Removal of the adult trematode from its confinement in the blood circulatory system was a change of environment which the fluke was unable to tolerate for an extended period of time. In 0. 7 percent physiological saline the trematode survived for a short time. After removal from the circulatory system the worm invariably rolled up on itself and could not be flattened. Use of even light coverslip pressure would cause rupture.

Adult blood flukes differ from other types of trematodes in their location in the definitive host, the lack of a cuticle of any significance, the complete absence of suckers, and the delicacy and complexity of their internal structures. The absence of a cuticle and suckers, presence of a small gut, and the inability to withstand extended periods of exposure are examples of the tendency toward retention in the adult blood fluke of those characteristics normally confined to larval trematodes.

Blood inhabiting trematodes of fresh water fish have all been

placed into a single genus Sanguinicola. McIntosh (1934) constructed a key to this genus which was expanded by Erickson and Wallace (1959) to include the species Sanguinicola davisi and Sanguinicola klamathensis. Although several fresh water species might fit more naturally into other genera, little effort has been made to put them there. Recently, S. davisi and S. klamathensis have been placed in the genus Cardicola by some authors, but most other workers retain them in the genus Sanguinicola. The major characteristic used at the present time for genus designation for the Sanguinicolidae is the number of follicles comprising the testes. The question then arises as to what can be considered a permanent lobe or follicle. Unless the specimen is studied in the living state, this lobing cannot be justifiably used as a distinguishing characteristic. Change in the number and size of the lobes of the testis of Cardicola alseae could be readily seen when the the live fluke was undergoing contractions. Folliculiform testes could be used as an important taxonomic characteristic if the testes were in separate and distinct follicles. This is usually not the case, making distinction between a follicle and a mere fold almost impossible. It appears questionable whether the entire group of sanguinicolids actually possesses more than one testis.

Due to a lack of extensive experimental studies with fish blood flukes, little effort has been made to compare age of these worms with degree of lobulation of the testis. Flukes removed from fish after

four weeks of development did not possess a testis which was as highly folded as those removed after ten weeks of infection. This is a rather common occurrence in trematodes, and found to be the case in another experimental study by the author with the eye fluke of birds Philophthalmus megalura (unpublished data). It seems reasonable that lobulation of testis is a characteristic in the Sanguinicolidae to be used with caution, and not the basic separating characteristic for genera.

Eggs of sanguinicolid trematodes are of two shapes, triangular and oval (Table 1). This feature is not so obscure as is the testes lobulation. Unfortunately, in a number of species eggs have never been located or recorded. In at least one instance some confusion in egg shape has been noted. Wales (1958) described S. klamathensis eggs as being spherical rather than oval. However, adult S. klamathensis produced experimentally in our laboratory possessed eggs in the ootype which were essentially the same as for Cardicola alseae. It appears that S. klamathensis eggs should be considered oval rather than spherical.

The majority of fish blood flukes possess a mouth located in the terminal position (Table 1). Members of the genus Cardicola have subterminal mouths while the majority of the genus Sanguinicola are terminal. However, if one was to place S. davisi and S. klamathensis into genus Cardicola, all members of Sanguinicola would have mouths terminal in position. Location of the mouth opening is a reasonably

satisfactory characteristic. Likewise, gut shape has proven to be a useful characteristic in taxonomy of the Sanguinicolidae. Three basic shapes occur; X-shaped, H-shaped, and single sac-like gut.

Occasionally in the literature special significance is given to the number of eggs present in the ootype. One poorly known species, Sanguinicola volgensis Rasin, was described with this as one of the essential characteristics. S. klamathensis and C. alseae usually have several eggs in the ootype. Little of taxonomic value can be assigned to this characteristic at the present time due to the incomplete knowledge for the majority of species.

Several fish blood trematodes are specific in their location in the definitive host including primarily S. inermis, S. armata, S. intermedia, S. chalmersi, S. occidentalis, and S. huronis found in the heart and gills. On the other hand, S. lophophora, S. argentinensis, and C. alseae are less specific and occur in the gills, heart, spleen, and liver. S. argentinensis has even been located in the coeloms of infected fish (Table 1). S. klamathensis has previously been reported in the efferent renal vein. However, during experimental study in our laboratory only two completely mature worms were in this vessel. Three partially grown flukes were in the vessels of the gills and liver. Due to the size of the S. klamathensis adult, it seems reasonable to assume that the efferent renal vein is the only vessel in a fingerling trout large enough to readily accommodate the mature

worm.

Important taxonomic characteristics in the family Sanguinicolidae include egg shape, body spination, gut shape, mouth position, and the number of eggs in the ootype. Degree of testes lobulation is of questionable value.

Present day trends in trematode classification have been to establish relationships on the basis of life history studies. The cercarial morphology here is of particular importance. Unfortunately, little in the way of experimental investigation has been conducted in the Sanguinicolidae. Getting cercariae to penetrate and mature in the vertebrate host has been accomplished only after considerable difficulty (Scheuring 1922) (Erickson and Wallace 1959). Once the conditions necessary for proper penetration were established in the present study, trematodes could be grown to mature adults. The cercariae maintained a strict specificity for salmonid fish. Attempts to infect fish of the genus Gambusia were unsuccessful. Likewise, amphibians, birds, and mammals when exposed to freshly emerged cercariae failed to elicit any type of response. The more difficult of the two major parts of the life history was to obtain and hatch miracidia and to infect snails experimentally. Only Scheuring (1922) was able to get miracidia to invade and develop in snails. Snails were successfully infected in the life history studies of Cardicola alseae. Efforts to obtain fully grown cercariae were not successful because Oxytrema

silicula hosts died before the infection was mature. However, the young sporocysts which were grown possessed the same characteristics as sporocysts found in nature. These latter were described in a previous section. Control snails maintained during this experiment were free from infection. The most obscure part of the snail experiment was the method by which the miracidium gained entrance into the exposed snail. Through careful observation of the miracidium and snail, it was determined that penetration was not through any part of the snail exposed to the exterior. The miracidia were seen disappearing into the mouth, but the exact mechanism of attraction could not be determined. It appeared that the snails did not ingest the miracidia. Some chemical attraction could possibly have induced them to enter the mouth. Miracidia could be found within a few hours in the intestinal lumen and in the mucosa of the intestinal epithelium. The miracidia at that time appeared more rounded than when free swimming, but the cilia, eyespot, and terebratorium were very prominent.

According to the present method of classifying the family Sanguinicolidae, Cardicola alseae differs from species of genus Sanguinicola as described by Plehn (1905) in several significant ways. She defined Sanguinicola as worms with numerous folliculiform testes in two irregular longitudinal rows, presence of a fusiform swelling in the esophagus, lack of heavy spination on the lateral margins of the body, presence in the uterus of only one egg with lateral projections,

and an X-shaped intestine. Characteristics of genus Paradeontacylix McIntosh (1934) which would distinguish it from C. alseae include a slender body of uniform diameter, rose-thorn shaped spines on the posterior end, and testes in two irregular longitudinal rows. The common middorsal genital pore in Selachohemecus Short (1954) is absent in C. alseae. Presence of a long sinuous esophagus, vitellaria around intestinal rami and over the periphery of the testes, and prominent uterus filling up the posterior third of the body separate Deontacylix Linton (1910).

In several ways the present genus resembles Psettarium Goto and Ozaki (1930). The presence "of a conical dorsal projection near posterior right margin and a distinct marginal notch in front of it" are obvious differences. Further differences include extensive diffuse testes and a ramified or two-winged ovary.

Except for a few very minor differences such as the length of uterus, restriction of the ovary to a median position, and highly folded testis, C. alseae is very similar to the genus Cardicola Short (1953). Considering the similarities it seems reasonable that C. alseae should be placed in the genus Cardicola. However, this present form is a parasite of fresh water and anadromous fish.

Two species of blood flukes have been described in salmonid fish in the northwest, Sanguinicola davisi Wales (1958) and Sanguinicola klamathensis Wales (1958). It seems on the basis of the descriptions

that both should be transferred to the genus Cardicola. Both worms differ considerably from Cardicola alseae in the morphology of the adult and in life cycle. S. davisi reaching a length of 0.85 is probably too small to be the same species. The X-shaped intestine, presence of a pharynx, and gourd-shaped ootype are major differences. The cercaria of S. davisi does not possess a dorsal keel and spine-tipped furcae according to Wales (1958).

S. klamathensis differs in several ways from C. alseae. The cercaria described by Wales (1958) as belonging to S. klamathensis is more than twice the length of the one for C. alseae. Snails we collected at the Klamath Hatchery shed large quantities of the cercariae described by Wales (1958). The shape and extent of the penetration glands, length and height of the dorsal keel, and flame cell formula are different from C. alseae. The presence of eggs of S. klamathensis in the main gill capillaries rather than in the secondary lamellae of the trout fingerlings is another difference. The larger size of the adult fluke, the location in the definitive host, number of rows of spines on the marginal surface, and simple non-bifurcate intestine are major characteristics of S. klamathensis adults which separate them from adults of C. alseae.

C. alseae differs from the other species of Cardicola in that the five outer rows of spines are much more prominent than those on the remainder of the body. Further differences include folded form of

the testis, small Y-shaped excretory bladder, and short uterus. The cercariae differ from those described by Wales (1958) for S. davisi which lack a keel and claws on the tips of the furcae and from S. klamathensis Wales (1958) which is much larger, has a shorter keel, smaller penetration glands, and a different flame cell formula.

Table 1. Species Comparison of Genus Sanguinicola

Fresh Water Sanguinicolidae.	Max. Body Length mm.	Pairs of Testes	Ootype Ratio	Egg Shape	Order Definitive Host	Location in Definitive Host	No. Caecal Lobes	Position Mouth
S. armata Plehn, 1905	1.5	10	1/7	triang.	Cypriniformes	heart, gills	5	terminal
S. inermis Plehn, 1905	1.0	15	1/7	"	"	"	4	"
S. chalmersi Odhner 1924	1.228	6-7	1/14	?	"	"	not lobed	"
S. intermedia Ejsmont 1926	1.0	10	1/7	triang.	"	"	4-5	"
S. occidentalis VanCleave and Mueller, 1932	1.3	15	1/11	?	Perciformes	"	4	"
S. huronis Fischthal 1949	.845	?	1/26	?	"	"	4	"
S. argentinensis Szidat 1951	1.7	Many	1/10	oval	Cypriniformes	Circ. system and coelom	4	"
S. davisi Wales 1958	0.85	Massive Irregular	1/11	oval	Clupeiformes	gills, heart	4	sub-term.
S. klamathensis Wales 1958	3.15	Saccular Irregular	1/8	oval	Clupeiformes	efferent renal vein	not lobed	sub-term.
S. lophophora Erickson and Wallace 1959	.525	17-18	1/10	?	Cypriniformes	gills, heart spleen, liver	4	terminal
Cardicola alseae	1.414	Saccular Irregular	1/12	oval	Clupeiformes	"	4	sub-term.

ootype ratio = ratio distance of ootype to posterior end of body to total body length

SUMMARY

Cardicola alseae is a blood dwelling trematode found in the fishes Salmo clarki henshawi and Salmo gairdneri gairdneri. Eggs which were ovoid in shape and non-operculate left the adult and passed to the gill capillaries of the secondary lamellae. The miracidium was ovoid, 0.070 mm. long by 0.052 mm. wide, covered with long cilia, and internally had an eyespot composed of 40 to 50 melanin granules. The miracidium was encased in an egg capsule which enlarged prior to eruption and release of the larva. Sporocysts were found in the visceral mass of the snail Oxytrema silicula (Gould). No mother sporocyst generation was identified. Percentage of infection was low, with infected snails having sporocysts of equal size. Usually one to three adult cercariae were present in each sporocyst, along with germinal balls in many stages of development. Cercariae were of the lophocercous, furcocercous, brevifurcate, apharyngeate type with furcae possessing claws on the tips. A delicate dorsal keel extended at least three-fourths the length of the body and reached its widest point at the dorsal bend of the body. If a cercaria touched the soft part of a potential host, it would attach, drop its tail, and penetrate within 15 to 20 minutes.

The adult fluke was removed from blood vessels of the gills, liver, mesenteries, and kidneys. It was covered with small spines,

possessed a highly saccular testis, subterminal mouth, an H-shaped intestine, and lacked a pharynx. The fluke appeared to possess the characteristics of the genus Cardicola. C. alseae differs from the other two blood flukes found in salmonid fish in the morphology of the cercaria, size of the adult worm, number of rows of marginal spines, form of the testis and ovary, and shape of the excretory bladder and intestine.

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PLATE

Figures 1 to 5.

Figure 1. Adult, ventral view.

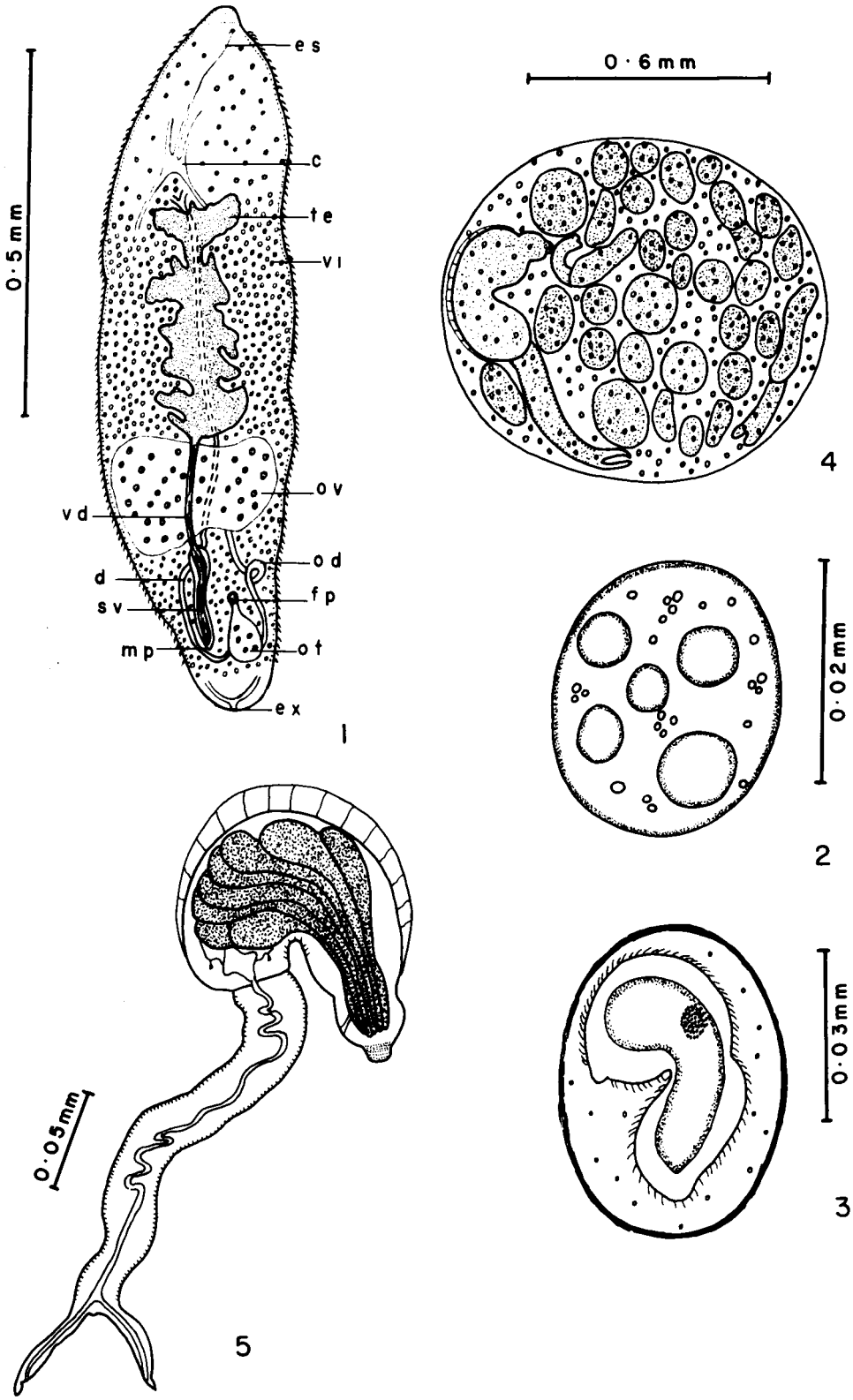
Abbreviations. C-intestinal caecum; D-common vitelline duct; ES-esophagus; EX-excretory vesicle; FP-female genital pore; MP-male genital pore; OD-oviduct; OT-ootype; OV-ovary; SV-seminal vesicle; TE-testis; VD-vas deferens; VI-vitellaria.

Figure 2. Egg.

Figure 3. Miracidium about to emerge from egg case.

Figure 4. Mature sporocyst.

Figure 5. Cercaria.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