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Biological studies on Cryptobia atraria SP.N. (Kinetoplastida: Cryptobiidae) in fishes from the Sevier River drainage, Utah

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BIOLOGICAL STUDIES ON *CRYPTOBIA ATRARIA* SP. N. (KINETOPLASTIDA: CRYPTOBIIDAE) IN FISHES FROM THE SEVIER RIVER DRAINAGE, UTAH

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INTRODUCTION

Fish culture for both food and sport utilization has been greatly increasing throughout the world. The editor of Fish Farming Industries (1973) predicted an increase by 1977 in the United States of 83% for catfish producers, 49% for trout farmers, and 91% for bait dealers. Concomitant with the renewed interest in fish culture has been a corresponding need to further understand fish diseases.

In the United States, *Cryptobia*, a biflagellated protozoan recorded in the blood of freshwater fishes, has caused significant mortality in rainbow trout *Salmo gairdneri* (Wales and Wolf, 1955) and king salmon *Oncorhynchus tshawytscha* (Becker and Katz, 1966) under hatchery conditions. This parasite has also been described from the blood of a marine fish (Strout, 1965).

Cryptobia has been reported in a population of cyprinid fishes in the Sevier River drainage in the central part of Utah (McDaniel, 1970). The objectives of this research were to determine a) the natural hosts, b) the vector, c) the prevalence within the Sevier River drainage near Richfield, Utah, and d) the taxonomic status of the parasite.

LITERATURE REVIEW

The genus *Cryptobia* was first proposed by Leidy (1846) for biflagellated protozoans occurring as parasites in the seminal vesicles of snails. Chalachnikow (1888) was the first to record the parasite in the blood of fishes, observing it in freshwater loaches in the U.S.S.R. Laveran and Mesnil (1901) established the genus *Trypanoplasma* for a biflagellated blood parasite from freshwater fishes in France. In 1909, Crawley states that *Cryptobia* from snails and *Trypanoplasma* from fishes were morphologically identical, and that *Cryptobia* had taxonomic priority. In defending the creation of the genus *Trypanoplasma*, Laveran and Mesnil (1912) argued that morphological similarities were not sufficient criteria for maintaining a single genus when strong biological differences, such as method of transfer, were evident. The parasites in snails were transferred directly during copulation while a leech vector was necessary to transfer the flagellate from the blood of one fish to another. Putz (1970) submitted that comparative biological studies between similar morphological types are necessary for a correct taxonomic classification. Use of the genus *Cryptobia* has, in most cases, emerged as the popular choice, and *Trypanoplasma* is generally recognized as a synonym in current works.

Four blood forms of *Cryptobia* have been reported in North America. Mavor (1915) first reported the parasite in a moribund white sucker, *Catostomus commersoni*, from Lake Huron. The flagellate was identified as *T. borreli* because of similarities with the species described by Laveran and Mesnil (1901). Katz (1951) recorded C. salmositica from the blood of silver salmon, O. kisutch, and C. lynchi from cottids in the state of Washington. Subsequent transmission studies showed C. lynchi to be a synonym of C. salmositica (Becker and Katz, 1965a). Laird (1961) described C. gurneyorum from the northern pike, Esox lucius, and from two salmonids, Coregonus clupeaformis and Salvelinus namaycush.

Another new species, C. cataractae, was described by Putz (1972a) from several cyprinid species in West Virginia. This record also included the first comprehensive study of a Cryptobia species encompassing comparative morphology, mode of transmission, natural and experimental hosts, in vivo and in vitro culture, histopathology, and cryopreservation. These criteria, along with an extensive comparison with C. salmositica from the West Coast, were used in the designation of C. cataractae as a valid species.

An ectoparasitic relationship of Cryptobia on goldfish maintained in aquaria was recorded by Swezy in 1919. In 1931 Wenrich also observed the external presence of the flagellate on the gills of carp in Pennsylvania.

The involvement of a vector in the transmission of Cryptobia was postulated by Mavor in 1915 when he stated, "the parasite without doubt is carried by a leech." In 1951 Katz observed developmental stages of Cryptobia from the gut of the leech Piscicola salmositica, thereby indicating this as the vector for C. salmositica. Subsequent experiments showed conclusively that the salmonid leech did function as a vector in the transfer of C. salmositica from fish to fish

(Becker and Katz, 1965a). In 1972b. Putz also showed a leech, *Cystobranchus virginicus*, to be the vector for *C. cataractae*.

Organisms of the genus *Cryptobia* have been reported as parasites in marine and freshwater fishes, salamanders, frogs, heteropods, planarians, siphonophores, chaetognaths, leeches, mole crickets, lizards, snails, and also as free living forms (Noble, 1968).

An undescribed species of *Cryptobia* was reported by McDaniel in 1970 from the blood of the Utah chub *Gila atraria* near Richfield, Utah.

MATERIALS AND METHODS

Geographical Area of Study

The collection site, located approximately three miles east of Richfield, Utah, was subdivided into three major areas (Fig. 1): the main Sevier River, northern spring ponds (Area 1), and southern spring ponds (Area 2). The ponds were located east of the Sevier River at the base of Bull Claim Hill. The springs were extremely rocky and contained large amounts of watercress and other aquatic plants. The river proper was always heavily silted and due to extensive use for irrigation it was almost dry during the summer. Fish were also examined from source waters of a producing rainbow trout hatchery in the northern spring area and from seven stations on the Sevier River south of the principal study area.

Field Collection and Examination of Fish

Fish were collected using fine mesh dip nets and a fifteen-foot minnow seine. A total of 181 fish encompassing five families and ten species were examined for the presence of *Cryptobia* using the "kidney strike" technique (Putz, 1970). This consisted of making a ventral incision in the fish and lifting the viscera forward and out of the body cavity. Hank's balanced salt solution was introduced into the body cavity and the kidney was punctured with a dissecting needle several times. After five minutes a drop of the solution was removed, placed on a glass slide and examined microscopically at a magnification

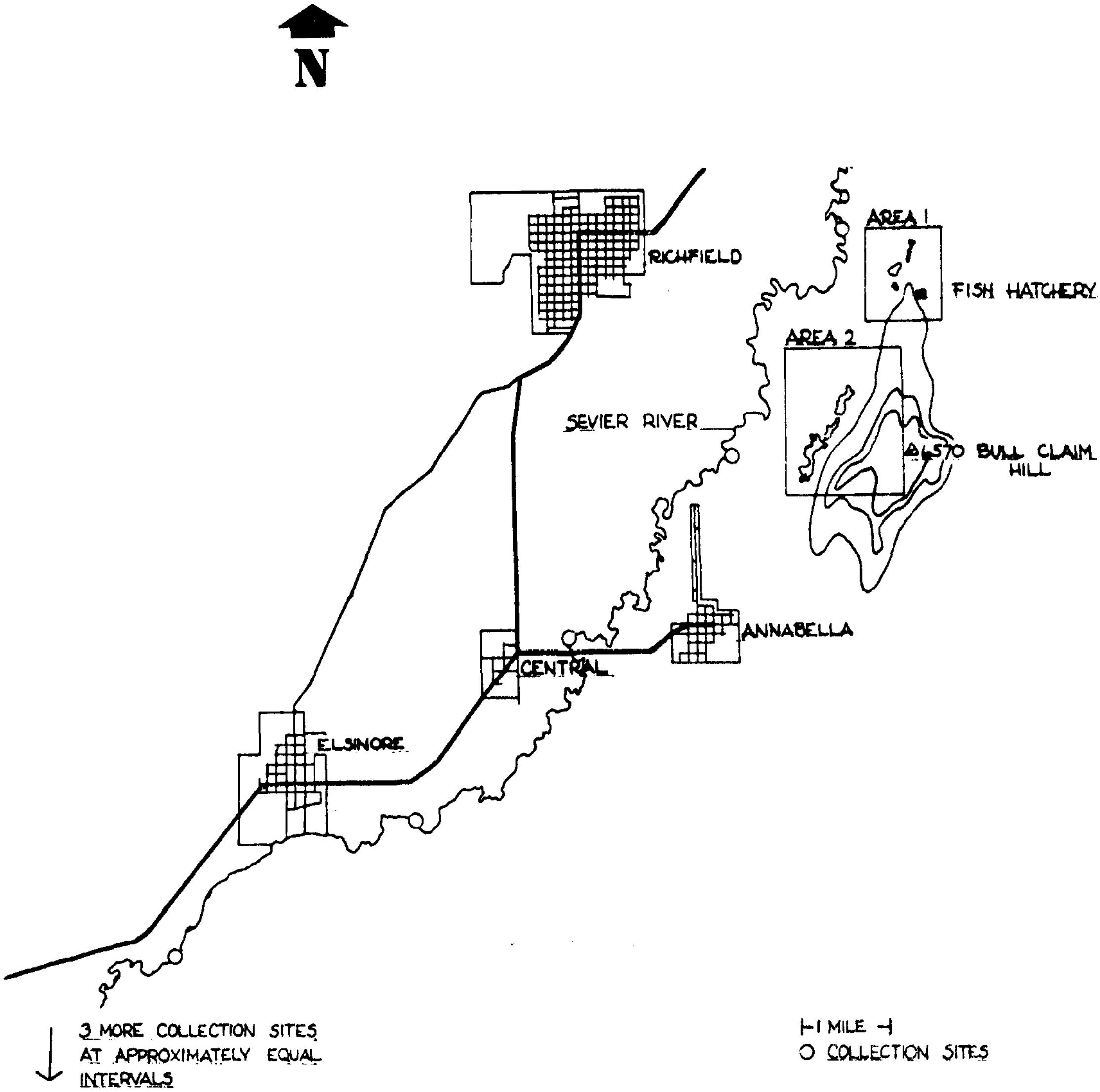

Figure 1. Map of the study area located near Richfield, Utah, showing the collection sites on the Sevier River and location of the spring ponds (Areas 1 and 2) near Bull Claim Hill.

of 100X. The presence of *Cryptobia* was detected by the characteristic whiplike motions of the protozoans. Observation of stained specimens at higher magnification confirmed the presence of the blood flagellate.

Collection and Identification of Leeches

Ectoparasitic leeches of fishes were collected from the undersides of rocks in the spring areas. These were identified to species using descriptions given by Hoffman (1967). Specimens were also sent to Dr. Roy W. Sawyer, Biology Department, College of Charleston, South Carolina, for confirmation. Leeches were maintained in the laboratory at 4°C in covered paper cups, in which they could be kept in good condition for up to three months.

Mounting and Staining Organisms

Blood from infected fishes was obtained from the caudal peduncle. A thin smear was prepared on a glass slide, air dried, and then fixed and stained with Giemsa as described by Humason (1967).

Preparation of stained smears from leeches was accomplished by mortaring them in a small amount of Hank's balanced salt solution. A smear was made from the solution and then stained following the same procedure used for the fish blood.

Photographs were taken with a Zeiss II photomicroscope of both the fish and leech stages of the parasite. Living *Cryptobia* were observed in wet mounts from the infected fish and the mortared leech solution to determine behavioral characteristics.

Morphometrics

Stained slides were examined at a magnification of 1000X. Measurements were recorded for anterior and posterior flagellar lengths, body length and width, kinetoplast length, and width of the nucleus. Fifty organisms were measured and the averages compared with existing measurements of other described species of *Cryptobia*.

RESULTS

Natural Hosts

Examination of 181 fish at fifteen stations revealed the presence of *Cryptobia* in the following species: Utah chub, *Gila atraria*; redside shiner, *Richardsonius balteatus*; speckled dace, *Rhinichthys osculus*. Seven fish species were negative for the blood flagellate: Utah sucker, *Catostomus ardens*; black bullhead, *Ictaluras melas*; rainbow trout, *Salmo gairdneri*; brown trout, *Salmo trutta*; leatherside chub, *Gila copei*; and a single mottled sculpin, *Cottus bairdi* (Table 1). The rainbow trout, carp, and Utah suckers all came from the northern spring ponds (Area 1), while the leatherside chubs, brown trout, and sculpin were only present in the Sevier River. Utah chubs and speckled dace were abundant in the springs, but only two chubs and a single dace were sampled from the Sevier River. The two balck bullheads were seined from the southern spring ponds (Area 2). The only species abundant at all collection sites was the redside shiner.

Recorded natural hosts and vectors of described species of *Cryptobia* from North America are given in table 2.

Prevalence of Cryptobia in the Richfield, Utah, Area

All of the infections of *Cryptobia* were, with one exception, located in the two spring areas along Bull Claim Hill (Table 1). This one exception was a speckled dace collected at an area where one of the northern springs empties into the Sevier River.

Table 1. Results of the examination of fish for the presence of *Cryptobia* from a) the main Sevier River, b) northern spring ponds, and c) southern spring ponds east of Richfield, Utah

Area	Fish Species	Number Examined	Positive Infections	Percent Positive
Main Sevier River	*G. copei*	10	0	---
	G. atraria	2	0	---
	R. balteatus	28	0	---
	R. osculus	1	1	100
	C. bairdi	1	0	---
	S. trutta	2	0	---
Northern Spring Ponds	*G. atraria*	20	20	100
	R. balteatus	20	20	100
	R. osculus	20	20	100
	S. gairdneri	10	0	---
	C. carpio	10	0	---
	C. ardens	10	0	---
Southern Spring Ponds	*G. atraria*	20	6	30
	R. balteatus	20	0	---
	R. osculus	5	0	---
	I. melas	2	0	---
Totals-All Areas	*G. copei*	10	0	---
	G. atraria	42	26	62
	R. balteatus	68	20	29
	R. osculus	26	21	81
	S. gairdneri	10	0	---
	C. carpio	10	0	---
	C. ardens	10	0	---
	S. trutta	2	0	---
	I. melas	2	0	---
	C. bairdi	1	0	---

Table 2. Natural hosts, vectors, and references of *Cryptobia* from freshwater fishes of North America

Species	Vector	Natural Hosts	References
Cryptobia atraria	*P. salmositica*	*Gila atraria*, *Richardsonius balteatus*, *Rhinichthys osculus*	This study
C. cataractae	*C. virginicus*	*R. cataractae*, *R. atratulus*, *E. maxillingua*, *C. anomalum*	Putz, 1970, 1972 a,b
C. salmositica	*P. salmositica*	*O. kisutch*, *C. rhotheus*, *C. aleuticus*, *S. gairdneri*, *O. tshawytscha*, *S. trutta*, *S. gairdneri gairdneri*, *C. snyderi*, *O. keta*, *O. gorguscha*, *P. williamsoni*, *C. bairdi*, *C. gubsus*, *C. beldingi*, *C. perplexus*, *C. asper*, *R. cataractae*, *Gasterosteus aculeatus*	Katz, 1951; Wales and Wolf, 1955; Becker and Katz, 1965b, 1966; Putz 1972 a,b
C. gurneyorum	None given	*C. clupeaformis*, *S. namaycush*, *E. lucius*	Laird, 1961
C. borreli	None given	*C. commersonii*	Mavor, 1915

In the northern spring ponds (Area 1) all individuals of the three host species were infected. At the southern springs (Area 2) the parasite was present in only 30% of the Utah chubs and was absent in the dace and shiners (Table 1). Microscopic examination of kidney fluids from the northern springs revealed three to four flagellates per field at 100X. Examination of several fields at the same magnification was necessary to observe a single parasite in the southern springs indicating a much lower level of infection in that area.

Vector

The parasitic leech recovered in the study area was identified as *Piscicola salmositica*, a common ectoparasite of fish in freshwater streams of the west coast of the United States. Microscopic examination of the mortared leech preparation revealed the presence of several developmental stages of *Cryptobia* which were all morphologically different from the parasite stage in the fish (Fig. 2).

Piscicola salmositica was observed only from the northern spring ponds with the exception of the northernmost spring in the southern springs area. Extensive search of the remainder of the southern springs and the Sevier River showed no specimens of the leech. Prevalence of the leeches was high in the fall and continued until peak numbers were observed in the middle of February. By late March to July 1972, only a small number of leeches were observed.

Rainbow trout, carp, Utah sucker, and Utah chubs were hosts for *P. salmositica*. Leeches were never observed feeding on redside shiners or speckled dace. Incidence of feeding appeared to be highest during the winter months.

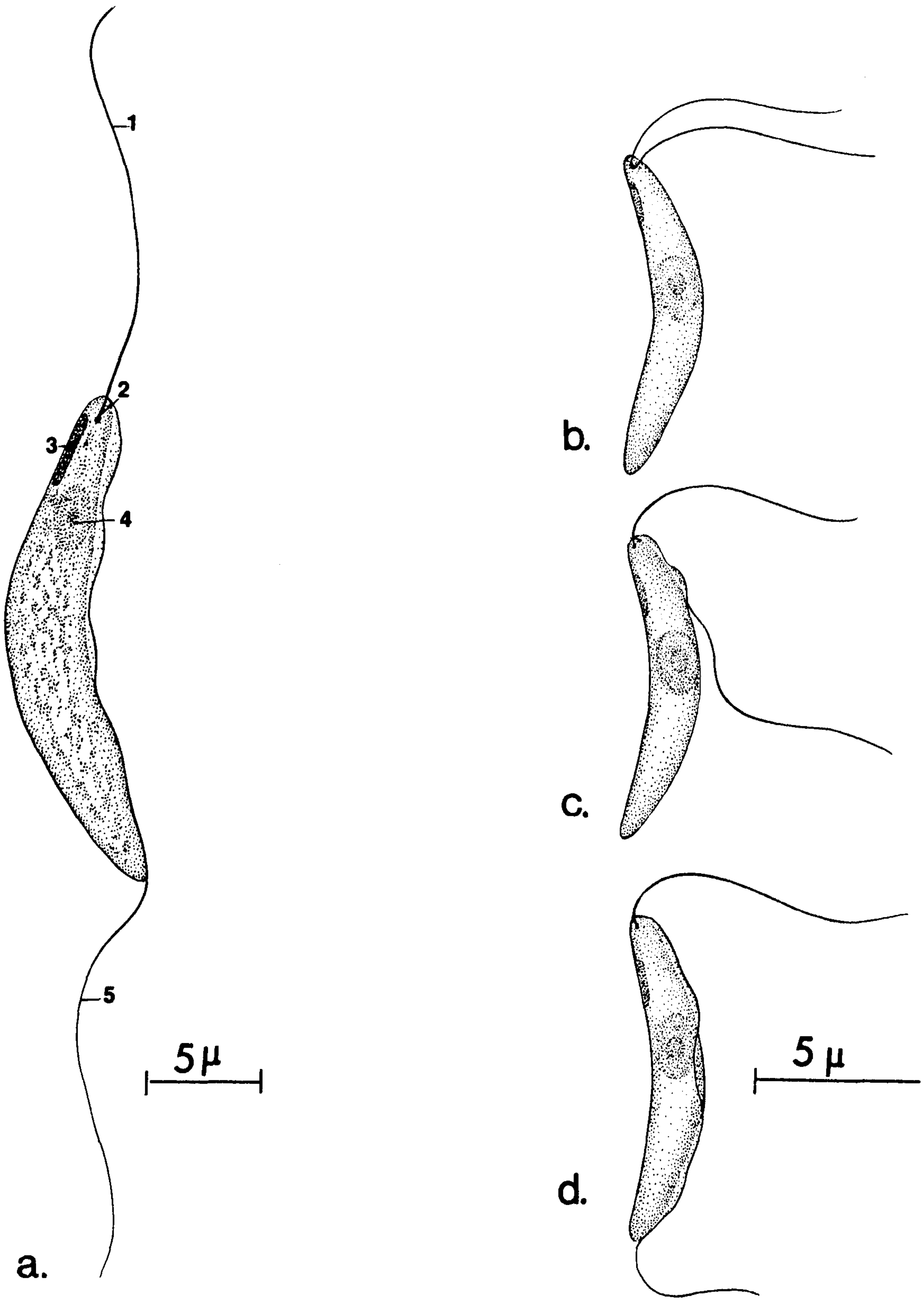

Figure 2. Morphological characteristics of Cryptobia atraria from fishes and a leech vector. a. Fish stage showing undulating membrane and: 1, anterior flagellum; 2, blepharoplast; 3, kinetoplast; 4, nucleus; 5, posterior flagellum. b. Leech stage with both flagella in anterior position. c. Leech stage with posterior migration of flagellum. d. Stage most commonly observed in leech with short posterior flagellum.

Morphometrics and Observed Behavior of *Cryptobia*

Average parameters in microns with ranges in parenthesis of fifty stained specimens of *Cryptobia* are as follows:

Body length: 30.5 (27-36)

Body width: 4.5 (3-7)

Length of anterior flagellum: 29.2 (23-34)

Length of posterior flagellum: 20.9 (15-24)

Nuclear width: 2.7 (2.0-3.5)

Kinetoplast length: 5.9 (4.5-7.0)

Morphometric comparison with other described species of *Cryptobia* from North America is shown in Table 3.

Cryptobia examined under phase microscopy revealed a high degree of polymorphism and constant whiplike undulatory movement. Stages in the leech maintained a quivering motion with much less distortion of body shape. The most prevalent stage visible in the leech had a very short posterior flagellum and was less than one half the size of that observed from the fish host (Fig. 2d, 5).

Table 3. Morphometric comparison of *Cryptobia atraria* with other described *Cryptobia* species from North American freshwater fishes (all measurements in microns)

Species	Body Length	Body Width	Length Ant. Flagella	Length Post. Flagella	Nuclear Width	Kinetoplast Length
Cryptobia atraria	30.5	4.5	29.1	20.9	2.0-3.5	5.9
C. cataractae	17	2	11	14	1.0-1.5	2.6-3.1
C. salmositica	14.94	2.46	16.05	8.96	1.5-3.5	4.58
C. gurneyorum	25.1	6.7	19	10	None given	None given
C. borreli	20-25	3-4	None given	None given	None given	None given

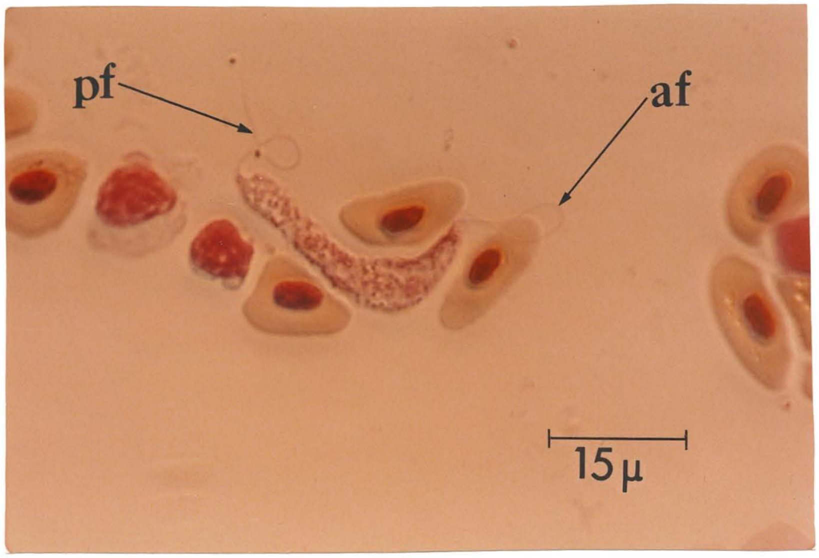

Figure 3. Cryptobia atraria from the blood of Utah chub, Gila atraria, showing anterior flagellum (af) and posterior flagellum (pf).

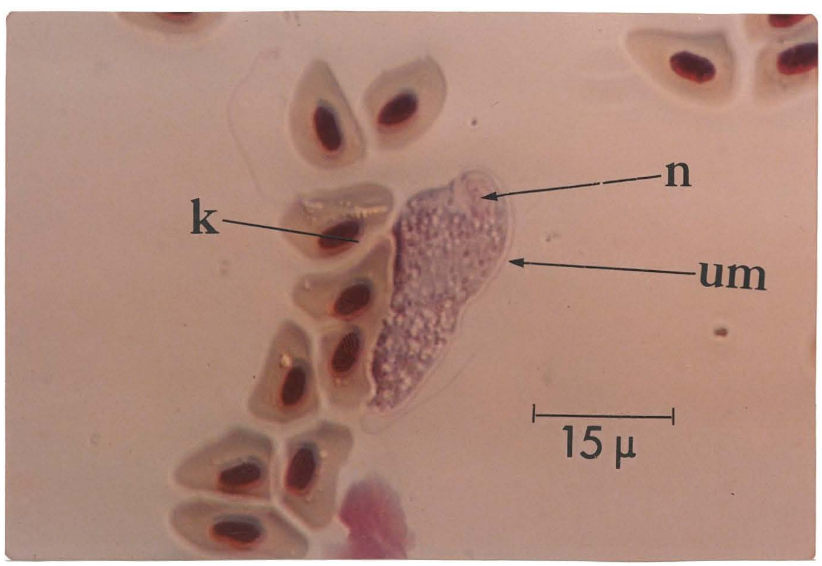

Figure 4. Cryptobia atraria from the blood of Utah chub showing kinetoplast (k), nucleus (n), and undulating membrane (um).

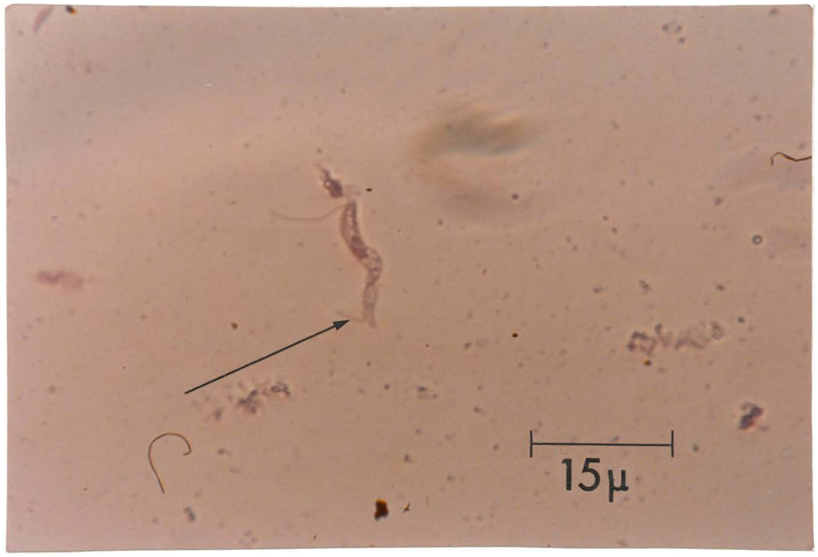

Figure 5. Cryptobia atraria from leech vector, Piscicola salmositica, showing common stage with short posterior flagellum (arrow).

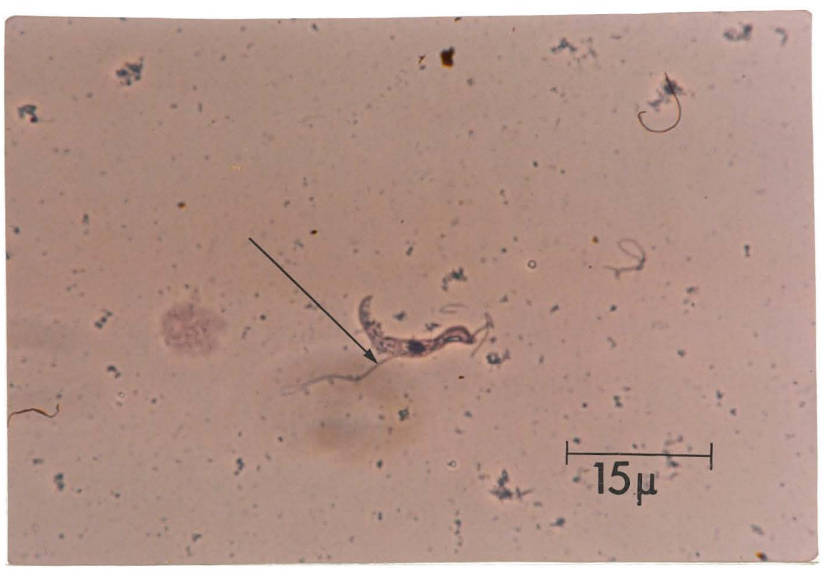

Figure 6. Cryptobia atraria from leech vector showing migration of posterior flagellum (arrow).

DISCUSSION

Published host records for *Cryptobia* in the blood of North American freshwater fishes show the presence of the parasite in 25 species (Putz, 1972a). *Cryptobia salmositica* is reported to parasitize 19 species of fishes, *C. cataractae* four, *C. gurneyorum* three, and *C. borreli* in only a single host species Table 2).

Results of this research show the presence of *Cryptobia* in three members of the family Cyprinidae: the Utah chub, the redside shiner, and the speckled dace, which are new host records for the hemoflagellate.

The only known vectors of *Cryptobia* are parasitic leeches. Two species have been demonstrated as vectors in North America: *Piscicola salmositica* as the vector of *C. salmositica* (Becker and Katz, 1965a), and *Cystobranchus virginicus* as the vector of *C. cataractae* (Putz, 1972a).

The salmonid leech, *Piscicola salmositica*, is probably the vector of the hemoflagellate in this study. No direct transmission experiments were conducted, but the leeches were observed parasitizing fishes at the collection sites and *Cryptobia* was observed in the gut of the leech. The protozoan appears to undergo developmental changes within the leech with the trailing flagellum migrating anterior to posterior forming the undulating membrane (Figs. 2, 5, 6). The size of the flagellate in the leech was about one-third to one-half that of the parasite in the fish host.

Becker and Katz (1965a) reported P. salmositica as endemic to the Pacific Coast of North America. Cope (1958) and Heckmann (1971) identified the presence of the salmonid leech on cutthroat trout in Yellowstone Lake, Wyoming. In 1960, Jones and Hammond reported P. salmositica at a rainbow trout hatchery in nothern Utah, establishing the range of the leech beyond the Pacific drainage. The finding of P. salmositica near Richfield, Utah, further expands the known range of the leech.

Piscicola salmositica was observed in large numbers only in the northern spring ponds. The northernmost spring in the southern springs area contained a small number of leeches. No leeches were observed at the remainder of the southern spring ponds or in the Sevier River. The northern and southern springs are connected indirectly by the Sevier River and it is possible that leeches could be transported on fish hosts. However, the distance involved, about two to three miles, would make this very unlikely. The absence of the leech in the Sevier River was probably due to the fluctuating water levels, temperature changes and the lack of a rocky substrate such as was present in the spring ponds. The salmonid leech prefers waters of low temperature and high dissolved oxygen in the presence of a graveled or rocky substrate (Becker and Katz, 1965c).

The occurrence of Cryptobia in the study area correlated with the occurrence of the leech vector. All members of the three host species in the northern springs were heavily parasitized by the hemoflagellate while only the Utah chub showed the presence of the parasite in the southern ponds. Samples from the Sevier River contained only one infected fish, a speckled dace, located at the point of entrance of a small stream from the northern springs.

Of equal importance to the finding of Cryptobia in three fish species is the absence of the parasite in the rainbow trout, Utah sucker, and carp. The ten fish examined from each of these species were collected from the northern spring ponds where incidence of infection with Cryptobia was 100% in the three hosts. Parasitism of P. salmositica on the rainbow, sucker, and carp was observed, indicating the fish were exposed to Cryptobia. The fact that the hemoflagellate was not present in rainbow trout, a reported host of C. salmositica, would strongly indicate that a different species is present.

Becker and Katz (1965b) advanced the concept that one distinct species of Cryptobia existed in a "geographically restricted area in association with one vector, regardless of the species of teleost in which the parasite may be found." Following this reasoning, all records of Cryptobia for the west coast drainages are recognized as C. salmositica with a common vector P. salmositica. Although the vector is the same in the Utah study, the absence of the Utah parasite in the rainbow trout together with the major differences in drainages and morphological characteristics of the protozoan, would justify a new species designation.

The author feels that sufficient evidence has been demonstrated for the description of a new species, Cryptobia atraria, in three cyprinids and a leech vector of Central Utah. Further surveys in the Utah area will be needed to determine the geographical limits of the parasite.

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J. Stephen Cranney

Department of Zoology

M.S. Degree, August 1974

ABSTRACT

A total of 181 fish, encompassing 10 species, was examined near Richfield, Utah, for the presence of *Cryptobia*, a biflagellated blood parasite. Utah chub (*Gila atraria*), redside shiner (*Richardsonius balteatus*), and speckled dace (*Rhinichthys osculus*) were found to be parasitized by this hemoflagellate, whereas rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), Utah sucker (*Catostomus ardens*), carp (*Cyprinus carpio*), leatherside chub (*Gila copei*), black bullhead (*Ictaluras melas*), and mottled scuplin (*Cottus bairdi*) did not harbor this parasite.

Developmental stages of *Cryptobia* were observed in the salmonid leech, *Piscicola salmositica*, thereby indicating a possible vector role for the organism. This leech is also the vector of *C. salmositica* in rainbow trout and other fishes from the west coast. The hemoflagellate studied herein was not detected in rainbow trout and stained specimens were nearly twice the size of *C. salmositica* suggesting that it should be considered a new species.

COMMITTEE APPROVAL:

VITA

J. Stephen Cranney