Great Basin Naturalist 56(2), © 1996, pp. 142–149

TRYPANOPLASMA ATRARIA SP. N. (KINETOPLASTIDA: BODONIDAE) IN FISHES FROM THE SEVIER RIVER DRAINAGE, UTAH

J. Stephen Cranney[1] and Richard A. Heckmann[2]

ABSTRACT.—A total of 181 fishes belonging to 10 species were captured near Richfield, Utah, and examined for parasites. A new species of hemoflagellate, *Trypanoplasma atraria* sp. n., was observed in 3 species: Utah chub (*Gila atraria* [Girard]), redside shiner (*Richardsonius balteatus* [Richardson]), and speckled dace (*Rhinichthys osculus* [Girard]). Seven other species of fishes examined in the study area were negative for *T. atraria* sp. n. The salmonid leech, *Piscicola salmositica* (Meyer), collected in the same area harbored developmental stages of *Trypanoplasma*, suggesting a possible leech vector for the hemoflagellate. Characteristics of *Trypanoplasma atraria* sp. n. place it near *T. salmositica*, but the new species is twice as large.

Key words: Trypanoplasma atraria *n. sp., blood parasites,* Gila atraria, *fish parasites.*

Trypanoplasma is a biflagellated protozoan found in the blood of freshwater fishes in the United States. It has caused significant mortality in rainbow trout (*Oncorhynchus mykiss* [Walbaum]) and king salmon (*O. tshawytscha* [Walbaum]) under hatchery conditions (Becker and Katz 1966, Wales and Wolf 1995). This genus has also been described from the blood of marine fish (Strout 1965). Another name for the blood biflagellate of salmonids described above is *Cryptobia*. There are differing opinions on the use of the two genera, *Cryptobia* and *Trypanoplasma*, but these differences have been recently clarified by Lom and Dykova (1992).

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 Russia. Laveran and Mesnil (1901) established the genus *Trypanoplasma* for a biflagellated blood parasite from freshwater fishes in France. In 1909, Crawley stated 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 infection, 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. Recently, Lom and Dykova (1992) used *Trypanoplasma* for biflagellated blood-inhibiting parasites of fishes in which a leech vector is involved. Thus, we adopted the classification scheme used by Lom and Dykova (1992).

Four species of *Trypanoplasma* from the blood of freshwater fishes have been reported in North America. Mavor (1915) found *T. borreli* in a moribund white sucker (*Catostomus commersoni* [Lacepe]) from Lake Huron. The identification of *T. borreli* was based on similarities with the species initially described by Laveran and Mesnil (1901). Katz (1951) recorded *C. (=Trypanoplasma) salmositica* from silver salmon (*O. kisutch* [Walbaum]) and *C. (=Trypanoplasma) 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. (=Trypanoplasma) gurneyorum* from northern pike (*Esox lucius* [Linnaeus]) and from 2 salmonids: lake whitefish (*Coregonus clupeaformis* [Mitchill]) and lake trout (*Salvelinus namaycush* [Walbaum]).

[1]Utah Division of Wildlife Resources, Duchesne, UT 84021.
[2]Department of Zoology, Brigham Young University, Provo, UT 84602.

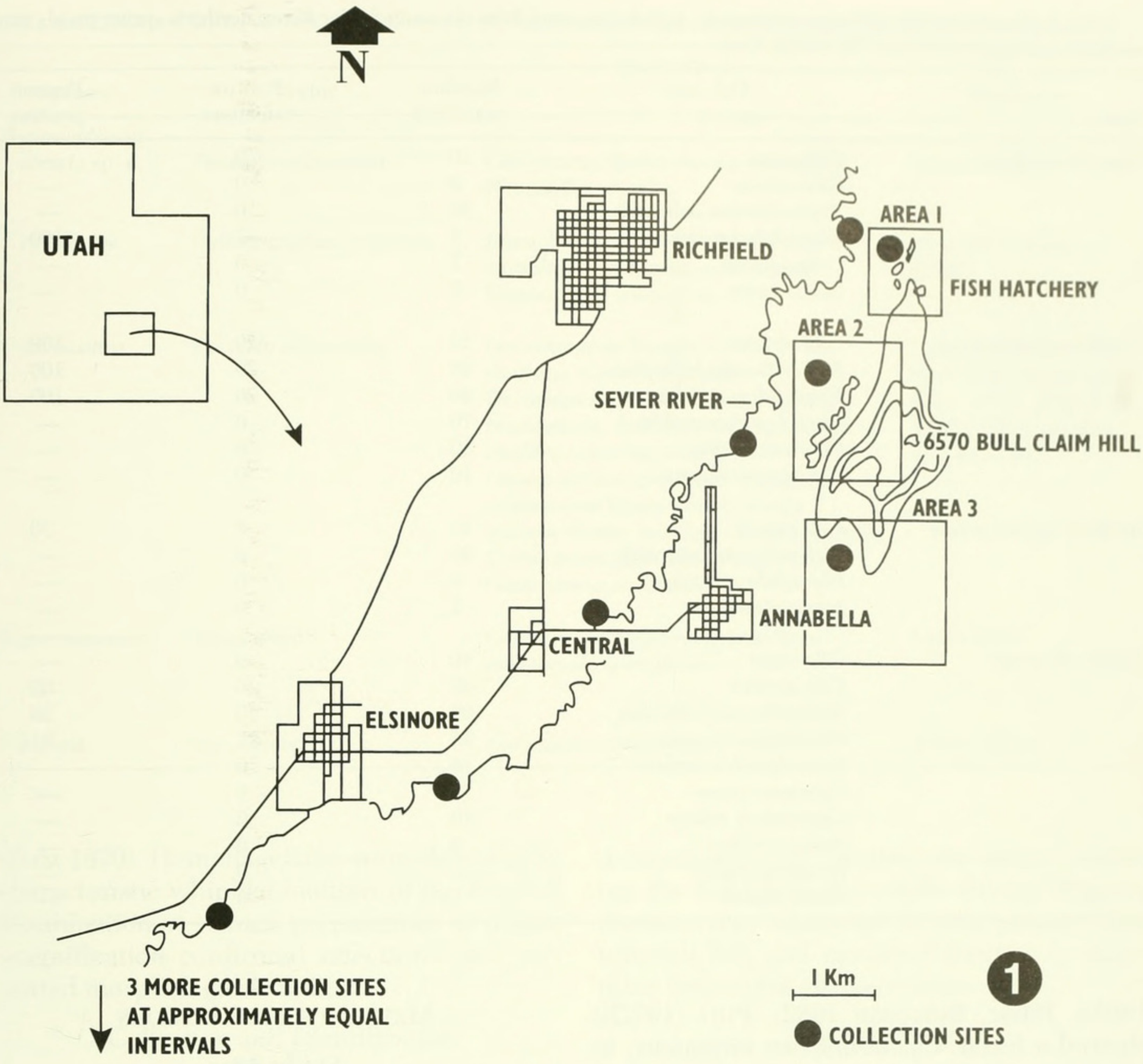

Fig. 1. Map of the study area near Richfield, Utah, showing collection sites on the Sevier River and location of the spring ponds (areas 1, 2, and 3) near Bull Claim Hill.

Another species, *C.* (=*Trypanoplasma*) *cataractae*, was described by Putz (1972a) from several cyprinids in West Virginia. This record also included the first comprehensive study of a *Cryptobia* (=*Trypanoplasma*) species that encompassed comparative morphology, mode of transmission, natural and experimental hosts, in viro and in vitro culture, histopathology, and cryopreservation. These criteria and extensive comparison with *T. salmositica* from the West Coast were used to justify designation of *T. cataractae* as a valid species.

An ectoparasitic relationship of *Trypanoplasma* on goldfish (*Carassius auratus* [Linnaeus]) maintained in aquaria was recorded by Swezy (1919). Wenrich (1931) also observed the presence of external flagellates on the gills of carp (*Cyprinus carpio* [Linnaeus]) in Pennsylvania. The use of the scientific name *Trypanoplasma* is accurate for these observations (Lom and Dykova 1992). Khan and Noble (1972) and Khan (1991) recently reported on another species of *Cryptobia*, *C. dahli*.

Involvement of a vector in transmission of *Cryptobia* (=*Trypanoplasma*) was postulated by Mavor (1915). Katz (1951) observed developmental stages of *Cryptobia* from the gut of the leech *Piscicola salmositica* and indicated it as a vector for *C. salmositica*. Subsequent experiments showed conclusively that the leech functioned as a vector in the transfer of *C. salmositica* from fish to fish (Becker and Katz

TABLE 1. Prevalence (%) of *Trypanoplasma* sp. in fish examined from the main Sevier River, northern spring ponds, and southern spring ponds east of Richfield, Utah.

Area	Fish species	Number examined	Positive infections	Percent positive
Main Sevier River	*Gila copei*	10	0	—
	Gila atraria	2	0	—
	Richardsonius balteatus	28	0	—
	Rhinichthys osculus	1	1	100
	Cottus bairdi	1	0	—
	Salmo trutta	2	0	—
Northern spring ponds	*Gila atraria*	20	20	100
	Richardsonius balteatus	20	20	100
	Rhinichthys osculus	20	20	100
	Oncorhynchus mykiss	10	0	—
	Cyprinus carpio	10	0	—
	Catostomus ardens	10	0	—
Southern spring ponds	*Gila atraria*	20	6	30
	Richardsonius balteatus	20	0	—
	Rhinichthys osculus	5	0	—
	Ameiurus melas	2	0	—
Totals—all areas	*Gila copei*	10	0	—
	Gila atraria	42	26	62
	Richardsonius balteatus	68	20	29
	Rhinichthys osculus	26	21	81
	Oncorhynchus mykiss	10	0	—
	Cyprinus carpio	10	0	—
	Catostomus ardens	10	0	—
	Salmo trutta	2	0	—
	Ameiurus melas	2	0	—
	Cottus bairdi	1	0	—

1965a, 1965c, Burreson 1982). Putz (1972b) showed a leech, *Cystobranchus virginicus*, to be a vector for *T. cataractae*.

Organisms of the genus *Cryptobia* and *Trypanoplasma* 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).

Woo and Wehnert (1983) and Bower and Margolis (1983) reported that *Trypanoplasma* and *Cryptobia* of many species of fish can be acquired directly via water and not only by leeches. Bower and Margolis (1984) and Woo (1987) also considered *Trypanoplasma* a synonym of *Cryptobia*, a view not helped by Becker and Katz (1966) or Lom (1979) prior to this time.

The species of *Trypanoplasma* described in this article was first observed by McDaniel in 1970 (personal communication) from Utah chub (*Gila atraria*) near Richfield, Utah. At that time it was considered a species of *Cryptobia*.

MATERIALS AND METHODS

Study Area

The primary collection site, located approximately 5 km east of Richfield, Utah, was subdivided into 3 major areas (Fig. 1): the main Sevier River (area 1), northern spring ponds (area 2), and southern spring ponds (area 3). The ponds are located east of the Sevier River at the base of Bull Claim Hill. The springs are rocky and contain dense stands of watercress and other aquatic plants. The river is heavily silted and almost dry during the summer. Fish were also examined from source waters of a fish hatchery in the northern spring area and from 7 stations on the Sevier River south of the principal study area to determine the local range of the hemoflagellate.

Collection and Examination of Fish

A total of 181 fish representing 5 families and 10 species were collected and examined for blood flagellates (*Trypanoplasma* and *Cryptobia*) using the "kidney strike" technique

TABLE 2. Natural hosts, vectors, and references of *Trypanoplasma* spp. from freshwater fishes of North America.

Species	Vector	Natural hosts (fish)	References
Trypanoplasma atraria sp. n.	*Piscicola salmositica*	*Gila atraria*, *Richardsonius balteatus*, *Rhinichthys osculus*	Present study
T. cataractae	*Cystobranchus virginicus*	*Rhinichthys cataractae*, *Rhinichthys stratulus*, *Exoglossum maxillingua*, *Campostoma anomalum*	Putz 1970, 1972a, 1972b
T. salmositica	*Piscicola salmositica*	*Oncorhynchus kisutch*, *Cottus rhotheus*, *Cottus aleuticus*, *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, *Salmo trutta*, *Catostomus snyderi*, *Oncorhynchus keta*, *Oncorhynchus gorbuscha*, *Prosopium williamsoni*, *Cottus bairdi*, *Cottus gulosus*, *Cottus beldingi*, *Cottus perplexus*, *Cottus asper*, *Rhinichthys cataractae*, *Gasterosteus aculeatus*	Katz 1951, Wales and Wolf 1995, Becker and Katz 1965b, 1966, Putz 1972a, 1972b, Becker and Katz 1977
T. gureneyorum	None given	*Coregonus clupeaformis*, *Salvelinus namaycush*, *Esox lucius*	Laird 1961
T. borreli	None given	*Catostomus commersoni*	Mavor 1915

(Putz 1970). Hemoflagellates were detected by characteristic whiplike motions of the flagella. Examination of stained preparations at higher magnification confirmed infections and permitted morphological studies.

Collection and Identification of Leeches

Ectoparasitic leeches of fishes were collected from the underside of rocks in the 2 spring areas and identified using Hoffman (1967). Specimens were confirmed by Dr. Roy W. Sawyer, Biology Department, College of Charleston, South Carolina. Leeches were maintained in the laboratory at 4° C in covered paper cups, where they could be kept in good condition for up to 3 mon.

Mounting and Staining

Blood was obtained from the caudal peduncle of infected fishes. Samples of hempoetic tissue were also taken directly from the kidney ("kidney strike"). A thin smear was prepared on a glass slide, air-dried, fixed with methyl alcohol (100%), and stained with Giemsa (Humason 1967).

Stained smears from leeches were prepared by mortaring each leech in a small amount of Hank's balanced salt solution (Hoffman 1967). A smear from the solution was stained following the fish blood procedure. Living *Trypanoplasma* were observed in wet mounts from infected fish and mortared leeches to determine behavioral characteristics.

Morphometrics

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

RESULTS

Natural Hosts

Examination of 181 fish at 15 stations revealed *Trypanoplasma* in Utah chub (*Gila atraria*), redside shiner (*Richardsonius balteatus*), and speckled dace (*Rhinichthyes osculus*). Seven species (Table 1) appeared to be negative for the blood flagellate: Utah sucker (*Catostomus ardens* [Jordan and Gilbert]), black bullhead (*Ameiurus melas* [Rafinesque]), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta* [Linnaeus]), carp (*Cyprinus*

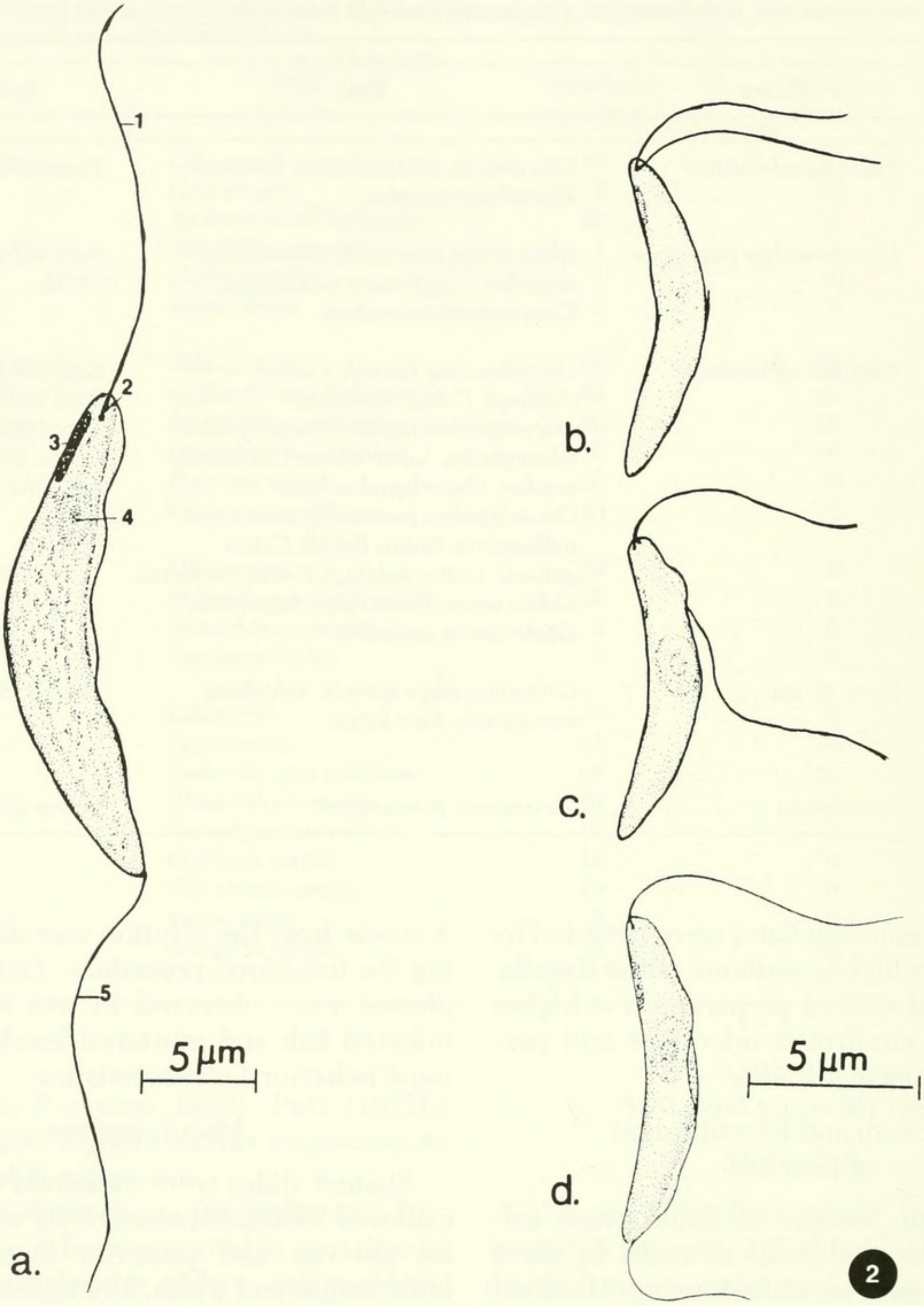

Fig. 2. *Trypanoplasma atraria* sp. n. from fishes (a) and a leech vector (b, c, d): 1, anterior flagellum; 2, blepharoplast; 3, kinetoplast; 4, nucleus; 5, posterior flagellum. (b) Both flagella in anterior position, (c) posterior migration of flagellum, (d) common stage in leech with short posterior flagellum.

capio), leatherside chub (*Gila copei* [Jordan and Gilbert]), and mottled sculpin (*Cottus bairdi* [Girard]). Rainbow trout, carp, and Utah sucker all came from the northern springs ponds (area 2), while the leatherside chub, brown trout, and mottled sculpin were only in the Sevier River. Utah chub and speckled dace were abundant in the springs, but only 2 chub and 1 speckled dace were collected from the Sevier River. The 2 black bullhead were from the southern spring ponds (area 3). Only redside shiner was abundant at all collection sites. Reported natural hosts and vectors of described species of *Trypanoplasma* and *Cryptobia* from North America are given in Table 2.

Prevalence of *Trypanoplasma* in the Richfield, Utah, Area

Fish infected with *Trypanoplasma* were, with 1 exception, obtained in the 2 spring areas along Bull Claim Hill (Table 1). One speckled dace was collected where 1 of the northern springs emptied into the Sevier River. In area 1, all individuals of the 3 host species were infected.

At area 2, the parasite was present in 30% of Utah chub and absent in speckled dace and redside shiner (Table 1). Microscopic examination of kidney fluids from northern spring fishes revealed 3–4 flagellates per field at 100X. For the southern springs, examination of several fields at the same magnification was necessary to locate a single parasite, 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 (Hoffman 1967). Microscopic examination of the mortared leech preparation revealed several developmental stages of *Trypanoplasma*, which were all morphologically different from the parasite stage in the fish (Fig. 2). This correlates with observations by other workers in the field (Lom and Dykova 1992).

Piscicola salmositica was observed from the northern springs ponds and the northernmost portion of the southern area. Extensive search of the remainder of the southern springs and Sevier River produced no additional specimens of the leech. Leech prevalence was high in autumn and continued until peak numbers were observed in the middle of February. By late March to July, only a small number of leeches were observed.

Rainbow trout, carp, Utah sucker, and Utah chub were hosts for *P. salmositica*. Leeches were never observed on redside shiner or speckled dace.

Description of *Trypanoplasma atraria* sp. n.
(Fig. 3)

Average parameters given in micrometers with ranges in parentheses of 50 stained specimens of *Trypanoplasma atraria* sp. n. 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 (2–3.5), kinetoplast length 5.9 (4.5–7). Type specimens including paratypes have been deposited (USNM Helminthological Collection Nos. 74436 and 74437), with additional paratypes in the junior author's collections. Morphometric comparisons with other described species of *Trypanoplasma* from North America are shown in Table 3.

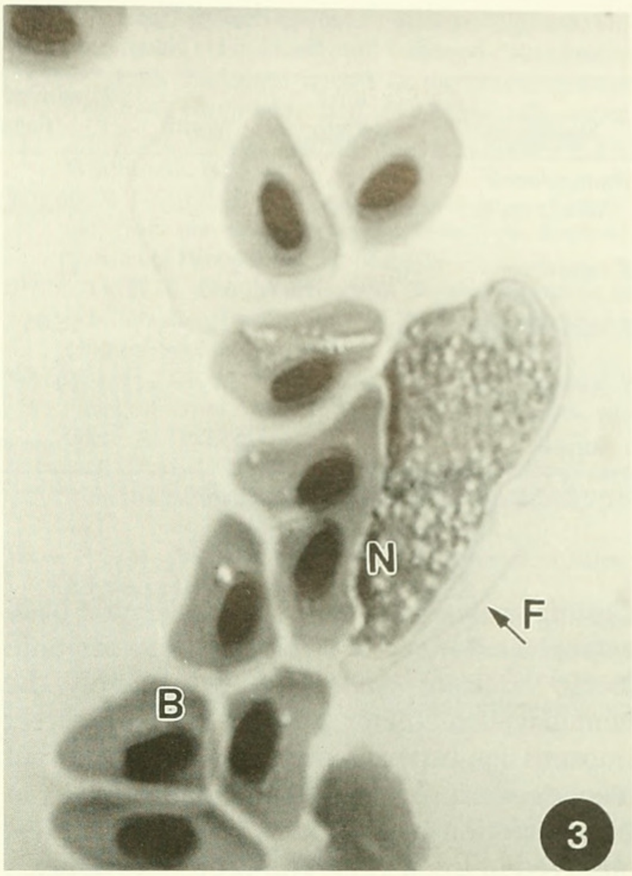

Fig. 3. *Trypanoplasma atraria* sp. n. Note erythrocyte (B), flagella (F), nucleus (N), and body of protozoan; 1000X magnification.

Trypanoplasma atraria sp. n. under phase microscopy revealed a high degree of polymorphism and constant whiplike undulatory movement. Stages in the leech exhibited a qivering motion with much less distortion of body shape. The most common stage visible in the leech had a short posterior flagellum and was less than 1/2 the overall size of that observed from the fish host (Fig. 2d).

DISCUSSION

Published host records for *Trypanoplasma* in North America include 25 species of freshwater fishes (Putz 1972a). *Trypanoplasma salmositica* is reported to parasitize 19 species, *T. cataractae* 4, *T. gurneyorum* 3, and *T. borreli* only a single host species. Results of this study showed *T. atraria* in 3 cyprinids: Utah chub, redside shiner, and speckled dace.

The only known vectors of *Trypanoplasma* are parasitic leeches. Two species have been demonstrated as vectors in North America: *Piscicola salmositica* as a vector of *T. salmositica* (see Becker and Katz 1965a) and

TABLE 3. Morphometric comparison of *Trypanoplasma atraria* sp. n. (ranges in parentheses) with other species of *Trypanoplasma*[a] described from the blood of North American freshwater fishes (all measurements in micrometers).

Species	Total length	Width	Length of anterior flagella	Length of posterior flagella	Nuclear width	Kinetoplast length
Tryanoplasma atraria sp. n.	30.5 (27–36)	4.5 (3–7)	29.1 (23–34)	20.9 (15–24)	2.7 (2.0–3.5)	5.9 (4.5–7.0)
T. cataractae	17	2	11	14	1.0–1.5	2.6–3.1
T. salmositica	14.94	2.46	16.05	8.96	1.5–3.5	4.58
T. gureneyorum	25.1	6.7	19	10	None given	None given
T. borreli	20–25	3–4	None given	None given	None given	None given

[a]There is a close relationship between the two blood flagellates: *Cryptobia* and *Trypanoplasma*. Species of *Trypanoplasma* are transmitted usually by a leech vector.

Cystobranchus virginicus as the vector of *T. cataractae* (see Putz 1972a). The salmonid leech, *Piscicola salmositica*, is probably the hemoflagellate vector in this study. No direct transmission experiments were conducted, but leeches were observed parasitizing fishes at the collection sites, and *Trypanoplasma* was observed in leech guts. The protozoan appears to undergo developmental changes within the leech with the trailing flagellum migrating anterior to posterior and forming the undulating membrane (Fig. 2). The size of the flagellate in the leech was about 1/3 to 1/2 that of the parasite in the fish host. Becker and Katz (165a) reported *P. salmositica* as endemic to the Pacific Coast of North America. Cope (1958) and Heckmann (1971) identified salmonid leeches from cutthroat trout in Yellowstone Lake. Direct transmission studies would clarify the role of the leech relative to fish infections with *T. atraria*.

LITERATURE CITED

BECKER, C. D., AND M. KATZ. 1977. Flagellate parasites of fish. Pages 357–416 *in* J. P. Kreier, editor, Parasitic protozoa. Volume 1. Academic Press, New York.

______. 1965a. Transmission of the flagellate *Cryptobia salmositica*, Katz, 1951, by a rhynchobdellid vector. Journal of Parasitology 51: 95–99.

______. 1965b. Infections of the hemoflagellate *Cryptobia salmositica* Katz, 1951, in freshwater teleosts of the Pacific Coast. Transactions of the American Fisheries Society 94: 327–333.

______. 1965c. Distribution, ecology, and biology of the salmonid leech, *Piscicola salmositica* (Rhynchobdellae: Piscicolidae). Journal of the Fisheries Research Board of Canada 22: 1175–1195.

______. 1966. Host relationships of *Cryptobia salmositica* (Protozoa: Mastigophora) in a western Washington hatchery stream. Transactions of the American Fisheries Society 95: 196–202.

BOWER, S. M., AND L. MARGOLIS. 1983. Direct transmission of the haemoflagellate *Cryptobia salmositica* among Pacific salmon (*Oncorhynchus* spp.). Canadian Journal of Zoology 61: 1242–1250.

______. 1984. Detection of infection and susceptibility of different Pacific salmon stocks (*Oncorhynchus* spp.) to the haemoflagellate *Cryptobia salmositica*. Journal of Parasitology 70: 273–278.

BURRESON, E. M. 1982. The life cycle of *Trypanoplasma bullocki* (Zoomastigophorea: Kinetoplastida). Journal of Protozoology 29: 72–77.

CHALACHNIKOW, A. P. 1888. Recherches sur les parasites du sang. Arkhives Veterinary Science, St. Petersburg 1: 65.

COPE, O. B. 1958. Incidence of external parasites on cutthroat trout in Yellowstone Lake. Proceedings of the Utah Academy of Science, Arts, and Letters 35: 95–100.

CRAWLEY, H. 1909. Studies on blood and blood parasites. II. The priority of *Cryptobia* Leidy, 1846, over *Trypanoplasma* Laveran and Mesnil, 1901. U.S. Department of Agriculture, Bulletin of the Bureau of Animal Industry 119: 16–20.

HECKMANN, R. A. 1971. Parasites of cutthroat trout from Yellowstone Lake, Wyoming. Progressive Fish Culturist 33: 103–106.

HOFFMAN, G. L. 1967. Parasites of North American freshwater fishes. University of California Press, Berkeley and Los Angeles. 486 pp.

HUMASON, G. L. 1967. Animal tissue techniques. Freeman and Company, San Francisco. 596 pp.

KATZ, M. 1951. Two new hemoflagellates (genus *Cryptobia*) from some western Washington teleosts. Journal of Parasitology 37: 245–250.

KHAN, R. A. 1991. Further observations on *Cryptobia dahli* (Mastigophorea: Kinetoplastida) parasitizing marine fish. Journal of Protozoology 38: 326–329.

KHAN, R. A., AND E. R. NOBLE. 1972. Taxonomy, prevalence, and specificity of *Cryptobia dahli* (Mobius) (Mastigophora: Bodonidae) in lumpfish, *Cyclopterus lumpus*. Journal of the Fisheries Research Board of Canada 29: 1291–1294.

LAIRD, M. 1961. Parasites from northern Canada. II. Haematozoa of fishes. Canadian Journal of Zoology 39: 541–548.

LAVERAN, A., AND F. MESNIL. 1901. Sur les flagelles à membrane ondulante des poissons (genres *Trypanosoma*

Fruby et *Trypanoplasma* n. gen.). Transactions of the French Academy of Science 133: 670–675.

______. 1912. Trypanosomes et Trypanosomiases. 2nd edition. Masson et Cie, Editeurs, Paris. 999 pp.

LEIDY, F. 1846. Descriptions of a new genus and species of Entozoa. Proceedings of the National Academy of Science, Philadelphia 3: 100–101.

LOM, J. 1979. Biology of the trypanosomes and trypanoplasms of fish. Pages 269–337 *in* W. H. R. Lumsden and D. A. Evans, editors, Biology of the Kinetoplastida. Volume 2. Academic Press, New York.

LOM, J., AND I. DYKOVA. 1992. Protozoan parasites of fishes. Developments in aquaculture and fisheries science, Volume 26. Elsevier Publishers, Amstersdam. 315 pp.

MAVOR, J. W. 1915. On the occurrence of a trypanoplasm, probably *Trypanoplasma borreli* Laveran et Mesnil, in the blood of the common sucker, *Catostomus commersonii*. Journal of Parasitology 2: 1–6.

MCDANIEL, D. W. 1970. Personal communication. U.S. Department of Interior. Fisheries. Springville, Utah.

NOBLE, E. R. 1968. The flagellate *Cryptobia* in two species of deep sea fishes from the eastern Pacific. Journal of Parasitology 54: 720–724.

PUTZ, R. E. 1970. Biological studies on the hemoflagellates (Kinetoplastida: Cryptobiidae) *Cryptobia cataractae* sp. n. and *Cryptobia salmositica* Katz, 1951. Unpublished doctoral dissertation, Fordham University, New York. 98 pp.

______. 1972a. *Cryptobia cataractae* sp.n. (Kinetoplastida: Cryptobiidae), a hemoflagellate of some cyprinid fishes of West Virginia. Proceedings of the Helminthological Society of Washington 39: 18–22.

______. 1972b. Biological studies on the hemoflagellates *Cryptobia cataractae* and *Cryptobia salmositica*. Technical Papers, Bureau of Sport Fisheries and Wildlife 63: 1–25.

STROUT, R. G. 1965. A new hemoflagellate (genus *Cryptobia*) from marine fishes of northern New England. Journal of Parasitology 51: 654–659.

SWEZY, O. 1919. The occurrence of *Trypanoplasma* as an ectoparasite. Transactions of the American Microscopical Society 38: 20–24.

WALES, J. H., AND H. WOLF. 1955. Three protozoan diseases of trout in California. California Fish and Game 41: 183–187.

WENRICH, D. H. 1931. A trypanoplasm on the gills of carp from the Schuylkill River. Journal of Parasitology 18: 133.

WOO, P. T. K. 1987. Cryptobia and cryptobiosis in fishes. Advances in Parasitology 26: 199–237.

WOO, P. T. K., AND WEHNERT, S. D. 1983. Direct transmission of a hemoflagellate, *Cryptobia salmositica* (Kinetoplastida: Bodonina), between rainbow trout under laboratory conditions. Journal of Protozoology 30: 334–337.

Received 1 September 1995
Accepted 19 January 1996