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Trypanosoma barbari, a new species from the newt Triturus torosus

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TRYPANASOMA BARBARI, A NEW SPECIES FROM THE NEWT TRITURUS TOROSUS

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TABLE OF CONTENTS

INTRODUCTION

While making studies of the parasites of fishes and amphibians at the Pacific Marine Station, Dillon Beach, California, during August, 1949, the writer encountered a hitherto unreported trypanasome from a female Triturus torosus. From the information obtained from the one smear, which was stained, the animal was presumed to be monomorphic. However, during February, 1950, a search was made expressly for the purpose of obtaining specimens of this Pacific Coast newt, and of the eight animals examined six were parasitized. Contrary to the primary observations, evidence was accumulated which proved beyond any doubt that the flagellate is polymorphic and that, evidently, it has not been reported or described. The name Trypanasoma barbari is proposed for this species.

I am indebted to Dr. Alden E. Noble, Director of the Pacific Marine Station, and to Miss Bertha Du Beau, Associate Professor of Bacteriology, College of the Pacific, whose suggestions and criticisms made the writing of this paper possible. I am also indebted to the staffs of the libraries of the California Academy of Sciences, and the Biology Library of the University of California.

Within the continental limits of the United States there are to be found numerous species of amphibians. The

state of California, alone, possesses some twenty species of urodeles and twenty-five species of salientia. Unfortunately, only a very few of the species of the American amphibians have been examined for trypanasomes (Table I), and the number of flagellates found, although not great, should provide sufficient stimulus for further work in the field (Table II). Four species of trypanasomes have been described as occuring in North American urodeles; however, the species from two of the genera have not been designated due to a lack of sufficient material.

Tobey (1906) described Trypanasoma diemyctyli from Triturus viridescens Rafinesque; Hegner (1921) observed a species of trypanasome in the lungless salamanders Plethodon cinerus Green and in Plethodon glutinosis Green; Hegner (1921), Roudabush and Coatney (1937) and Nigrelli (1945) reported a species of trypanasome from Necturus maculosus Rafinesque; and, finally, Cryptobranchus alleganiensis (Daudin) was reported by Roudabush and Coatney to be the host of a flagellate which was designated Trypanasoma cryptobranchi.

TABLE I

SPECIES OF AMERICAN AMPHIBIANS EXAMINED FOR TRYPANASOMES

ANIMAL	EXAMINER, YEAR, AND RESULT
Acris gryllus (Le Conte)	Nigrelli, 1944; T. grylii
Ambystoma maculatum (Shaw)	Nigrelli, 1945; negative
Ambystoma opacum (Gravenhorst)	Nigrelli, 1945; negative
Ambystoma tigrinum (Green)	Nigrelli, 1945; negative
Ambystoma tigrinum (Green)	Roudabush and Coatney, 1937; negative
Aneides flavipunctatus (Stauch)	Lehmann, 1950; negative
Batrachoseps a. attenuatus (Eschscholz)	Wood and Wood, 1936; negative
Batrachoseps a. attenuatus (Eschscholz)	Lehmann, 1950; negative
Batrachoseps pacificus major Camp	Wood and Wood, 1936; negative
Bufo americanus Holbrook	Fantham, Porter and Richardson, 1942; T. lavalia
Bufo americanus Holbrook	Fantham, Porter and Richardson, 1942; T. gaumontis
Bufo americanus Holbrook	Fantham, Porter and Richardson, 1942; T. montrealis
Bufo boreas halophilus Baird and Girard	Wood and Wood, 1936; negative
Bufo canorus Camp	Wood and Wood, 1936; negative

TABLE I (CONTINUED)

ANIMAL	EXAMINER, YEAR, AND RESULT
Bufo fowleri (Hinckley)	Brandt, 1936; T. rotatorium
Bufo woodhousii Girard	Roudabush and Coatney, 1937; negative
Cryptobranchus alleganiensis (Daudin)	Roudabush and Coatney, 1937; T. cryptobranchi
Desmognathus fuscus (Rafinesque)	Nigrelli, 1945; negative
Desmognathus fuscus (Rafinesque)	Hegner 1921; negative
Ensatina e. eschscholtzii (Gray)	Lehmann, 1950; negative
Hyla andersoni Baird	Nigrelli, 1945; Trypanasoma sp.
Hyla cinera (Schneider)	Nigrelli, 1945; negative
Hyla crucifer Wied	Brandt, 1936; T. rotatorium
Hyla crucifer Wied	Nigrelli, 1945; Trypanasoma sp.
Hyla femoralis Latreille	Nigrelli, 1945; negative
Hyla regilla Baird and Girard	Wood and Wood, 1936; negative
Hyla regilla Baird and Girard	Lehmann, 1950; negative
Hyla squirella Latreille	Nigrelli, 1945; negative
Hyla versicolor (Le Conte)	Nigrelli, 1945; Trypanasoma sp.

TABLE I (CONTINUED)

ANIMAL	EXAMINER, YEAR, AND RESULT
Necturus maculosus (Raf.)	Hegner, 1921; Trypanasoma sp.
Necturus maculosus (Raf.)	Roudabush and Coatney, 1937 Trypanasoma sp.
Necturus maculosus (Raf.)	Nigrelli, 1945; Trypanasoma sp.
Plethodon cinereus (Green)	Hegner, 1921; Trypanasoma sp.
Plethodon glutinosis (Green)	Hegner, 1921; Trypanasoma sp.
Pseudacris brimleyi (Brandt and Walker)	Brandt, 1936; T. rotatorium
Pseudotriton rubra (Latreille)	Nigrelli, 1945; negative
Rana boylii boylii Baird	Wood and Wood, 1936; negative
Rana aurora draytonii Baird and Girard	Wood and Wood, 1936; negative
Rana aurora draytonii Baird and Girard	Lehmann, 1950; negative
Rana catesbeiana Shaw	Brandt, 1936; T. rotatorium
Rana catesbeiana Shaw	Fantham et al, 1942; T. rotatorium
Rana catesbeiana Shaw	Nigrelli, 1945; T. rotatorium
Rana catesbeiana Shaw	Fantham et al, 1942; T. inopinatum
Rana catesbeiana Shaw	Hegner, 1920; Trypanasoma sp.
Rana clamitans Latreille	Stebbins, 1907; T. climatae

TABLE I (CONTINUED)

ANIMAL	EXAMINER, YEAR, AND RESULT
Rana clamitans Latreille	Kudo, 1922; T. parvum
Rana clamitans Latreille	Kudo, 1922; T. rotatorium
Rana clamitans Latreille	Fantham et al, 1942; T. rotatorium
Rana clamitans Latreille	Nigrelli 1945; T. rotatorium
Rana clamitans Latreille	Stebbins, 1907; Trypanasoma sp.
Rana clamitans Latreille	Hegner, 1920; Trypanasoma sp.
Rana palustris Le Conte	Nigrelli, 1945; negative
Rana pipens Schreber	Kudo, 1922; T. rotatorium
Rana pipens Schreber	Packchanian, 1934; T. rotatorium
Rana pipens Schreber	Fantham et al, 1942; T. rotatorium
Rana pipiens Schreber	Fantham et al, 1942; T. inopinatum
Rana pipiens Schreber	Nigrelli, 1945; Trypanasoma sp.
Rana sphenocephala (Cope)	Brandt, 1936; T. rotatorium
Rana sylvatica Le Conte	Fantham et al, 1942; negative
Rana sylvatica Le Conte	Nigrelli, 1945; negative
Rana virgatipes Cope	Nigrelli, 1945; negative

TABLE I (CONTINUED)

ANIMAL	EXAMINER, YEAR, AND RESULT
Scaphiopus holbrook (Harlan)	Brandt, 1936; negative
Spelerpes bislineatus (Green)	Hegner, 1921; negative
Triturus torosus Rathke	Lehmann, 1950; *T. barbari*
Triturus viridescens (Raf.)	Tobey, 1906; *T. diemyctyli*
Triturus viridescens (Raf.)	Hegner, 1921; *T. diemyctyli*
Triturus viridescens (Raf.)	Nigrelli, 1929; *T. diemyctyli*
Triturus viridescens (Raf.)	Nigrelli, 1929; *T. diemyctyli*

TABLE II

TRYPANASOMES FROM NORTH AMERICAN AMPHIBIANS

TRYPANASOME	HOST
T. barbari Lehmann (1950)	Triturus torosus Rathke
T. climatae Stebbins (1907)	Rana clamitans Latreille
T. cryptobranchi Roudabush and Coatney (1937)	Cryptobranchus alleganiensis (Daudin)
T. diemyctyli Tobey (1906)	Triturus viridescens (Raf.)
T. gaumontis Fantham, Porter, and Richardson (1942)	Bufo americanus Holbrook
T. grylli Nigrelli (1944)	Acris gryllus (Le Conte)
T. inopinatum Sergent and Sergent (1904)	Rana catesbeiana Shaw Rana pipiens Schreber
T. lavalia Fantham et al (1942)	Bufo americanus Holbrook
T. montrealis Fantham et al (1942)	Bufo americanus Holbrook
T. parvum Kudo (1922)	Rana clamitans Latreille
T. rotatorium (Mayer 1843)	Bufo fowleri (Hinckley) Hyla crucifer Wied Pseudacris brimleyi Brandt and Walker Rana catesbeiana Shaw Rana clamitans Latreille Rana pipiens Schreber Rana sphenocephala (Cope)

MATERIALS AND METHODS

Of the eight salamanders examined by the writer, six, all of which were parasitized, were obtained from Rolands Pond, Dillon Beach. Two, neither of which was parasitized, were collected from Armstrongs Woods State Park in Sonoma County.

Rolands Pond is a permanent body of water located in a cattle and sheep pasture. During the rainy season it is approximately 150 yards in length, 35 yards in width, and from 5 to 6 feet in depth. During the summer and autumn drought, however, the pond recedes in size until the dimensions are 50 to 75 yards in length, 15 to 20 yards in width, and not more than three feet in depth. The bottom of the pond is covered with a growth of green algae, interspersed with clear sandy areas. Upon occasion free swimming leeches have been noted, and the author has been informed that several specimens of Triturus torosus have been taken with leeches attached to them. It has been demonstrated by numerous investigators that hirudineans may act as intermediate hosts in the transmission of trypanasomes to aquatic fishes, amphibians, and reptiles. Newts taken from Armstrong Woods State Park were found in pools of a small, clear stream. No aquatic vegetation was observed, nor were leeches found.

In all animals which were killed, blood for the thin films was taken directly from either the heart or lungs, while the forms which were to be used in further experimental work were bled by clipping the end of the tail with a scalpel.

Blood films were immediately stained by Wrights method and observed for the presence of flagellated bodies. Splenic smears from the killed animals were prepared by the same method and examined for intracellular leishman bodies, which, according to Carini (1912) and Nigrelli (1929), are present in Triturus viridescens parasitized by Trypanasoma diemyctyli and in Leptodactylus scelatus, a South American amphibian infected with Trypanasoma leptodactyli. These bodies, according to the above named authors, are involved in one of thetwo methods of reproduction, the other method being binary fission of the flagellated form in the blood stream. Reproduction by leishman bodies is also a characteristic of Trypanasoma cruzi Chagas, the causative agent of Chagas disease. However, in this species binary fission in the blood stream is not existant. Studies of mononuclear leucocytes in splenic smears from Trypanasoma barbari failed to reveal the presence of leishman bodies.

In preparations which were to be used for the study of living flagellates, a drop of fresh blood was mixed equally

with saline solution (.75%) containing 1% sodium citrate. The preparation was covered with a number 1 coverslip ringed with vaseline.

Slides were prepared for temperature-activity experiments in the manner described in the previous paragraph and were placed under oil immersion upon a heating stage. The thermostatic controls were manipulated so as to attain the desired temperature.

Cultivation of Trypanasoma barbari was attempted in: (1) Rogers medium, a mixture of normal saline solution to which is added 8 per cent sodium citrate and acidified, when necessary, with citric acid; (2) dextrose agar, which contains ten parts of 1 per cent dextrose agar and ninety parts of normal saline; (3) dextrose blood agar, a variation of dextrose agar, to which is added fresh amphibian blood in half the amount of the dextrose agar.

Unfortunately, no growth was observed in or on any of the media, and microscopical examinations confirmed the fact. It was ascertained, however, that in the vaseline ring blood preparations the trypanasomes remained alive for as long as forty-eight hours.

DESCRIPTION OF TRYPANASOMA BARBARI N. SP.

Two forms of Trypanasoma barbari have been recognized in both stained and living preparations. The first is a small, slender form while the second is a large broad one. Stained specimens of the small, slender form of T. barbari (Figs. 4 and 5, plate I) measures 26(20-30) microns in length and 1.9(1-2.5) microns in width. In the anterior one half of the body is the large, oval, mottled nucleus, the average size being 2.5 x 4 microns; no endosome was observed. The parabasal body is large (1.5 microns), round, deeply stained, and situated from 7 to 12 microns from the posterior end. Arising from the parabasal body is the axoneme which follows the edge of the narrow, folded undulating membrane until it becomes a free flagellum at the anterior end of the body; its length is approximately that of the body. All forms have sharply pointed ends; the anterior being bayonet-shaped, while the posterior, behind the parabasal body, is thin, drawn out, and resembles a needle. There is no apparent variation in the morphology of the slender, thin forms, nor is there any marked contortion in the body shape, most individuals being shallowly U-shaped.

Stained preparations of the large, broad forms (Figs. 1 and 2, plate I) of the trypanasome reveal great variation among the individuals. The length is 71(63-81) microns,

and the width is 7.5(6-10.5) microns. The nucleus is large (8 x 6 microns), oval, and in the anterior one half of the body; it is mottled, as in the smaller forms; however, upon one occasion it was observed in an early stage on mitosis (fig. 2, plate I). The possession of a parabasal body does not appear to be constant; but, when present, it is dark and smaller in proportion than is the same structure found in the narrow flagellate; it is located from 4 to 14 microns fro m the posterior extremity. A broad, folded undulating membrane is present in approximately 90 per cent of the broad forms, the axoneme of which becomes a short, free flagellum at the anterior end. It will be noted, however, in the description of the living animal, that under certain experimental conditions the flagellum can be demonstrated as being extensible for approximately two-thirds the length of the body. Body shape varies from conspicuously folded forms on one hand to broad leaf-shaped ones on the other. Both ends are pointed, and the posterior end is usually needle shaped; however, upon occasion, it may be bayonet-shaped. Clear vacuolated areas can occasionally be seen in the extreme anterior and posterior portions, but at no time was the parabasal body observed in a posterior area.

COMPARISONS

To the author's knowledge there are only two other species of trypanasomes reported from representatives of North America urodeles. These species are Trypanasoma diemyctyli Tobey (1906) which is found in Triturus viridescens (Rafinesque), and Trypanasoma cryptobranchi Roudabush and Coatney (1937) from the hellbender Cryptobranchus alleganiensis (Daudin).

The parabasal body of T. diemyctyli is described as small, difficult to see, located in the center of one of several round, unstained spaces at the posterior extremity, and connected to the axoneme. In addition to the connection between the parabasal body and the axoneme there may be one or two lines connecting the parabasal body with the edge of the clear space. Roudabush and Coatney (1937) state that the parabasal body of T. cryptobranchi is elliptical and perpendicular to the long axis of the body. Conversely, the parabasal body of T. barbari is large, round and readily observable; it is not perpendicular to the long axis of the body; it is not situated in a clear area in the cytoplasm, nor are there accessory lines. Furthermore, the posterior end of T. barbari only occasionally contains round, unstained spaces, and these were observed only in the large, broad forms.

Tobey (1906) describes the nucleus of T. diemyctyli as lying near but usually posterior to the center of the body, while the nucleus in T. cryptobranchi is approximately in the middle of the body. In T. barbari, on the other hand, the nucleus was found to be within the anterior one half of the body.

An additional characteristic which may serve to differentiate T. barbari from the trypanasomes of other Caudata is the long, thin, narrow, and drawn out posterior portion. Comparative measurements of the three species are presented (Table III); but, due to the extreme polymorphism of amphibian trypanasomes, size alone cannot be considered a reliable specific characteristic.

TABLE III

COMPARATIVE MEASUREMENTS

SPECIES	LENGTH (Microns)	WIDTH (Microns)	FLAGELLUM (Microns)
T. barbari			
Narrow Forms	20-30	1-2.5	Body length
Broad Forms	63-81	6-10.5	2/3 body length in living forms
T. cryptobranchi	46.8-77.4	1.8-5.84	31.61
T. diemyctyli			
Tobey	45-50	.....	24
Hegner	38.5-75.3	1.9-4.4	.....
Nigrelli			
Slender Forms	38-57	1.9-4.5	Very long
Broad Forms	63.5-79.4	5.2-9	.....

OBSERVATIONS ON THE LIVING ANIMAL

In each form of T. barbari the living flagellate is colorless, the periplast is well defined, and the nucleus and undulating membrane are readily observable. Neither the parabasal body nor the flagellum can be seen.

Movement is accomplished by a rhythmical motion of the undulating membrane; it arises at the anterior and progresses to the posterior end of the body. Locomotion is very limited in the large, broad forms as the body is continually writhing in such a manner as to dissipate the differential of force caused by the resistance of the medium to the undulating membrane and flagellum. However, in the small, slender flagellates such marked, hindering contortions are not as noticible, and the animal proceeds through the medium at a relatively rapid rate.

When the temperature of the slide containing the living preparation was raised from the 18°C. room temperature to 23°C. the animal slightly increased its action; between 26° and 33° C. a maximum of both undulation and forward progress was noted; however, motion decreased as the temperature rose toward 38° C. At 41° C. the flagellate remained relatively quiet for a greater percentage of the time, slowly moving the undulating membrane, with an occasional sporadic burst of motion involving the entire body. All

motion ceased after the temperature reached approximately 46° C., and subsequent lowering of the temperature did not result in further motion.

Although it was possible to see the complete flagella in stained preparations of the slender forms, the structure was only rarely observed in stained preparations of the large, broad forms, and even then it appeared quite short. However, during the periods of relative quiet, which occured between the temperatures of 41° to 47° C., it was possible to observe a slowly waving flagellum that was approximately two-thirds as long as the body in the large, broad forms, and equal to the body length in the small slender flagellates.

T. barbari apparently has no phototropic tendencies, as motion at room temperature (18° C.) remained constant regardless of the source and amount of light; and further experimentation, which involved an adjustment of the mirror so that half of the animal could be illuminated while the other half was in a darkened portion of the field, showed that the trypanasome entered the illuminated section of the field as often as it passed into the dark area.

CROSS INFECTION

Attempts were made to infect *Hyla regilla*, *Batrachoseps attenuatus attenuatus*, *Aneides flavipunctatus*, and *Ensatina escholtzii escholtzii* by injecting each animal, intraperitoneally, with .12 cc of citrated blood containing *T. barbari*. Examination of the animals at various times, up to two weeks after the attempted infection, revealed that the parasite was not able to become established in the new hosts.

DISCUSSION

Upon exclusively morphological bases it might be possible to regard the small, slender and the large, broad forms of *T. barbari* as representing two distinct species. However, size and shape alone are not good diagnostic characteristics in establishing the specificity of amphibian trypanasomes, due to the extreme polymorphism displayed by many members of the group. It is the opinion of the author that the position of the nucleus and the size, shape, and position of the parabasal body, which are constant features of both forms, are sufficient criteria for the inclusion of both forms in a single species. The occurence of polymorphic forms among amphibian trypanasomes is a common phenomenon, and, of the relatively large number of flagellates reported from amphibians, only a very few have been accepted as valid species (Table II) because of outstanding and unique characteristics. It will not be until cultivation, cross infection, and serological tests have been completed that these reported, but unnamed, forms may be properly relegated into either distinct species or considered variations of previously known forms.

T. barbari has been shown in the comparisons to be a distinct species from *T. diemyctyli* and *T. cryptobranchi*; it is also possible that serological differentiation of *T.*

barbari from Triturus torosus and T. cryptobranchi from Cryptobranchus alleganiensis may prove of some value. Boyden and Noble (1933) demonstrated that the genera Triturus and Cryptobranchus are not related serologically, and according to Nigrelli, there appears to be a correlation between serological reactions and the species of amphibian trypanasomes. Should appropriate tests be conducted, it might then be possible to distinguish T. barbari and T. cryptobranchi by means of serological tests as well as morphological differences.

In the opinion of the author the primary objective which should be attained, before further experimental work is attempted with T. barbari, is cultivation of the flagellate. When this goal has been reached, it will be possible to (1) watch the changes occuring in the morphology of the parasite during the course of infection in laboratory raised animals, (2) determine the pathology, if any, caused by the infection, and (3) attempt passive and active immunization. The intermediate host of the trypanasome should also be determined and the course of its infection studied. When all of the above information has been compiled and analyzed, it should provide an excellent background for further workers in the field, and it will fill in many of the gaps in our knowledge regarding blood parasites of poikilothermal animals.

SUMMARY

1. A new species of trypanasome, T. barbari, is described from the newt Triturus torosus. Tables of North American amphibians which have been examined for trypanasomes are presented, together with tables of the trypanasomes reported from them.

2. Observations upon living specimens of T. barbari are recorded, including data on morphology, locomotion, tropisms and effects of temperature changes.

3. Attempted, but unsuccessful, methods of cultivation in artificial media are reported, together with suggestions for further experiments along these lines.

4. Cross infection experiments gave negative results.

EXPLANATION OF PLATE

Figs. 1 and 2 Large, broad forms

Fig. 4 Erythrocyte

Figs. 3 and 5 Small, narrow forms

EXPLANATION OF LETTERING

AX - Axoneme

E - Erythrocyte

EM - Mitotic Stage of Endosome

F - Flagellum

N - Nucleus

PB - Parabasal body

U - Undulating membrane

LITERATURE CITED

Boyden, Alan and G. K. Noble, "The Relationships of Some Common Amphibia as Determined by Serological Reactions", American Museum Novitates, 1933, 606: 1-24.

Brandt, B. B., "Parasites of North Carolina Salientia", Ecological Monograph, 6:491-532, 1936.

Brumpt, E., "Un nouveau trypanasome, Trypanasoma neveu-lemairei sp. nov.", Annales de Parasitologie, 6:18-22, 1928.

Carini, A., "Uber Schizogonien bei Trypanasomen", Orchiv fur Protistenkunde, 24:80-83, 1912.

Fantham, H. P., Annie Porter and L. R. Richardson, "Some Haematazoa Observed in Vertebrates in Eastern Canada", Parasitology, 34:199-226, 1942.

Hegner, R. W., "Measurements of Trypanasoma diemyctyli from Different Hosts and Their Relation to Specific Identification, Heredity and Environment", Journal of Parasitology, 7:105-113, 1921.

Kudo, R. R., "On the Protozoa Parasitic in Frogs", Transactions of the American Microscopical Society, 41: 59-76, 1922.

Nigrelli, Ross F., "On the Cytology and Life History of Trypanasoma diemyctyli and the Polynuclear Count of Infected Newts (Triturus viridescens)", Transactions of the American Microscopical Society, 48:366-387, 1929.

....., "Trypanasomes from North American Amphibians with a Description of Trypanasoma grylii Nigrelli (1944) from Acris gryllus (Le Conte)", New York Zoologica, 30:47-56, 1945.

Packchanian, A., "On the Cultivation of Seven Species of Trypanasomes in Vitro", Science, 80(2079):407-408, 1934.

Roudabush, Robert L., and G. Robert Coatney, "On Some Blood Protozoa of Reptiles and Amphibia", Transactions of the American Microscopical Society, 56:291-297, 1937.

Sergent, Ed. and S. Sergent, "Sur un trypanasome nouveau parasite de la grenouille verte", Compte Rendu Society Biologie, 56:123-124, 1904.

Stebbins, J. H. Jr., "On the Occurence of Trypanasomes in the Blood of Rana clamita", Transactions of the American Microscopical Society, 27:26-30, 1907.

Tobey, E. N., "Trypanasomiasis of the Newt", Journal of Medical Research, 10:147-148, 1906.

Wood, S. F. and F. D. Wood, "Occurence of Hematazoa in Some California Cold Blooded Vertebrates", Journal of Parasitology, 22:518-520, 1936.

PLATE I