Observations on Some Intestinal Protozoa in Oklahoma Lizards, With the Description of a New Genus, *Biflagella*[1]

ROSE M. ZIMMERMAN, Okmulgee High School, Okmulgee, and HARLEY P. BROWN, University of Oklahoma, Norman

Lizards are promising animals for a study of intestinal protozoa, since they consume large numbers of insects, and insects are known to harbor many forms of protozoa. It is to be expected that some of these protozoa might become established in the lizards. It is surprising, therefore, that so little has been published on this subject in the United States—or in the world. A handful of scattered papers constitutes the useful literature on the subject in America. This report represents the results of a study of seventy-five specimens of common Oklahoma lizards belonging to five species (exclusive of twenty-one newly-hatched specimens).

The lizards used in this study were collected from two areas: the vicinity of the University of Oklahoma Biological Station on Lake Texoma near Willis, and Oliver's Woods, near Norman. The Lake Area is a dry, sandy habitat in which *Cnemidophorus sexlineatus* was found in abundance. Also present in this area were *Sceloporus undulatus* and *Scincella* (*Leiolopisma*) *laterale*. *Phrynosoma cornutum* occurs there, but none was used in this study. The Oliver's Woods Area is a moist area, frequently flooded. *Eumeces fasciatus*, *Scincella laterale*, *Cnemidophorus sexlineatus*, and *Sceloporus undulatus* were obtained from this habitat and *Phrynosoma cornutum* from the surrounding area.

The lizards were killed by using carbon tetrachloride fumes or by snipping off their heads. The alimentary canal was quickly removed and divided into three sections: stomach, intestine, and cloaca. Fresh smears were examined in dilute saline solution with a phase microscope. Permanent smears from each section were fixed and stained with Heidenhain's iron hematoxylin. Both the fresh and permanent smears were examined for trophozoites and cysts with the 4 mm. objective.

Trophozoites were found in the intestine and cloaca. Cysts were found most abundantly in the cloaca and feces. The stomachs contained unidentified cysts which were also present in the proctodeal contents of some of the grasshoppers consumed as food.

Identification of the protozoa was based largely upon Kudo (17) and Wenyon (22).

Leptomonas. Leptomonad and leishmanial forms, though not found in the Lake area, were found in all the lizards from the Norman area: *Eumeces fasciatus*, *Phrynosoma cornutum*, *Cnemidophorus sexlineatus*, *Scincella laterale*, *and Sceloporus undulatus*.

Herpetomonas, which is closely related to *Leptomonas*, has been reported by Fantham (6) and Franchini (7) in *Chamaeleon vulgaris*, and by Bayon (2) in *Chamaeleon pumilus*. *Herpetomonas* and *Leptomonas* are similar; both occur in insects, and it seems not improbable that they should also occur in lizards which feed upon such insects. Thus, although these genera are defined as occurring only in invertebrates, the use of the host (vertebrate, invertebrate) as the basis for classification of this group of protozoa is hardly a valid one. (A forthcoming article by H. P. Brown will elaborate upon this point.)

[1] Based upon a Master's Thesis submitted to the Graduate College of the University of Oklahoma in August, 1942, by Rose M. Zimmerman.

These leptomonads were obtained from the intestine and cloaca. Smears taken from the lower intestine produced heavy concentrations, while specimens in the cloacal contents were less concentrated.

The flagellum is long, often exceeding the body length. The blepharoplast and parabasal body are located anterior to the nucleus and at the extreme anterior end of the body. The nucleus is also in an anterior position. The body is slender, tapering to a blunt point posteriorly. The maximum body width is about three microns. The body length varies from about fifteen microns in the elongate forms to a small, rounded leishmanial form. The body size and flagellar length closely resemble *Herpetomonas mansoni*, described by Bayon (2) from *Chamaeleon pumilus* of Robben Island. Wenyon (22) believed this to be *Leptomonas*.

The rounded leishmanial forms were found from the lower intestine to the cloaca—mostly in the cloaca.

Various mosquitoes, flies, and other soft-bodied insects abundant in Oliver's Woods were not found in the Lake Texoma Area. The fact that *Leptomonas* was so abundant in lizards from Oliver's Woods and was not found in lizards from the Lake Texoma Area seems significant on the assumption that lizards adopt, at least in part, the intestinal fauna of the insects upon which they feed. Confirmation of this hypothesis will necessitate further research upon the intestinal fauna of the insects serving as food for the lizards. A start has been made at the biological station, in the form of a survey of the food habits of the local lizards.

The genus *Leptomonas* has not previously been reported from lizards in the United States.

Proteromonas lacertae-viridis. *Proteromonas lacertae-viridis* was first seen and described by Grassi, in 1879 (11). Since then it has been reported from lizards by Prowazek (20), Dobell (4), Bělař (3), Grassé (8), Hirschler (15), and Wood (23). Recently, it was reported from both lizards and snakes by Moskowitz (19). It has been listed under a variety of names. Grassi (11) included this species in the genus *Monocercomonas*. Prowazek (20) and Bělař (3) included it in the genus *Bodo*, while Alexeieff (1) considered it *Heteromita*, but later created for it the genus *Prowazekella*, which is now considered synonymous with *Proteromonas*, first described by Kunstler (Kudo, 17).

Proteromonas lacertae-viridis is the most abundant and most frequently-occurring protozoon in the lizards of both localities studies. It is an elongated pyriform organism having two flagella of unequal length. The position of the flagella seems to vary. The longer flagellum, about three times the body length, is always directed forward, while the shorter one of about twice the body length or less may be directed anteriorly or may trail along the body. The nucleus is in the anterior end, its membrane surrounded by parabasal bodies.

The swimming motion of *Proteromonas* in the dilute saline solution was a flopping one, the posterior end being very pliable.

Cysts were abundant in the stomach with the partly-digested food, but, as would be expected, no motile forms were present. The active flagellates were abundant in the intestine, gradually decreasing in number toward the cloaca, whereas the cysts increased in number toward the cloaca, being most abundant in the rectum. Freshly evacuated fecal material yielded many of the cysts.

An entire intestine of *Cnemidophorus* was cut in short lengths, imbedded in paraffin, and sectioned. The sections show an increase in the number of the flagellates just posterior to the duodenum. They form a composite mass in the ventral half of the proctodeum. None is present in

the dorsal half. The folds of the intestine are completely destroyed; the tissue seems to be eroded down to the outer wall. Where numbers of flagellates are low, erosion has not taken place, but where the flagellates are massed, considerable damage to the host has occurred. It is apparent that Proteromonas may be definitely pathogenic.

The lizards infected with this parasite were *Eumeces fasciatus*, *Phrynosoma cornutum*, *Scincella laterale*, *Sceloporus undulatus*, and *Cnemidophorus sexlineatus*.

Biflagella texoma, nov. gen., nov. sp. The second most abundant protozoon in the lizard intestines studied at Lake Texoma, though not found in the Norman area, apparently represents a new genus and is here described as such (Figs. 1-4).

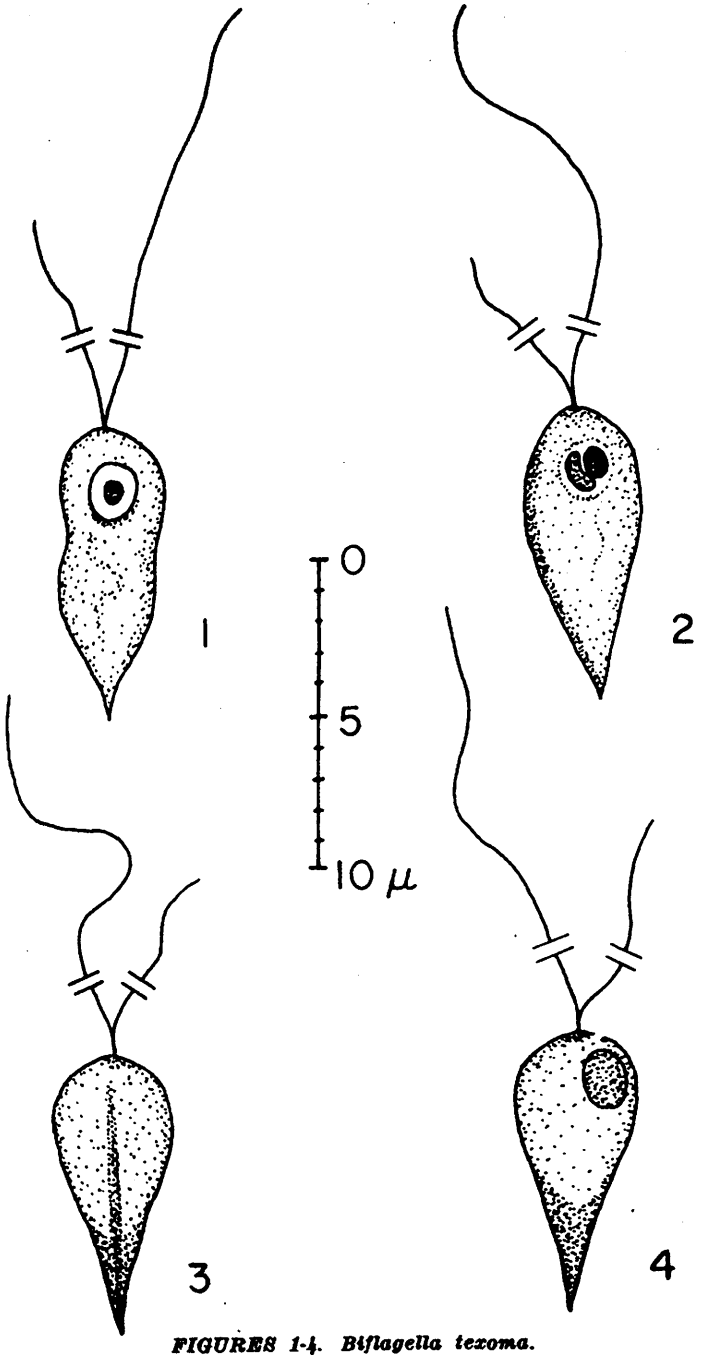
Biflagella texoma is pyriform, with a maximum width of 3.1 to 4.6 microns and a length of 7.8 to 9.4 microns. It possesses a rigid posterior end which resembles a trichomonad axostyle. This rigid section extends three-fourths the distance up one side of the animal. The nucleus is anterior and eccentric in position, occurring on the side opposite the "axostyle." The parabasal body is located beside the nucleus. Two flagella of unequal length are present, one about five times the body length and the other about three times the body length (their full length is not shown in the figures). Both flagella are directed anteriorly.

Biflagella swims with a rolling motion, and occurs abundantly from the duodenum to the cloaca and rectum. It was found in *Cnemidophorus sexlineatus*, *Scincella laterale*, and *Sceloporus undulatus*, from the Lake Texoma Area only.

This organism appears to be closely related to *Trichomonas*, in the order Polymastigina. However, since it possesses only two flagella it must be placed in the Order Protomonadina. Here it would key down (in Kudo) to the Family Monadidae, although it is probably more closely related to members of the Family Bodonidae. Until the classification of the Subclass Zoomastigina is revised, confusion of this sort will probably continue. A great forward step in this revision has been taken by Grassé (10). Tentatively, the authors suggest that *Biflagella* be placed in the Family Proteromonadidae, recently created by Grassé (10), despite the fact that neither of its flagella is a trailing flagellum. Employing the classification of Kudo (17) or Hall (12), it would be placed in the Family Bodonidae.

Retortamonas (*Embadomonas*). Very little literature is available on this genus, which was first described by Grassi (Kudo, 17). Mackinnon (Kudo, 17) gave it the name *Embadomonas*, and later Wenyon (22), working with the tortoise, *Testudo argentina*, was able to culture a flagellate which he indicated was structurally similar to *Retortamonas intestinalis*, but which he called *Embadomonas*. Grassé (8) noted a new species, *Retortamonas saurarum*, in the lizard, *Anguis fragilis*, but gave no description. Moskowitz (19) gave a thorough description of a form he found in five species of snakes, employing the name *Retortamonas saurarum*. However, Moskowitz did not report finding it in any of the lizards he examined.

The species observed by us in *Cnemidophorus sexlineatus*, *Phrynosoma cornutum*, and *Eumeces fasciatus* was probably that described by Moskowitz. It is ovoid-pyiform, with the posterior end more or less pointed and occasionally drawn out to a rigid point or caudal spike. Its size ranged from four to eight microns in width and six to twelve microns in length, the smaller dimensions being very common. The two flagella are of unequal length. The longer one is thinner and usually exceeds the body length, while the shorter one is less than body length, is thicker, and may lie in the anterior region of the oral pouch or the distal end may protrude laterally or posteriorly. The nucleus is spherical and is located anteriorly.



FIGURES 1-4. Biflagella texoma.

(1) Biflagella texoma from a prepared slide stained with Heidenhain's iron hematoxylin. The full length of the short flagellum is about 25 microns, that of the long flagellum about 40 microns.

(2) Biflagella texoma from a prepared slide stained with Heidenhain's iron hematoxylin. The darkened area along the left side of the drawing at different levels of focus. (3) shows the axostyle-like structure and indicates the axostyle-like structure.

(3) and (4) Biflagella texoma drawn from a living specimen observed with a phase microscope. These figures represent a single specimen viewed (4) shows the nucleus.

Cercomonas. Cercomonas is generally considered a coprozoic organism, although several species have been described from the stomachs of cattle (Wenyon, 22). Both trophozoites and cysts were found in *Cnemidophorus sexlineatus*, *Sceloporus undulatus*, and *Eumeces fasciatus*, and occurred in both localities studied. *Cercomonas* was fairly common in the upper intestine and in the cloaca.

As seen under the phase microscope in the living condition, this organism is usually pear-shaped. However, the body shape varies, depending upon the length of time out of the intestine and in the saline solution. The usual length is about twice the width, with the posterior end of the body drawn out into a short, blunt point. Sometimes these flagellates from a freshly-killed lizard exhibited such a high degree of plasticity as to appear amoeboid. Their bodies were capable of becoming quite flattened on one side of an object, or quickly becoming a curlicue, or drawing out the posterior end to a knob on a stalk. The nucleus is anterior in position, with a central karyosome. The nuclear membrane is extended into the rhizoplast and continues to the two flagella. One flagellum is directed forward while the other usually becomes a trailing flagellum. Each of the two flagella is about twice the body length. The two minute blepharoplasts are in the shape of a granule at the extreme anterior end of the body.

Bodo. The genus *Bodo* is characteristic of stagnant or polluted water. Perhaps this was the source of the lizard infections observed. It was found in the rectal contents of *Cnemidophorus sexlineatus* and *Scincella laterale* from both localities studied, and of *Eumeces fasciatus* and *Phrynosoma cornutum* from the Norman Area.

This flagellate has a somewhat flattened but usually ovoid body with the posterior end drawn out so that the length is about one and a half times the width. The nucleus is central in position. The kinetoplast consists of a parabasal body and two blepharoplasts that stain black in iron hematoxylin. The two flagella are unequal in length, and may appear curled at the distal ends. Both are capable of anteriorly-directed movement, but one is usually directed anteriorly, the other trailing. The former is the shorter one, being about body length, while the trailing flagellum is about twice the body length.

Entamoeba ctenosaura. Amebas resembling *Entamoeba coli* of the human intestine have been reported from the Egyptian lizards, *Lacerta agilis* and *Agama stellio*, by Wenyon (21). The trophozoites were very similar to those of *E. coli*, and eight-nucleated cysts occurred which were indistinguishable from those of the human parasite. Dobell (10) reported a similar organism from the wall lizard, *Lacerta muralis*. Apparently Franchini (7) found a similar ameba in *Lacerta ocellata*.

Hegner and Hewitt (14) describe *Entamoeba ctenosaura*, found in *Ctenosaura acanthura*, as a *coli*-like species having cysts with one to sixteen (usually two to eight) nuclei, and lacking the splinter-like chromatoid body.

It is apparently this ameba which was observed in *Cnemidophorus sexlineatus* and *Scincella laterale* from both localities, and in *Eumeces fasciatus* and *Phrynosoma cornutum* near Norman.

Both trophozoites and cysts were found in the lower intestine. The trophozoites were mononucleate and very sluggish in movement. The cysts contained four to eight nuclei, and no chromatoid bodies.

Endamoeba cuautlae. This is another ameba described by Hegner and Hewitt (14) from the Mexican lizards, *Ctenosaura acanthura* and *Sceloporus clarkii.* They describe the cyst as being eight to fourteen microns in diameter, characteristically containing four nuclei and one to sixteen rod-shaped chromatoid bodies either single or in pairs.

Trophozoites and cysts of what appeared to be this species were observed in the lower intestine and cloaca of *Cnemidophorus sexlineatus* and *Sceloporus undulatus* from the Lake Texoma area, and in *Cnemidophorus sexlineatus*, *Sceloporus undulatus*, *Eumeces fasciatus*, and *Phrynosoma cornutum* from the Norman area.

The trophozoites have the eruptive formation of lobopodia and the ringlike nucleus typical of *Entamoeba histolytica.* In no case were they numerous. The cysts are spherical, five to ten microns in diameter, and contain one rod-shaped chromatoid body which may be absorbed as the cyst grows older. The nucleus, which is eccentric and with a bead-like membrane, may divide twice to produce the four-nucleate type of cyst which is often present in the same smear with the mononucleate type.

Nyctotherus. Nyctotherus is a large ciliate, possessing an oral groove with an undulating membrane. It is commonly found in frogs and toads, but may inhabit various insects as well as other vertebrates. It has been reported from lizards by Grassé (9) as *Nyctotherus haranti* from a gecko in Tunisia, by Johnston (6) as *trachysaurai* from *Trachysaurus rugosus*, by Wood (23) as *Nyctotherus* sp. from California lizards, and by Hegner (13) as *N. beltrani* from an iguana.

Since the organism was observed on only one occasion in a fresh smear, no description will be given here. It was observed in *Phrynosoma cornutum* taken near Norman.

General Discussion

Not all the lizards collected were good material for study. The smaller lizards had a smaller faunal content and very frequently had none. Baby (newly-hatched) lizards were found to have no protozoan inhabitants, which is what would be expected if we assume that lizards acquire their intestinal parasites primarily from the insects eaten as food. The intestines of baby lizards (*Eumeces fasciatus*) hatched in the laboratory exhibited the same living nematodes as were found in the sawdust in which the baby lizards were kept.

Only a few lizards failed to produce motile forms. Three lizards that were killed in the field but autopsied and examined promptly yielded negative results. The little brown skink, *Scincella laterale*, was most often negative.

Adult males appeared to be more heavily infected than adult females. Perhaps this observation is correlated with a greater food consumption by the males.

Vital stains were unnecessary in using the phase microscope; it showed the flagella and nuclei distinctly.

TABLE I

Intestinal Protozoa of Oklahoma Lizards

Host	Number Examined	Parasites	Number of Hosts Infected	Degree of Infection
Cnemidophorus sexlineatus	30	*Endamoeba cuautlae*	6	light
		Entamoeba ctenosaura	5	light
		Bodo	20	light
		Cercomonas	20	light
		Leptomonas	12	heavy
		Proteromonas lacertae-viridis	24	heavy
		Retortamonas	16	light
		Biflagella texoma	12	heavy
Eumeces fasciatus	12	*Endamoeba cuautlae*	2	light
		Entamoeba ctenosaura	2	light
		Bodo	12	light
		Cercomonas	9	light
		Leptomonas	12	heavy
		Proteromonas lacertae-viridis	12	heavy
	(21	"babies" not infected)		
Phrynosoma cornutum	7	*Endamoeba cuautlae*	2	light
		Entamoeba ctenosaura	2	light
		Bodo	5	light
		Cercomonas	4	light
		Leptomonas	7	heavy
		Proteromonas lacertae-viridis	7	heavy
		Nyctotherus	1	very light
Sceloporus undulatus	11	*Endamoeba cuautlae*	1	light
		Entamoeba ctenosaura	1	light
		Cercomonas	3	light
		Leptomonas	7	heavy
		Proteromonas lacertae-viridis	11	heavy
		Biflagella texoma	4	heavy
Scincella (Leiolopisma) laterale	15	*Entamoeba ctenosaura*	1	light
		Bodo	2	light
		Leptomonas	7	heavy
		Proteromonas lacertae-viridis	9	heavy
		Biflagella texoma	2	heavy

The lizards found in the two areas of study were the same, with the exception of *Eumeces fasciatus*, which was not encountered in the Lake Texoma area.

The intestinal protozoan fauna differed in the two areas only in the presence of *Leptomonas* in the lizards of the Norman area but not in the Lake Texoma Area, and in the presence of *Biflagella* in the lizards of the Lake Texoma area but not in the Norman area. *Biflagella* was also found in the proctodeal contents of a grasshopper taken from the habitat of the infected lizards.

The appended table shows the number of specimens of each lizard species examined and the number of individuals parasitized by each species of Protozoa.

Summary

Seventy-five lizards, representing five species, were examined for intestinal protozoa. Specimens of three of the species, *Cnemidophorus*

sexlineatus, *Sceloporus undulatus*, and *Scincella* (*Leiolopisma*) *laterale*, were collected both from the vicinity of Norman, Oklahoma and from the vicinity of the University of Oklahoma Biological Station at Lake Texoma. Specimens of *Phrynosoma cornutum* and *Eumeces fasciatus* were taken only from the vicinity of Norman.

The intestinal protozoa found were *Proteromonas lacertae-viridis*, *Leptomonas*, *Biflagella texoma*, nov. gen., nov. sp., *Bodo*, *Cercomonas*, *Retortamonas*, *Endamoeba cuautlae*, *Entamoeba ctenosaura*, and *Nyctotherus*.

Proteromonas was the most abundant and the most prevalent protozoon found. Serial sections of the small intestine of *Cnemidophorus* show a pathological erosion of the mucosa and submucosa in the presence of great numbers of *Proteromonas*.

Leptomonas was abundant in the material from near Norman, but was not observed in lizards of the lake area. This may be correlated with a difference in the insect fauna of the two areas.

Biflagella texoma, described herein as a new genus and species, is a trichomonas-like form possessing two flagella and a sort of axostyle, but lacking an undulating membrane. Thus far it has been found only in lizards of the Lake Texoma area.

Leptomonas, *Bodo*, and *Cercomonas* have not hitherto been reported from American lizards.

Additional work is suggested upon the intestinal protozoa of the arthropods which serve as food for lizards, since they probably represent the major source of infection for the lizards.

The need for taxonomic revision within the protozoan Subclass Zoomastigina is pointed out.

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