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## ***Trimitus parvus* Grasse (Protozoa, Mastigophora) from a garter snake, *Thamnophis radix***

By L. H. SAXE AND EUGENE M. SCHMIDT

### INTRODUCTION

Reptiles collected in the vicinity of the Iowa Lakeside Laboratory, Dickinson County, Iowa, during the summer of 1950, were examined for helminths by Burnham (1951) and for protozoa by Schmidt (1954). *Monocercomonas colubrorum*, *Trichomonas batrachorum* and *Haemogregarina* sp. were found in both *Thamnophis radix* and *T. ordinatus parietalis*. An enteric flagellate of the genus *Trimitus* was found in a single specimen of *T. radix*.

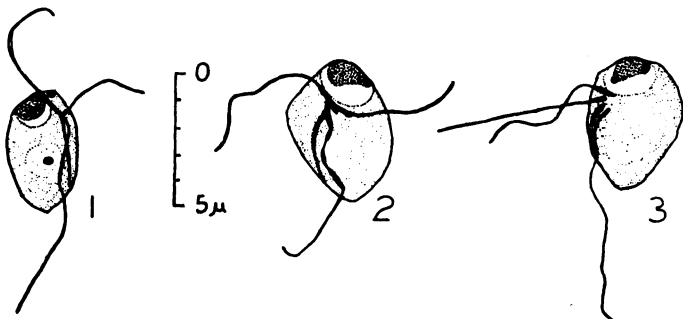
Special consideration is herein given to *Trimitus* because though this genus has been reported from North American amphibians and reptiles it appears that neither descriptions nor figures have been published, and also, because there is some uncertainty concerning the characterization of the genus.

### METHODS

For the study of enteric protozoa smears of gut contents were fixed in Schaudinn's fluid with 5% glacial acetic acid added and either stained with Heidenhain's hematoxylin or impregnated by the protein-silver method of Moskowitz (1950).

### OBSERVATIONS

*Trimitus* from *T. radix* is ovoid, with the anterior end usually somewhat broader than the posterior end. The size ranges from 4 to 6 microns in length and from 3 to 5 microns in width.



Figures 1-3. *Trimitus parvus* from *Thamnophis radix*, fixed in Schaudinn's fluid with 5% glacial acetic acid added and impregnated by the protein-silver method of Moskowitz. Figures were drawn by Schmidt with the aid of a camera lucida at an initial magnification of 4,000.

The vesicular nucleus is spheroidal in shape and located at the extreme anterior end of the body (see figures). The endosome is large and usually in contact with the nuclear membrane in the region at which the nuclear membrane is contiguous with the body surface. One or two dense granules are commonly seen within the endosome. The perieosomal space appears to be devoid of granules and may be an artifact of fixation.

There are two anterior flagella of unequal length, that have an origin near the nucleus. The longer of these two flagella is approximately the length of the body or slightly longer. The shorter flagellum is generally less than the length of the body. In protein-silver material, the flagella appear to be of uniform diameter, except for a proximal portion which is about one micron in length and about twice as thick as the rest of the filament.

Also originating in the anterior of the body is the posterior flagellum, which has an enlarged proximal portion similar to that of the anterior flagella. The posterior flagellum is approximately twice the length of the body and is appressed to the body surface.

Originating near the nucleus, along with the flagella, is a chromatic rod-like structure, which extends posteriorly in the cytosome. It may be in juxtaposition with the posterior flagellum or independent of it. In either case, it does not protrude through the cytosomal surface. It is analogous, if not homologous, to the structure in *Enteromonas* that Nie (1950) called the funis.

The cytoplasm is vacuolated, with endocyttoplasmic granules occasionally present.

The authors have been unable to distinguish a blepharoplast with certainty. In silver-protein preparations the structure probably does not impregnate (see figure 3); in Heidenhain's hematoxylin material, it is obscured by a chromaticity of the cytoplasm surrounding the nucleus. In their *Trimitus* material the authors have failed to find either a parabasal body or an axostyle.

#### DISCUSSION

Alexeieff (1910) found, in the digestive tract of a marine fish, *Onos* (= *Motella*) *tricirrata*, a small flagellate for which he proposed the name *Trimitus motellae*. He described the organism as being 6 to 8 microns in length; ovoid and slightly flattened in form with the posterior end drawn out and the anterior end often truncated. The mid-region of the body was filled with ingested bacteria and surrounded by a layer of more dense peripheral cytoplasm. The nucleus was found in the extreme anterior of the body. It was, said Alexeieff, the flagella which characterized this organism. There

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were two anterior flagella of unequal length (the one shorter, the other a bit longer than the body) and also a long trailing flagellum (up to four times the body length) that was joined, in its proximal portion, to the body surface.

Duboscq and Grassé (1923) described flagellates from *Kaloterme*s which they placed in the genus *Trimitus*. Kirby (1930) erected the genus *Tricercomitus* to include these organisms and de Mello (1932) also classified *Trimitus*-like flagellates from a termite as *Tricercomitus*. Among differences which distinguish *Tricercomitus* Kirby from *Trimitus* Alexeieff are the presence in the former of three equal anterior flagella, a parabasal body and an axostyle. Grassé (1952), however, considered *Tricercomitus* to be a synonym of *Trimitus*.

Grassé (1932), from *Rana temporaria* and *Bufo vulgaris*, described *Trimitus parvus* with a trailing posterior flagellum about twice the body length and two anterior flagella, one shorter, the other the same length or longer than the body. Many individuals were described to have a third anterior flagellum. A rod shaped parabasal body and a lightly staining filiform axostyle were also described.

Wenrich in the course of culture experiments has identified *Trimitus parvus* from *Rana pipiens* (1945, 1947) and *Triturus viridescens* (1947), and *Trimitus* sp. from *Pseudemys floridana mobilensis* (1947). *Trimitus* was previously reported from a turtle by Knowles and Das Gupta (1930).

Wenrich and Saxe (unpublished) found *Trimitus* in a specimen of *Thamnophis sirtalis* captured in Delaware County, Pennsylvania. Moskowitz (personal communication), in his study of the flagellates of Squamata (1951), recognized but did not record the presence of *Trimitus* in his material.

In the present study the authors confirm the generic characterization given by Alexeieff (1910). No structure identifiable as a parabasal body was found even in material impregnated by a protein-silver technique. Silver impregnation procedures are widely recognized as the most satisfactory of methods for the demonstration of Janicki-type parabasal bodies and the technique employed successfully revealed the parabasal apparatus of *Trichomonas batrachorum*, present along with *Trimitus*. Also, the authors have failed to distinguish an axostyle in *Trimitus* from either *Thamnophis* or *Rana*.

In the present material, *Trimitus* with more than two anterior flagella were found only when division was obviously in progress.

In examining material from *Rana pipiens* an occasional specimen of *Monocercomonoides*, possibly *M. rotunda* (Bishop), was encountered. This finding suggests that some "*Trimitus*" with more than two anterior flagella may have been mistakenly identified. In this connection the resemblance of Grassé's figures (1952) of *Trimitus blattae* to *Monocercomonoides orthopterorum* is striking.

The presence of a funis in *Trimitus* is considered evidence of a relationship to *Enteromonas*. These two genera differ significantly only in the number of anterior flagella, two in *Trimitus*, three in *Enteromonas*. If *Trimitus* truly lacks an axostyle and parabasal body, which the present investigation has not revealed, the genus must be removed from the Trichomonadida where it is placed by Grassé (1952) and join *Enteromonas*, possibly in the Cercomonadidae as suggested by Kirby (1930).

A comparison of flagellates of the genus *Trimitus* from *Thamnophis radix* and *Rana pipiens* has shown that these organisms are of the same species, *Trimitus parvus*. The snake might well have acquired its infection by feeding upon frogs, as larger garter snakes commonly do.

#### SUMMARY

1. Observations have been made that confirm Alexeieff's characterization of the genus *Trimitus*.
2. The same species, *Trimitus parvus*, may occur in both *Thamnophis radix* and *Rana pipiens* and the former host may acquire its infection from its prey.
3. *Trimitus* is closely related to *Enteromonas* and does not belong in the order Trichomonadida.

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