Morphologic Aspects of *Tetratrichomonas didelphidis* Isolated from Opossums *Didelphis marsupialis* and *Lutreolina crassicaudata*

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Tetratrichomonas didelphidis *(Hegner & Ratcliffe, 1927) Andersen & Reilly, 1965 is a flagellate protozoan found in the intestine, cecum, and colon of* Didelphis marsupialis. *The parasitic protozoa used in this study was found and isolated in the intestine of opossums in Pavlova starch-containing medium in Florianópolis, State of Santa Catarina, Brazil, from* D. marsupialis *and* Lutreolina crassicaudata. *The strains were cultivated in Diamond medium without maltose and with starch solution, pH 7.5 at 28°C. The specimens were stained by the Giemsa method and Heidenhain's iron hematoxylin. The light microscopy study of the trophozoites revealed the same morphologic characteristics as specimens previously described.*

Key words: *Tetratrichomonas didelphidis - Didelphis marsupialis - Lutreolina crassicaudata* - flagellate protozoan - morphology

Tetratrichomonas didelphidis is a parasitic protozoan found in the intestine, cecum and colon of the opossum *Didelphis marsupialis.* The protozoan is a flagellate belonging to the family Trichomonadidae, subfamily Trichomonadinae (Honigberg 1963). The taxonomy of trichomonads has a complex history. This species was first described by Hegner and Ratcliffe (1927a) under the name *Trichomonas didelphidis*. Afterwards, Andersen and Reilly (1965) redescribed the species as *Tetratrichomonas*. To date, there is only one paper on the occurrence and anatomy of trichomonads from opossum *D. marsupialis*. This is the first report on the occurrence of *T. didelphidis* in another opossum species, *Lutreolina crassicaudata.* Nothing is known about the transmission, epidemiology, pathogenicity, biochemistry and immunologic features, nor about the culture requirements of the parasites.

Many of the cecal trichomonads look alike, and cross-transmission studies have shown that many of them can be easily transmitted from one host species to another. Further and extensive studies are needed to establish the correct names and host spectra of many trichomonads (Levine 1973).

The aim of this work was to describe the detailed morphology of this trichomonad isolated from *D. marsupialis* and *L. crassicaudata* and to compare the data with that of Andersen and Reilly (1965).

MATERIALS AND METHODS

Isolation - The *T. didelphidis* strains (TDM86 and TDLC01) used in this study were isolated in Florianópolis, State of Santa Catarina, Brazil by Dr Mário Steindel, in Pavlova starch-containing medium (Pavlova 1938). The TDM86 strain was isolated in the rectal glands from *D. marsupialis* while the TDLC01 strain was isolated from the swabbed rectum of *L. crassicaudata.*

Cultivation - Microscopic observation of trichomonads in Pavlova starch-containing media was difficult due to the large quantity of starch granules. Diamond's (1957) modified trypticase-yeast extract-starch (TYS) medium, without maltose and with starch solution (5 mg/ml) pH 7.5 (Tasca et al. 1999) supported *T. didelphidis* growth well. The strains were cultured *in vitro* at 28ºC (± 0.5) in TYS medium supplemented with 10% (v/v) heat inactivated bovine serum, penicilin (1000 UI/ml) and streptomycin sulfate (1 mg/ml). Isolates were subcultured every 72 h in TYS starch-containing media. Certain samples were frozen and maintained at -196°C with 5% (v/v) of dimethyl

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sulfoxide (DMSO) as previously described by Honigberg et al. (1965).

Light microscopy - In order to make a comparison with the mensural data given by Andersen and Reilly (1965) the specimens were fixed with methanol and stained by the Giemsa method and Heidenhain's iron hematoxylin (De Carli 1994). Material for the stained smears was obtained from a 72 h old culture in TYS medium. All measurements were taken from 100 specimens (except when indicated in Table I) using an Olympus AX70 photomicroscope connected to a video camera and to a computer with the Image-Pro Plus 4.1 program. The significance of the variation between the mean measurements of the structures was analyzed using the ANOVA Test. The F values were significant when probability was equal to or lower than 0.05.

RESULTS

Microscopic observation of trichomonads from Pavlova's medium was difficult due to the large number of starch granules and bacteria. In this case the flagellates presented numerous vacuoles that distorted the body shape and masked the morphology (Figs 1, 2). The vacuoles occupied about 24% ± 13.5 (5 to 53%) of the entire body area of the protozoa. The organisms grew well in Diamond medium without maltose (TYS) and with starch solution, enabling the morphologic study.

TABLE I

Measurements (in µm) of Giemsa-stained specimens of *Tetratrichomonas didelphidis* isolated from opossums *Didelphis marsupialis* (TDM86) and *Lutreolina crassicaudata* (TDLC01) and comparisons with data of other authors (n = number of specimens investigated)

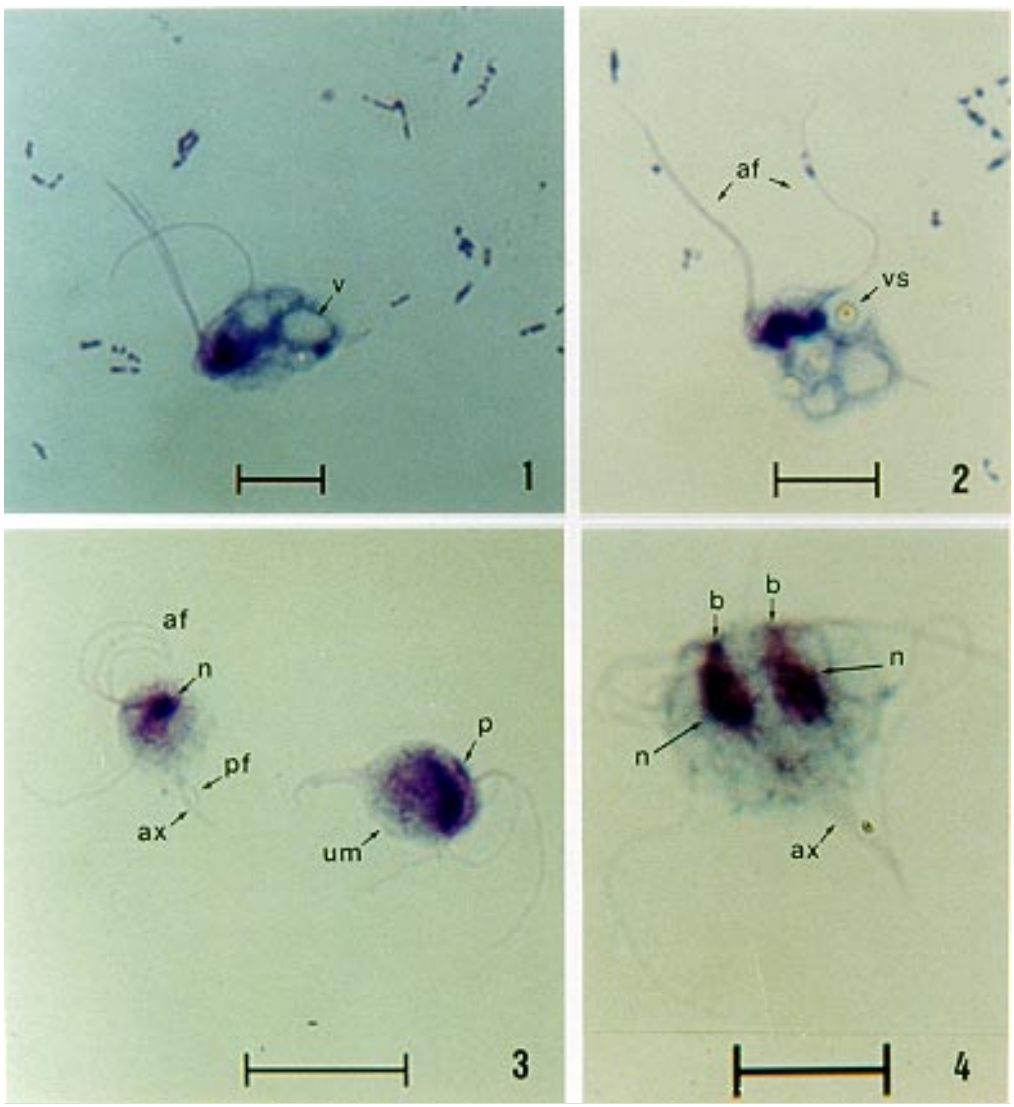
	Andersen & Reilly 1965 n =100	Present study, strain TDM86 n =100	Present study, strain TDLC01 n =100	Comparison between media: F test
Structure	Media ± standard deviation (variation)			
Body length	6.6 ± 0.6 (5.6 - 8.0)	7.3 ± 1.4 (4.2 - 11.1)	7.7 ± 1.3 (5.1 - 11.17)	22.52 *b*
Body width	3.9 ± 0.4 (2.8 -4.8)	5.9 ± 1.4 (2.7 - 8.8)	5.0 ± 1.2 (3.2 - 8.8)	87.55 *b*
Nucleus length	2.9 ± 0.4 (2.0 - 4.0)	2.7 ± 0.8 (1.1 - 4.9)	2.5 ± 0.5 (0,3 – 3.9)	15.12 *b*
Nucleus width	2.2 ± 0.3 (1.6 - 2.8)	2.3 ± 0.8 (7.4 - 4.5)	1.9 ± 0.4 (1.1 - 2.6)	20.72 *b*
Parabasal body length	1.3 ± 0.2 (0.8 - 1.8)	1.6 ± 0.7 (a)*a* (0.9 - 6.3)	1.6 ± 0.3 (0.8 - 2.5)	14.84 *b*
Parabasal body width	1.2 ± 0.2 (0.8 - 1.6)	1.0 ± 0.3 (b)*a* (0.5 - 2.0)	0.9 ± 0.2 (0,5 - 1.6)	32.10 *b*
Anterior flagella				
Number 1	17.4 ± 2.0 (12.0 - 20.8)	18.2 ± 5.5 (7.5 - 34.6)	15.9 ± 2.4 (5.2 - 16.4)	9.82 *b*
Number 2	13.5 ± 1.8 (9.2 - 17.2)	13.3 ± 2.9 (7.2 - 21.6)	14.5 ± 2.3 (8.3 - 17.8)	7.82 *b*
Number 3	10.2 ± 1.7 (6.8 - 14.8)	11.1 ± 2.7 (5.5 - 17.8)	12.4 ± 2.2 (9.3 - 19.2)	22.32 *b*
Number 4	7.6 ± 1.3 (3.6 - 11.2)	8.2 ± 2.5 (c)*a* (2.6 - 13.1)	10.3 ± 2.3 (f)*a* (12.1 - 21.1)	44.15 *b*
Posterior free flagellum	6.5 ± 1.3 (3.2 - 9.2)	8.0 ± 5.8 (d)*a* (2.1 - 26.7)	4.4 ± 1.7 (0.4 - 8.5)	28.32 *b*
Protruding part of axostyle	4.3 ± 0.9 (2.4 - 6.0)	4.7 ± 2.1 (1.0 - 13.6)	2.2 ± 0.9 (0.2 - 7.0)	94.39 *b*
Undulating membrane length	-	12.8 ± 3.9 (e)*a* (3.1 - 23.4)	17.2 ± 3.8 (11.0 - 29.2)	

a: number of specimens different from 100: (a)=71; (b)=71; (c)=93; (d)=61; (e)=77; (f)=98; *b:* significance level lower than 0.01

Direct microscopic examination of smears from culture media revealed large numbers of mobile flagellate protozoa. These were classified as trichomonads because of their elongated ellipsoid shape. Accurately counting only became possible when the trichomonads had slowed down or stopped moving. The body is very plastic but not particularly ameboid. Most of these morphological features could be recognized in air-dried smears of culture media fixed in methanol and stained with Giemsa, which produced better results than Heidenhain's iron hematoxylin. Measurements are described in Table I. Trichomonads observed were piriform, whereas living specimens in culture media or stained specimens were usually ellipsoidal, ovoidal, or spheroidal.

There were four anterior flagella unequal in size in the mature individuals (Figs 3, 5). The flagella appear to be applied closely to one another for a short distance anterior to the point at which they emerge from the body. In many permanent preparations they were arranged in two pairs being somewhat longer than the other.

In several specimens a well-developed undulating membrane extended almost the entire length of the body (Figs 3, 6, 7, 8, 9). The undulating membrane often exhibits several bold waves and the outer margin of the membrane consists of the



Figs 1-4: morphology of *Tetratrichomonas didelphidis*, TDM86 strain in Giemsa-stained specimens. Bar = 10 µm. af: anterior flagella; ax: axostyle; b: blepharoplast; n: nucleus; p: pelta; pf: posterior flagellum; um: undulating membrane; v: vacuoles; vs: vacuoles with starch

accessory filament, which extends into a free posterior flagellum (Figs 3, 8, 9).

The prominent costa generally extended for the entire length of the body (Figs 6, 7). It originated in the area of the blepharoplast but its exact origin was obscured by the parabasal body.

There was a spheroidal, ellipsoidal, or ovoid nucleus situated in the anterior portion of the body (Figs 3, 6, 7), clearly discernible in most living and stained specimens. The left ventral surface of the nucleus was applied to the spatulate axostylar capitulum. The anterior part of the capitulum was continuous with the pelta, a crescent-shaped membranous structure that surrounds the area of emergence of the anterior flagella from the cell (Figs 3, 6). Anterior to the nucleus was a blepharoplast where the flagella were inserted (Fig. 9).

Posterior to the nucleus the capitulum continues as a slender, hyaline, somewhat attenuating axostylar trunk that courses near the anteroposterior axis of the organism, its terminal segment projecting approximately one-third the cell length beyond the posterior surface of the flagellate (Figs 3, 8).

The parabasal body was typically disc-shaped with a well-defined constant central granule (Figs 6, 8).

Figs 4 and 10 show a trophozoite of *T. didelphidis* in binary division.

Comparison between the average structural measurements obtained from our work and the data of Andersen and Reilly (1965) (Table II) revealed that their measurements differed significantly (t test > 1.96) from those of the TDM86 and TDLC01 strains. There were significant differences (t test > 1.96) between the TDM86 and TDLC01 strains in nucleus length, nucleus width, parabasal body length, anterior flagella numbers 1, 2 and 3 lengths, posterior free flagellum as well as the protruding part of axostyle length.

DISCUSSION

There are several species of trichomonads found in domestic animals and man, but nomenclature and host-parasite relations still remain unclear. They have been found in the cecum and colon of almost every species of mammal or bird examined, and they also occur in reptiles, amphibia, fishes, and many invertebrates (Levine 1973).

There are other species belonging to the genus *Tetratrichomonas,* the type species *T. prowazeki*, described by Alexeieff (1911), is widely distributed among amphibians and squamate reptiles, in which it inhabits the large intestine.

The most studied species from this genus is *T. gallinarum.* It has worldwide distribution, being harbored by a variety of gallinaceous birds, including chicken, turkey, guinea fowl, quail, and chukar partridge (Bondurant & Honigberg 1994).

T. anatis, reported by Kotlan (1923) occur in the posterior part of the intestinal tract of domestic ducks and the body is broadly beet-shaped.

Two species were reported by Hegner and Ratcliffe (1927a,b) to occur in the mouths of dogs (*T. canistomae*) and cats (*T. felistomae*). The diagnoses of these trichomonads are incomplete, but, as pointed out by Levine (1973), the two organisms actually may belong to a single species.

Hegner and Ratcliffe (1927a) described *T. macacovaginae* from the vagina of a rhesus monkey; *T. anseri,* was observed by Hegner (1929) in the cecal contents of a domestic goose; *T. ovis* occurs in the cecum and rumen of domestic sheep (Robertson 1932). *T. buttrey* was described by Hibler et al. (1960) as occurring in the cecum and colon of pigs and oxen and occasionally in the rumen (cattle) and small intestine (pigs); *T. pavlovi* was originally described by Pavlov and Dimitrov (1957) as occurring in the large intestine of oxen in Bulgaria, and was named for Pavlov by Levine (1961).

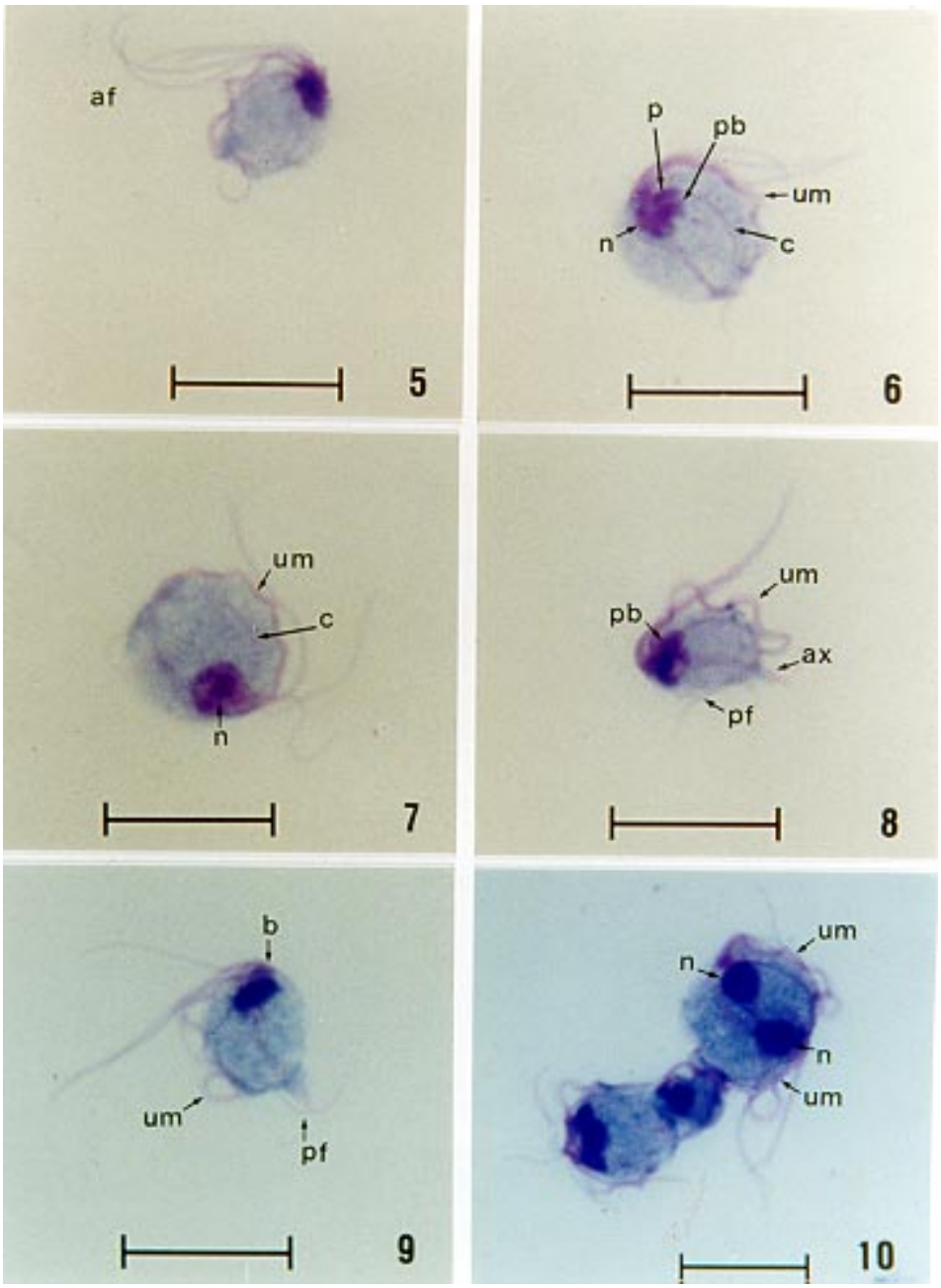
A comparison between the morphology of species of the genus *Tetratrichomonas* showed that *T. didelphidis* is very similar to the others. All species are found in the intestinal tract in their respective hosts, except *T. canistomae, T. felistomae* and *T. macacovaginae.* Furthermore, the species are probably mutualistic symbiontes; however, there is no satisfactory evidence to suggest that any of the species which inhabit the intestinal tract of their hosts are pathogenic. In the light of the presently available information it appears that the only trichomonad species containing frankly pathogenic strains are found in sites other than the intestine (Honigberg 1963).

The evolution of several genera in the subfamily Trichomonadinae is marked by the increased number of anterior flagella accompanied by differences in the structure of the other mastigont organelles. Two genera, *Tetratrichomonas* and *Trichomitus,* may be considered as representing the first evolutionary step. Hence, particularly in actively dividing populations, young organisms with three flagella may be found, and it seems that evolutionary history is repeated in the development of the individual flagellates. Each of the genera is characterized by certain features which they share with *Trichomitus* (Honigberg 1963).

The genus *Tetratrichomonas* is different from *Trichomonas* not only with respect to minor morphological characteristics (parabasal body typically disc-shaped; trunk of axostyle more often slender, relatively stout in some species) but also in the structure of the well-developed undulating membrane, the outer margin of which continues into a

free posterior flagellum. It is the last attribute which most facilitates the distinction between *Trichomitus* and *Tetratrichomonas* on one side and *Trichomonas* on the other. Although it is nearly impossible to be certain which genera, *Tetratrichomonas* or *Trichomonas*, or if both of them, came directly from a *Trichomitus*-type flagellate, it seems that in most features, morphological as well as physiological (for example, the primitive site in the hosts), *Tetratrichomonas* is closer to the main line of evolution (Honigberg 1963). The phylogenetic analysis of *T. didelphidis* is at present, the aim of study of our group.

In the present paper differences were observed between the structural measurements of the TDM86 and TDLC01 strains and the data of



Figs 5-10: morphology of *Tetratrichomonas didelphidis*, strain TDLC01 in Giemsa-stained specimens. Bar = 10 µm. af: anterior flagella; ax: axostyle; b: blepharoplast; c: costa; n: nucleus; p: pelta; pb: parabasal body; pf: posterior flagellum; um: undulating membrane

TABLE II

Comparison between the different strains of *Tetratrichomonas didelphidis* isolated from opossums *Didelphis marsupialis* (TDM86) and *Lutreolina crassicaudata* (TDLC01) and data of other authors by *Post Hoc* Test

Structure	Andersen & Reilly 1965		Present study, strain TDM86		Present study, strain TDLC01	
	Strain TDM86	Strain TDLC01	Andersen & Reilly 1965	Strain TDLC01	Strain TDM86	Andersen & Reilly 1965
Body length	8.81[a]	2.85[a]	8.81[a]	1.45	1.45	2.85[a]
Body width	11.49[a]	2.94[a]	11.49[a]	1.89	1.89	2.94[a]
Nucleus length	10.40[a]	9.38[a]	10.40[a]	6.45[a]	6.45[a]	9.38[a]
Nucleus width	13.49[a]	12.52[a]	13.49[a]	11.49[a]	11.49[a]	12.52[a]
Parabasal body length	13.54[a]	7.10[a]	13.54[a]	8.73[a]	8.73[a]	7.10[a]
Parabasal body width	3.36[a]	2.56[a]	3.36[a]	0.95	0.95	2.56[a]
Anterior flagella						
Number 1	11.89[a]	1.91	11.89[a]	10.69[a]	10.69[a]	1.91
Number 2	6.49[a]	2.62[a]	6.49[a]	4.07[a]	4.07[a]	2.62[a]
Number 3	6.27[a]	4.43[a]	6.27[a]	2.02[a]	2.02[a]	4.43[a]
Number 4	8.54[a]	8.09[a]	8.54[a]	0.62	0.62	8.09[a]
Posterior free flagellum	15.37[a]	3.89[a]	15.37[a]	13.54[a]	13.54[a]	3.89[a]
Protruding part of axostyle	10.37[a]	0.31	10.37[a]	10.15[a]	10.15[a]	0.31
Undulating membrane length	—	—	—	0.65	0.65	—

a: significance level lower than 0.01

Andersen and Reilly (1965). When compared the microphotographs of those authors and the present work, it seems to be the same flagellate protozoa in both studies. The discrepancy between the measurements observed was probably due to the different types of fixatives and stains. Often the shape of trichomonads is variable in fresh and in fixed and stained preparations. Physicochemical conditions (e.g., pH, temperature, oxygen tension, and ionic strength) affect the shape of trichomonads (Honigberg & Brugerolle 1990). In the present study the measured specimens were fixed in methanol and stained with the Giemsa method, while Andersen and Reilly (1965) used the protargol stain. Furthermore, the differences between the TDM86 and TDLC01 strains and the data of Andersen and Reilly (1965) is due to sample variation and the measurements could not be used as a systematic criterion.

Finally, in the present investigation it was observed that *T. didelphidis* found in the intestine content of opossums *D. marsupialis* and *L. crassicaudata* had the same morphological characteristics as those previously described by Andersen and Reilly (1965) in *D. marsupialis*, being the same protozoan species in both different host species.

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