PROTOZOAN INQUILINES FROM REPTILES. I. MONOCERCOMONAS NEOSEPSORUM N. SP. FROM THE SAND SKINK, NEOSEPS REYNOLDSI STEJNEGER.

EUGENE C. BOVEE AND SAM R. TELFORD, JR.¹ University of Florida

The sand skink, *Neoseps reynoldsi* Stejneger, is a small lizard of the family *Scincidae*. It is endemic to central Florida, and has been found only there in sandy mounds of rosemary scrub² and sandhills in Lake, Polk and Highlands counties (Telford, 1959). It is morphologically adapted to existence in loose sand, and has limbs reduced in size and number of digits, a pointed flattened snout, with countersunk lower jaw. Its diet is highly specialized, consisting mainly of termites which inhabit the scrub and sandhills.

Its protozoan inquilines include a hypermastigid flagellate of the genus *Monocercomonas* (= *Eutrichomastix*), but one clearly distinct from the *Monocercomonas colubrorum* Hammerschmidt (1844) of vagile and aquatic reptiles; and also distinct from, though somewhat similar to, *Monocercomonas axostylis* Kirby (1931) described from the termite *Nasutitermes kirbyi*.

MATERIALS AND METHODS

Cloacal feces and gut content were taken from eleven specimens of *Neoseps reynoldsi*. Some material was placed in covered dishes in 25 ml. of Trager's solution (Trager, 1934), to accelerate the growth of trophic protozoa. It was examined at intervals after 24 hrs. Fresh fecal material was diluted with Trager's solution or reptilian Ringer's solution and examined.

Slides were prepared with 4% formalin, AFA, Noland's or Bouin's fixatives, and air dried. Some were observed unstained by phase contrast and interferometric microscopy. Others were stained with Giemsa's hematoxylin or Gomori's hematoxylin as

¹ Present address: University of California, Los Angeles, Zoology Department.

 $^{^{2}}$ The classification of habitats referred to here is that of A. M. Laessle (1958).

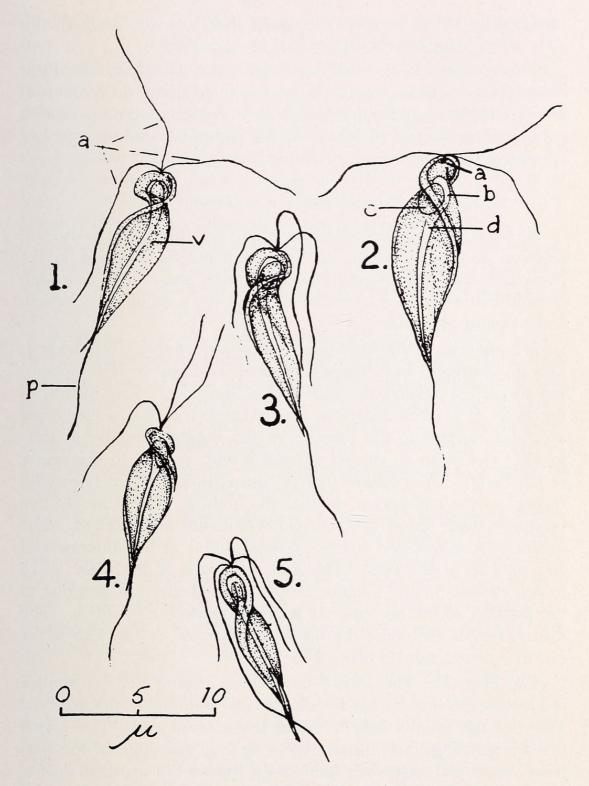


Fig. 1. a. The three anterior swimming flagella. v. vacuole-like central body present in some individuals. p. posterior trailing flagellum.
Fig. 2. a. Kinetosomal complex (mastigont) of the flagella. b. The capitulum of the axostyle. c. The nucleus. d. The shaft of the axostyle.
Figs. 3-5. Other specimens of Monocercomonas neosepsorum n. sp.

modified by Melander and Wingstrand (1953), or were stained with chloroform-extracted methyl green, or congo red.

Microscopic examinations were done also with bright-field microscopy, as well as phase contrast and interferometric systems, at 100x to 1000x magnifications. All measurements were made with calibrated ocular micrometers, or ocular grids; and are for live specimens, unless otherwise indicated.

Results and Observations

Monocercomonas neosepsorum n. sp. was found in fresh fecal material of two specimens of *Neoseps reynoldsi* captured in the rosemary scrub near Winter Haven, Florida; and also in a severalday-old culture from a specimen taken in the sandhills of southern Lake County, Florida.

Morphology of the Protozoan: Size and Shape: The body is laterally flattened and distinctly twisted, with an axostyle appearing to project beyond the rear end of the body. The torsion of the body is marked anteriorly in the region of the nucleus and axostylar capitulum, the rotation of the anterior end being 190° to 210° clockwise as viewed anteriorly with respect to the center of the body. The posterior half is also twisted, 90° or more with respect to the mid-region, so that the body is twisted nearly two complete turns about the axostyle within the body length. The organism is a flattened twisted spindle, blunt at the anterior end and pointed at the rear. The greatest width of the body is 2.5 to 3 μ (widest in the longest, largest individuals) just anterior to the position of the nucleus. It is approximately 1.5μ thick. The overall length, including the tip of the axostyle, is 7 to 12.5 μ , the majority of individuals being 9 to 10 μ long.

The Flagella: There are four of these, arising from a complex of kinetosomes (mastigont) anterior to the capitulum of the axostyle. Three of the flagella beat freely at the anterior end. The fourth is a trailing flagellum, usually lying against one edge of the flattened body and extending behind the tip of the axostyle during locomotion. The trailing flagellum is not firmly attached to the body, however; and it may, and sometimes does, beat freely and independently, particularly as the organism changes direction when swimming.

The exact lengths of the flagella could not be clearly deter-

mined, even with phase microscopy. The tips of all are so slender that they cannot be resolved with a light microscope. In the live protozoan the 3 anterior flagella are about ³/₄ as long as the body (*i.e.* 7 to 8.5 μ long), being somewhat the longer in the larger individuals. They measure about 0.35 μ diameter at the bases, and taper gradually to the tips, which cannot be clearly resolved, and are less than 0.2 μ diameter. In stained specimens these flagella appear thicker, 0.5 μ at the bases, and shorter, about ²/₃ the body length. In stained specimens also, these flagella appear to be acronematic. The trailing flagellum is somewhat thicker, about 0.4 μ at the base in the live organism, 0.6 μ when fixed and stained. It is at least 15 to 17 μ long on the live animal; but appears only 12-14 μ long on the fixed and stained specimens. It is also apparently acronematic, when stained.

Parabasal Apparatus: The parabasal body is difficult to see. It sometimes appears to be a slender rod 4.5 x 1.5 μ , other times a round structure 0.7 μ diameter, adjacent to, and apparently attached to the kinetosomal complex. The ball-like contracted form may be an artifact due to fixing and staining. The parabasal apparatus was not seen in the living organism.

We could find no sign of the presence of a cresta, or pelta, or costa, nor of an undulating membrane.

The Axostyle: The capitulum of the axostyle is about 0.85 μ wide by 1.5 μ long, ovate and flattened. The shaft of the axostyle is narrower, about 0.4 μ just behind the capitulum, tapering to a slender tip less than 0.3 μ diameter at its terminus. It is 6 to 12 μ long. The slender tip does not actually project through the cell membrane, but sometimes appears to do so, and plainly is adherent to it where the tip seemingly projects.

The Nucleus: This is adjacent to the axostylar capitulum, is broadly ovate, slightly broader than thick $(0.4 \times 0.55 \mu)$ and is 1.25-1.45 μ long. When stained with acid-methyl green it is of a homogeneous shade. In the nucleus of certain specimens stained with Gomori's or Giemsa's hematoxylins a small dense nucleolus could sometimes be seen peripherally, usually near the anterior end of the nucleus. It is about 0.2 μ in diameter, barely visible.

Other Inclusions: There were no distinct vacuoles in the living organism. In stained specimens the cytoplasm sometimes appeared areolar. Usually, however, the cytoplasm appeared slight-

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ly granular when stained, with the larger granules in approximate rows along either side of the axostyle, and with some of the larger granules (0.3 to 0.4 μ diameter) in the region surrounding the capitulum and the nucleus. Some of the larger granules appeared spherical, others irregular and perhaps crystalline.

DISCUSSION

The genus Monocercomonas Grassi (1879) is presumably synonymous with Eutrichomastix Kofoid and Swezy (1915) and with Trichomastix Blochmann (Kudo, 1954). Its placement in higher taxa is disputed. Grassé (1953) places it in the subfamily Monocercomonadidae, family Trichomonadidae, order Trichomonadida. Reichenow (1952) recognizes the same genus, family and subfamily, but not the ordinal rank, placing the family in the order Poly-Kudo (1954) recognizes Eutrichomastix in lieu of mastigina. Monocercomonas, family Polymastigidae, order Polymastigina. Hall (1953) recognizes both the orders Trichomonadida and Polymastigida, disagreeing with Kudo by employing the generic name Monocercomonas, in a family Monocercomonadidae, order Trichomonadida. Kirby (1931) uses Eutrichomastix, family Trichomonadidae; and Honigberg (1947) employes Monocercomonas, placing it in a trichomonad family. Calkins recognizes all three genera, and places each separately in group one (including Monocercomonas), and group two (including Eutrichomastix) of a tribe, Monozoa, in the order Polymastigida.

Whatever the placement, the members of the genus more closely resemble the trichomonads in most morphological respects, and are plainly closely related to them, if not perhaps strictly belonging to the same family taxon. They differ mainly from "true" trichomonads in the absence of an undulating membrane with its attendant costa or cresta. A pelta is presumably present in some species of *Monocercomonas* (Grassé, 1953).

Monocercomonas neosepsorum n. sp. differs from other members of the genus, being smaller in size, except for those species found in termites (Kirby 1931; Bernstein, 1928) and beetles (Travis and Becker, 1931; Travis, 1932), and with greater torsion of the body. Monocercomonas colubrorum, the only other species reported from reptiles, is less twisted and much larger. The host of Monocercomonas neosepsorum, the sand skink, Neoseps reynoldsi, is distinctive, also, restricted to an endemic xeric habitat in Florida. Since the host feeds on termites, and perhaps other small insects in its restricted habitat, termites were examined for the presence of *Monocercomonas* spp. in their intestinal tracts. None from the habitats of the lizards harbored any. Hence it is not likely that the organism was surviving in the lizard after digestion of a presumed isopteran host by the lizard. Furthermore, as the protozoan is coprophilic, growing well in fecal suspension in Trager's solution, it could invade by ingestion of the feces.

Diagnosis: Monocercomonas neosepsorum n. sp., inquilinic in the intestinal tract of the Florida sand skink, Neoseps reynoldsi. Length 7-15 μ ; greatest breadth, 3-4.5 μ . Shape flattened, clockwise-twisted spindle around a central axostyle. Four flagella, 3 anterior, 7-8.5 μ long, 0.35 μ diameter at bases, tapering to less than 0.2 μ at tips; one trailing flagellum, adjacent, not attached to body, 15-17 μ long, 0.4 μ thick at base, tapering to less than 0.2 μ at tip. Kinetosomes of flagella form a mastigont. Parabasal body rod-shaped to round, 0.2 x 0.5 μ , or 0.7 μ diameter. Axostyle single, central, with ovate capitulum, 0.85 x 1.5 μ , with nucleus adjacent to capitulum; shaft of axostyle straight, tapering to a point, 0.4 μ diameter at capitulum, 6-12 μ long. Nucleus broadly ovate, 0.4 x $0.55 \times 1.3 \mu$ diametric dimensions, vesicular, without endosome, but with small peripheral nucleolus 0.2μ diameter. Cytoplasm with small granules, no distinct vacuoles. No pelta, costa, nor undulating membrane. From rosemary scrub and sandhills of Lake and Polk Counties, Florida.

SUMMARY

1. A new species of trichomonad flagellated protozoan, *Monocercomonas neosepsorum* n. sp., inquilinic in the intestinal tract of the Florida sand skink, *Neoseps reynoldsi* Stejneger is described and depicted.

2. This new species differs from other members of the genus in being smaller and having greater torsion of the body than *Monocercomonas colubrorum*, the species commonly found in reptiles, and smaller than other species, except for certain ones found in termites, the latter also having less torsion of the body than the new species.

3. *Neoseps reynoldsi* is an endemic lizard, restricted in habitat and diet, and therefore likely to harbor a specialized and restricted group of protozoan inquilines.

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