PROTOZOAN INQUILINES FROM FLORIDA REPTILES. III. *RIGIDOMASTIX SCINCORUM* N. SP.; *CERCOBODO STILOSOMORUM* N. SP.; AND *CRYPTOBIA GECCORUM* N. SP.

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A study of protozoan inquilines from reptiles in Florida not previously examined has revealed a number of new species. Some of these have already been described in an earlier paper (Bovee and Telford, 1962).

We here describe and depict three more species: (1) an ameboflagellate of the genus *Rigidomastix* from the endemic Florida sand skink, *Neoseps reynoldsi* Stejneger. (2) An ameboflagellate of the genus *Cercobodo* from the short-tailed snake, *Stilosoma extenuatum arenicolor* Highton. (3) A flagellate of the genus *Cryptobia* from the reef gecko, *Sphaerodactylus notatus* Baird.

MATERIALS AND METHODS

Fecal samples were taken from reptiles by methods previously discussed (Bovee and Telford *loc. cit.*). Included were: Eleven *N. reynoldsi* from sandy mounds of Polk County, Florida; two newly captured adult *Stilosoma e. arenicolor*, one from Citrus County, the other from Hillsborough County, Florida; and five adult S. *notatus* from Miami, Florida.

Each fecal sample was placed in a separate Stender dish with 15 ml of Trager's (1934) solution A. The *Rigidomastix* were found after 48 hours in material from stomach and intestine of an *N. reynoldsi* from Winter Haven, Florida; and in a sample from one from Lake County, Florida. The *Cercobodo* were seen, after 48 hours, in a sample from the *Stilosoma e. arenicolor* from Hillsborough County, and after 160 hours in the sample from the snake from Citrus County. One of the five *S. notatus* had numerous *Cryptobia*.

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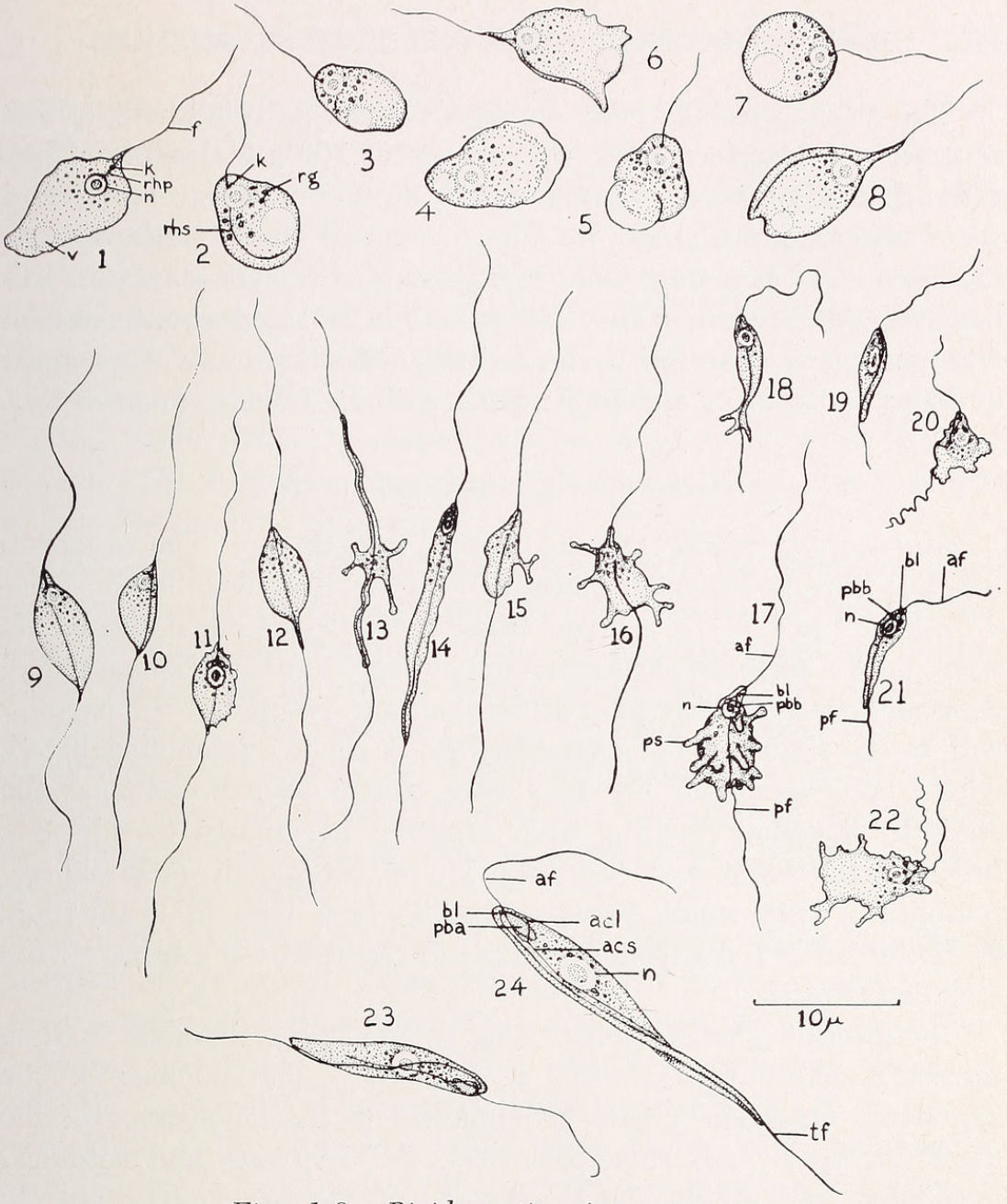

Figs. 1-8. *Rigidomastix scincorum* n. sp.

Fig. 1. Ameboflagellate form. Fig. 2. Flagellated, with a rhizostyle. Fig. 3. An ovate flagellate. Fig. 4. Completely ameboid. Fig. 5. A pyriform flagellate with a large vacuole. Fig. 6. An ameboflagellate. Fig. 7. A spherical flagellate without a rhizostyle. Fig. 8. Another ameboflagellate with a rhizostyle. *f* - flagellum; *k* - kinetosome of flagellum; *rhp* - rhizoplast from flagellum to nucleus; *rhs* - rhizostyle; *n* - nucleus; *v* - vacuole; *rg* - refractile granule.

Figs. 9 - 22. *Cercobodo stilosomorum* n. sp.

Figs. 9, 10, 11, 12. Flagellates, drawn from live specimens. Figs. 13, 14, 15, 16, 17. Ameboflagellates, also from live specimens. Figs. 18, 19, 21. Flagellates, fixed and stained. Figs. 20, 22. Ameboflagellates, fixed and stained. *af* - anterior flagellum; *bl* - blepharoplast; *pbb* - parabasal body; *n* - nucleus; *pf* - posterior flagellum.

Figs. 23 - 24. *Cryptobia geccorum* n. sp.

Fig. 23. A small one, shaped like a slightly twisted spindle. Fig. 24. A large somewhat flattened individual. *af* - anterior flagellum; *acl* - acicular loop; *acs* - acicular shaft; *bl* - blepharoplast; *pba* - parabasal apparatus; *n*- nucleus; *tf* - trailing flagellum.

The organisms all were observed alive by brightfield, phase contrast and interferometric microscopy at 100 x to 1000 x. They were then fixed in 4% formalin and air dried to coverslips, and were observed unstained by phase contrast and interferometric microscopy. Then they were lightly stained in modified Gomori's hematoxylin (Melander and Wingstrand, 1953) and counterstained with eosin, and observed by brightfield. Measurements were made at 400 x, 430 x, 970 x and 1000 x with calibrated ocular micrometers.

Rigidomastix scincorum n. sp.

Morphology of the Protozoan: *Size and Shape*: The organism has five forms: (a) oval, slightly pyriform and flagellated, 7.2—9.7 μ long and 5.5—7.2 μ broad (Figs. 2, 5); (b) spherical and flagellated, 6—8 μ diameter, averaging 6.8 μ (Fig. 7); (c) ameboflagellated, generally ovate, but irregular at the rear, and pointed at the flagellar end, 9—10 μ long, 6—7 μ broad (Figs. 6, 8); (d) ameboflagellated, whole body ameboid, 10—12 μ long, and 6—8 μ broad near the anterior, flagellum bearing end, tapering irregularly to 2.5—3 μ broad at the blunt rear end (Fig. 1); (e) completely ameboid, resembling a very small dientamoeba, 9—12 μ long in locomotion, 6—8 μ broad at the advancing end, and about 2.5—3 μ broad at the rear end (Fig. 4).

Inclusions: *The Nucleus*: This is 1.7—1.9 μ diameter with a refractile endosome 0.75—0.9 μ diameter. The nucleus of the flagellated organism is anterior, attached to the kinetosome of the flagellum by a short rhizoplast (Fig. 1). In ovate and spherical flagellated individuals, the kinetosome of the flagellum is next to the nucleus (Fig. 2).

Vacuoles: A clearly defined spherical vacuole is near the rear end, 2—3.6 μ diameter. If it be a contractile vacuole, its cycle of formation and expulsion is extremely slow, and was not observed. No other vacuoles were seen.

Rhizostyle: A slender, curved, acicular fibril attached to the flagellar kinetosome is present in some of the ovate and semi-ameboid organisms, but was not seen in most. It is extremely delicate, about ¾ as long as the body, barely resolvable in live organisms, and seen clearly only by phase microscopy in fixed but unstained organisms. It was not seen in the ameboid forms nor the spherical forms of the protozoan. (Figs. 2, 6, 8).

Crystals and Granules: A number of crystalloid, highly refractile granules are usually located near the nucleus. They are 0.3—0.6 μ diameter. Others, scarcely resolvable, generally distributed, are also present (Figs. 2, 7).

Flagellum: This is anterior, with kinetosome adjacent to the nucleus, or very near it, and attached to the nucleus by a slender rhizoplast (Fig. 1). The flagellum is very slender and slightly longer than the body, 8—10 μ. It is 0.4 μ diameter at its base tapering to a tip which is barely resolvable, less than 0.2 μ in the live organism. The flagellum beats rapidly and vigorously, but it is not used as a swimming organelle.

Discussion: This ameboflagellate appears to be midway in morphology between *Rhizomastix gracilis* (MacKinnon, 1914) and *Rigidomastix coprocola* (Alexieff, 1929). It also resembles the *Histomonas meleagridis* figured by Bishop (1938), but is only about half the diameter of that organism. Dobell (1940) reported that *Dientamoeba fragilis* has a similar flagellated stage, as well as an ameboid form. The fully ameboid form of this ameboflagellate resembles the figures of the ameboid form of *Dientamoeba fragilis* (Dobell, *loc. cit.*), but is smaller.

Alexeiff (1929) established the genus *Rigidomastix* to include the ameboflagellate *R. coprocola*, found in horse manure. Apparently none other than Alexeiff has since reported it, nor any other protozoan assignable to the genus. Neither genus nor species is generally recognized. Protozoology texts usually ignore it (Grell, 1956; Hall, 1953; Jahn and Jahn, 1949; Kudo, 1954; Reichenow, 1952). Grassé (1953) mentions the organism, calling it a flagellate of uncertain status, perhaps a colorless chrysomonad.

The protozoan cannot be placed taxonomically with accuracy. We assign it to the genus *Rigidomastix* because it is a coprophile, has several forms, ameboid and flagellate, and it more nearly resembles *R. coprocola* Alexeiff than it does any other protozoan we have found described in the literature.

Its affinities above the generic level are so indefinite we believe it presently best to adhere to Grassé's (1953) assignment of it as a flagellate of uncertain status. We do not agree, however, with Grassé's (*loc. cit.*) question-marked statement that *Rigidomastix* spp. may be colorless chrysomonads. They are ameboflagellates, wherever those may be considered to reside, taxonomically.

Cercobodo stilosomorum n. sp.

Morphology: *Size and Shape*: Alive, this protozoan is ovate, tapering abruptly to an acute tip at either end, the anterior tip being slightly rounded at the apex, the posterior tip pointed. It is 6—8.5 μ long, and 2.5—3.5 μ broad (Figs. 9-12). It takes this ovate form when swimming, which it does infrequently, or when creeping on a surface, its usual mode of progress. The body is very ameboid and metabolic, and may briefly become elongate, 15 μ long by slightly more than 0.1 μ broad (Figs. 14, 21). It forms pseudopods, 0.5—3.0 μ long, digitate in form, slightly tapered from base to tip, 0.4—0.5 μ diameter at the bases, and 0.25—0.3 μ diameter at the tips. When these tips touch the substrate they flatten and broaden, ovate to disc-like, about 0.5 μ diameter. Pseudopods are usually single; but two, and sometimes three, may extend from a common basal "stalk" (Figs. 13, 16, 18). Pseudopods may also be sent out along and surrounding the two flagella to lengths of 1—10 μ (Figs. 13, 14), being 0.5 μ or less in breadth. When disturbed, the organism releases its pseudopods from attachment to the substrate and swims, slowly retracting them (Fig. 17).

The Flagella: Two are present. One is anterior, beating above or ahead of the body; the other trails, usually under the body as it moves. Each is about 18—20 μ long in the live animal. The anterior one is 0.45 μ diameter at its base, tapering to a barely resolvable tip, less than 0.2 μ diameter. When fixed and stained the flagella either contract, or the tips break off, and they measure about 0.5 μ diameter and 6—12 μ long. Both arise from a kinetosomal complex at the anterior end.

Inclusions: *The Parabasal Complex*: There are two parabasal bodies, nearly spherical, or short rods with rounded ends. These rest against the nucleus. Each is attached to the kinetosomal complex ("blepharoplast") of the flagella. In fixed organisms the nucleus appears to be attached to the blepharoplast by a rhizoplastic fibril of fibrils. The parabasal bodies are about 0.5 μ long and 0.35—0.45 μ broad. Fibrils which attach them to the blepharoplast are barely resolvable.

The Nucleus: This organelle is anterior, just behind the parabasal bodies and blepharoplast, to which it is attached. It is broadly ovate, nearly spherical, somewhat elastically plastic 1.25—

1.5 μ diameter, with a nucleolar, central endosome 0.5—0.75 μ diameter.

Refractile bodies: Some 15—20 amorphous, perhaps crystalline bodies are present in the anterior third of the body, near the nucleus. Each is 0.3—0.5 μ diameter. Many other tiny granules, too small to be resolved clearly, give the cytoplasm a hazy appearance under phase microscopy.

Vacuoles: None were seen.

Movement and Locomotion: The organism changed form swiftly, from that in Fig. 9 to that of Fig. 12 in three seconds; to that form in Fig. 13 in the next seven seconds; to that in Fig. 14 in another five seconds; to that of Fig. 15 in still another five seconds; then to the form of Fig. 16 in the succeeding twelve seconds. It remained mastigamebid (Fig. 16) for several minutes, was disturbed and swam off, retracting pseudopods. Its non-locomotive movements are very actively ameboid. Locomotion is mainly by the action of the anterior flagellum. As it swims, that flagellum beats at about an angle of 40° to the directional path of progress, the body gyrating in an erratic spiral around the path of advance. The flagellate moves 10—15 μ per second. Swimming is rare, however. The protozoan usually glides along the substrate, with the anterior flagellum acting as a *tractellum*, similar to that of *Petalomonas* or *Peranema*. Only the most anterior ¼ of the flagellum is in motion. The other flagellum trails passively, except during a change of direction. Then it may whip sharply once or twice, swinging the body into a new direct orientation behind the anterior tractile flagellum. The pseudopods are not used in locomotion, and rarely are present when the animal is in progress.

Discussion: Most authors accept *Cercobodo* Krassilstschick as the genus for these semi-ameboid bodonids, assuming *Cercomonas* Dujardin to be a synonym (Hollande, 1953; Pascher and Lemmermann, 1914; Reichenow, 1953; Skuja, 1948). Others accept *Cercomonas* as the generic name (Kudo, 1954; Manwell, 1961). Still others consider both to be distinct and valid genera (Calkins, 1926; Hall, 1953; Jahn and Jahn, 1949). Either genus, when recognized is usually assigned to the family Bodonidae. Many species have been described (Pascher and Lemmermann, *loc. cit.;* Hollande, 1942, 1953; Skuja, 1948, 1956).

The general shape of the body of *C. stilosomorum* n. sp. resem-

bles that of *C. crassicauda*; but *C. stilosomorum* is shorter and smaller, with proportionately longer flagella. The ameboid propensities of *C. stilosomorum* are equalled or exceeded only by *C. draco* Skuja, and *C. cometa* Hollande. *C. stilosomorum* also grossly resembles *C. heimi* Hollande, and has a paired parabasal apparatus similar to that of *C. heimi*, but differs in being more ameboid, and in having longer flagella than *C. heimi*.

C. stilosomorum was found only in the short-tailed snake, though we examined fecal material from over 200 snakes from a wide variety of locales, including 65 species. This rarity, and its presence only in this highly endemic snake further indicates the distinctness of *C. stilosomorum* as a species.

Cryptobia geccorum n. sp.

Morphology: *Size and Shape*: The body is a long slender spindle with about 190° of torsion, slightly to noticeably flattened, 15.2—23 μ long and 2.8—3.2 μ wide at the widest point (Figs. 23, 24). The blunt end of the spindle is the anterior flagellar pole of the body, and the acute end is the rear.

Flagella: two of these arise from the blunt end of the body and extend from the flagellar kinetosomes, which together form a blepharoplast 0.6 μ diameter. The flagella are very slender, 0.3 μ diameter at the bases, and scarcely resolvable at the tips. The anterior one is 12—15 μ long; and the other, apparently attached to the body surface and following the line of torsion along one edge, trails behind another half body length, being 25—30 μ long.

Inclusions: *The Parabasal Apparatus*: This is sausage-shaped in the live animal, 0.45 μ wide, and about 2.3 μ long; and it is attached to the blepharoplast. When stained it is ovate, slightly longer than broad.

The Aciculum: This is needle shaped, 0.3 μ diameter and 8—12 μ long. The posterior end is barely resolvable. The anterior "eye" of the needle is a loop, rounded at the base where it is attached to the blepharoplast and tapering so that its fibrils join at an acute angle to form the shaft of the aciculum. The open part of the loop is 0.45 μ at its widest, and is 0.8 μ long. The loop surrounds the blepharoplast.

The Nucleus: This is vesicular, broadly oval. Its broader aspect is 2.6 x 3.2 μ; and its narrower, 2.3 x 3.2 μ. In fixed specimens

it is practically spherical, 3.3—3.5 μ diameter. It has a dark staining central nucleolar endosome which is approximately spherical, 1.0 μ diameter. The nucleus is located just forward of the center of the body.

Refractile Bodies: About 20 of these, 0.3—0.9 μ diameter are in the forward half of the body. A few are in the rear half. These appear similar to the neutral red stained bodies described in *Cryptobia helicis* by Kosloff (1948). Many smaller granules are visible in fixed and stained specimens.

Vacuoles: None were seen.

Striations and "Myonemes": These structures, reported by Belar (1916) for *C. helicis*, were not seen.

Discussion: The biflagellated protozoa of the genus *Cryptobia* Leidy are inquilines, perhaps parasitic, in the seminal vesicles of snails (Leidy, 1846; Collin, 1914; Fantham and Porter, 1910; Kosloff, 1948), and in the intestinal tracts of leeches (Hesse, 1910), planarians (Fantham and Porter, *loc. cit.*), marine fish (Elmhurst and Martin, 1910; Leger, 1905; Alexeiff, 1910, 1912) and chaetognaths (Hovasse, 1924). Other genera which are sometimes considered synonymous with *Cryptobia* (Hollande, 1953; Kudo, 1954), or distinct (Hall, 1953; Reichenow, 1952) include: (a) *Trypanoplasma* Laverin and Mesnil in the blood of freshwater fish (Laveran and Mesnil, 1910; Leger, 1904; Keysselitz, 1904; Minchin, 1909), with leeches as the vector (Leger, *loc. cit.*; Keysselitz, *loc. cit.*; Robertson, 1911); (b) *Trypanosomoides* Martin in the stomach of marine fish; and (c) *Trypanophysis* in siphonophores (Dubosque and Rose, 1926; Keysselitz, *loc. cit.*). Hollande (1953) also considers the free living genus, *Phanerobia* (Skuja, 1948) to be allied. There are also reports of *Cryptobia* spp. from a salamander (Rankin, 1937) and a frog (Walker, 1910).

C. geccorum n. sp. closely resembles *C. helicis* Leidy in general morphology and size, but the aciculum of the new species is longer, and the parabasal body is shorter and thicker than those structures in *C. helicis*.

No other reptile is reported to be host to *Cryptobia* spp. The reef gecko lives in a habitat well populated by other animals which do harbor *Cryptobia* spp.; *i. e.* a tropical hammock with many species of snails, leeches, fish and amphibians, and could very well have acquired its own cryptobian. Whether the protozoan is an obligate or facultative inquiline we cannot say.

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