Pleurotricha. It has 3 RMC, a bipartite macronucleus, and six small caudal cirri.

Coniculostomum monilata (Dragesco & Njiné, 1971) Njiné, 1979: Hemberger (loc. cit.) transferred this species to the genus *Pleurotricha*. The habitat (temporal waters in the tropical Cameroon) is similar to that of *P. indica*; however, it has a multipartite macronucleus with 9–16 nodes, at least three RMC (Njiné (10) describes 5–6), and a large number of ventral cirri (16, if the presence of 3 RMC is assumed). Although we observed in *P. indica* in some cases some additional cilia between dorsal kineties 4 and 5 and at the right margin of the cell, the number of dorsal kineties of *P. monilata* (11–12) is much higher.

Laurentia macrostoma Dragesco, 1966: This species has some characters in common with *P. indica* (size, number of macronuclear parts) and differs only slightly in the number of RMC and LMC; however, the number of AZM (50–60) is lower in *L. macrostoma*. This species differs also in the number of FVT (37–47) from *P. indica* and in the number of dorsal kineties (3, with numerous scattered single cilia; Hemberger, loc. cit.).

In our opinion this comparison shows clearly that *P. indica* is a true species of the genus *Pleurotricha*, which can be readily identified by its morphological features.

LITERATURE CITED

1. Ammermann, D. & Schlegel, M. 1983. Characterization of two sibling species of the genus *Stylonychia* (Ciliata, Hypotricha): *S. mytilus* Ehrenberg, 1838 and *S. lemnae* n. sp. I. Morphology and reproductive behavior. *J. Protozool.*, **30**: 290–294.

2. Ammermann, D., Steinbrueck, G., von Berger, L. & Hennig, W. 1974. The development of the macronucleus in the ciliated protozoan *Stylonychia mytilus*. *Chromosoma*, **45**: 401–429.

3. Borror, A. C. 1972. Revision of the order Hypotrichida (Ciliophora, Protozoa). *J. Protozool.*, **19**: 1–23.

4. Dragesco, J. & Njiné, T. 1971. Compléments à la connaissance de ciliés libres de Cameroun. *Ann. Fac. Sci. Cameroun* **7–8**: 97–140.

5. Fernandez-Leborans, G. 1984. The morphology of the infraciliature and fibrillar systems of the marine ciliate *Pleurotricha planensis* sp. nov. *J. Nat. Hist.*, **18**: 939–948.

6. Gelei, J. 1954. Über die Lebensgemeinschaft einiger temporärer Tümpel auf einer Bergwiese im Börzonygebirge (Oberungarn). III. Ciliaten. *Acta Biol. Hung.*, **5**: 259–343.

7. Jeffries, W. B. & Mellott, J. L. 1968. New observations on the anatomy of *Pleurotricha lanceolata*. *J. Protozool.*, **15**: 741–747.

8. Jerka-Dziadosz, M. & Frankel, J. 1969. An analysis of the formation of ciliary primordia in the hypotrichous ciliate *Urostyla weissei*. *J. Protozool.*, **16**: 612–637.

9. Martin Gonzalez, A., Serrano, S. & Fernandez-Galiano, D. 1984. New aspects of the morphology of *Pleurotricha lanceolata* (Ciliophora, Hypotrichida): cirral and membranellar patterns and fibrillar systems. *J. Protozool.*, **31**: 347–351.

10. Njiné, T. 1979. Compléments à l'étude des ciliés libres du Cameroun. *Protistologia*, **15**: 343–354.

Received 9 XII 85; accepted 5 IX 86

J. Protozool., 34(1), 1987, pp. 34–39

A New Species of *Perezia* (Microsporida: Pereziidae) from the Argentine Grasshopper *Dichroplus elongatus* (Orthoptera: Acrididae)[1]

CARLOS E. LANGE[2]

Rangeland Insect Laboratory, Montana State University, U.S. Department of Agriculture, Bozeman, Montana 59717-0001

ABSTRACT. The new microsporidium (Microsporida: Pereziidae), *Perezia dichroplusae* n. sp., infects the epithelial cells of the Malpighian tubules of the Argentine grasshopper *Dichroplus elongatus*. Characteristics of the pathogen include the following: development in direct contact with the host cell cytoplasm; bi-, tetra-, and sometimes multinucleate diplokaryotic meronts, rounded or elongate in shape; unikaryotic sporonts and sporogonial plasmodia, elongate in shape; sporoblasts and spores uninucleated; spores highly variable in size (1.6–6.7 by 1.0–2.7 μm, $\bar{x}$ = 3.5 ± 0.09 by 1.5 ± 0.02, n = 100) with eight or fewer polar tube coils and showing a posterior electron-dense inclusion body.

THE widespread Argentine grasshopper *Dichroplus elongatus* Tos is one of the several species responsible for important agricultural losses in Central Argentina (3, 8, 9, 14). During the summers of 1984 and 1985, while searching for natural enemies of *D. elongatus*, a new microsporidium was found in their Malpighian tubules. Based on light and electron microscopy, the pathogen is assigned to the genus *Perezia* Léger & Duboscq, 1909.

MATERIALS AND METHODS

Naturally infected *D. elongatus* were collected during January–March, 1984 and 1985, in natural pastures near Brandsen, Buenos Aires Province, Argentina. For light microscopy studies, smears of healthy and infected tissues were stained with Giemsa (4). Fresh mounts, using saline, agar, or immersion oil were used to measure and photograph spores. Measurements were made either with a filar micrometer or by the photographic method.

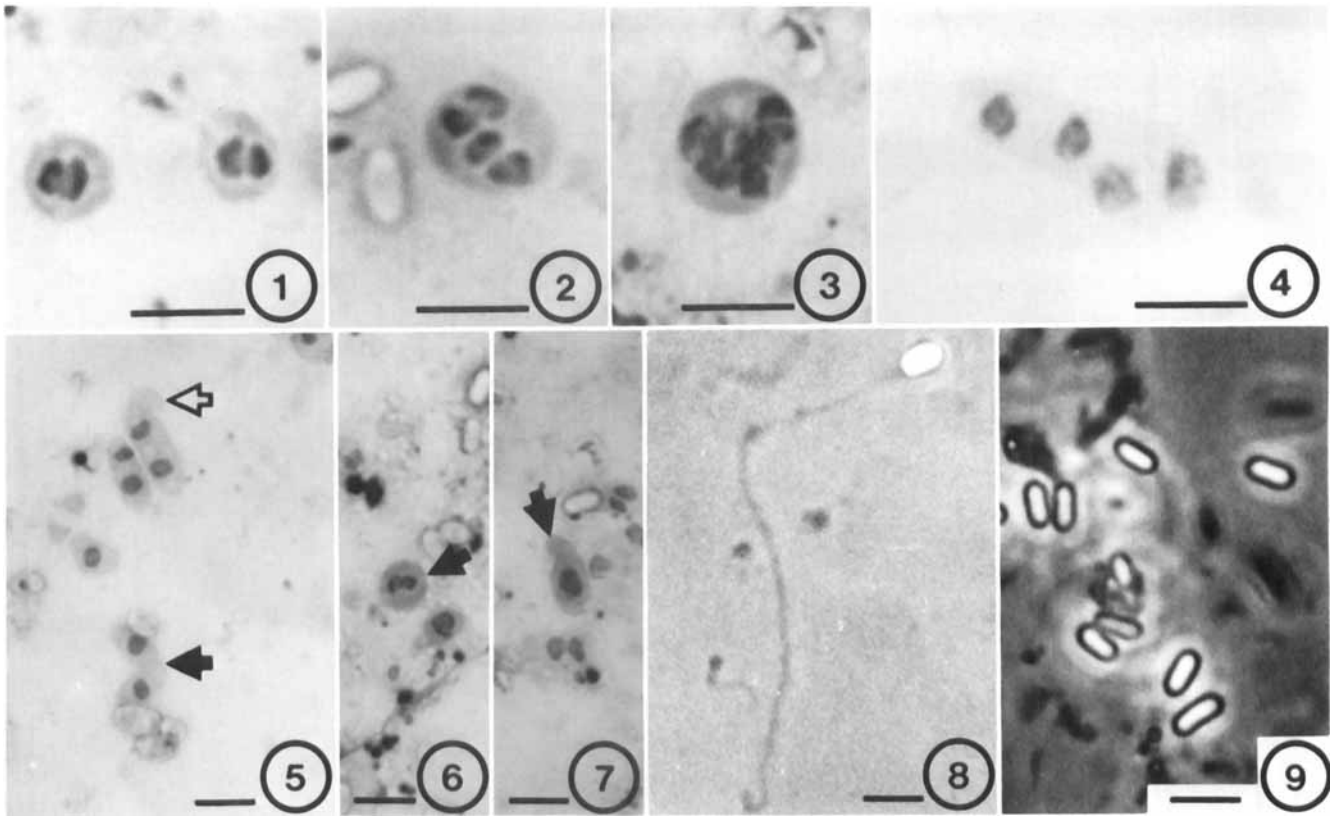
For electron microscopy, small pieces of infected tissues were fixed in 2.5% (v/v) glutaraldehyde buffered with 0.1 M cacodylate buffer, pH 7.4. Postfixation was in 1% (w/v) OsO_4 and was followed by dehydration in an ethanol series. Both a mixture of Epon-Araldite (11) and Spurr's low-viscosity resin (18) were used for embedding. Silver sections were stained with uranyl acetate followed by lead citrate (13) and photographed with a JEOL JEM 100 CX electron microscope at an accelerating voltage of 100 kV.

RESULTS

Light microscopy studies. There were no obvious external signs of infection. Infections were observed only in the epithelial cells of the Malpighian tubules of *D. elongatus*. The earliest stages seen were rounded, sometimes elongated, diplokaryotic

[1] The author wishes to thank Drs. J. E. Henry, D. A. Streett, R. A. Ronderos, and J. J. García for their assistance and advice. Part of this research was sponsored by the World Bank McNamara Fellowship Program, to which the author is thankful.

[2] Fellow from "Comisión de Investigaciones Científicas," Buenos Aires Province, Argentina. Present address: "Centro de Estudios Parasitológicos y de Vectores," Calle 2 No. 584, (1900) La Plata, Argentina.



Figs. 1–9. Developmental stages of *Perezia dichroplusae* n. sp. in Giemsa-stained smears (Figs. 1–7) and fresh mounts (Figs. 8, 9). Bar = 5 μm. 1. Diplokaryotic meronts. 2. Diplokaryotic meront with four nuclei. 3. Multinucleate diplokaryotic meront. 4. Sporogonial plasmodium with four isolated nuclei. 5. Sporogonic stages with one and two isolated nuclei. Cytoplasmic constriction (black arrow) and tail-like projection (empty arrow) are seen. 6. Stage undergoing either dissociation or nuclear division (arrow). 7. Uninucleate sporogonial stage showing large tail-like end (arrow). 8. Spore with extruded polar filament. 9. Spores.

meronts (Fig. 1). Meronts with two diplokarya were common (Fig. 2) while meronts with more diplokarya were infrequent (Fig. 3). Merogony usually occurred by binary fission of tetranucleated diplokaryotic meronts.

Stages in sporogony stained less intensely than meronts and could be distinguished by having unpaired nuclei (i.e., not in the diplokaryotic arrangement). Most stages in sporogony were uni-, bi-, or tetranucleated (Figs. 4–7) although some stages with additional nuclei were occasionally observed. Binucleated stages undergoing cytoplasmic constriction (Fig. 5) and uni- and binucleated stages exhibiting tail-like projections (Figs. 5, 7) were seen. Stages showing nuclear rearrangement, either nuclear division or nuclear dissociation, were also observed (Fig. 6).

The fresh ovocylindrical spores (Figs. 8, 9) varied greatly in

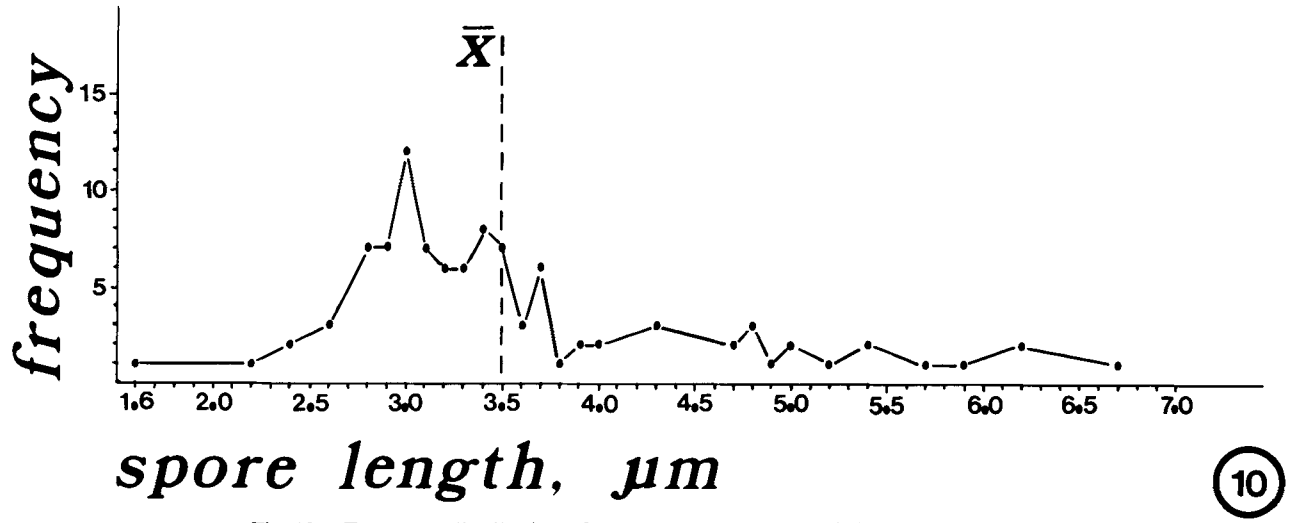
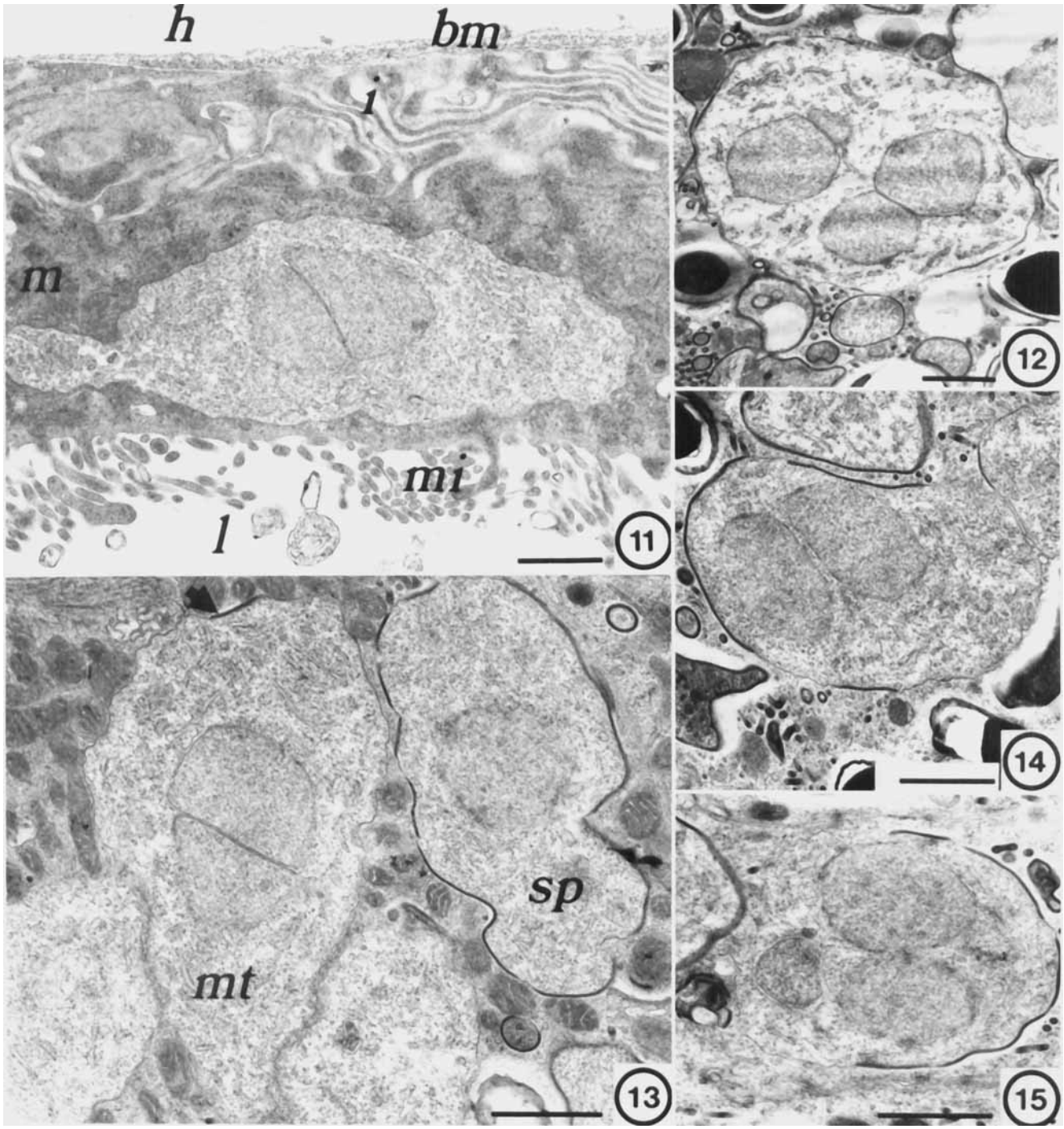


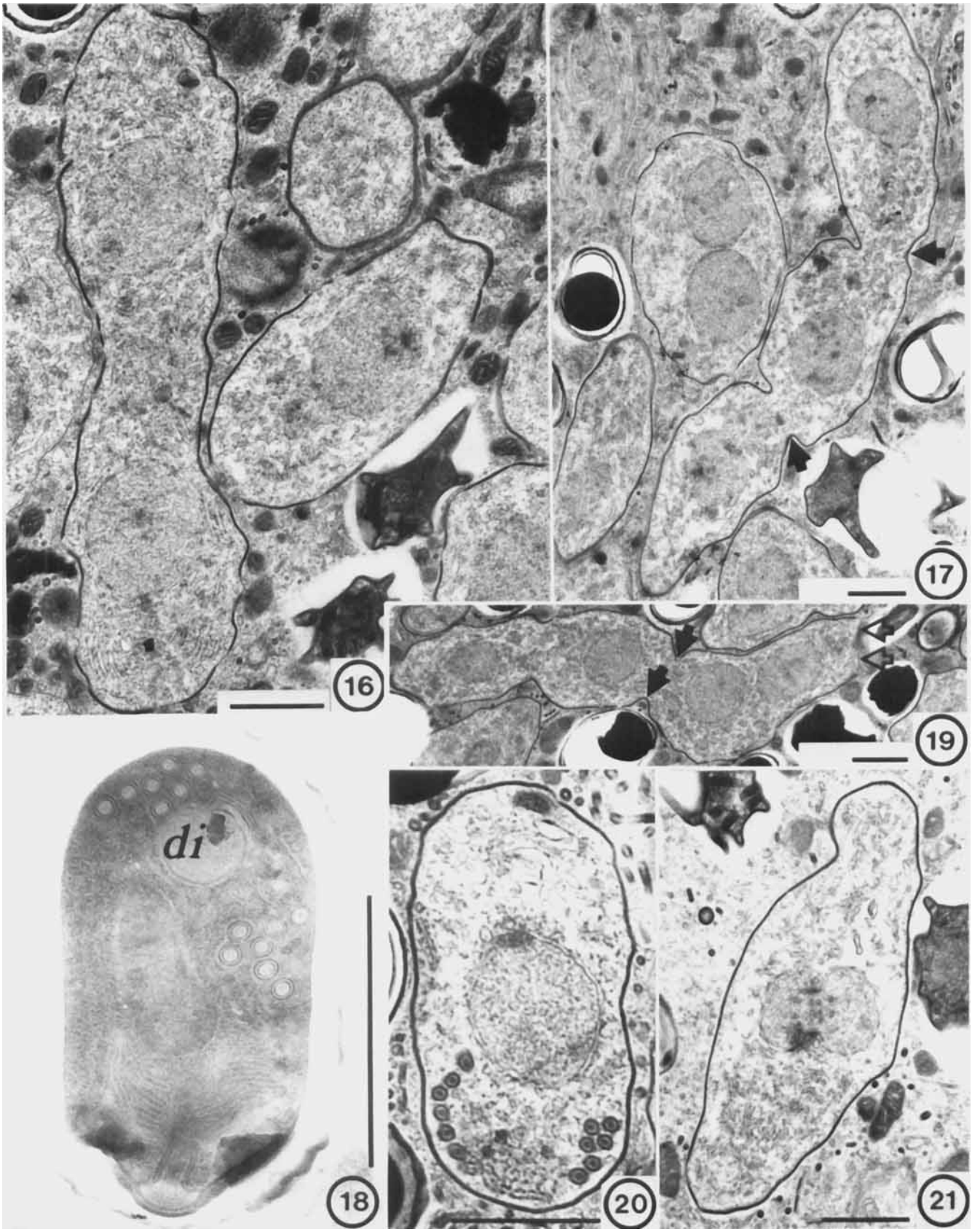
Fig. 10. Frequency distribution of spore lengths in *Perezia dichroplusae* (n = 100).



Figs. **11–15**. Transmission electron micrographs of *Perezia dichroplusae* n. sp. Bar = 1 μm. **11**. Dividing meront in a Malpighian tubule cell. (bm) basement membrane, (h) hemocoel, (i) infoldings, (l) lumen, (m) mitochondria, (mi) microvilli. **12**. Meront showing two diplokarya and somewhat electron-lucent cytoplasm. **13**. Diplokaryotic meront (mt) in which the plasma membrane is beginning to thicken (arrow) and a sprogonial stage (sp) showing one nucleus. **14**. Transitional form showing two nuclei that appear to be separate, also with thickening of the plasmalemma. **15**. Early sporont undergoing nuclear dissociation.

→

Figs. **16–21**. Ultrastructure of late sporogonic stages, sporoblasts, and spores of *Perezia dichroplusae* n. sp. Bar = 1 μm. **16**. Stage showing one nucleus (probably a cross-section of a moniliform plasmodium) and a binucleate stage. **17**. Binucleate form and a moniliform plasmodium showing three isolated nuclei and apparently undergoing cytokinesis (arrows) to form uninucleate stages. **18**. Uninucleate spore. (di) dense inclusion. **19**. Moniliform plasmodium showing four isolated nuclei. Area marked with empty arrows suggests that the plasmodium is longer and passing



out of the section. Cytoplasmic constriction is also seen (black arrows). **20**. Uninucleate sporoblast or young spore. **21**. Stage undergoing nuclear division.

size. They had dimensions (means ± standard error of the mean) of 3.5 ± 0.09 μm by 1.5 ± 0.02 μm, with a range of 1.6–6.7 μm by 1.0–2.1 μm (n = 100). There was no evidence that the extreme variation in length was due to dimorphic development (Fig. 10).

Electron microscopy studies. All stages of the parasite were observed in the cytoplasm of the principal Malpighian cells (10) (Fig. 11) and were in direct contact with the cytoplasm of the host cell. Meronts containing one or two diplokarya were observed (Figs. 11–13). Meront shapes ranged from round (Fig. 12) to elongate (Figs. 11, 13). Two events marked the transition between merogony and sporogony: a thickening of the plasmalemma (Figs. 13–15) and a dissociation of the paired nuclei (Figs. 14, 15, 17). Sporogonial stages, differentiated by their thickened plasmalemma, contained one to four unpaired nuclei (Figs. 13, 16, 17, 19) and always appeared somewhat elongated. Sporogonial stages undergoing nuclear division were observed (Fig. 21). The surface of the stages seen did not present hair-like outgrowths. Sometimes, tubule-like structures were seen in the host cell cytoplasm (Figs. 12, 14, 20, 21) but they did not show physical continuity with the parasite's surface. Synaptonemal complexes were not seen in nuclei of sporogonial stages. Sporoblasts appeared to originate by multiple fission of moniliform sporogonial plasmodia (Figs. 17, 19).

The sporoblasts (Fig. 20) and spores (Fig. 18) contained a single nucleus. Spores also contained a posterior, electron-dense inclusion body and eight or fewer coils of the polar tube.

DISCUSSION

The genus *Perezia* Léger & Duboscq, 1909, was originally established for a hyperparasitic microsporidium of a gregarine from tunicates in France (7). Later, Sprague & Vernick (17) treated it as a junior synonym of *Nosema* Naegeli, 1857. Youssef (23), based on a light microscopy study of a microsporidium pathogen of the alfalfa weevil, *Hypera postica,* restored *Perezia.* Soon after, Sprague (15) again assigned most of the previously described species of the genus, including Youssef's *P. hyperae,* to *Nosema.* Also, he suggested that *Perezia* and *Unikaryon* Canning, Lai & Lie, 1974, may be very similar.

Ormières et al. (12), in an ultrastructural study on *Perezia lankesteriae* Léger & Duboscq, 1909, the type species for the genus, finally clarified the confused situation surrounding the genus. They stated that *Perezia* is a valid genus that should be maintained, and they provided the following characteristics: meronts with diplokaryotic nuclei and polysporoblastic sporonts with isolated nuclei. This kind of sporogony is different from the disporoblastic type that both *Nosema* (16) and *Unikaryon* (2, 19, 20) exhibit. Also, the presence of diplokarya in *Perezia* clearly differentiates it from *Unikaryon,* which has isolated nuclei through all its development (2, 19, 20).

Ameson Sprague, 1977, has a developmental pattern similar to *Perezia* and is also placed in the family Pereziidae. Synaptonemal complexes were observed in early sporonts of *Ameson* (21, 22), and the genus was distinguished from *Perezia* in having hair-like appendages on its sporulation stages (22). Synaptonemal complexes were not observed in *Perezia* by Ormières et al. (12), but these authors only examined late stages in sporulation.

The main characteristics of the microsporidium in the Malpighian cells of *D. elongatus* (i.e. diplokaryotic meronts, sporonts and sporogonial plasmodia with isolated nuclei, polysporous sporogony, uninucleated sporoblasts and spores) clearly allow its placement in the family Pereziidae. Other characteristics, like the lack of synaptonemal complexes and appendages during sporogony and the presence of a posterior, electron-dense inclusion body in spores, similar to that one described by Ormières et al. (12), permit its assignment to the genus *Perezia.* As *P. lankesteriae* is a parasite of gregarines and *Perezia nelsoni* (Sprague, 1950) Vivares & Sprague, 1979, the only other microsporidium lately included in the genus (22), is a parasite of penaeid shrimps, it appears highly unlikely that the microsporidium described here from Argentine grasshoppers is identical with either species. Accordingly, it is considered a new species and the name *Perezia dichroplusae,* after the host genus, is proposed. A wide range of spore lengths is considered to be a peculiar characteristic of *P. dichroplusae.* The final spore size appeared to depend on the length of the cell following multiple fission of moniliform sporogonial plasmodia. Since Ormières et al. (12) redescribed the genus, this is the first report of a *Perezia* microsporidium parasitizing an insect host. Three other microsporidia described from Acrididae (1, 5, 6) have been assigned to the genus *Nosema* and can be differentiated from *P. dichroplusae* by their diplokaryotic sporogonial stages and binucleate sporoblasts and spores.

Perezia dichroplusae n. sp.

Host species. Dichroplus elongatus (Orthoptera: Acrididae).

Type locality. Brandsen, Buenos Aires Province, Argentina.

Host site. Principal epithelial cells of the Malpighian tubules.

Relation to host cell. All stages in direct contact with host cell cytoplasm.

Merogony. Rounded and elongate diplokaryotic stages.

Sporogony. Polysporous. Moniliform plasmodia with isolated nuclei undergo multiple fission to form uninucleate sporoblasts.

Spore. Free and uninucleate. Ovocylindrical in shape and highly variable in size (1.6–6.7 μm by 1.0–2.1 μm; $\bar{x}$ = 3.5 ± 0.09 μm by 1.5 ± 0.02 μm). Polar tube with eight or fewer coils.

Type material. Slides will be sent to the International Protozoan Type Slide Collection, National Museum of Natural History, Washington, D.C. Also, slides will be deposited in the "Centro de Estudios Parasitológicos y de Vectores," University of La Plata, Argentina, and in the USDA/ARS Rangeland Insect Laboratory, Bozeman, Montana.

LITERATURE CITED

1. Canning, E. U. 1953. A new microsporidian, *Nosema locustae* n. sp., from the fat body of the African migratory locust, *Locusta migratoria migratorioides* R. & F. *Parasitology,* **43**: 287–290.

2. Canning, E. U. & Nicholas, J. P. 1974. Light and electron microscope observations on *Unikaryon legeri* (Microsporida, Nosematidae), a parasite of the metacercaria of *Meigymnophallus minutus* in *Cardium edule. J. Invertebr. Pathol.* **23**: 92–100.

3. Hayward, K. J. 1958. Insectos tucumanos perjudiciales. *Rev. Ind. Agr. Tuc.,* **42**: 3–144. (in Spanish)

4. Hazard, E. I., Ellis, E. A. & Joslyn, D. J. 1981. Identification of microsporidia, *in* Burges, H. D., ed., *Microbial Control of Pests and Plant Diseases, 1970–1980.* Academic Press, New York, pp. 163–182.

5. Henry, J. E. 1967. *Nosema acridophagus* sp. n., a microsporidian isolated from grasshoppers. *J. Invertebr. Pathol.,* **9**: 331–341.

6. ——— 1971. *Nosema cuneatum* sp. n. (Microsporida: Nosematidae) in grasshoppers (Orthoptera: Acrididae). *J. Invertebr. Pathol.,* **17**: 164–171.

7. Léger, L. & Duboscq, O. 1909. *Perezia lankesteriae* n. g., n. sp., microsporidie parasite de *Lankesteria ascidiae* (Ray-Lank.). *Arch. Zool. Exp. Gen.,* **41**: 89–93.

8. Liebermann, J. 1970. The current state of the locust and grasshopper problems in Argentina. Proc. Int. Study Conf. Current and Future Problems of Acridology, London, pp. 191–198.

9. Liebermann, J. & Schiuma, R. 1946. Las "tucuras" más perjudiciales de nuestra agricultura y ganadería. *Ins. San. Veg., Min. Agr. Nac.,* **2**: 1–39. (in Spanish)

10. Martoja, R. & Ballan-Dufrançais, C. 1984. The ultrastructure of the digestive and excretory organs, *in* King, R. C. & Akai, H., eds., *Insect Ultrastructure.* Plenum Press, New York, **2**: 199–268.

11. Mollenhauer, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.*, **39**: 111–114..

12. Ormières, R., Loubès, C. & Maurand, J. 1977. Etude ultrastructurale de la microsporidie *Perezia lankesteriae* Léger et Duboscq, 1909. Espece-type: validation du genre *Perezia*. *Protistologica*, **13**: 101–112.

13. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.*, **17**: 208–211.

14. Ronderos, R. A. 1959. Identificación de las especies de tucuras más comunes de la provincia de Buenos Aires. *Agro*, **1**: 1–31. (in Spanish)

15. Sprague, V. 1977. Annotated list of species of microsporidia, *in* Bulla, L. A. & Cheng, T. E., eds., *Comparative Pathology*. Plenum Press, New York, **2**: 31–510.

16. ——— 1978. Characterization and composition of the genus *Nosema*. *Misc. Publ. Entomol. Soc. Am.*, **11**: 5–16.

17. Sprague, V. & Vernick, S. H. 1971. The ultrastructure of *Encephalitozoon cuniculi* (Microsporida, Nosematidae) and its taxonomic significance. *J. Protozool.*, **18**: 560–569.

18. Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, **26**: 31–43.

19. Toguebaye, B. S. & Marchand, B. 1983. Développement d'une microsporidie du genre *Unikaryon* Canning, Lai et Lie, 1974 chez un coléoptère Chrysomelidae, *Euryope rubra* (Latreille, 1807): étude ultrastructurale. *Protistologica*, **19**: 371–383.

20. ——— 1984. Etude ultrastructurale de *Unikaryon mattei* n. sp. (Microsporida: Unikaryonidae) parasite de *Nisotra* sp. (Coleoptera: Chrysomelidae) et remarques sur la validité de certaines *Nosema* d'insectes. *J. Protozool.*, **31**: 339–346.

21. Vávra, J., Canning, E. U., Barker, R. J. & Desportes, I. 1981. Workshop No. 10. Characters of microsporidian genera. *Parasitology*, **82**: 131–142.

22. Vivares, C. P. & Sprague, V. 1979. The fine structure of *Ameson pulvis* (Microspora, Microsporida) and its implications regarding the classification and chromosome cycle. *J. Invertebr. Pathol.*, **33**: 40–52.

23. Youssef, N. N. 1974. Biology and pathogenicity of the microsporidian *Perezia hyperae* sp. n., a pathogen of the alfalfa weevil, *Hypera postica*. *J. Invertebr. Pathol.*, **24**: 282–292.

Received 16 VI 86; accepted 7 IX 86

J. Protozool., 34(1), 1987, pp. 39–47

Structure and Operation of the Feeding Apparatus in a Colorless Euglenoid, *Entosiphon sulcatum*[1]

RICHARD E. TRIEMER and LAWRENCE FRITZ

Department of Biological Sciences and Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08901

ABSTRACT. *Entosiphon sulcatum* is a phagotrophic euglenoid. The tubular ingestion apparatus, called a siphon, is composed of three microtubular rods extending the length of the cell. Within the tube are four large striated vanes arranged much like the blades in a pinwheel. The vanes arise from the microtubular rods and curve towards the center of the feeding apparatus. Sheets of endoplasmic reticulum are positioned adjacent to each of the vanes and surround the perimeter of the apparatus. A cap, supported by a scaffold and anchored into the cytoplasm, covers the opening of the siphon. An elongate invagination of the plasma membrane is positioned adjacent to the edge of the cap and extends downward into the siphon forming the opening. The vanes converge at the anterior end of the siphon and surround the invagination. During feeding, the siphon protrudes from the cell. As the apparatus protrudes the cap is withdrawn to the side, opening the siphon. The vanes spread apart expanding the invagination of the plasma membrane into a large cavity into which ingested food particles are taken.

THE euglenoids are often thought of as a group of photosynthetic flagellates; however, many members of the Euglenida are colorless phagotrophs. These latter organisms are of particular interest if, as suggested by Leedale (2), they are the predecessors of the pigmented euglenoids. Recently, Kivic & Walne (1) suggested that the euglenoids had their origins in the zooflagellates, probably in the Kinetoplastida. One of the criteria used to reach this conclusion was comparative ultrastructure of the feeding apparatus. Unfortunately, few complete descriptions of the feeding apparatus are available in the literature. For this reason we are elucidating the ultrastructure and determining the mechanism of operation of the feeding apparatus in a number of colorless euglenoids.

Perhaps the most elaborate feeding apparatus is found in the genus *Entosiphon*, a common, phagotrophic, fresh-water euglenoid. The general organization of the apparatus has been described in part by Mignot (3). This report elaborates on earlier observations and proposes a mechanism for the operation of this organelle.

MATERIALS AND METHODS

Cells of *Entosiphon sulcatum* (Duj.) Stein were isolated from the Delaware/Raritan canal in New Brunswick, NJ, in the spring of 1985. Cultures were grown in a soil-water medium at 18°C on a 16:8, light/dark cycle with illumination of 25–35 $\mu E/m^2/sec$ provided by 40-watt, cool-white fluorescent bulbs. Cells were quick-fixed in 1% (v/v) glutaraldehyde and 1% OsO_4 (w/v) in 0.05 M sodium phosphate buffer, pH 6.8, for 8 min at room temperature. Following three washes in buffer, samples were post-fixed in 2% OsO_4 in the same buffer for 30 min, rinsed in buffer, and dehydrated. Samples for scanning microscopy were critical point-dried and sputter-coated prior to viewing on a Hitachi S 450 scanning electron microscope. Samples for transmission electron microscopy were infiltrated and embedded in epoxy resin and polymerized at 60°C. Silver-gold sections were stained with uranyl acetate and lead citrate prior to viewing on a Philips 300.

RESULTS

Structure of the feeding apparatus. Entosiphon is a biflagellate, colorless, phagotrophic euglenoid. The flagella and feeding

[1] The authors wish to acknowledge the National Science Foundation (BSR 84-07360) and the Charles and Johanna Busch Fund of the Bureau of Biological Research at Rutgers University for their support of this research.