

LITERATURE CITED

- Augustine, P. C. & Vetterling, J. M. 1984. Effect of Concanavalin A-binding sites on invasion of cultured cells by *Eimeria tenella* sporozoites. *Proc. Helminthol. Soc. Wash.*, **51**:171-172.
- Bristol, J. R., Pinon, A. J. & Mayberry, L. F. 1983. Interspecific interactions between *Nippostrongylus brasiliensis* and *Eimeria nieschulzi* in the rat. *J. Parasitol.*, **69**:372-374.
- Bristol, J. R., Upton, S. J., Mayberry, L. F. & Rael, E. D. 1989. Lack of phytohemagglutinin induced splenocyte proliferation during concurrent infection with *Eimeria nieschulzi* and *Nippostrongylus brasiliensis*. *Experientia*, **45**:752-753.
- Castro, G. A. & Duszynski, D. W. 1984. Local and systemic effects on inflammation during *Eimeria nieschulzi* infection. *J. Protozool.*, **31**:283-287.
- Dubremetz, J. F., Ferreira, E. & Dissous, C. 1989. Isolation and partial characterization of rhoptries and micronemes from *Eimeria nieschulzi* zoites (Sporozoa, Coccidia). *Parasitol. Res.*, **75**:449-454.
- Duszynski, D. W., Russell, D., Roy, S. A. & Castro, G. A. 1978. Suppressed rejection of *Trichinella spiralis* in immunized rats concurrently infected with *Eimeria nieschulzi*. *J. Parasitol.*, **64**:83-88.
- Files, J. G., Paul, L. S. & Gabe, J. D. 1987. Identification and characterization of the gene for a major surface antigen of *Eimeria tenella*. In: Agabian, N., Goodman, H. & Noguiera, N. (ed.), Molecular Strategies of Parasitic Invasion, UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 42. Alan R. Liss, Inc., New York, New York, pp. 713-723.
- Hosek, J. E., Todd, K. S. & Kuhlenschmidt, M. S. 1988. Improved method for high-yield excystation and purification of infective sporozoites of *Eimeria* spp. *J. Protozool.*, **35**:583-589.
- Jenkins, M. C. & Dame, J. B. 1987. Identification of immunodominant surface antigens of *Eimeria acervulina* sporozoites and merozoites. *Mol. Biochem. Parasitol.*, **25**:155-164.
- Jenkins, M. C., Danforth, H. D., Lillehoj, H. S. & Fetterer, R. H. 1989. cDNA encoding an immunogenic region of a 22 kilodalton surface protein of *Eimeria acervulina* sporozoites. *Mol. Biochem. Parasitol.*, **32**:153-162.
- Laemml, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*, **227**:680-685.
- Thompson, J. A., Lau, A. L. & Cunningham, D. D. 1987. Selective radiolabeling of cell surface proteins to a high specific activity. *Biochemistry*, **26**:743-750.
- Tilahun, G. & Stockdale, P. H. G. 1982. Sensitivity and specificity of the indirect fluorescent antibody test in the study of four murine coccidia. *J. Protozool.*, **29**:129-132.
- Tomavo, S., Dubremetz, J. F. & Entzeroth, R. 1989. Characterization of a surface antigen of *Eimeria nieschulzi* (Apicomplexa, Eimeriidae) sporozoites. *Parasitol. Res.*, **75**:343-347.
- Towbin, H., Staehelin, T. & Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA*, **76**:4350-4354.
- Upton, S. J., Mayberry, L. F., Bristol, J. R., Favela, S. H. & Sambrano, G. R. 1987. Suppression of peripheral eosinophilia by the coccidium *Eimeria nieschulzi* (Apicomplexa: Eimeriidae) in experimentally infected rats. *J. Parasitol.*, **73**:300-308.
- Whitmire, W. M., Kyle, J. E. & Speer, C. A. 1989. Protein 20, an immunodominant surface antigen of *Eimeria bovis*. *Inf. Immun.*, **57**:289-290.
- Wisher, M. H. 1986. Identification of the sporozoite antigens of *Eimeria tenella*. *Mol. Biochem. Parasitol.*, **21**:7-15.
- Wisher, M. H. & Rose, M. E. 1987. *Eimeria tenella* sporozoites: the method of excystation affects the surface membrane proteins. *Parasitology*, **95**:479-489.

Received 8-16-89; accepted 11-22-89

J. Protozool., 37(2), 1990, pp. 90-99
© 1990 by the Society of Protozoologists

Nolleria pulicis N. Gen., N. Sp. (Microsporida: Chytridiopsidae), a Microsporidian Parasite of the Cat Flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae)

CHARLES B. BEARD,*** JERRY F. BUTLER* and JAMES J. BECNEL***

*Entomology and Nematology Department, University of Florida, Institute of Food and Agricultural Sciences,
3105 McCarty Hall, Gainesville, Florida 32611,

**Yale MacArthur Center for Molecular Parasitology, Department of Internal Medicine, Yale University School of Medicine,
P.O. Box 3333, New Haven, Connecticut 06510, and

***Insects Affecting Man and Animals Laboratory, U.S. Department of Agriculture,
P.O. Box 14564, Gainesville, Florida 32604

ABSTRACT. A new species of microsporidium, *Nolleria pulicis*, is described and named here from the cat flea, *Ctenocephalides felis*. The genus *Nolleria* is created and placed within the family Chytridiopsidae. The family is slightly modified to accommodate certain features of intracellular development seen in *N. pulicis*, which is otherwise very similar to other species in the family Chytridiopsidae. Sporulation is described from ultrastructural analysis of infected midgut epithelial cells of adult *C. felis*. The term "multiple division by vacuolation" is proposed for describing sporogony as it occurs in this species and certain related species of microsporidia. The probable mode of transmission and apparent absence of merogony are discussed.

Key words. Development, host-parasite relationship, microsporidium, systematics, taxonomy, ultrastructure.

IN a recent study of the prevalence of endosymbionts in fleas from dogs and cats in north central Florida, a microsporidium was observed in approximately one percent of the fleas examined [2]. Parasites were seen in the midgut epithelial cells of *Ctenocephalides felis* and *Pulex simulans*. The purpose of this paper is to describe, name, and classify what we have determined to be a new species of microsporidium in the cat flea, *C. felis*.

MATERIALS AND METHODS

Fleas were collected off dogs and cats belonging to residents of the Gainesville, Florida area or accessed at the Gainesville Animal Shelter. Specimens were combed directly into plastic bags or removed with forceps and brought back to the laboratory where they were anesthetized with carbon dioxide and attached

with an adhesive to the wall of a glass depression slide. The fleas were then dissected in a modified Insect Ringers' solution composed of 7.5 g NaCl, 0.35 g KCl, and 0.21 g CaCl₂ in 1 L potassium phosphate monobasic sodium hydroxide buffer, 0.06 M, pH 7.00.

Dissections were carried out with the aid of a stereoscopic dissecting scope and various tissues observed by phase contrast microscopy. Selected tissues were fixed for electron microscopy in a solution of 2.5% (v/v) glutaraldehyde and 2.0% (v/v) formaldehyde in 0.2 M sodium cacodylate buffer, pH 7.4, postfixed in 1.0% (w/v) osmium tetroxide, dehydrated in graded ethanol solutions and embedded in Spurr's resin [13]. Thin sections were cut on a LKB Ultratome III microtome and poststained in an aqueous solution of 1.0% (w/v) uranyl acetate and in Reynold's lead citrate. The sections were viewed and micrographs taken with a Hitachi HU-11E electron microscope.

RESULTS

Relation to host cell. Infections were observed in epithelial cells in all regions of the midguts of both male and female fleas (Fig. 1). Frequently, a high percentage of epithelial cells in a particular host was infected. No obvious cytopathology was observed. Although in some instances the nucleus of the cell was displaced peripherally (Fig. 2), there was no clear evidence of a special association between the parasite and the host cell nucleus. In some cases, two sites of infection occurred in what seemed to be the same host cell.

Various stages of development were observed in different cells of a single host, including sporonts in the early stages of nuclear division (Fig. 3), multinucleate sporogonial plasmodia (Fig. 4), sporoblasts (Fig. 5), and mature spores (Fig. 6). No vegetative stages or evidence of a merogonial sequence were observed in infected adult fleas.

A delicate membrane, presumably of host origin, lay just outside the parasite plasmalemma. This membrane persisted throughout the entire developmental cycle from early sporont to mature polysporophorous vesicle (Fig. 7–9). Host microtubules were frequently observed running parallel and adjacent to this membrane. In places these microtubules actually appeared to arise from the membrane (Fig. 7, 8).

Sporogony and development of the polysporophorous vesicle. The nuclei of early sporonts were variable in size and shape (Fig. 4). A depression in the nuclear membrane was observed frequently. As nuclear division proceeded, cytokinesis was achieved by the formation of irregularly shaped vacuoles which seemed to enlarge and coalesce (Fig. 10, 11). The membranes of these expanding vacuoles appeared to fuse together, enclosing the partitioned cytoplasm and individual nuclei into primordial sporoblasts (Fig. 12). Other portions of the membranes appeared to push outward toward the sporont plasmalemma giving rise to a 2nd exterior limiting membrane which, together with the plasmalemma, formed the polysporophorous vesicle (Fig. 11). Frequently, small round secondary vacuoles, approximately 0.2–0.5 μm , were formed presumably from membrane fragments cleaved during cytokinesis. These vacuoles appeared to migrate outward and fuse with the newly formed polysporophorous vesicle (Fig. 13). These round vacuoles frequently contained small dense granular or vesicular material.

Sporogenesis and maturation of the polysporophorous vesicle. In some cases, more than one nucleus could be seen within early sporoblasts (Fig. 14), suggesting that nuclear division may continue after the primordial sporoblasts have been formed by the enlarging and coalescing vacuoles. The elements forming the spore extrusion apparatus 1st appeared as membrane-bound clusters of tiny vesicles within uninucleate sporoblasts (Fig. 15,

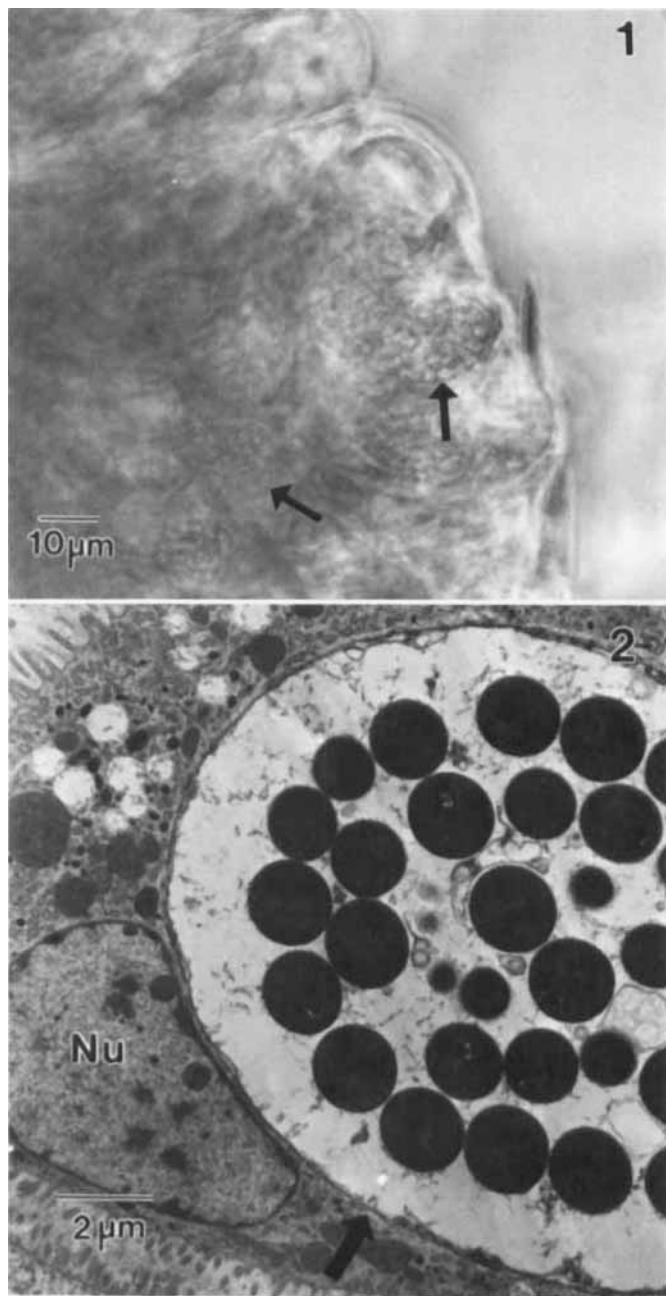


Fig. 1, 2. Polysporophorous vesicles (arrows) of *Nolleria pulicis* in midgut epithelial tissue of *Ctenocephalides felis*. 1. Phase contrast photomicrograph. 2. Electron micrograph showing polysporophorous vesicle and host cell nucleus (Nu).

16). As the spores approached maturity, the inner membrane layer of the polysporophorous vesicle seemed to lose definition in most cases and become dispersed throughout the episporontal space, frequently enclosing individual spores (Fig. 2, 6). Small tubules, approximately 40–50 nm in diameter were also dispersed in the episporontal space (Fig. 2, 6, 17). These tubules along with some filamentous material could sometimes be seen attached to the walls of individual spores (Fig. 17). Polysporophorous vesicles containing mature spores were roughly spherical, measuring approximately 15–20 μm (Fig. 18). As many

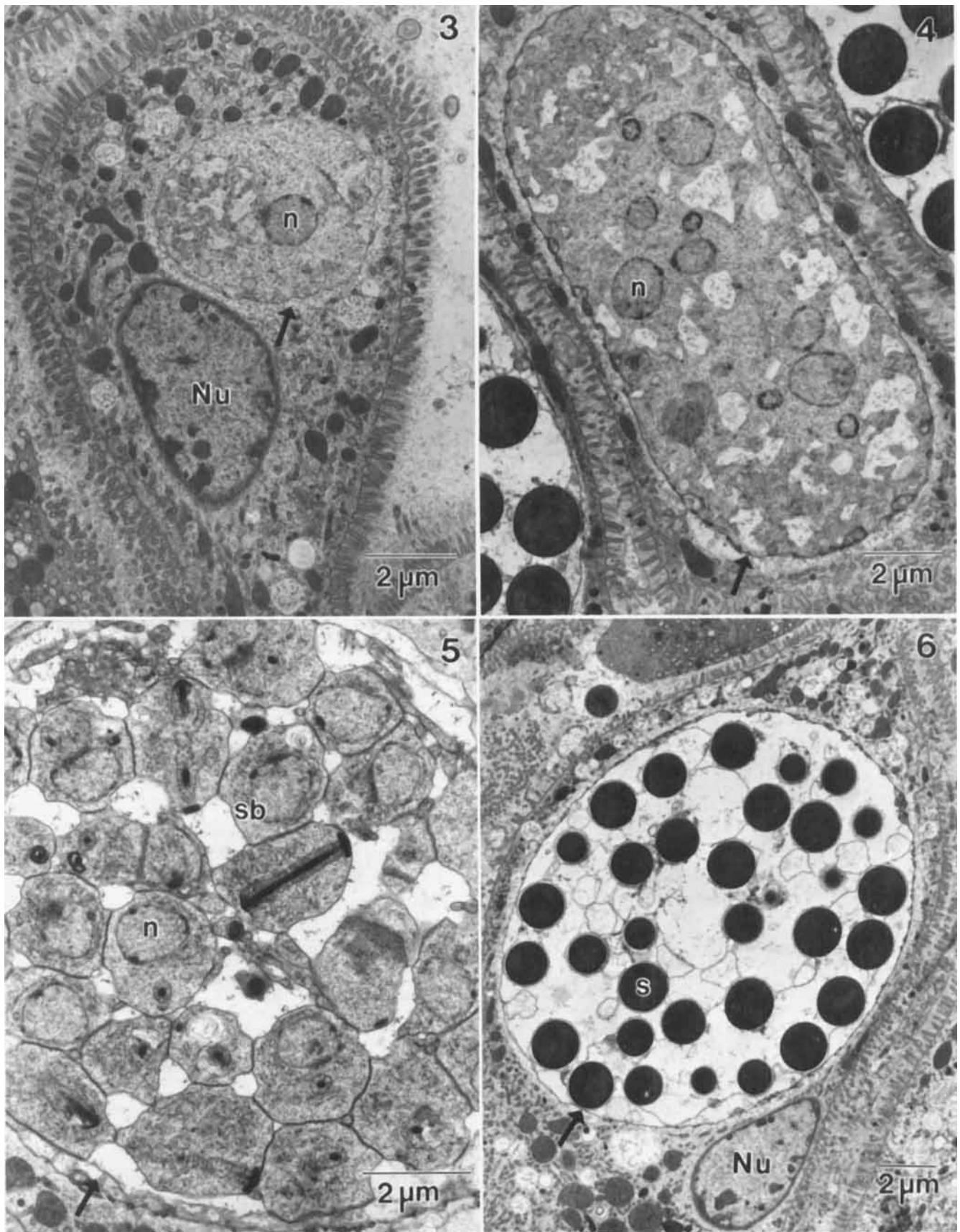
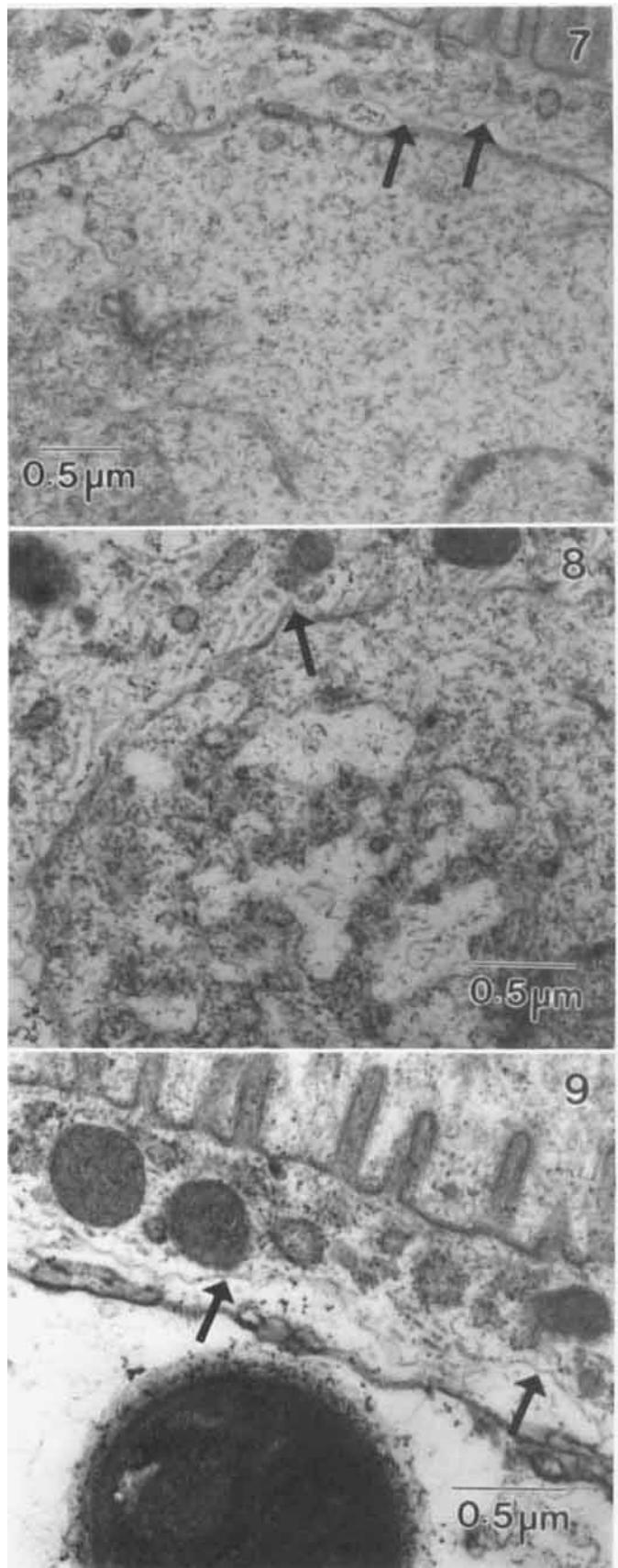


Fig. 3-6. Electron micrographs demonstrating developmental cycle within midgut epithelial cells. Abbreviations: (arrow) parasite, (n) parasite nucleus, (Nu) host cell nucleus, (s) spore, (sb) sporoblast. 3. Early plasmodium at onset of nuclear division. 4. Multinucleate plasmodium. 5. Early sporoblasts. 6. Mature spores within polyporous vesicle.



as 50 spores were counted in a single thin section of a polyporophorous vesicle, and between 100 and 150 spores per polyporophorous vesicle were estimated using light microscopy.

Spore morphology. The spores were spherical and highly refractile as seen by phase contrast microscopy (Fig. 19). Fixed specimens measured in the range of 1.9–2.5 μm in diameter ($\bar{x} = 2.12$, SD = 0.138, n = 32) (Fig. 20–23). A thin electron-dense exospore was present but no endospore evident (Fig. 20). The anchoring disc was crescent-shaped and comprised of a highly electron-dense anterior layer and a less dense posterior layer (Fig. 21). The polar tube was short and thick, approximately 275–300 nm in diameter. It extended posteriorly from the anchoring disc, made two turns around the centrally located nucleus, and terminated in the posterior vacuole. It had an electron-dense core, inconspicuous inner and outer filament tubes and a prominent "honeycomb" layer (as a similar structure in the Chytridiopsidae has been called), consisting of rows of regularly arranged vesicles or alveoli (Fig. 20–23). The honeycomb or alveolar layer was enclosed by an outer membrane and appeared in cross-section as a ring with 9 chambers. The alveolar layer covered the polar tube from its anterior end to its region of entry into the posterior vacuole. There the alveoli appeared to give rise to a system of wavy membranes that encircled the polar tube as it terminated in the posterior vacuole (Fig. 20, 22). The posterior vacuole was rather large, having a diameter approximately one third that of the spore.

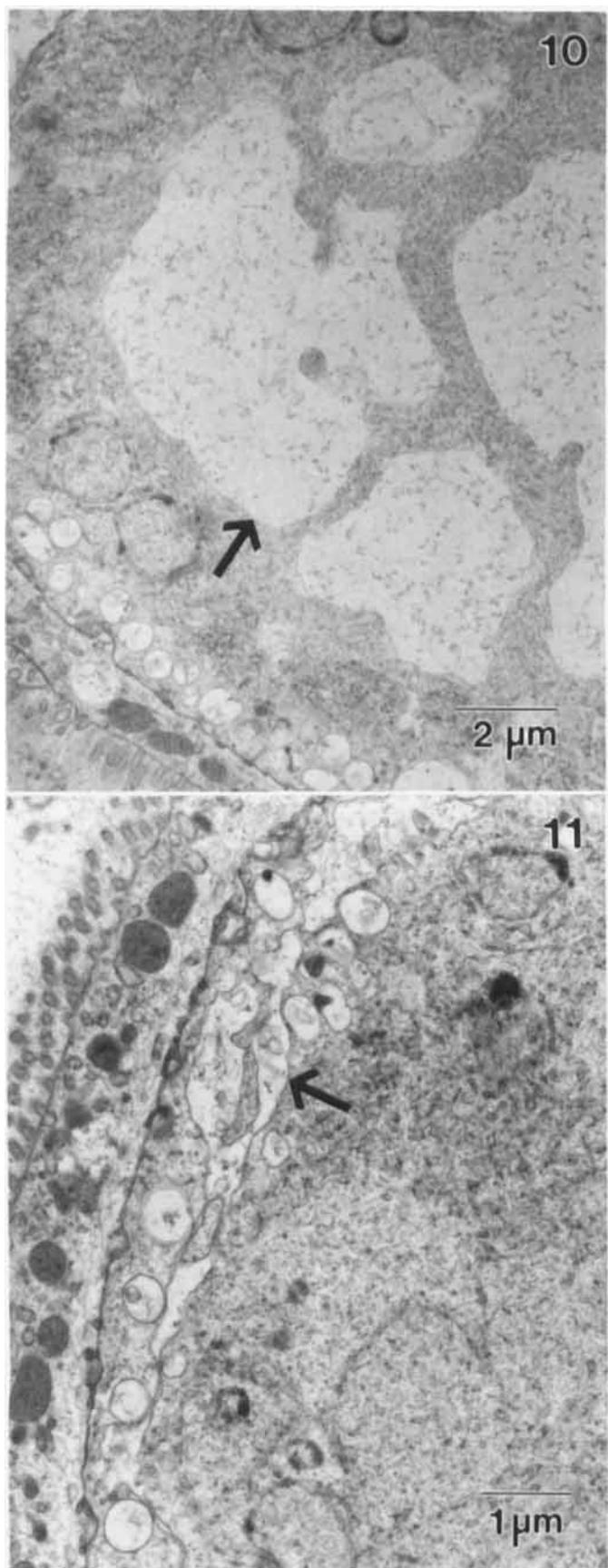
DISCUSSION

Transmission. Although there are no direct data relating to the mode of transmission, a probable mode is apparent. Adult fleas are obligate hematophagous insects. When they locate a suitable host and begin feeding, they defecate particles of dried semi-digested blood. In many flea species, including *C. felis*, this material is actively sought after by larval fleas, constitutes a critical portion of their diet, and serves as a vehicle for transmission of microorganisms from adult fleas to their progeny [1, 2]. These special habits of the flea would insure transmission efficiency and obviate the need for a thick-walled "cyst" as in *Chytridiopsis socius* [12].

The mechanism whereby infective spores contaminate the feces of the flea presumably proceeds as follows: As the spores reach maturity, the inner membrane of the polyporophorous vesicle breaks down, resulting in a loss of structural integrity; the infected midgut epithelial cells eventually rupture releasing the polyporophorous vesicle; the polyporophorous vesicle also ruptures, releasing the mature spores into the midgut lumen where some may infect other epithelial cells of the same gut and others are eliminated with the feces. While this process is speculative, it is supported by the observation of individual mature spores within the midgut lumen of the infected fleas.

Relation to host cell. From the earliest stages observed, probably sporoplasms developing into sporonts (Fig. 3), there was a halo around the parasite which probably indicates some kind of interaction between parasite and host cell cytoplasm. A system of host-derived microtubules was observed consistently in the region of this halo, just external to the polyporophorous vesicle (Fig. 7–9). While the exact significance of these micro-

Fig. 7–9. Electron micrographs showing host membrane and associated microtubules (arrows), exterior to parasite plasmalemma, at different stages in parasite development. 7. Early plasmodium. 8. Multinucleate plasmodium. 9. Mature polyporophorous vesicle.



tubules is not known, their presence is suggestive of a complex host-parasite interaction.

In similar species of the genus *Chytridiopsis*, a close association of the developing parasite with the host cell nucleus is considered a taxonomical feature. The evidence for such an association was not so compelling in the present study. In many sections, stages of the parasite were observed closely adjacent to a host cell nucleus, resulting in distortion of the nucleus. This effect appeared to be due to the extensive size of the polysporophorous vesicle which compressed the nucleus within a limited cytoplasmic compartment.

Apparent absence of merogony. It is significant that the infected cell harbored only one or very few individual parasites and that the parasites were always in some stage of a sporulation sequence. These observations suggest that a merogonic sequence does not occur, at least in adult fleas. The absence of merogony could be explained by the efficiency of transmission. Perhaps microsporidia such as these, that have a very efficient means of transmission, have no need for complicated life cycles and large numbers of spores.

If a merogonic sequence does occur, the larval tissues seem a likely site since it is this stage that acquires the infection. The involvement of other tissues besides midgut epithelium is also a possibility, however.

Type of sporogony. The term "multiple division by vacuolation" is proposed here to describe the type of sporogony observed in the present species. This type of division does not appear to be unique to this species, but has also been reported in a few other microsporidia, notably, *C. typographi* [10], *Buxtehudea scaniae* [7], *Enterocytozoon bieneusi* [3], and *Pseudopleistophora szollosii* [11]. The small round secondary vacuoles observed in this study, presumably representing membrane fragments remaining after cytokinesis, may function in the removal of metabolic products of sporogony. Such a function is suggested by their apparent outward migration, their frequent contents of granular and/or vesicular material, and their fusion to form the inner membrane of the polysporophorous vesicle. The function of vacuoles such as these in the release of metabolic products has been suggested in other species of microsporidia [4].

The completion of sporogenesis appeared to coincide with the degeneration of the inner membrane of the polysporophorous vesicle. Small tubules and filamentous material, apparently remnants in part of the inner membrane, were frequently seen within the episporontal space and around the mature spores (Fig. 2, 6, 17). Purrini & Weiser reported similar observations in *C. typographi* which they referred to as a fixed system of membranes [9].

Spore morphology. The spores observed in this study were similar in their basic ultrastructure to the thin-walled spores of the genus *Chytridiopsis*. Only a single spore type was observed, however, rather than both thin and thick-walled spores as seen in *C. typographi* [10]. The absence of a thick-walled spore in *Nolleria* could be explained in view of the presumed high transmission efficiency. It was suggested by Vavra that the alveolar layer of the polar tube of members of the genera *Chytridiopsis* and *Steinhausia* represents a "special kind of polaroplast" [14]. The membranes that continue posteriorly from the alveolar layer in the present species are very similar to membranes ex-

←
Fig. 10, 11. Electron micrographs of multinucleate sporogonial plasmodia with irregularly shaped vacuoles (arrows). 10. Expanded vacuoles. 11. Coalesced vacuoles.

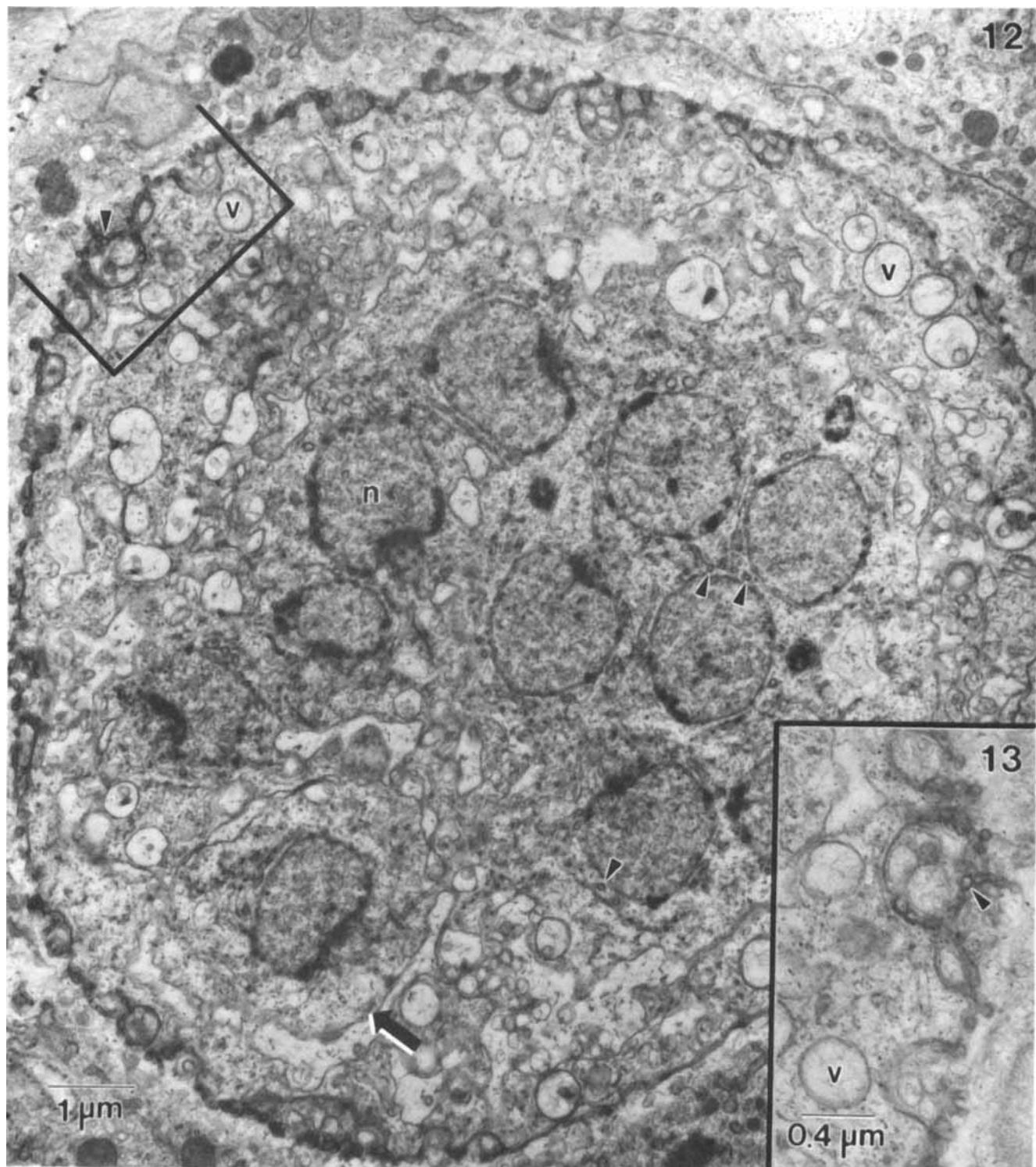


Fig. 12, 13. Electron micrographs of multinucleate sporogonial plasmodium in early stages of multiple division by vacuolation. 12. Large irregularly shaped vacuoles coalesce around an individual parasite nucleus (n) giving rise to a primordial sporoblast (arrow). Smaller secondary vacuoles (v) appear to migrate outward and fuse with the parasite plasmalemma, expelling metabolic products (pointer). 13. Enlargement of secondary vacuole fusing with the parasite plasmalemma.

tending posteriorly in many typical polaroplasts. These similarities suggest that this system of membranes is homologous to the polaroplast of other species in the sense that both structures are modifications of the sheath of the polar tube and apparently function in extrusion of the spore contents.

SYSTEMATICS

Two other species of microsporidia have been described from fleas and both assigned to the genus *Nosema*. Nöller [8] named *Nosema pulicis* from adult gut epithelium, malpighian tubules, fat body, salivary glands, and ovaries of *C. felis*. Korke [5], unaware of Nöller's paper, also named a species of microsporidia from *C. felis* as *Nosema pulicis*. Kudo [6] renamed the latter species *Nosema ctenocephalides*. Although the taxonomic position of these two species as members of the genus *Nosema* is uncertain, they obviously are far removed from the present species which strongly resembles members of the family Chytridiopsidae. A single obstacle stands to placing this species in that family. The present definition of the family Chytridiopsidae contains the statement that the "Sporogonial plasmodium develops in intimate contact with the host cell nucleus" [12]. This obstacle could be removed justifiably by modifying that statement in a redefinition of the family. The justification is that the resemblances of the present species to the members of that family are so overwhelming as to override a difference that may be more apparent than real. The following redescription of the family Chytridiopsidae incorporates suggestions to us by Dr. Victor Sprague.

Family CHYTRIDIOPSIDAE Sprague, Ormieres & Manier, 1972, emend.

Merogony lacking. Development limited to a sporulation sequence. Haplokaryotic throughout. Polysporophorous vesicles present, two types (one a thick wall and the other a fragile membrane) in some species, only one type (the fragile membrane) in others. Spores small, spherical or oval, uninucleate, with thin exospore, little or no endospore, polar tube isofilar, thick, and short to medium in length. No typical polaroplast, but with the outer sheath of the polar tube comprised of an alveolar layer which may be homologous to the polaroplast. Parasites of host cytoplasm, typically one or very few individuals occurring in a host cell. Parasites typically develop while intimately associated with the host cell nucleus, lying in a more or less cupulate depression, rarely occurring inside the nucleus. Genera: *Chytridiopsis* Schneider, 1884; *Steinhausia* Sprague, Ormieres & Manier, 1972; *Nolleria* n. g.

Nolleria n. g.

With basic characters of the family Chytridiopsidae. Lacks the thick-walled polysporophorous vesicle seen in *Chytridiopsis*. Distinguished from *Steinhausia* by being parasites of terrestrial insects rather than aquatic molluscs, by being restricted to midgut epithelium, and by having a different type of sporogony.

Type of sporogony. Multiple division by vacuolation.

Etymology. Name derived from that of W. Nöller, who first reported the occurrence of microsporidia in fleas.

Type species. *Nolleria pulicis* n. sp.

Nolleria pulicis n. sp.

With the basic characters of the genus *Nolleria*.

Type host. *Ctenocephalides felis* (Bouché, 1835) (Siphonaptera: Pulicidae).

Site of infection. Midgut epithelial cells.

Relation to host cell. In cytoplasm within a host-derived vacuole, typically one parasite per cell, often close to host cell nucleus and possibly attracted to it. No apparent pronounced hypertrophy of host cell or other cytological changes.

Development. Sporogony via multiple division by vacuolation, resulting in approximately 150–200 sporoblasts. Plasmalemma of parasite, transformed in process involving vacuolation, develops into a polysporophorous vesicle with delicate inner and outer membranes.

Spore. Spore spherical, 1.9–2.5 μm (fixed), uninucleate, with thin exospore, no endospore. Typical polaroplast absent. Polar tube isofilar, thick, short, turning twice around the nucleus, and terminating in or near the posterior vacuole. Outer sheath of the polar tube characterized by an anterior alveolar region covering most of the tube and a lamellar region covering a short distal portion of the tube before terminating in the posterior vacuole. Posterior vacuole large, having a diameter about one third that of the spore.

Type locality. Gainesville (Alachua County), Florida, USA.

Transmission. Per os (by inference).

Specimens deposited. A type slide (syntype) and a host voucher specimen have been deposited at the International Protozoan Type Slide Collection, Smithsonian Institution (assessment number 41851).

ACKNOWLEDGMENTS

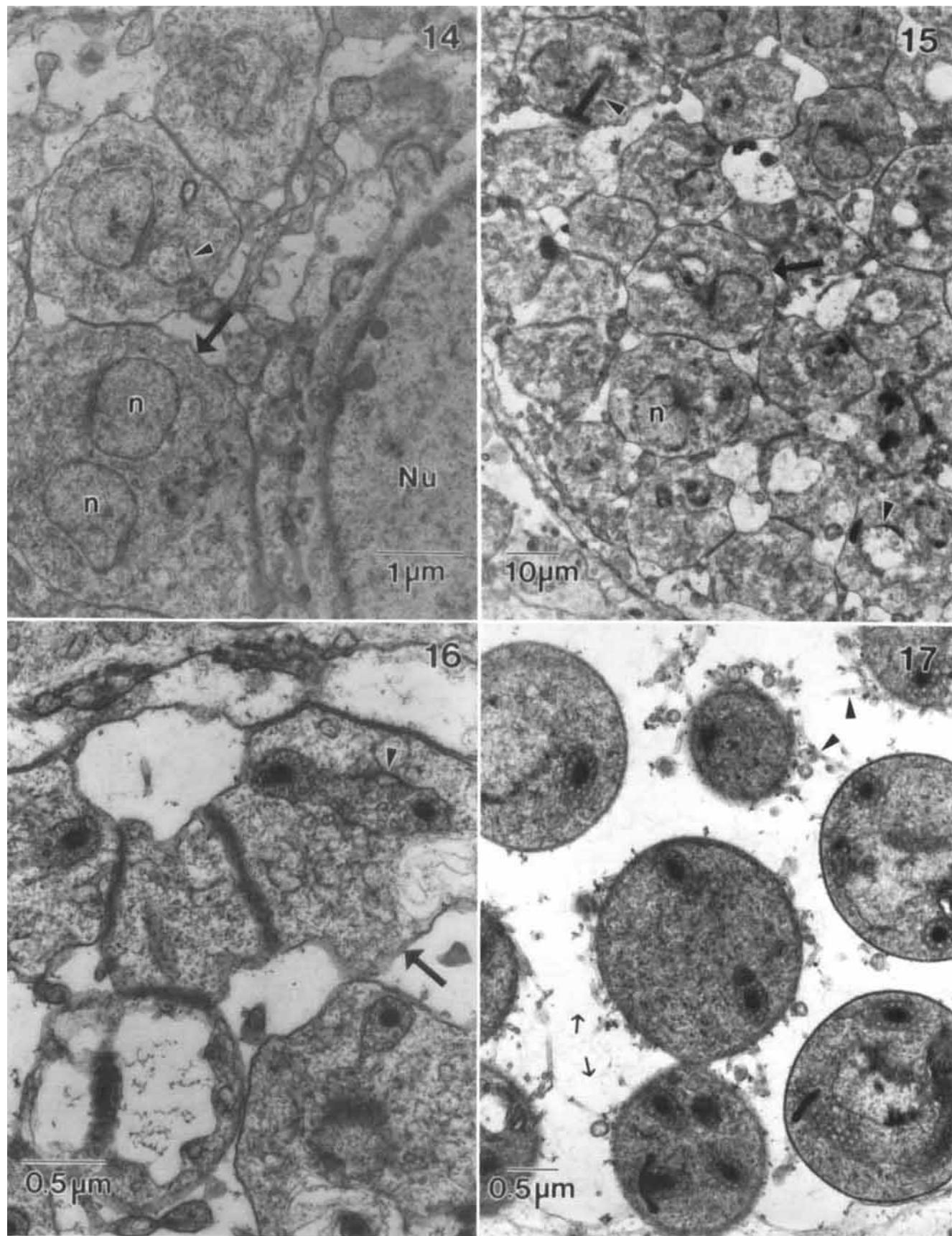
The authors thank Ms. Diana Simon and Debra Boyd for their technical assistance, and the personnel at the IFAS Electron Microscopy Facility, namely Drs. Henry Aldrich and Greg Erdos and Ms. Donna Williams and Debra Akin. The authors also thank Dr. Victor Sprague for reviewing the manuscript. This article is Florida Agriculture Experiment Station Journal Series number R-00101.

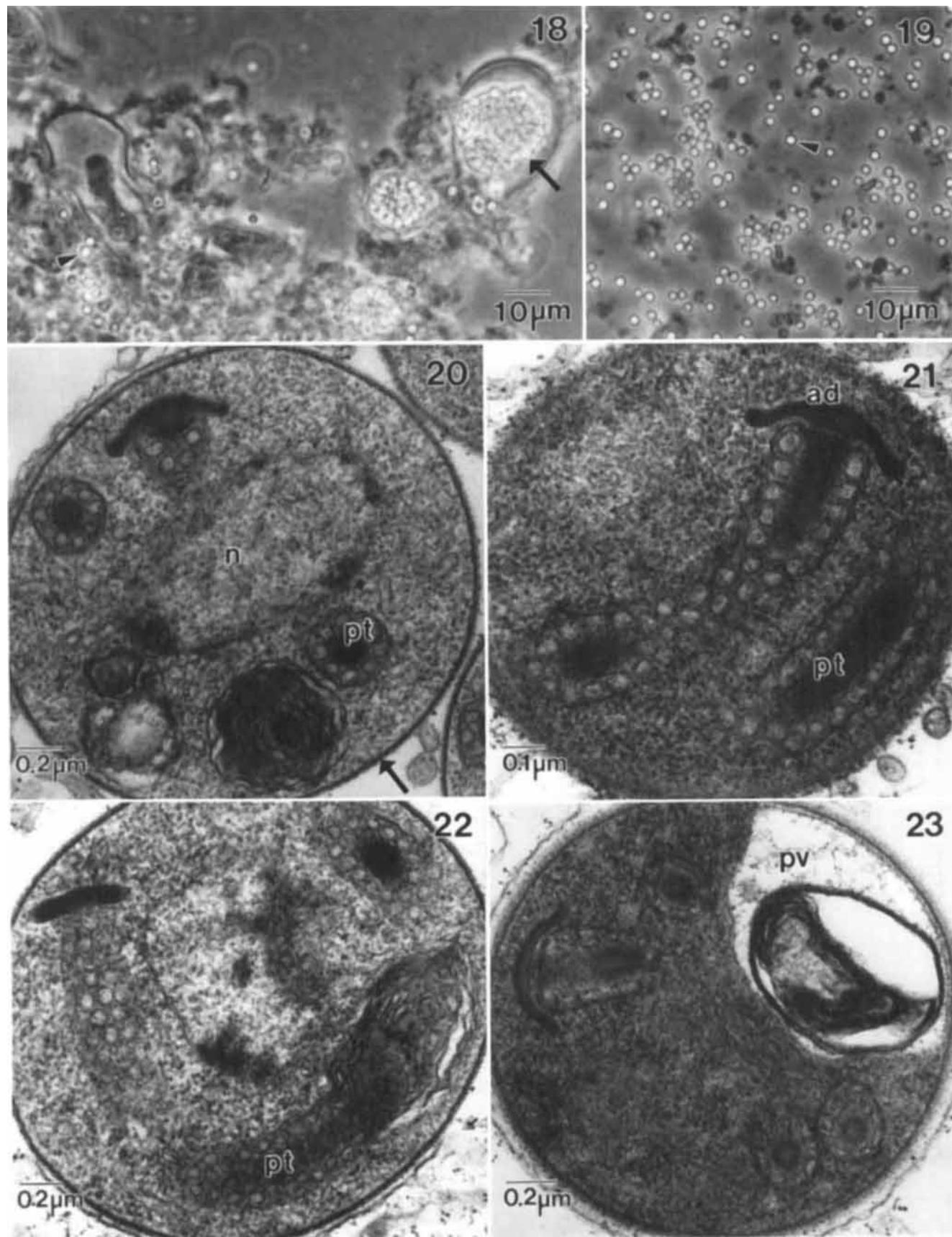
LITERATURE CITED

1. Askew, R. R. 1971. Parasitic arthropods. American Elsevier Publishing Company, Inc., New York, New York.
2. Beard, C. B., Butler, J. F. & Hall, D. W. 1989. Prevalence and biology of endosymbionts of fleas from dogs and cats in Alachua County Florida. *Fla. Entomol.* (in press).
3. Desportes, I., Le Charpentier, Y., Galian, A., Bernard, F., Cochand-Priollet, B., Lavergne, A., Ravisse, P. & Midigliani, R. 1985. Occurrence of a new microsporidian: *Enterocytozoon bieneusi* n. g., n. sp., in the enterocytes of a human patient with AIDS. *J. Protozool.*, 32: 250–254.
4. Hazard, E. I. & Federici, B. A. 1985. Ultrastructure and description of a new species of *Telomyxa* (Microspora: Telomyxidae) from the semiaquatic beetle, *Ora texana* Champ. (Coleoptera: Helodidae). *J. Protozool.*, 32:189–194.
5. Korke, V. T. 1916. On a *Nosema* (*Nosema pulicis* n.s.) parasitic in the dog flea (*Ctenocephalus felis*). *Ind. J. Med. Res.*, 3:729–730.
6. Kudo, R. R. 1924. A biologic and taxonomic study of the Microsporidia. *Ill. Biol. Monogr.*, 9:76–344.
7. Larsson, R. 1980. Insect pathological investigations on Swedish Thysanura II. A new microsporidian parasite of *Petrobius brevistylis*

Fig. 14–17. Electron micrographs showing sporogenesis. 14. Two nuclei (n) are seen within an early sporoblast (arrow). Elements of the extrusion apparatus (pointer) are seen in an adjacent sporoblast. The host cell nucleus (Nu) is to the right of polysporophorous vesicle. 15, 16. Differentiating uninucleate sporoblasts (arrow) with nucleus (n) and developing extrusion apparatus (pointer). 17. Spores and associated tubular (pointers) and filamentous (small arrows) material within episporontal space of polysporophorous vesicle.

Fig. 18–23. Polysporophorous vesicles and spores. 18, 19. Phase contrast photomicrographs of spores (pointers) and polysporophorous vesicles (arrow). 20–23. Electron micrographs of mature spores. Abbreviations: (ad) anchoring disc, (n) nucleus, (pt) polar tube with alveolar layer, (pv) posterior vacuole, (arrow) exospore.





- (Microcoryphia, Machilidae); description of the species and creation of two new genera and a new family. *Protistologica*, **16**:85–101.
8. Nöller, W. 1912. Über Blutprotozoen eingeheimischer Nagetiere und ihre Übertragung. *Berl. Klin. Woch.*, **49**:524–525.
 9. Purrini, K. & Weiser, J. 1984. Light- and electron microscopic studies of *Chytridiopsis typographi* (Weiser 1954) Weiser 1970 (Microspora), parasitizing the bark beetle *Hylastes cunicularius*. *Er. Zool. Anz., Jena*, **212**:369–376.
 10. Purrini, K. & Weiser, J. 1985. Ultrastructural study of the microsporidian *Chytridiopsis typographi* (Chytridiopsida: Microspora) infecting the bark beetle, *Ips typographus* (Scolytidae: Coleoptera), with new data on spore dimorphism. *J. Invertebr. Pathol.*, **45**:66–74.
 11. Sprague, V. 1977. Annotated list of species of Microsporidia. *In: Bulla, L. A. & Cheng, T. C. (ed.), Comparative Pathobiology, Vol. 2, Systematics of the Microsporidia. Plenum Press, New York, New York*, pp. 31–334.
 12. Sprague, V., Ormières, R. & Manier, J. F. 1972. Creation of a new genus and a new family in the Microsporidia. *J. Invertebr. Pathol.*, **20**:228–231.
 13. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, **26**:31–43.
 14. Vávra, J. 1976. Structure of the microsporidia. *In: Bulla, L. A. & Cheng, T. C. (ed.), Comparative pathobiology, Vol. 1, Biology of the Microsporidia. Plenum Press, New York, New York*, pp. 1–85.

Received 7-19-89; accepted 11-14-89

J. Protozool., **37**(2), 1990, pp. 99–107
© 1990 by the Society of Protozoologists

Uronychia transfuga (O. F. Müller, 1786) Stein, 1859 (Ciliophora, Hypotrichia, Uronychiidae): Cortical Structure and Morphogenesis during Division

BRUCE F. HILL

Department of Biology, Mount Vernon College, Washington, D.C. 20007

ABSTRACT. Morphogenesis of cell division was investigated in *Uronychia transfuga* utilizing both light microscopy of living and stained specimens and SEM of preserved specimens. The cortical morphogenetic pattern of *Uronychia* is similar in several respects to that of the members of the family Euplotidae. These features include: the de novo development of the opisthe oral primordium in a subcortical pouch; the development of frontoventral and transverse cirri for both the proter and opisthe from 5 cirral primordia that form de novo within a single latitudinal developmental zone; and the absence of right marginal cirri. The members of the genus *Uronychia* also show a number of unique characteristics: development of a proter oral primordium that causes partial replacement of the parental adoral zone of oral polykinetids during development of the proter; a large oral membrane that is divided into a right and left component; large caudal cirri that bend to the left; and dorsal kineties comprised of closely set paired-kinetosome kinetids. When compared to the other euplotid-like ciliates, these unique features support the placement of the genus *Uronychia* in a separate family, Uronychiidae.

Key words. Cortical morphogenesis, hypotrich, infraciliature, taxonomy, *Uronychia*.

CHARACTERISTIC ciliary structures such as the adoral zone of oral polykinetids, the endoral and paroral membranes, and different functional groups of cirri have been important in descriptive taxonomic studies of ciliates. Differences in morphogenetic patterns of ciliary structures during cell division involving the extensive alteration of the cortex, including resorption of parental organelles, growth and development of new organelles in daughter cells, and the migration of kinetidal organelles complexes, have also proven useful in taxonomic considerations [1, 4–6, 9, 17, 22–25, 30, 35–37]. Several studies on cortical events of predivision morphogenesis in the euplotids *Euploites* [29, 43], *Aspidisca* [13, 14, 18], *Certesia* [41], *Diophysys* [11, 19], *Discocephalus* [40] and *Euplotaspis* [Hill, B. F. 1979. *J. Protozool.*, **26**:18A] have led to a better understanding of taxonomic relationships among the members of the order Euplotida within the subclass Hypotrichia of the class Nassophorea [30].

The members of the genus *Uronychia* are among the most distinctive ciliates of the order Euplotida in both structure and behavior. First to describe a *Uronychia*-like protozoon was O. F. Müller [27] who named it *Trichoda transfuga*. The genus *Uronychia*, which now contains 6 nominal species [4], was erected by Stein [32, 33] and was most recently placed by itself into its own family, Uronychiidae, by Jankowski [20, 21] even though it shares a common predivision morphogenetic pattern with other euplotids assigned to different families [4, 9, 13, 18, 19, 29, 30, 41].

Uronychia, with its elaborate oral membranes and giant posterior cirri, has been used to investigate cortical morphogenetic processes [7, 12, 34]. Wallengren's [38] classical investigation of *U. transfuga* confirmed that ciliary organelles of the parental cell are resorbed during fission while a new set simultaneously develops for each of the daughter cells. Knowledge of the cortical reorganization process of regeneration [7, 12, 44] was summarized and extended by Taylor [34]; however, since these original studies, only three incomplete observations of the process of predivision morphogenesis have been made [4, 42, Hill, B. F. 1978. *J. Protozool.*, **25**:28–29A]. Among these accounts of cortical morphogenesis, there are differences in the interpretation of the origin of several of the ciliary structures. According to Wilbert & Kahan [42], the left marginal cirri (as termed by Borror [5] and Hill [18] on topographical and developmental characteristics) are in fact ventral cirri because they develop de novo. Wilbert & Kahan [42] also report that the morphogenesis of *U. transfuga* differs from all other euplotid genera in that the 5 cirral primordia of the frontoventral and transverse cirral systems develop by the proliferation of kinetosomes from the parental transverse cirri rather than forming de novo, as has been reported in other euplotid-like ciliates [13, 18, 19, 41].

The study of the cortical structure and ontogeny of the ciliary organelles in *Uronychia* presented in this paper clarifies aspects of the morphogenetic processes involved and also the taxonomic position of the genus, lending support to Jankowski's [20, 21] idea of a monotypic family, Uronychiidae.