

A New Microsporidian Parasite, *Flabelliforma montana* n.g., n.sp., Infecting *Phlebotomus ariasi* (Diptera, Psychodidae) in France

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Flabelliforma montana, a new microsporidian genus and species, is described from the sandfly *Phlebotomus ariasi*. The parasite was found in sandflies collected in the commune of Roquedur, Gard, France, and represents the first microsporidium recorded from Old World sandflies. Meronts lie in direct contact with the host cell cytoplasm and divide by binary fission or plasmotomy. Sporogonial plasmodia divide by multiple fission within a sporophorous vesicle, passing through a lobed fan-like stage before division into uninucleate sporoblasts. All stages have unpaired nuclei. Transmission is direct. *Flabelliforma* is one of nine genera of microsporidia with multisporous sporogony, all but one of which are currently considered to belong to one family, Pleistophoridae, but in reality are not closely related. The characters which differentiate *Flabelliforma* from the other multisporous genera are discussed and a key is provided for identification of the genera. © 1991 Academic Press, Inc.

KEY WORDS: *Phlebotomus ariasi*; *Flabelliforma montana* n.g. n.sp.; Microspora; Pleistophoridae; direct transmission; life cycle; multisporous sporogony; key to genera.

INTRODUCTION

Although microsporidia are known to be common parasites of mosquitoes (Diptera, Culicidae), there are few published records of infections in sandflies (Diptera, Psychodidae). Ward and Killick-Kendrick (1974) found an unidentified microsporidium in the midgut of *Psychodopygus lainsoni* from Brazil. Lainson et al. (1976) reported developmental stages and spores of a microsporidium in the Malpighian tubules of *Psychodopygus complexus* and commented that microsporidia had been encountered in other sandfly species. Lainson et al. (1977) reported four microsporidioses during routine dissections for *Leishmania* promastigotes in a species of *Lutzomyia* of the *anduzei* group also from Brazil: of these, the two in the Malpighian tubules, which were probably the same species, were attributed to the genus *Pleistophora* and the two different types in the gut wall were attributed to *Thelohania* and *Microsporidium*, respectively. Canning (1977) described some of the stages of the *Thelohania*-like parasite from this sandfly, then identified as *Psychodopygus maripaensis*.

Finally, Lawyer (1984) reported massive infections of unidentified microsporidia in the hemocoele of 3–8% of female *Lutzomyia diabolica* collected in Texas. When fed spores, larvae of the same species failed to become infected.

We have examined specimens of *Phlebotomus ariasi* collected in the Cévennes mountains in southern France. In 1976 and 1977, 6 adult female *P. ariasi* were found infected with microsporidia, 5 in the midgut and 1 probably in the fat body but prevalence figures were not obtained. Of 400 *P. ariasi* examined in 1982, 5 were infected with microsporidia in the midgut, 1 in the Malpighian tubules, and 1 in the fat body. None of these infections was examined in detail nor were the parasites identified. In 1987, 5 out of 74 *P. ariasi* examined were infected with the midgut parasite. The spores were fed to the progeny of an infected female fly and studies on its development and on the infectivity of the parasite are reported here.

MATERIALS AND METHODS

P. ariasi adult female flies, caught in CDC miniature light traps in July, 1987,

were fed on a dog 4–5 days later and were caged for 10 days. They were then transferred individually to tubes and maintained on a 50% sucrose solution. One fly was moribund later and on dissection was found infected with microsporidia in the midgut wall. The gut and terminalia were put into a pot lined with moistened plaster of Paris and kept at 10–24°C. The fly had laid 56 eggs which, after 6 days, were put into the pot containing the remains of the fly, together with 5 eggs laid by another fly. Larval food, consisting of a matured mixture of equal parts of rabbit feces and commercially available rabbit pelleted food, was added after 4 days when the eggs began to hatch. About 50 first instar larvae counted the next day were still alive 5 days later and survived transit to England over the next 3 days. All were second instars when examined the next day. Two larvae were examined on each of five occasions during the third and fourth instars, in Giemsa-stained smears of dissected midguts. For electron microscopy, the dissected midguts were fixed in Karnovsky's fixative for 10 min at room temperature then cut into smaller pieces and transferred to fresh fixative at 4°C for 1 hr. After washing in two changes of 0.1 M cacodylate buffer, pH 7.4, for 30 min, the tissue was postfixed in 2.5% OsO₄ in 0.1 M cacodylate buffer for 1 hr. Still at 4°C, it was rinsed in two changes of 0.1 M sodium acetate, transferred to 0.25% aqueous uranyl acetate for 1 hr, rinsed again in

0.1 M sodium acetate, and passed through 35 and 50% uranyl acetate overnight. Subsequent dehydration in acetone was at room temperature and the tissue was embedded in Spurr's low viscosity resin. Although fixation for electron microscopy was not completely satisfactory in that there was loss of some membranous structure, sufficient detail was preserved to permit a description of the new microsporidium.

RESULTS

Light Microscopy

The transition from merogony to sporogony was not clear by light microscopy as stages with nuclear numbers varying from 1 to 32 were seen. However, stages with up to eight nuclei were believed to be meronts which could divide at the binucleate, tetranucleate, or octonucleate stages (Figs. 3, 6). Uninucleate stages (Figs. 1, 4) measured 2–3.5 μm in diameter, binucleate stages (Figs. 2, 4) varied from 3.5 to $7 \times 4.5 \mu\text{m}$ in diameter, and tetranucleate stages (Fig. 5) and octonucleate stages (Fig. 7) measured 6 $\times 4.5 \mu\text{m}$ up to $11 \times 5.5 \mu\text{m}$.

The stages with numbers of nuclei greater than 8 (Figs. 8, 9) were interpreted as sporogonial plasmodia, although there may have been some overlap with merogonic stages. Rounded or ellipsoid stages with more than 30 nuclei measured up to $11 \times 8 \mu\text{m}$. Division could commence in stages with fewer nuclei. Sporogonial

FIGS. 1–19. Light micrographs from Giemsa-stained smears. Bar = 10 μm .

FIG. 1. Uninucleate meront.

FIG. 2. Binucleate meront.

FIG. 3. Dividing meront.

FIG. 4. Uninucleate and binucleate meronts.

FIG. 5. Tetranucleate meront.

FIG. 6. Dividing octonucleate meront.

FIG. 7. Octonucleate stage, a meront or early sporont.

FIGS. 8 AND 9. Multinucleate sporonts.

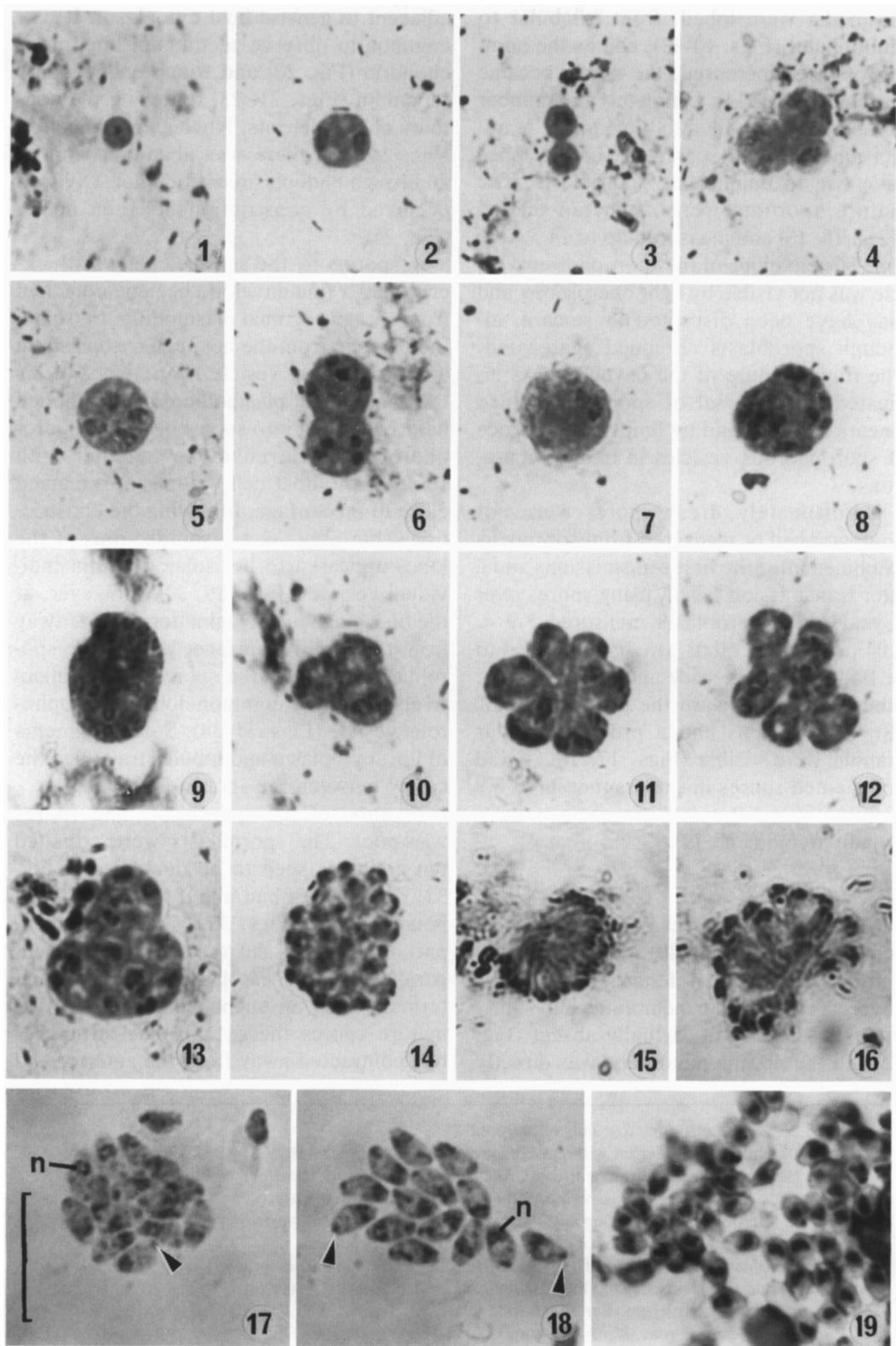
FIGS. 10–13. Lobed multinucleate sporonts.

FIG. 14. Sporont in the final stages of division.

FIGS. 15 AND 16. Fan-shaped sporonts immediately before separation of sporoblasts.

FIGS. 17 AND 18. Clusters of sporoblasts showing nuclei (n) and polar granule (arrows).

FIG. 19. Spores.



plasmodia were lobed, from trilobular to multilobular (Figs. 10–13), and as the number of lobes increased, the nuclei became peripheral (Fig. 14). Ultimately the number of lobes corresponded to the number of nuclei and the final separation of the lobes gave rise to uninucleate sporoblasts. The mature sporonts were often fan-shaped (Figs. 15, 16) and measured up to 14.5×10 μm . The envelope of the sporophorous vesicle was not visible by light microscopy and may have been disrupted in smears, although sporoblasts remained aggregated. The fragile nature of the envelope was indicated by dispersal of spores in stained smears (Fig. 19) and by limited persistence of sporophorous vesicles in fresh preparations.

Unfortunately, fresh spores were not photographed or measured from specimens examined after the first transmissions and a later transmission failed, using spores over 1 year old. Sporoblasts measured $3.9 \pm 0.04 \mu\text{m} \times 2.1 \pm 0.03 \mu\text{m}$. They tended to be flattened on one side and convex on the other, tapering toward the poles (Figs. 17, 18). The nucleus and a prominent polar granule were visible (Figs. 17, 18). Fixed and stained spores in smears measured $2.9 \pm 0.04 \mu\text{m} \times 1.9 \pm 0.03 \mu\text{m}$. Spores were broadly ovoid (Fig. 19).

Electron Microscopy

Meronts lay in direct contact with host cell cytoplasm (Fig. 20). Usually a thin amorphous electron-dense surface coat covered the plasma membrane but sometimes this layer was virtually absent (Fig. 22) and the plasma membrane was directly

adjacent to general host cytoplasm. It was common to observe sections of host mitochondria (Fig. 20) and rough endoplasmic reticulum (Figs. 21, 23) following the contours of the meronts. Nuclei were unpaired (Fig. 24) and there was abundant smooth and rough endoplasmic reticulum. Division occurred by constriction between nuclei (Fig. 25).

In sporogony the surface coat was thickened and a fine envelope became detached from the sporogonial plasmodium to isolate the parasite from the host cell cytoplasm in a sporophorous vesicle (Figs. 26, 27). As the sporogonial plasmodium became lobed before dividing into sporoblasts, the sporophorous vesicle envelope together with some of the host cell cytoplasm remained close to the surface, following the constrictions (Fig. 28). As a result sections of the lobes appeared to be isolated within individual vesicles (Figs. 29, 30). However, at the final stage the invaginations broke away from the parasite surface, leaving the sporoblasts (and later the spores) lying collectively within a common lobed sporophorous vesicle (Figs. 31–34). Some fragments of host cytoplasm and tubules traversed the cavity between the spores.

Fixation of the sporoblasts and spores was poor. The sporoblasts were crenated but could be seen to be uninucleate (Fig. 32). The spores had about 3.5 coils of the polar filament (Figs. 33, 34), the retilinear part of which was surrounded by prominent parallel membranes of the polaroplast and terminated in an anchor-like polar sac. In mature spores the cytoplasmic structures had contracted away from the anterior and

FIGS. 20–25. Electron micrographs of meronts.

FIG. 20. Uninucleate meront with closely associated host mitochondria (arrows). Bar = 0.5 μm .

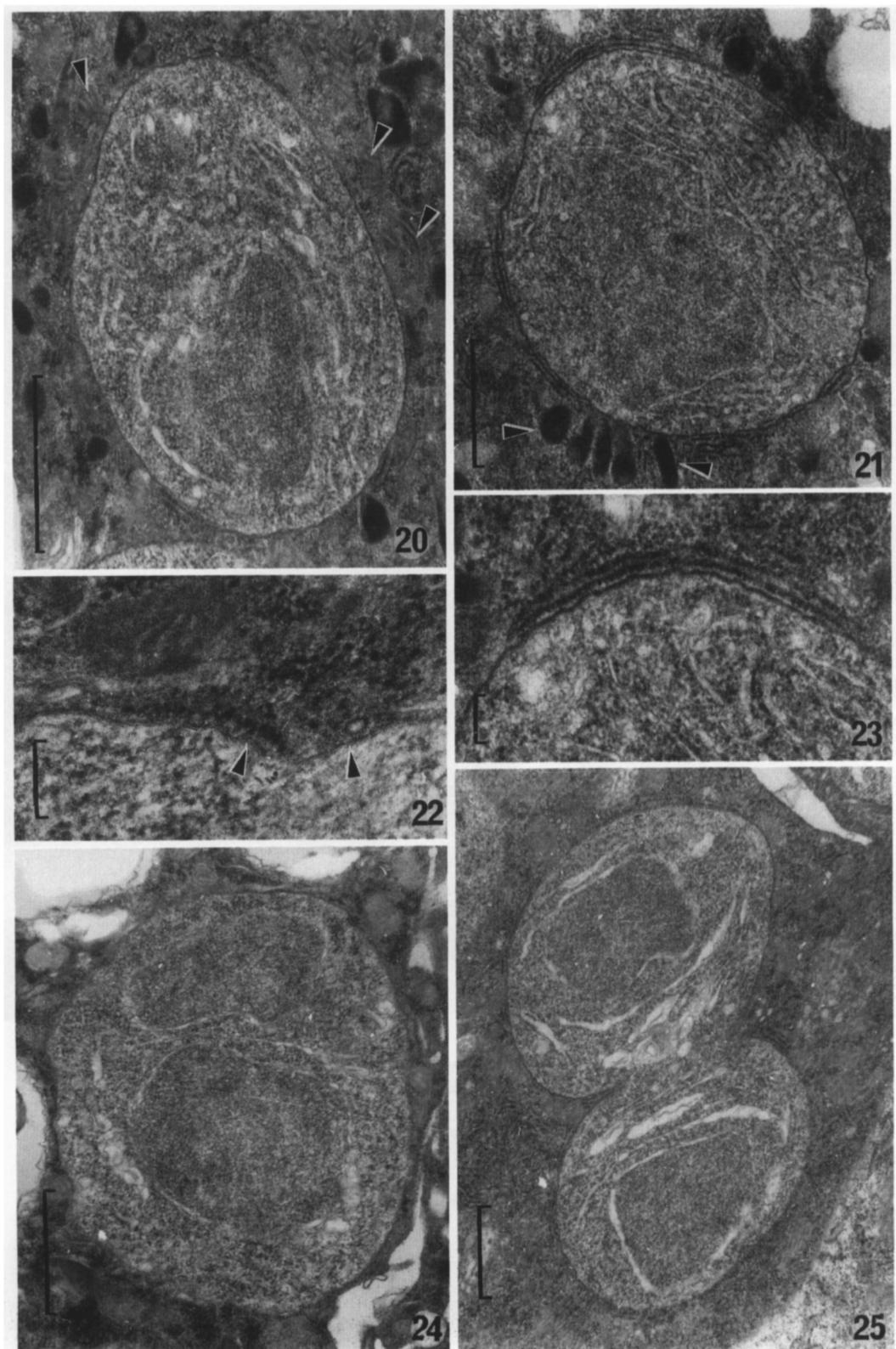
FIG. 21. Uninucleate meront with host rough endoplasmic reticulum and unidentified electron-dense bodies (arrows) closely associated with the surface. Bar = 0.5 μm .

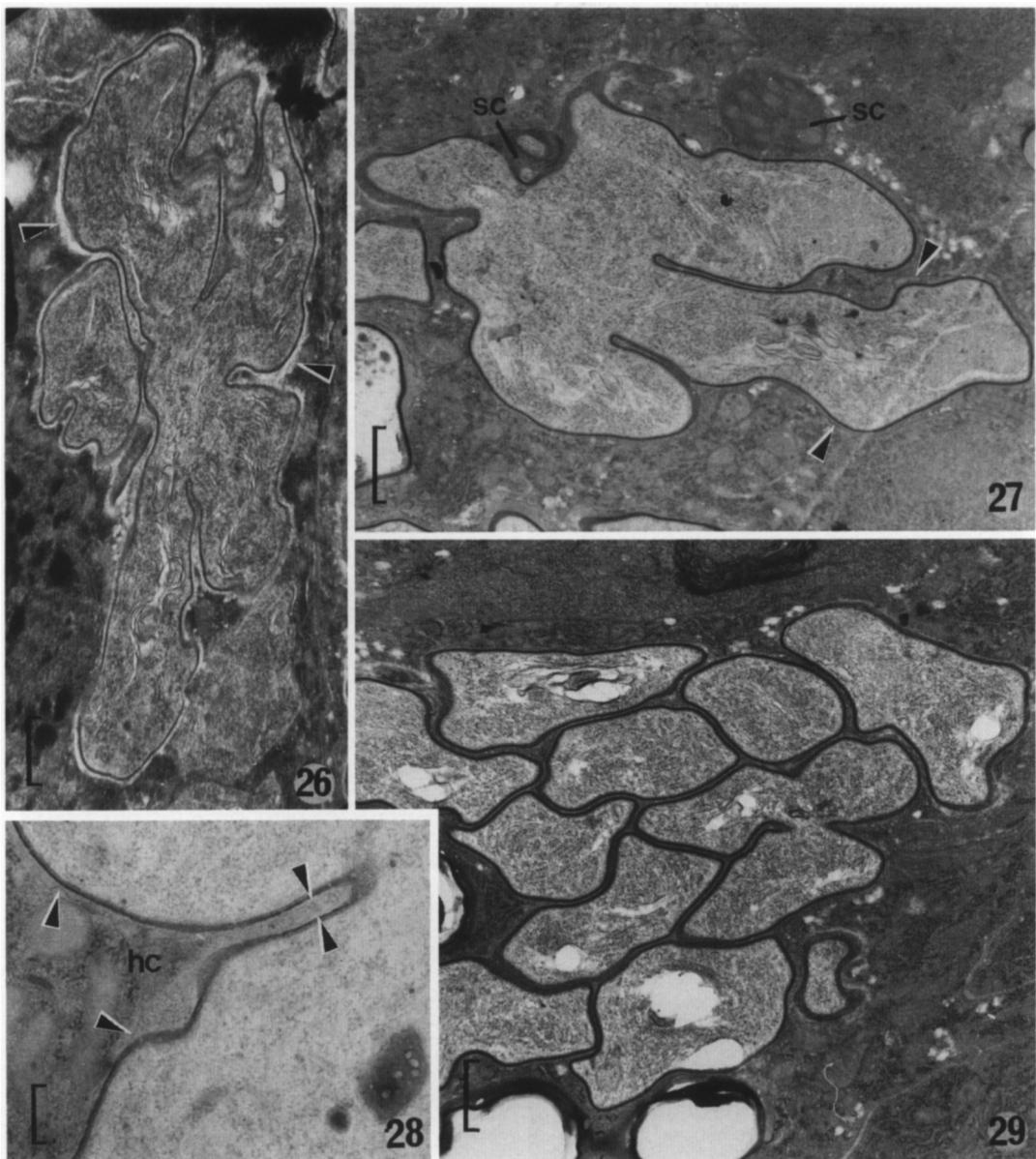
FIG. 22. Meront surface showing unthickened plasma membrane (single arrow) and thickened membrane (double arrows) with associated host ribosomes. Bar = 0.1 μm .

FIG. 23. Enlargement of part of Fig. 21 showing thin electron-dense surface coat on the plasma membrane and surface-associated host endoplasmic reticulum. Bar = 0.1 μm .

FIG. 24. Binucleate meront. Bar = 0.5 μm .

FIG. 25. Dividing meront. Bar = 0.5 μm .





FIGS. 26-29. Electron micrographs of sporonts.

FIGS. 26 AND 27. Deeply invaginated sporonts within sporophorous vesicles (arrows). In Fig. 27 some areas cut tangential to the surface show the nonhomogeneous nature of the surface coat (sc). Bar = 0.5 μm .

FIG. 28. Surface of dividing sporont showing electron-dense surface coat and sporophorous vesicle (arrows), accompanied by some host cell cytoplasm (hc) following the invagination of the sporont. Bar = 0.1 μm .

FIG. 29. Almost completely divided sporont showing thick surface coat on the apparently separate sporoblasts. Bar = 0.5 μm .

posterior ends. Laterally the electron-dense exospore was of equal thickness to the electron-lucent endospore.

Transmission

Spores from the original infected female *P. ariasi* had been fed to her own progeny and to larvae hatching from five eggs from another female. All larvae examined at the third and fourth instars were infected. Infections indicated by opacity of tissue were confined to the midgut. Even when infections were heavy, the majority, if not all, of the larvae pupated successfully and emerged as adults. As the possibility existed that the infection had been transmitted transovarially rather than by ingestion of spores, eggs from an uninfected female *P. ariasi* were added to the pot containing spores. Again all the larvae became infected. In contrast, when larvae of *Phlebotomus papatasi* (Pune strain) were similarly exposed to spores, only 2 of 22 examined were infected and these were light infections involving small patches of midgut.

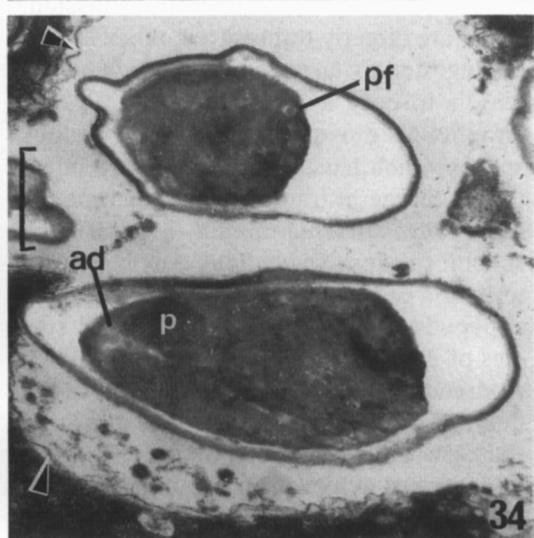
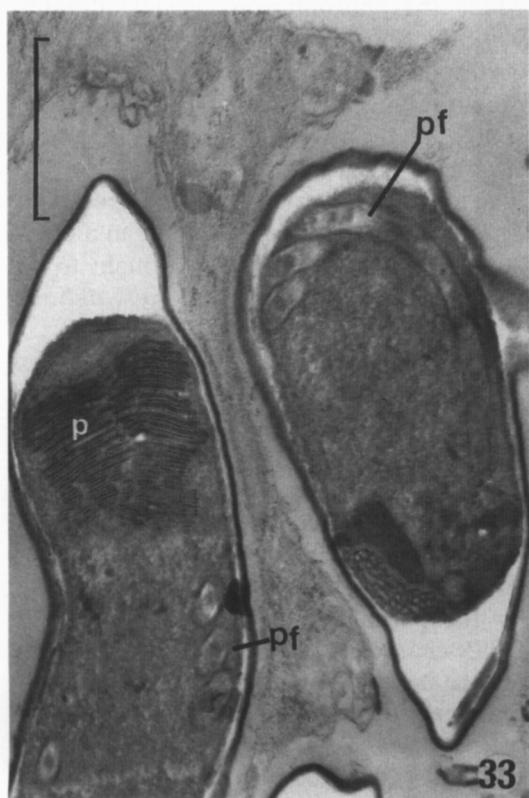
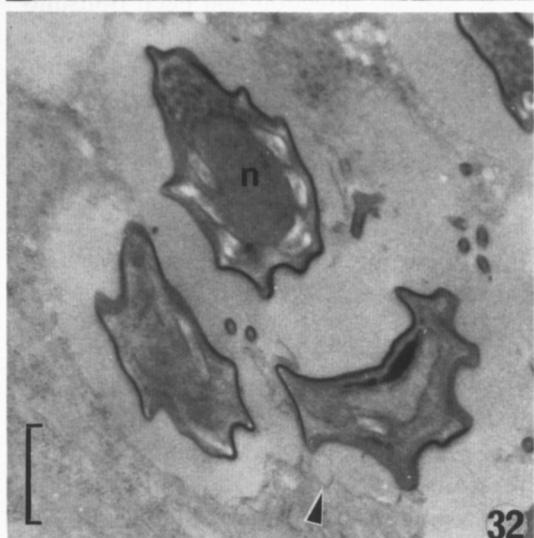
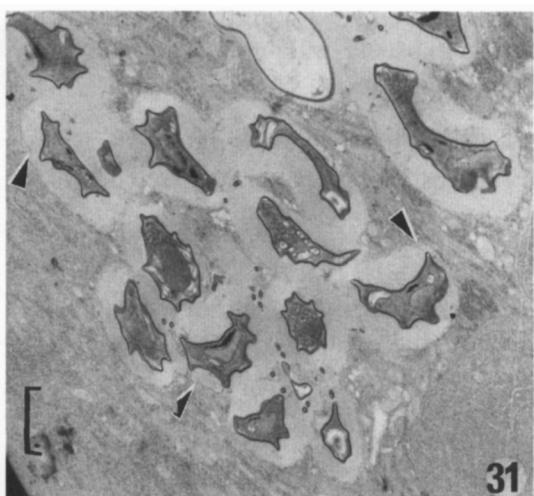
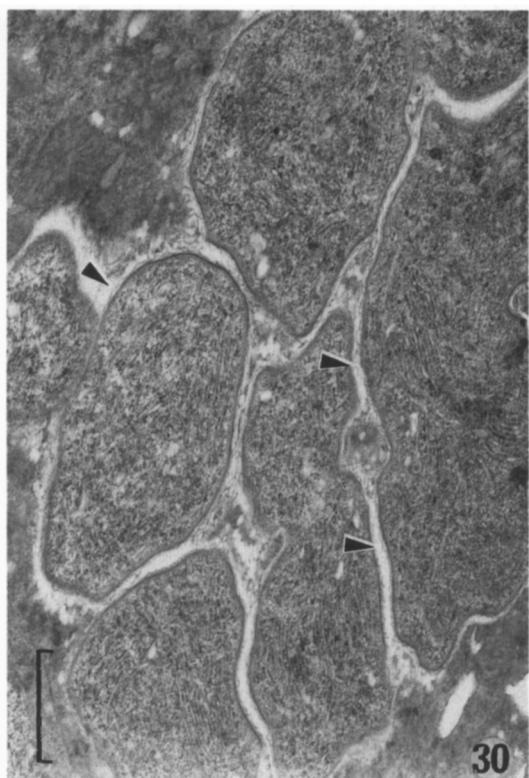
DISCUSSION

This report of a microsporidian infection in *P. ariasi* is the first from the genus *Phlebotomus*, as all previous reports are of infections in New World sandflies. Microsporidia in dipteran hosts fall into two categories in terms of their transmission. Some are directly transmitted, when spores are ingested by larvae, e.g., *Vavraia culicis* which infects a range of culicine and anopheline mosquitoes. Others produce spores which have not been directly infective to larvae in transmission experiments, e.g., *Polydispyrenia simulii*, a parasite of several species of *Simulium*. An obligate alternation of hosts between mosquitoes and copepods has been shown for several species of *Amblyospora* (Sweeney et al., 1985; Andreadis, 1985). The species in *P. ariasi* belongs to the first category, as shown by the ease with which infections of *P. ariasi* larvae were achieved in the laboratory. It may have a wide host range with differen-

tial virulence to various species but *P. papatasi* was the only other sandfly tested. The species investigated by Lawyer (1984) may be of the other type since the author failed to transmit it to larvae of the same species.

The genus *Pleistophora* was established by Gurley (1893) to accommodate microsporidia which sporulate within an envelope, the sporophorous vesicle (a term suggested by Canning and Hazard (1982) in preference to pansporoblast membrane), to give rise to a large and variable number of spores. It is now known that the species of multisporous microsporidia are not all congeneric but have a diversity of biological characters which has necessitated the establishment of several more genera. This "*Pleistophora complex*" today embraces nine genera and at least three species which should be transferred to existing or new genera. Other multisporous microsporidia belong to the *Tuzetia* complex (Larsson, 1983). This is a group of four genera in which the sporophorous vesicle divides with the sporogonial plasmodia, so that each sporoblast lies in a separate sporophorous vesicle which persists around the spore. As the invaginations of the sporophorous vesicle envelope break up in the final stages of sporulation of the species in *P. ariasi*, leaving the sporoblasts in a common vesicle, this species is thought to be more closely allied to the *Pleistophora* complex.

The genera of the *Pleistophora* complex are separated on a range of characters, the most important of which the authors believe to be whether the nuclei are paired or unpaired or alternate between the two states and whether the envelope that surrounds the sporogonic phases is host derived (parasitophorous vacuole) or secreted by the parasite (sporophorous vesicle). Other characters used to differentiate genera are the presence or absence of a sporophorous vesicle precursor around the meronts and the events of the sporogonic division sequence. Apart from *Pseudopleis-*



tophora, for which the family Pseudopleistophoridae was established (Sprague, 1977), the tendency has been to place all multisporous genera which sporulate in a common vesicle in the family Pleistophoridae established by Stempell (1909). This is clearly untenable in the light of new ultrastructural data on nuclear arrangement and nature of the vesicle enveloping the spores. Some species requiring new generic emplacements have been placed temporarily in the genus *Pleistophora* by their authors, without naming the species, e.g., *Pleistophora* sp. of Sandars and Poinar (1976) and *Pleistophora* sp. of Percy et al. (1982). Yet others have been placed temporarily in the collective group *Microsporidium*, e.g., *Microsporidium itiiti* of Malone (1985) and *Microsporidium novacastriensis* of Jones and Selman (1985). These two species have characters in common with the newly established genera *Cystosporogenes* of Canning et al. (1985) and *Endoreticulatus* of Brooks et al. (1988) and should probably be transferred to one or the other of these genera.

The parasite of *P. ariasi* cannot be attributed to any of the established genera. In lacking diplokaryotic nuclei during merogony and sporulation, it can be distinguished from *Pseudopleistophora*, *Polydispyrenia*, *Ovavesicula*, and the unnamed species placed temporarily in the genus *Pleistophora* by Sandars and Poinar (1976) and Percy et al. (1982). *Glugea* is an unsuitable emplacement because of its development in a xenoma and differences in the sporogonic sequence. *Baculea* is also not suitable be-

cause of its rod-like spores among other distinguishing characters. The membrane-like sporophorous vesicle of the species in *P. ariasi* is unlike the thick amorphous layer present around the meronts and which becomes the sporophorous vesicle of *Pleistophora* and *Vavraia*. The formation of a true sporophorous vesicle as opposed to the parasitophorous vacuole distinguishes the species parasitizing *P. ariasi* from *Endoreticulatus*. The genus *Cystosporogenes* resembles *Endoreticulatus* in that all stages of merogony as well as sporogony take place within a membrane-bound vesicle. In *Endoreticulatus* it was established that the membrane was of host origin and thus that the parasite developed in a parasitophorous vacuole. In *Cystosporogenes* the origin of the membrane was not determined but it remains possible that it is of host origin. Whatever its origin it does not pass through a lobed phase around the dividing sporogonial plasmodium. We, therefore, consider the parasite of *P. ariasi* as a new species of a new genus, for which the name *Flabelliforma montana* n.g. n.sp. is proposed, the generic name referring to the fan-shaped sporonts viewed by light microscopy and the specific name referring to the mountainous region from which the host and its parasite were recovered.

This genus is included in a proposed dichotomous key to the genera of the *Pleistophora* complex outlined below. The key is intended as a guide to the identification of the genera and in no way serves to indicate relationships between genera.

FIGS. 30-34. Electron micrographs of sporoblasts and spores.

FIG. 30. Part of a dividing sporont showing sporoblasts apparently separated from one another by the sporophorous vesicle envelope (arrows) and some host cell cytoplasm. Bar = 0.5 μm .

FIG. 31. Crenated sporoblasts lying in a lobed sporophorous vesicle (arrows) with remnants of invaginated host cell cytoplasm in the vesicle cavity. Bar = 1 μm .

FIG. 32. Enlargement of part of Fig. 31 showing envelope of sporophorous vesicle (arrow) and remnants of host cell cytoplasm and tubular structures in episporontal cavity. Bar = 0.5 μm .

FIG. 33. Maturing spores within envelope of the sporophorous vesicle: persisting invagination of the sporophorous vesicle envelope and host cell cytoplasm form an incomplete barrier between spores. One spore shows three coils of the polar filament (pf) and the lamellar polaroplast (p). Bar = 0.5 μm .

FIG. 34. Part of a sporophorous vesicle showing mature spores in a continuous cavity bounded by the vesicle envelope (arrows). One spore shows 3.5 coils of the polar filament; the other shows the lamellar polaroplast (p) and anchoring disc (ad). Bar = 0.5 μm .

KEY TO THE GENERA OF THE *Pleistophora* COMPLEX

- 1 Nuclei alternating between unpaired and paired (diplokaryotic) arrangements during life cycle
- Nuclei either unpaired or diplokaryotic throughout life cycle
- 2 Sporogonic phases develop in a parasitophorous vacuole derived from host endoplasmic reticulum.

Pleistophora sp. of Percy et al. (1982)

- Sporogonic phases develop in a sporophorous vesicle of parasite origin
- 3 Sporophorous vesicle envelope fine and membrane like
- 4 Sporophorous vesicle ovoid with thick, two-layered and persistent envelope enclosing 32 spores.

Ovavesicola Andreadis and Hanula, 1987

- 4 Separation of diplokaryon nuclei occurs in multinucleate plasmodia. Unpaired nuclei undergo meiosis.

Polydispyrenia Canning and Hazard, 1982

- Separation of diplokaryon nuclei occurs in sporont with a single diplokaryon. Sporoblasts derived by repeated nuclear and cytoplasmic divisions.

Pleistophora sp. of Sandars and Poinar (1976)

- 5 Nuclei unpaired throughout life cycle
- Nuclei diplokaryotic throughout life cycle. Sporogonial plasmodium interdigitates with host cell cytoplasm; division of sporogonial plasmodium into diplokaryotic sporoblasts by fusion of internal vesicles with plasma membrane

Pseudopleistophora Sprague, 1977

- 6 Sporogonic (and merogonic stages) develop within a vacuole bounded by a unit membrane of host or of unknown origin
- Sporogonic stages develop in a sporophorous vesicle of parasite origin
- 7 Ribbon-like sporogonial plasmodia
- Rounded sporogonial plasmodia
- 8 Sporogonial plasmodia give rise to chains of sporoblasts coiled within parasitophorous vacuoles, which become progressively more rounded. Parasitophorous vacuole is fragile; spores ovoid.

Microsporidium ititi Malone, 1985

(and possibly *M. novacastriensis* Jones and Selman, 1985)

- Sporogonial plasmodia give rise to sporoblast mother cells which undergo further division into sporoblasts; persistent parasitophorous vacuoles become navicular during sporoblast and spore formation. Spores rod-like.

Baculea Loubes and Akbarieh, 1978

- 9 Parasitophorous vacuole persistent, origin of vacuolar membrane unknown.

Cystosporogenes (Canning et al. (1985)

- Parasitophorous vacuole easily ruptured, vacuolar membrane of host origin.

Endoreticulatus Brooks et al., 1988

- 10 Sporophorous vesicle apparent only during sporogony
- Precursor of sporophorous vesicle present on and dividing with the meronts
- 11 Sporophorous vesicle amorphous, very thick and permeated by channels; division of sporogonial plasmodium by progressive segmentation into sporoblasts; parasites of vertebrates.

Pleistophora Gurley, 1893

- Sporophorous vesicle amorphous, moderately thick, not permeated by channels; division of sporogonial plasmodium by rosette formation into sporoblasts; parasites of invertebrates.

Vavraia Weiser, 1977

- 12 Meronts completely surrounded by endoplasmic reticulum; sporophorous vesicle membrane-like, separating via a series of blisters from the sporogonial plasmodium; sporogonial plasmodium divides into sporoblast mother cells, which each give rise to two sporoblasts. Development within a xenoma.

Glugea Thelohan, 1891

- Meronts not surrounded by endoplasmic reticulum; membrane-like sporophorous vesicle separates from surface of sporogonial plasmodium without blister formation; fan-shaped sporogonial plasmodium divides directly into sporoblasts by multiple fission; no xenoma formation.

Flabelliforma n.g.

TAXONOMIC SUMMARY

Flabelliforma n.g.

Nuclei are unpaired at all stages of the life cycle. Meronts lie in direct contact with the host cell cytoplasm, have numbers of nuclei probably not exceeding eight, and divide by binary fission or plasmotomy.

Sporogony is multisporous. Sporogonial plasmodia are encased in a sporophorous vesicle, have up to 32 nuclei and divide by forming lobes which become progressively more numerous, until, with peripheral nuclei, they become fan-shaped before division into sporoblasts. The sporophorous vesicle wall is fine and membrane-like, first adhering closely to the surface of the sporogonial wall and following the indentation of the lobes, and finally persisting as a lobed vesicle around the group of sporoblasts and spores. Transmission direct.

Flabelliforma montana n.sp. (type species)

Meronts measure 2 μm in diameter to $11 \times 5.5 \mu\text{m}$. Sporogonial plasmodia measure up to $14.5 \times 10 \mu\text{m}$ at the fan-shaped stage. Spores are ovoid, measure $2.9 \pm 0.04 \times 1.9 \pm 0.03 \mu\text{m}$ in Giemsa-stained smears and have about four coils of the polar filament.

Type host is *Phlebotomus ariasi* Tonnoir 1921. Infection limited to the midgut.

Type locality is the commune of Roquedur, Gard in France.

REFERENCES

- ANDREADIS, T. G. 1985. Experimental transmission of a microsporidian pathogen from mosquitoes to an alternate copepod host. *Proc. Natl. Acad. Sci. USA.*, **82**, 5574-5577.
- ANDREADIS, T. G., AND HANULA, J. L. 1987. Ultrastructural study and description of *Ovavesicula popilliae* n.g., n.sp. (Microsporidia: Pleistophoridae), from the Japanese beetle, *Popillia japonica* (Coleoptera, Scarabaeidae). *J. Protozool.*, **34**, 15-21.
- BROOKS, W. M., BECNEL, J. J., AND KENNEDY, G. G. 1988. Establishment of *Endoreticulatus* n.g. for *Pleistophora fidelis* (Hostounsky & Weiser, 1975) (Microsporidia: Pleistophoridae) based on the ultrastructure of a microsporidium in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *J. Protozool.*, **35**, 481-488.
- CANNING, E. U. 1977. New concepts of microsporidia and their potential in biological control. In "Parasites Their World and Ours: Proceedings of the 18th Symposium of the Royal Society of Canada" (A. M. Fallis, Ed.), pp. 101-140.
- CANNING, E. U., BARKER, R. J., AND NICHOLAS, J. P. 1982. Genus *Glugea* Thelohan 1891 (Phylum Microspora): Redescription of the type species (*Glugea anomala* (Moniez, 1887) and recognition of its sporogonic development within sporophorous vesicles (pansporoblastic membranes) *Protistologica*, **18**, 193-210.
- CANNING, E. U., AND HAZARD, E. I. 1982. Genus *Pleistophora* Gurley 1893: An assemblage of at least three genera. *J. Protozool.*, **29**, 39-49.
- CANNING, E. U., AND NICHOLAS, J. P. 1980. Genus *Pleistophora* (Phylum Microspora): Redescription of the type species, *Pleistophora typicalis* Gurley, 1893 and ultrastructural characterization of the genus. *J. Fish Dis.*, **3**, 317-338.
- GURLEY, R. R. 1893. Classification of the Myxosporidia, a group of protozoan parasites infesting fishes. *Bull. U.S. Fish. Comm.*, **11**, 407-420.
- JONES, A. A., AND SELMAN, B. J. 1985. *Mircosporidium novacastriensis* n.sp., a microsporidian parasite of the grey field slug, *Deroceras reticulatum*. *J. Protozool.*, **32**, 581-586.
- LAINSON, R., KILICK-KENDRICK, R., CANNING, E. U., SHAW, J. J., WARD, R. D., LEANEY, A. J., AND NICHOLAS, J. P. 1977. Microsporidia of Brazilian sandflies. *Trans. R. Soc. Trop. Med. Hyg.*, **71**, 381. [Abstract]
- LAINSON, R., WARD, R. D., YOUNG, D. G., SHAW, J. J., AND FRAIHA, H. 1976. Preliminary entomological and parasitological studies in Humboldt, Anipuana, Mato Grosso State, Brazil. *Acta Amazonica*, **6**, 55-60.
- LARSSON, R. 1983. A revisionary study of the taxon *Tuzetia* Maurand, Fize, Fenwick and Michel, 1971, and related forms (Microspora, Tuzetiidae). *Protistologica*, **19**, 323-355.
- LAWYER, P. G. 1984. "Biology and Colonization of the Sandfly *Lutzomyia diabolica* (Hall) (Diptera, Psychodidae) with Notes on Its Potential Relationship to Human Cutaneous Leishmaniasis in Texas, U.S.A." Ph.D. Thesis, University of Florida, 1984.
- LOUBÈS, C., AND AKBARIEH, M. 1978. Étude ultrastructurale de la microsporidie *Baculea daphniae* n.g. n.sp., parasite de l'épithélium intestinal de *Daphnia pulex* Leydig, 1860 (Crustacé, Cladocère). *Protistologica*, **14**, 23-38.
- MALONE, L. A. 1985. A new pathogen, *Microsporidium ititi* n.sp. (Microsporidia), from the Argentine stem weevil, *Listronotus bonariensis* (Coleoptera, Curculionidae). *J. Protozool.*, **32**, 535-541.
- PERCY, J., WILSON, G., AND BURKE, J. 1982. Development and ultrastructure of a microsporidian parasite in midgut cells of the larch sawfly, *Pristiphora erichsonii* (Hymenoptera: Tenthredinidae). *J. Invertebr. Pathol.*, **39**, 49-59.
- SANDARS, R. D., AND POINAR, G. O. 1976. Development and fine structure of *Pleistophora* sp. (Cnidosporea: Microsporida) in the mosquito *Aedes sierrensis*. *J. Invertebr. Pathol.*, **28**, 109-119.
- SPRAGUE, V. 1977. Classification and phylogeny of the microsporidia. In "Comparative Pathobiology" (L. A. Bulla and T. C. Cheng, Eds.) Vol. 2, pp. 1-30. Plenum, New York.
- STEMPELL, W. 1909. Über *Nosema bombycis* Nägelei. *Arch. Protistenkd.*, **16**, 281-358.
- SWEENEY, A. W., HAZARD, E. I., AND GRAHAM, M. F. 1985. Intermediate host for an *Amblyospora* sp. infecting the mosquito *Culex annulirostris*. *J. Invertebr. Pathol.*, **46**, 98-102.
- THÉLOHAN, P. 1891. Sur deux sporozoaires nouveaux, parasites des muscles des Poissons. *C.R. Soc. Biol.*, **112**, 168-171.
- WARD, R. D., AND KILICK-KENDRICK, R. 1974. Field and laboratory observations on *Psychodopygus lainsoni* Fraiha & Ward and other sandflies (Diptera, Phlebotomidae) from the Transamazônica highway, Pará State, Brazil. *Bull. Entomol. Res.*, **64**, 213-221.
- WEISER, J. 1977. Contribution to the classification of microsporidia. *Vestn. Cesk. Spol. Zool.*, **41**, 308-320.