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The taxonomy of three species of microsporidia (Protozoa: Microspora) from an oakwood population of winter moths *Operophtera brumata* (L.) (Lepidoptera: Geometridae)

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Summary

Three species of microsporidia from a population of winter moths, *Operophtera brumata* (L.), were examined by light microscopy. *Pleistophora operophterae* (Canning, 1960) is redescribed. The stages with one or two nuclei which were originally termed presporonts are now interpreted as fragments of disrupted vacuolated sporonts or prematuraly separated sporoblasts. The range of spore numbers within sporophorous vesicles is modified to 4 to c.60, rather than 10 to c.100. This species cannot be retained in the genus *Pleistophora* Gurley, 1893, redefined by Canning & Nicholas (1980), but ultrastructural studies are required before taxonomic reassignment is attempted.

Nosema operophterae Canning, 1960 is also redescribed. Merogony consists mainly of binary fission of binucleate and tetranucleate stages but large irregular-shaped multinucleate meronts were also present. Ribbon-shaped sporonts with two, four or eight linearly-spaced nuclei give rise, via binucleate segments, to chains of uninucleate sporoblasts. The species is transferred to a new genus *Orthosoma* n.g. as *Orthosoma operophterae* (Canning, 1960) since its isolated nuclei and polysporoblastic sporogony are not characteristic of the genus *Nosema* Nägeli, 1857.

The third species *Nosema operophterae* Purrini & Skatulla, 1979 is renamed *N. wistmansi* nom.nov. because the name given by Purrini & Skatulla was preoccupied and is now a synonym of *O. operophterae*. Details of the developmental stages of *N. wistmansi* are given, which show that it is typical of the genus *Nosema*, with nuclei in diplokaryon arrangement and with disporoblastic sporogony.

All species are shown to have stages in the eggs of their hosts, which are thought to play a part in generation to generation transmission.

Introduction

Winter moth, *Operophtera brumata* (L.), can be serious defoliating pests of deciduous trees, especially in orchards in temperate countries. Their microsporidian parasites are of interest because they are likely to play an important part in the natural regulation of populations. Considerable confusion surrounds the nomenclature and identity of the several species of microsporidia, which have been recorded from populations in Germany and England. Material collected from a small oak wood, Wistman's Wood, on Dartmoor in the south-west of England (Ordnance Survey map reference NGRSX6176/6177), has provided the opportunity to sort out the nomenclatorial difficulties and give adequate descriptions of some of the parasites, from which future identifications can be made.

The first species to be described was an octosporous species developing within sporophorous vesicles (pansporoblast membranes) in the fat body. It was recorded from Darmstadt, Federal Republic of Germany (DFR) and named *Thelohania cheimatobiae* by Krieg (1956). Purrini & Skatulla (1979) examined *O. brumata* populations from forests in the Spessart mountains of Bavaria (DFR). In spite of differences in spore measurements they referred an octosporoblastic species that they found in the fat body, lymphocytes and tracheal matrix to *T. cheimatobiae*. This species was not found in the present study

Canning (1960) described two other species from larvae and pupae collected near Oxford, England. One of these, which produced variable numbers of small spores measuring 2.3–3.0 × 1.0–1.5 μm in sporophorous vesicles, was named *Plistophora operophterae* Canning, 1960. Its primary site of infection was the silk glands but infections also spread to the gut epithelial cells, Malpighian tubules, fat body and muscles. Since Sprague (1971) correctly pointed out that the generic name *Pleistophora* has priority, the species should be known as *Pleistophora operophterae* (Canning, 1960) as used by Sprague (1977). The second species was found only twice and was also a parasite of the silk glands: few stages other than spores were found and since the spores were free and appeared to be derived individually from sporonts, the species was placed in the genus *Nosema*, in which at that time the sporont was thought to give rise to a single spore. It was named *Nosema operophterae* Canning, 1960 but the absence of nuclei in diplokaryon arrangement does not allow its inclusion in the genus *Nosema*, as redefined by Sprague & Vernick (1971). The mode of transmisson of these two species was not fully elucidated, though some spread of *P. operophterae* was thought to occur among larvae of the same generation, when spores from infected gut cells passed out in the faeces and contaminated the trees (Canning, 1960).

Purrini & Skatulla (1979) also found microsporidia, which were polysporous in sporophorous vesicles, in *O. brumata* and *Erannis defolaria* Clerck. The spore size was given as 2.0–2.1 × 1.3–1.7 μm and the sites of infection were the fat body, lymphocytes, oenocytes and gut epithelium. They identified the parasite of the gut as *P. schubergi* Zwölfer, 1927 and, as they were not sure whether or not the parasite of the fat body belonged to the same species because it lacked sporophorous vesicles with more than 32 spores, they did not name it pending cross-infection experiments. All references to *P. schubergi* and its various subspecies from Lepidoptera give gut epithelium as the only site of infection (see Sprague, 1977).

Purrini & Skatulla (1979) also found a species of *Nosema* in the anterior part of the gut and in the fat body near this site. Pear-shaped or oval, binucleate spores, usually measuring 6.3–6.6 × 1.8–2.5 μm were described but no details were given of vegetative stages. They named the species *N. operophterae*, unaware that this name was preoccupied.

The winter moth population from Wistmans Wood, examined by one of us (PJW) from 1973–76 for virus infections, was found to harbour three species of microsporidia which we identified as *P. operophterae* (Canning, 1960), *Nosema operophterae* Canning, 1960 and the *Nosema* sp. with large, pear-shaped spores seen by Purrini & Skatulla (1979) in the Bavarian population. Evidence of transovarial transmission was provided by discovery of vegetative and sporogonic stages in eggs laid by adults, which were subsequently shown to harbour infections of each of the three species (Wigley, 1976).

The appearances of infected silk glands and some characteristic features of stages of the three parasites in larval tissues and in eggs have been described briefly by Canning (1981) and are amplified in the present work.

Until recently all microsporidian species, in which sporogony results in the development of a variable and usually large number of uninucleate spores contained within sporophorous vesicles, were assigned to a single genus *Pleistophora* Gurley, 1893. The genus *Pleistophora* was redefined by Canning & Nicholas (1980), on the basis of the ultrastructure of the type species *Pleistophora typicalis* Gurley, 1893. Although mature sporophorous vesicles of the numerous polysporous species have a superficial similarity, it is now known that there may be fundamental differences during develop-

ment, in organization and ploidy of nuclei, mode of separation of the sporoblasts from the sporogonial plasmodium and in the nature and even the origin of the sporophorous vesicle wall (Canning & Hazard, 1982). The origin of the sporophorous vesicle wall can only be determined on ultrastructural evidence. A preliminary electron microscopic examination (Canning, 1981) has shown that the fine sporophorous vesicle wall of *P. operophterae* does not resemble the thick wall formed as an amorphous secretion in *Pleistophora* and that the uninucleate sporoblasts are formed from the plasmodium directly as buds in *P. operophterae*, in contrast to their progressive formation through multinucleate segments in *Pleistophora*. Nevertheless in order to avoid possible confusion at a later stage, no taxonomic change is being recommended for this species until ultrastructural studies are complete. Nomenclatorial and taxonomic changes are proposed for the other species as follows:

(a) *Nosema operophterae* Canning, 1960 to be transferred to a new genus for which the name *Orthosoma* n.g. is proposed and becomes *Orthosoma operophterae* (Canning, 1960).

(b) The replacement name *Nosema wistmansi* n.sp. is proposed for *N. operophterae* Purrini & Skatulla, 1979, as this name (now a synonym of *O. operophterae*) is preoccupied.

Materials and methods

The material collected in 1973-76 was prepared primarily for investigation of virus infections and microsporidia were only encountered incidentally. Larvae were collected in bulk and were stored at $-30°$ before thawing and preparation of smears. The thinly spread smears were stained by a differential method for the major groups of insect inclusion body viruses (Wigley, 1980) to produce areas stained by Giemsa alone and areas pretreated by picric acid or by picric acid and naphthalene black. Microsporidia were easily detected in the areas stained by Giemsa alone.

Larvae collected in 1978 were maintained for a few days on oak twigs bearing half-opened leaves. During this time larvae, mainly in the 3rd to 5th instars, were examined for microsporidia. The silk glands were dissected out and examined in distilled water at $120\times$ magnification. Small segments of gland which showed signs of infection by opacity of their cells were examined under a coverslip at $1000\times$ to identify the species. Small pieces of the midgut wall were similarly examined.

After identification of species on presence or absence of sporophorous vesicles (pansporoblasts) and size and shape of spores, smears were made of silk glands, gut wall, Malpighian tubules and fat body. These were dried, fixed in methanol and stained in 10% Giemsa. Measurements of stained stages were made against an ocular micrometer. Fresh spores were measured after photographic enlargement against a 10 μm scale similarly enlarged.

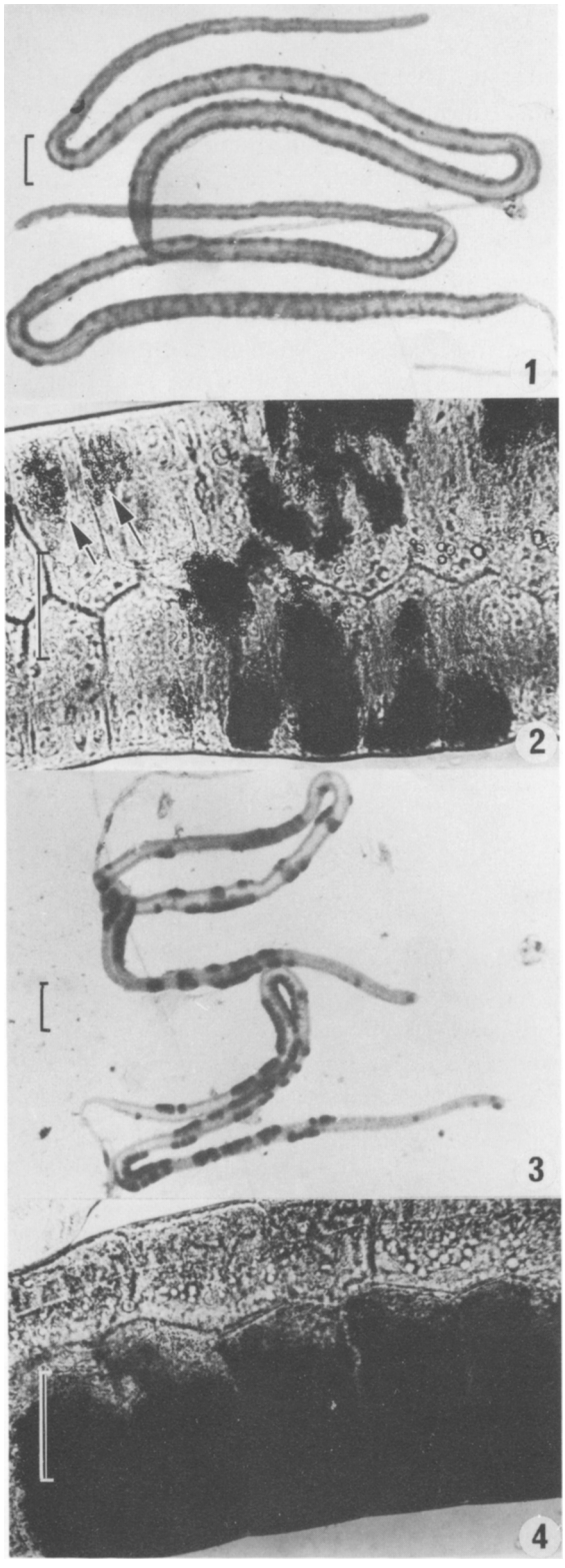
Adult female moths, collected in winter were allowed to lay eggs in 30 ml plastic pots lined with filter paper and containing an additional piece of filter paper folded into a concertina. Most of the eggs were laid between the folds of the filter paper. Adults and the corresponding eggs were smeared, stained with Giemsa and examined for infection.

Results

Prevalence and signs of infection

In the earlier collections of larvae (1973–76) of all instars, prevalences of 30 to 40% were obtained for *O. operophterae* but *P. operophterae* and *N. wistmansi* were found in only about 2% of the populations. Of the collection made in 1978, 200 larvae were examined. There were 48 infected with *O. operophterae* alone, seven with *P. operophterae* alone and three with *N. wistmansi* alone. There was also one double infection of *O. operophterae* and *P. operophterae*, two double infections of *O. operophterae* and *N. wistmansi* and one double infection of *P. operophterae* and *N. wistmansi*.

The appearance of the infect silk glands was characteristic for the two species which infected them. *P. operophterae* invaded the cells in a very regular manner, so that almost every cell of the region affected and eventually of the whole gland



was involved: the cells themselves were only slightly hypertrophied and the lumen of the gland was unobstructed (Figs. 1, 2). In contrast, the invasion of cells by *O. operophterae* was irregular, with clumps of infected cells interspersed with healthy cells: infected cells were grossly hypertrophied causing considerable enlargement of the affected region and occlusion of the lumen (Figs. 3, 4). The silk glands were not infected by *N. wistmansi*: this species infected the gut wall, which became less robust than normal and was easily broken during dissection, and spread to the fat body and Malpighian tubules.

Descriptions of the species

Pleistophora operophterae (Canning, 1960)

Stages in larvae and adults

Stages found in larvae or adults were similar to those found by Canning (1960). There were plasmodia with varying numbers of nuclei, of which those with dense homogeneous cytoplasm and low numbers of nuclei (Figs. 5, 6, 7) were, probably, meronts capable of division by multiple fission (Fig. 8). Sporogonial plasmodia were difficult to differentiatie from meronts except possibly by the greater number of nuclei (Figs. 10, 11) until they had begun their separation into sporoblasts. The nuclei of all stages were isolated and showed a ring-shaped distribution of chromatic material around a pale area. The occasional occurrences of paired nuclei in sporogonial plasmodia (Fig. 12) were probably indicative of telophases in rapid division sequences rather than of true diplokaryon arrange-

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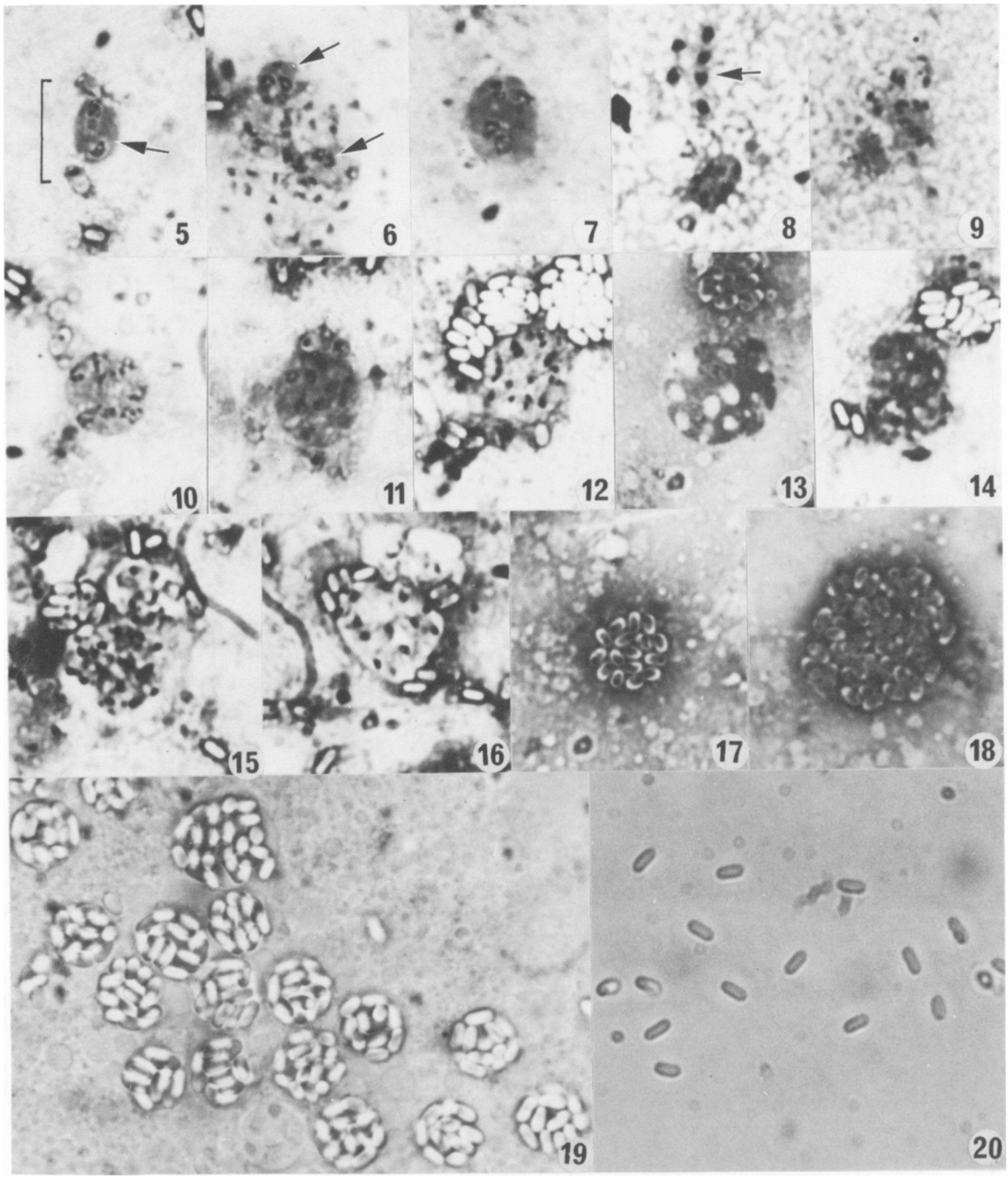
Figs. 1, 2. Fresh preparations of silk glands of *Operophtera brumata* infected with *Pleistophora operophterae*. Almost every cell is infected, although in some cells the foci are quite small (arrows). Infected cells are not particularly hypertrophied.

Figs. 3, 4. Fresh preparations of silk glands of *O. brumata* infected with *Orthosoma operophterae*. Distribution of infected cells is patchy with long stretches of the glands free of infection. Infected cells are grossly hypertrophied giving a bumpy outline to the gland.

Figs. 1, 3. Scale bar = 1 mm. Figs. 2, 4 Scale bar = 100 μm.



Figs. 5–18. ***Pleistophora operophterae.*** **Stages of development in larvae and adult** *Operophtera brumata.* **Giemsa-stained.**
Fig. 5. Binucleate meront (arrow). Fig. 6. Meronts (arrows) among uninucleate elements of a disrupted sporogonial plasmodium. Fig. 7. Tetranucleate meront. Fig. 8. Uninucleate division products of merogony (arrow). Fig. 9. Disrupted plasmodium showing paired nuclei, probably representing mitotic telophases. Figs. 10, 11. Multinucleate plasmodia probably of the sporogonic sequence. Fig. 12. Sporogonial plasmodium showing compact nuclei, including some in pairs resembling mitotic telophases. Fig. 13. Vacuole formation in sporogonial plasmodium, initiating sporoblast formation. Figs. 14, 15. Sporoblast formation within sporophorous vesicle; peripheral nucleated buds are connected by strands of cytoplasm. Fig. 16. Free or almost free sporoblasts in sporophorous vesicle. Fig. 17. Group of 16 spores. Fig. 18. Group of about 60 spores.
Fig. 19. Fresh sporophorous vesicles, containing 8, 16 and more spores.
Fig. 20. Fresh spores liberated from pansporoblast. Scale bar for all figures = 10 μm.

ments. Some binucleate segments were observed in merogonial plasmodia disrupted in smears (Fig. 9).

Division of sporogonial plasmodia into sporoblasts was mediated by the formation of large vacuoles in the cytoplasm which disrupted the homogeneous cytoplasm of the earlier stages (Fig. 13). The vacuoles, presumably by fusion with the surface membrane, gave rise to the sporoblast buds which remained temporarily connected in the centre of the plasmodium (Figs. 14, 15) by anucleate strands of cytoplasm. At the time of their separation, the uninucleate, pale-staining sporoblasts were loosely arranged within the now obvious sporophorous vesicles (Fig. 16). Later, when the spore walls had formed, the resulting spores were tightly packed in spheres (Figs. 17, 18). The number of spores derived from the sporogonial plasmodia ranged from four to about 60 with eight, 12 and 16 the most common numbers. (Fig. 19). Fresh spores were elongate ellipsoid, symmetrical about both axes and without visible posterior vacuole. Measurements of $2.4\pm0.6 \times 1.2\pm0.2$ μm (n = 30) for stained spores accord with those given in the original description. Fresh spores measured $2.5\pm 0.2 \times 1.2\pm0.1$ μm (n = 25) and fresh sporophorous vesicles ranged from about 4.0 to 12.0 μm diameter.

Stages in eggs

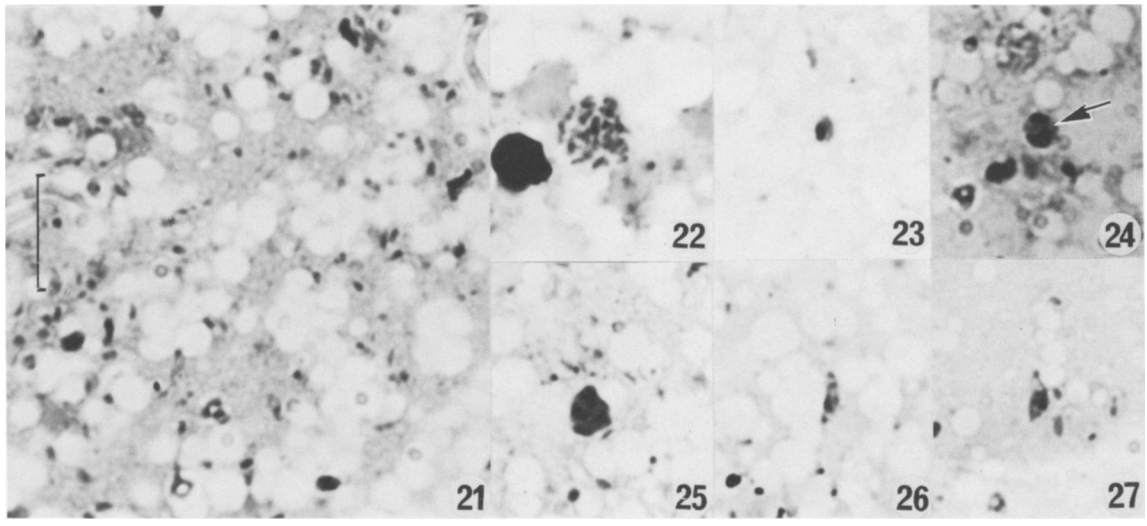
Stages in smears of non-embryonated eggs were mostly free spores, which were sometimes abundant (Fig. 21). Sporophorous vesicles (Fig. 22) were rare and large, unsporulated plasmodia were not seen. A few uninucleate, binucleate and tetranucleate stages were seen (Figs. 23, 24, 25) but vegetative stages were mostly absent. The only other stages seen were fusiform bodies, with unevenly stained cytoplasm and dense polar regions, which measured about 4.5×1.5 μm (Figs. 26, 27).

Orthosoma operophterae (Canning, 1960)

Stages in larvae and adults

Meronts were usually uni-, bi- or tetra-nucleate. (Figs. 28–33). Measurements in smears were highly variable, according to the degree of smearing. The following mean measurements of 25 examples of each stage from one smear serve to indicate the relative sizes of the meronts: 4.0×4.1 μm (uninucleate), 4.4×5.5 μm (binucleate) and 7.3×4.4 μm (tetranucleate).

With Giemsa stain, the cytoplasm was a uniform



Figs. 21–27. *Pleistophora operophterae*: stages in eggs. Giemsa-stained. Fig. 21. Free-spores. Fig. 22. Sporophorous vesicle with spores. Fig. 23. Uninucleate meront. Fig. 24. Binucleate meront (arrow). Fig. 25. Tetranucleate meront. Figs. 26, 27, Uninucleate elongate stages, possibly early sporonts. Scale bar = 10 μm.

deep blue and the nuclei, which were always isolated, consisted of a pale area enclosing red-staining, ring-shaped or reticulate chromatin. Division was by binary fission of tetra- and bi-nucleate stages, during which normally spherical meronts became elongate, then constricted in the centre into spherical division products (Figs. 32, 33). Multinucleate bodies of bizarre shapes, which were less common than the rounded stages described above were also attributed to the merogonic cycle (Figs. 34, 35, 36).

There were many characteristically spindle-shaped stages with a single central nucleus and the same staining reactions as the meronts (Figs. 37, 38). It is possible that these represented the first step in the sporogonic sequence. They measured 5.9 × 3.0 μm.

Sporonts were elongate stages, which when mature were rarely more than 2 μm wide. Immature stages were about 3 μm wide. Nuclear division accompanied elongation. Mature sporonts contained two, four, eight or rarely 12 nuclei strung out in a single line (Figs. 37–42). Sporonts with odd numbers of nuclei were rare. The deep blue colour of the immature stages was lost during maturation so that mature sporonts had pale, unevenly stained cytoplasm.

Binucleate sporonts divided into two uninucleate sporoblasts (Fig. 40). Tetranucleate and octonucleate sporonts divided first into binucleate stages with polar nuclei (Figs. 40, 41), then immediately into uninucleate sporoblasts (Figs. 38, 42). The nuclei moved towards the centre in the sporoblasts. The sporoblasts were held together in chains of two, four or eight according to the number of nuclei in the sporont. The size of the sporoblasts was highly variable (Fig. 43) and depended on the length and number of nuclei of the sporont: in general those derived from octonucleate sporonts were shortest and those derived from binucleate sporonts were longest. Mean measurements of sporonts were 9.1 × 1.8 μm (binucleate) 14.0 × 2.4 μm (tetranucleate) and 25.8 × 1.9 μm (octonucleate). Mean measurements for sporoblasts were 4.2 × 1.8 μm. The cytoplasm of sporoblasts was pale, often streaked with blue and the single nucleus lay slightly eccentrically with the appearance of a narrow band, separate dots (Figs, 39, 43) or a reticulum staining red in a clear area.

Spores, which were uninucleate like the sporoblasts, were elongate ellipsoid in shape and measured $3.2 \pm 0.8 \times 1.3 \pm 0.1$ μm (n = 50) in stained smears and $3.5 \pm 0.4 \times 1.3 \pm 0.2$ μm (n = 50) when fresh (Fig. 44). The length of the spores was unusually variable for microsporidia with some more than twice the length of others, the range being 2.5 × 1.0 μm to 6.0 × 1.5 μm for 50 spores measured fresh. This variation may be caused in some cases by unequal splitting of the sporont between unevenly spaced nuclei but more usually by the different lengths of the sporonts and the number of sporoblasts derived from them. Giant spores of greater length and width measuring 7.5 × 2.0 μm were also occasionally found which were probably teratogenic, resulting from incomplete separation of the binucleate elements into sporoblasts. Spores were not held together in chains.

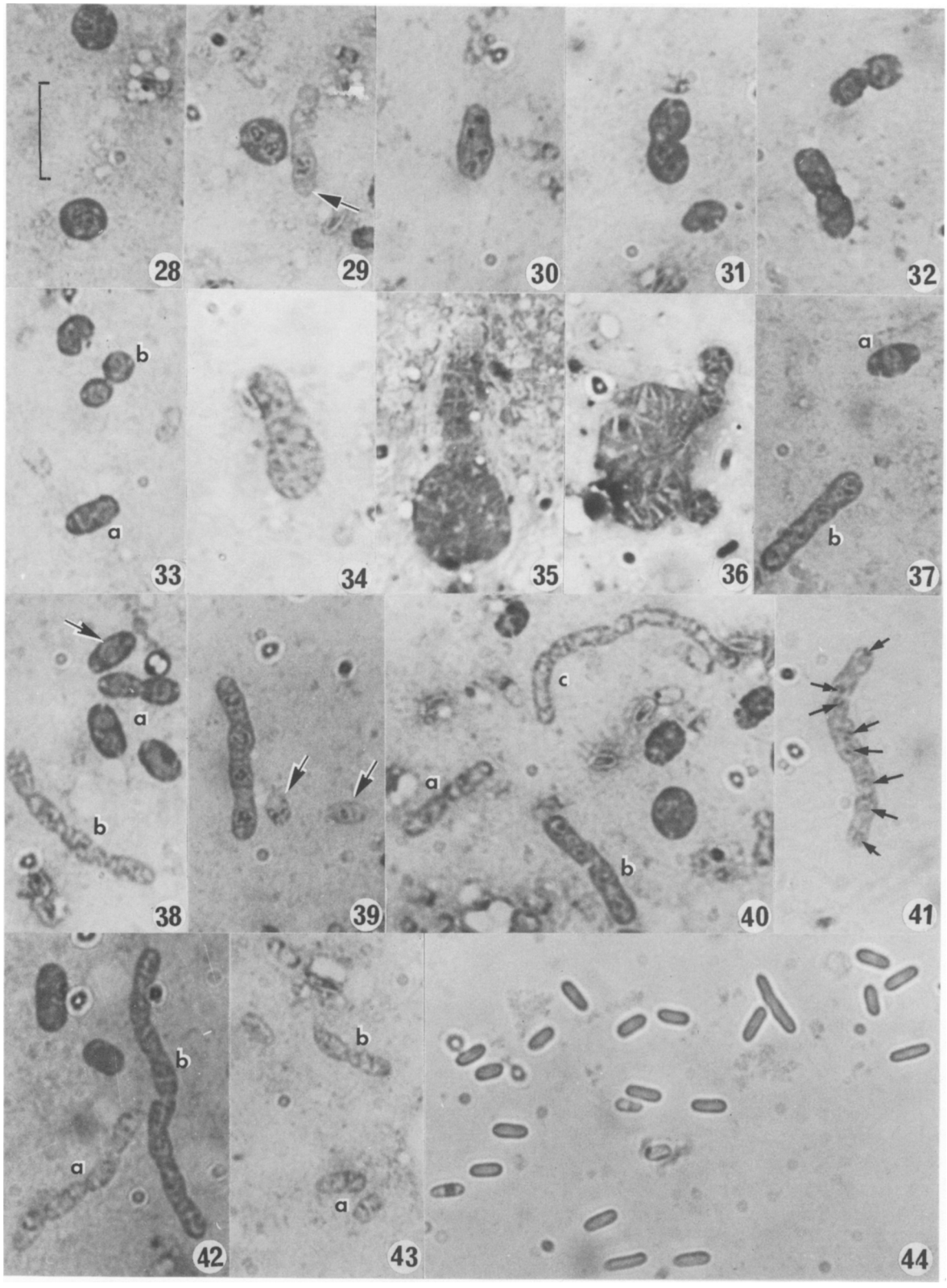
Stages in eggs

Nearly all stages found in eggs were sporogonic. A few rounded uninucleate, binucleate and tetranucleate meronts were present (Figs. 45, 46) and it was presumed that most of the proliferation had ceased when the eggs were smeared a few days after having been laid. Sporonts were often misshapen, sometimes grossly abnormal (Figs. 47, 49). Sporonts gave rise to sporoblasts by binary fission, or by division into short chains (Figs. 50–53). Spores were abundant.

***Nosema wistmansi* nom. nov.**

Stages in larvae and adults

Meronts were typically rounded cells, mostly binucleate or tetranucleate with nuclei in diplokaryon arrangement (Figs. 54–57). Some had higher numbers of diplokarya (Figs. 58, 59): several were seen with four and eight and there was even one with 11 diplokarya. A few stages were seen apparently with a single nucleus (Figs. 55, 56, 60) but there was no means of telling wether or not this was an artefact of viewing one nucleus superimposed on another.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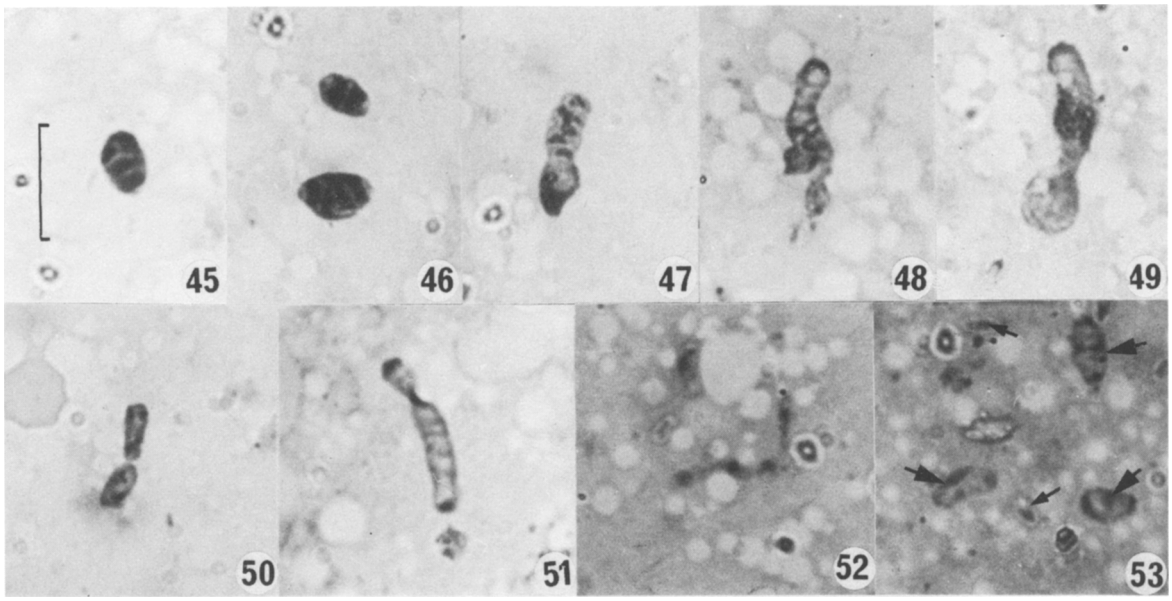

Figs. 45–53. *Orthosoma operophterae*: stages in eggs. Giemsa-stained. Figs. 45, 46. Binucleate and tetranucleate stages, probably meronts. Figs. 47, 48, 50, 51, 52. Multinucleate, elongate stages, probably of the sporogonic sequence. Fig. 49. Giant uninucleate stage, one of many abnormal forms found in eggs. Fig. 53. Sporonts? (large arrows) before elongation and sporoblast production and spores (small arrows). Scale bar = 10 μm.

Nuclei were either compact and homogeneous or composed of a central deeply-stained body (nucleolus?) surrounded by a reticulum (Figs. 54, 61, 62). Division stages were rarely seen: there was no evidence of multiple fission and even the multinucleate stages were thought to divide by binary fission (plasmotomy) into smaller meronts. In one smear binucleate meronts ranged from 5.5 to 8.0 μm diameter, tetranucleates from 7.5 μm diameter to 11.0 × 9.0 μm and a single octonucleate stage measured 15.0 × 14.0 μm.

Sporonts were elongate cells, either fusiform or ellipsoid. They were initially binucleate (Fig. 63) and later tetranucleate with polar nuclei. Mature sporonts measured a mean of 9.6 × 2.8 μm. Sporogony was completed by division of the tetranucleate sporonts into binucleate sporoblasts (Fig. 64), which matured into spores. Sporoblasts measured $5.9 \pm 0.6 \times 1.6 \pm 0.3$ μm (n = 50). Spores were binucleate (Figs. 65, 66). In stained smears they looked ellipsoid (Figs 67) and measured $6.6' \pm 0.8 \times 3.0 \pm 0.1$ μm (n = 70). When fresh

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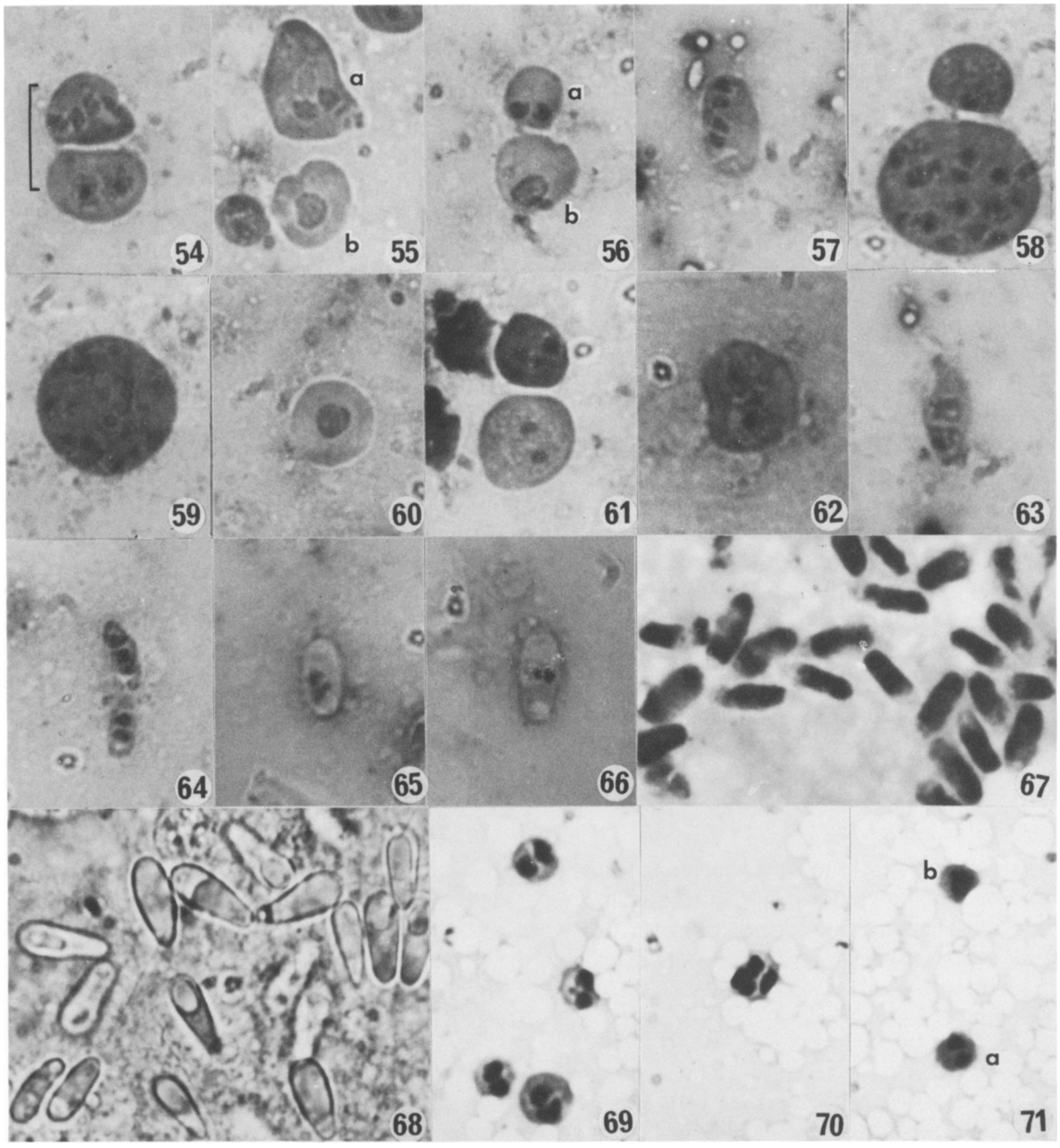
Figs. 28–43. *Orthosoma operophterae*: stages of development in larvae and adults of *Operophtera brumata*. Giemsa-stained.
Figs. 28, 29. Binucleate meronts; the ring-like distribution of chromatic material in the nuclei is distinct; an elongate sporont in Fig. 29 (arrow) shows the difference in cytoplasmic staining between meronts and sporonts. Fig. 30. Tetranucleate meront. Fig. 31. Dividing tetranucleate meront. Fig. 32. Dividing tetranucleate and binucleate meronts. Fig. 33. Elongate binucleate meront (a) and almost complete separation of another (b) into uninucleate products. Figs. 34–36. Multinucleate irregular stages of the merogonic cycle. Fig. 37. Possible pre-sporont stage (a) with central large nucleus, and tetranucleate sporont (b) in which the nuclei may be dividing. Fig. 38. Group of meronts (a) with a pre-sporont (arrow), and a sporont (b) dividing into 4 sporoblasts. Fig. 39. Tetranucleate sporont, and 2 isolated sporoblasts (arrows), in which the nucleus appears as separate granules. Fig. 40. Division of sporonts: a — binucleate sporont into 2 sporoblasts; b — tetranucleate sporont into 2 binucleate segments; c — octonucleate sporont into 4 binucleate segments. Fig. 41. Octonucleate sporont dividing into 4 binucleate segments – the polar positions of the nuclei in the segments are clear (arrows). Fig. 42. Division of tetra- (a) and octo- (b) nucleate sporonts into uninucleate sporoblasts. Fig. 43. Sporoblasts of unequal sizes (a) with band-like nuclei, and disporoblastic sporont (b).
Fig. 44. Fresh spores showing the wide size range. Scale bar for all figures = 10 μm.



Figs 54–67. *Nosema wistmansi*: stages of development in larvae and adults of *Operophtera brumata*. Giemsa-stained.
Fig. 54. Tetranucleate and binucleate meronts; note nuclear structure of the binucleate stage with reticulate background and 'nucleolus'. Figs. 55, 56. Binucleate (a) and uninucleate (b) meronts. Fig. 57. Tetranucleate meront. Fig. 58. Meront with 8 diplokarya. Fig. 59. Meront with 11 diplokarya. Fig. 60. Uninucleate meront. Figs 61, 62. Binucleate meronts, showing reticulate nuclei with central chromatic 'nucleolus'. Fig. 63. Binucleate sporont. Fig. 64. Division of tetranucleate sporont into diplokaryotic sporoblasts. Figs. 65, 66. Diplokaryotic spores. Fig. 67. Densely stained ovoid spores.
Fig. 68. *N. wistmansi* Fresh spores; note pyriform shape and posterior vacuole.
Figs. 69–71. *N. wistmansi* stages in eggs. Giemsa-stained.
Fig. 69. Binucleate meronts. Fig. 70. Tetranucleate meront. Fig. 71. Binucleate (a) and possibly uninucleate (b) meronts.
Scale bar = 10 μm.

they were elongate pyriform (Fig. 68) with a prominent vacuole at the wider end: they measured $8.0 \pm 0.68 \times 3.0 \pm 0.3$ μm (n = 50).

Stages in eggs

Only meronts were found in smears of undifferentiated eggs. Most were rounded binucleate cells measuring 3.5–5.0 μm diameter. A few appeared to be uninucleate and one tetranucleate stage was found (figs. 69, 70, 71).

Discussion

The polysporous microsporidia which sporulate within sporophorous vesicles and were originally assigned to the genus *Pleistophora*, have recently been shown to belong to four distinct genera: *Tuzetia* Maurand, Fize, Fenwick & Michel; *Pleistophora* Gurley; *Vavraia* Weiser; and *Polydispyrenia* Canning & Hazard (Loubès & Maurand, 1976; Canning & Hazard, 1982). In two of these, *Tuzetia* and *Polydispyrenia*, the diplokarya of young plasmodia undergo meiosis at the onset of sporogony giving haploid spores. In contrast, in *Pleistophora* and *Vavraia*, the nuclei are always isolated and these genera are differentiated from one another by the thickness of the sporophorous vesicle wall and mode of division of the sporogonial plasmodium. All four genera have sporophorous vesicle walls of parasite origin.

Percy, Wilson & Burke (1982) have reported on a *Pleistophora* sp. from the larch sawfly, *Pristiphora erichsonii* Hartig in which the membrane surrounding the groups of spores was derived from host cell endoplasmic reticulum. Their generic diagnosis was tentative and will require reassessment in the light of the host cell origin, rather than parasite origin, of the membrane round the spores.

On the evidence available, including ultrastructure (Canning, 1981), '*Pleistophora*' *operophterae* accords in some of its features with the genus *Vavraia*, as defined by Canning & Hazard (1982): the nuclei are isolated throughout development and the sporogonial plasmodium undergoes multiple fission to produce uninucleate sporoblasts via peripheral buds. However the sporophorous vesicle wall is finer than would be expected for *Vavraia* and looks more like a membrane than a layer of secretions as described for *Vavraia*. It may be that a new genus will be required to accommodate *P. operophterae*. Additional ultrastructural studies are required to clarify the taxonomy and compare *P. operophterae* with the *Pleistophora* sp. from the larch sawfly described by Percy *et al.* (1982).

In the original description (Canning, 1960) stages were described which were elongate with the extremities drawn out into cytoplasmic processes curled back towards the thick region of the body. They were binucleate or uninucleate and it was proposed that they were pre-sporonts in which autogamy took place as a prelude to sporogony. Clumps of similar bodies with tenuous cytoplasm were also seen in the present study but it is now believed that they were produced by disruption of the vacuolated sporogonial plasmodia at a stage when telophase nuclei were still present or by disruption of the cytoplasmic strands holding sporoblasts together. The manner in which the cytoplasmic processes were curled towards one another as originally illustrated is suggestive of disrupted vacuoles.

Sporophorous vesicles with as few as four spores were seen in the present study, which showed that division could commence in the sporogonial plasmodia before the ten-nucleate stage previously noted. It is more difficult to determine the upper limit to the number of spores but it is doubtful whether more than about 60 can be attained.

Purrini & Skatulla (1979) also reported microsporidia producing spores in sporophorous vesicles from the gut and fat body of *O. brumata* and the large winter moth, *Erannis defolaria*. They identified the species in the gut as *Pleistophora schubergi* Zwölfer but were uncertain whether the parasite of the fat body was the same or a new species. They made reference in passing to *P. operophterae* but apparently did not consider whether the parasites of the gut and fat body might be identified with it rather than with *P. schubergi*, which, in all its host records, is restricted to the gut epithelium. The limited data given in Purrini & Skatulla's paper would not exclude this possibility.

The present light microscopic study has enabled us to propose more accurate taxonomic and nomenclatorial assignments for two of the microsporidia described from winter moths, than those previously published (Canning, 1960; Purrini & Skatulla, 1979).

The parasite named *Nosema operophterae* by Canning (1960) can now be removed from this genus with certainty, on the basis of its isolated nuclei and ribbon-shaped sporonts with two, four or eight nuclei (rarely 12) giving chains of unikaryotic sporoblasts. There are no sporophorous vesicles and the chains of sporoblasts break up into free spores. The two apansporoblastic genera with similarly shaped sporonts, namely *Perezia* and *Ameson* differ by virtue of their diplokarya in merogony. A new genus *Orthosoma* has been proposed, with *Orthosoma operophterae* (Canning, 1960) as the type species.

Taxonomic summary

Genus *Orthosoma* n.g.

Nuclei are isolated in all stages of the life-cycle.
Sporogony is apansporoblastic.
Meronts are rounded, usually uni, bi- or tetranucleate with division by binary fission; occasionally multinucleate.

Sporonts are ribbon-shaped, with two, four or eight, rarely 12, linearly spaced nuclei. Sporogony is bi-, tetra- or octosporoblastic.

Sporoblasts are held together in chains but spores are free.

Derivation of Name. From Greek *orthos* = straight and *soma* = body, referring to sporonts.

Type Species. Orthosoma operophterae (Canning, 1960) n. comb. parasitic in the silk glands, gut and other tissues of larvae and adults of winter moth, *Operophtera brumata.* Also in eggs. With characters of the genus. Spores ovoid, very variable in length but of constant width, measuring $3.5 \pm 0.4 \times 1.3 \pm 0.2$ μm fresh.

The stages with polar nuclei, referred to as sporonts, in the original material obtained from Oxford (Canning, 1960) are now recognized as sporoblasts. None of the ribbon-shaped sporonts was seen in the Oxford material and they were often absent from smears of larvae and adults examined in the present study. This is suggestive of sporadic rather than continuous sporogony. The frequent appearance of the nucleus as separate dots in the sporoblasts and spores accounts for the previous observation that the mature spores had two nuclei, lying close together at the edge of a polar vacuole.

The third parasite found in the Wistman's Wood population of winter moths is clearly identical with the species named *Nosema operophterae* by Purrini & Skatulla (1979). These authors correctly assigned the parasite to the genus *Nosema*, as has been confirmed by the present study of the merogonic and sporogonic sequences. Unfortunately Purrini and Skatulla used a specific name which was preoccupied and which is now a synonym of *Orthosoma operophterae.* We have suggested a new specific name *Nosema wistmansi* from the name of the woodland where we found it. The species is notable for its large size. We did not find three distinct sizes of spores, as recorded by Purrini & Skatulla. It is possible that the means of the three sizes of spore that they recorded would agree with the measurements for the Wistman's Wood isolate but the means cannot be calculated from their data.

The demonstration that all three of these microsporidia have stages present in non-embryonated eggs throws some light on the transmission between generations of winter moths, which have a long non-feeding period from pupation in mid-summer until the eggs hatch the following spring. However the actual mechanism by which the parasites invade the cells of the new generation of larvae has yet to be elucidated since Canning & Barker (1982) have reported that stages of *O. operophterae*, which were widespread in unembryonated eggs, were absent from the tissues of newly hatched larvae of the same batch and that spores were concentrated in the meconium.

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