

**Pleistophora oncoperae sp.n. (Protozoa: Microsporida) from
Oncopera alboguttata (Lepidoptera: Hepialidae)
in Australia**

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Pleistophora oncoperae sp.n. is described from adults and larvae of *Oncopera alboguttata* and *O. rufobrunnea*. The main site of infection was muscle, though fat body and connective tissue were also infected. Fresh pansporoblasts measured about 25 μm in diameter and contained 16 to 32 or more spores with a mean size of $5.9 \times 3.1 \mu\text{m}$. Macrospheres measuring $7.7 \times 4.4 \mu\text{m}$ were also seen. The mean polar filament length was $158 \mu\text{m}$; ultrastructural studies showed that the filament is normally arranged in 14 coils (range, 13 to 20) at an angle of 53.5° to the axis of the spore. The species was found to be distinct from all previously described *Pleistophora* reported from Lepidoptera.

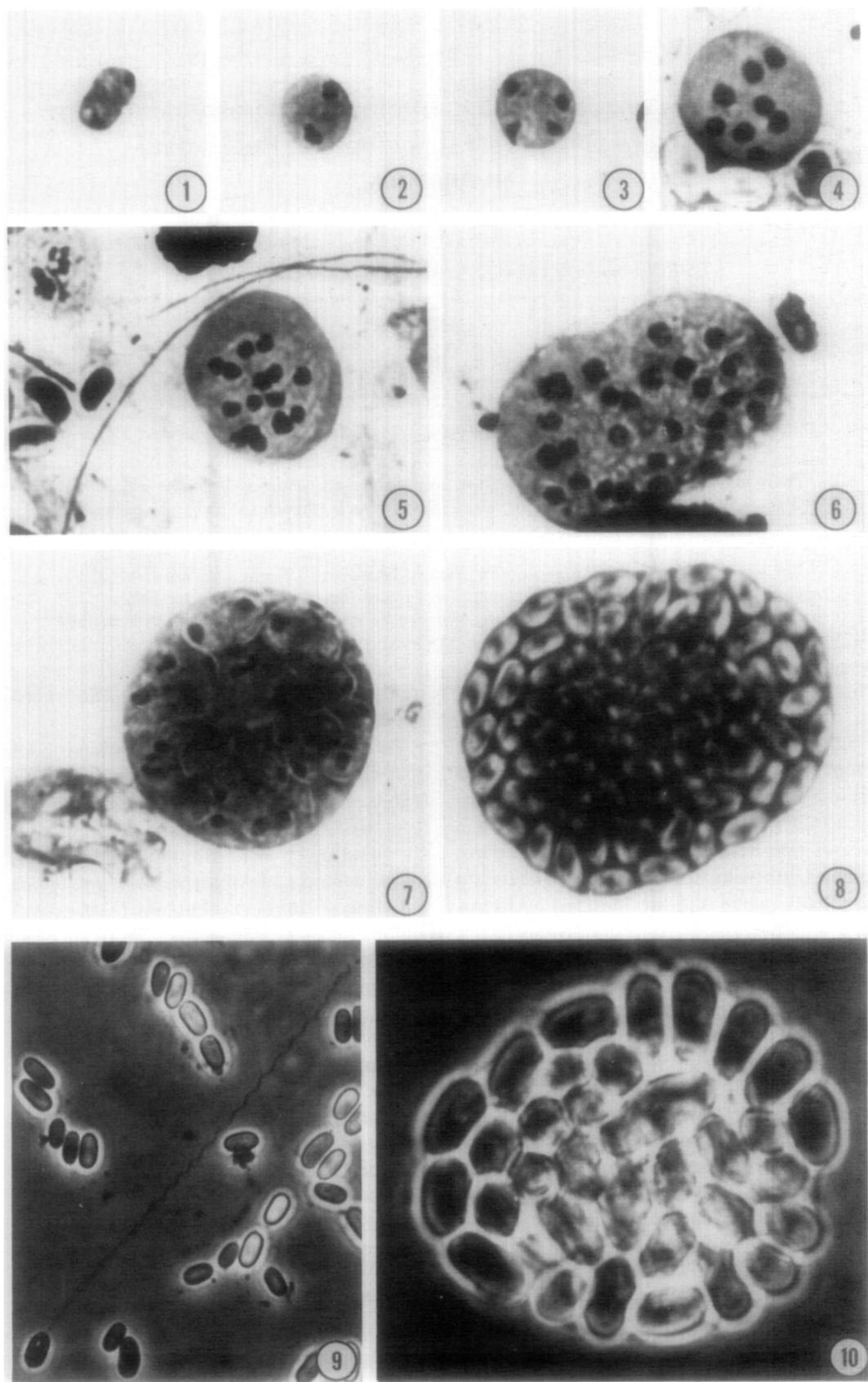
INTRODUCTION

Oncopera alboguttata (Lepidoptera: Hepialidae) is a severe pest of pastures in Ebor/Dorriga region of New South Wales, Australia (Bourke, 1966), where it occurs in association with *O. rufobrunnea* (Common, 1966). Outbreaks of *O. alboguttata* develop every few years and are induced by favorable weather and pasture conditions (R. J. Roberts, pers. comm.). The collapse of the recent outbreak was found to be correlated with high incidences of a microsporidian and an entomopoxvirus (Milner, 1977). Thus, on the average, 48% of the 1035 larvae and 127 adults of *O. alboguttata* collected during 1971-1974 were infected with a microsporidian, and an average of 24% of larvae with an insect poxvirus. Double infections were very common. Adult moths were frequently infected with the microsporidian but were never infected with the virus. In addition, 259 larvae and 29 adult *O. rufobrunnea* were examined. The virus was not found, but 15 larvae (6%) and 1

moth (3%) were infected with the microsporidian.

To date, only fungal diseases have been described from *Oncopera* spp.; Martyn (1960) recorded a *Cephalosporium* sp. (tentative identification) and a *Cordyceps* sp. infecting *O. intricata* while Elder (1971) found a strain of *Metarrhizium anisopliae* infecting *O. mitocera* but not *O. brachyopilla* in North Queensland. More recently a *Nosema* sp. (Protozoa: Microsporida) was found infecting *O. tindalei* at Walcha, New South Wales, and a strain of *M. anisopliae* was isolated from *O. alboguttata* at Ebor (Milner, unpubl.).

This study forms part of a program to evaluate candidate microbial control agents for pasture pests in Southeastern Australia. Previous publications have been concerned with a new variety of *Bacillus popilliae* (milky disease) from *Rhopaea verreauxi* (Coleoptera: Scarabaeidae) (Milner, 1974) and a virus from *Othonius batesi* (Coleoptera: Scarabaeidae) (Milner and Lutton, 1975).



MATERIALS AND METHODS

Only field-infected insects were studied as all attempts to infect larvae in the laboratory failed. However, larvae were successfully maintained in the laboratory using the peat and carrot method devised for pasture scarabaeids (Milner, 1974). Smears were studied either by phase contrast or by fixing in methanol followed by Giemsa's stain. For sectioning, insects were fixed in Carnoy-Lebrun, embedded in paraffin, sectioned at 5–10 μm , and stained with Giemsa's stain. For transmission electron microscopy, infected tissues were fixed in 4% glutaraldehyde in Na-cacodylate buffer, pH 7.4, and, after washing in the buffer, were postfixed in buffered 2% osmium tetroxide. In addition, pure spores, which had been stored frozen at -20°C , were fixed in 1.2% potassium permanganate and postfixed in 1% osmium tetroxide (Gassouma and Ellis, 1973). The material was then embedded in Spurr's (1969) medium, and thin sections cut with a diamond knife. These were stained with lead citrate and uranyl acetate before examination with JEM 100C and Siemens Elmiskop I electron microscopes.

Measurements of the stages of the microsporidan were made either with a filar micrometer eyepiece or by the photographic method. To evaginate the polar filament the wet-dry-wet method of Kramer (1960) was used in conjunction with mechanical pressure.

RESULTS

Pleistophora oncoperae sp.n.

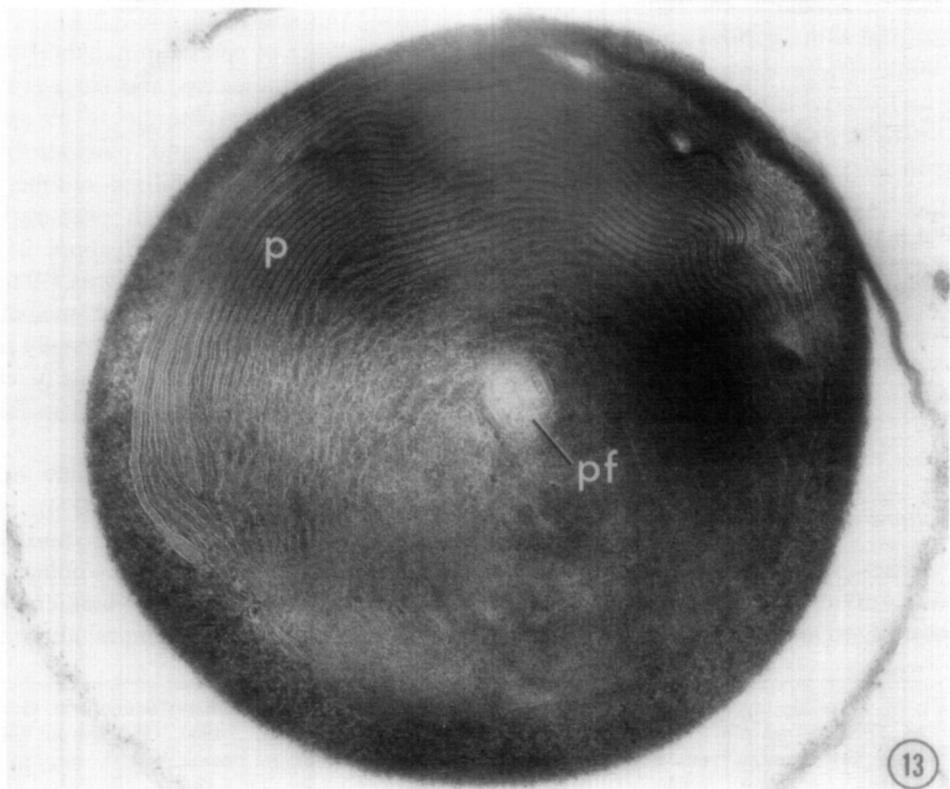
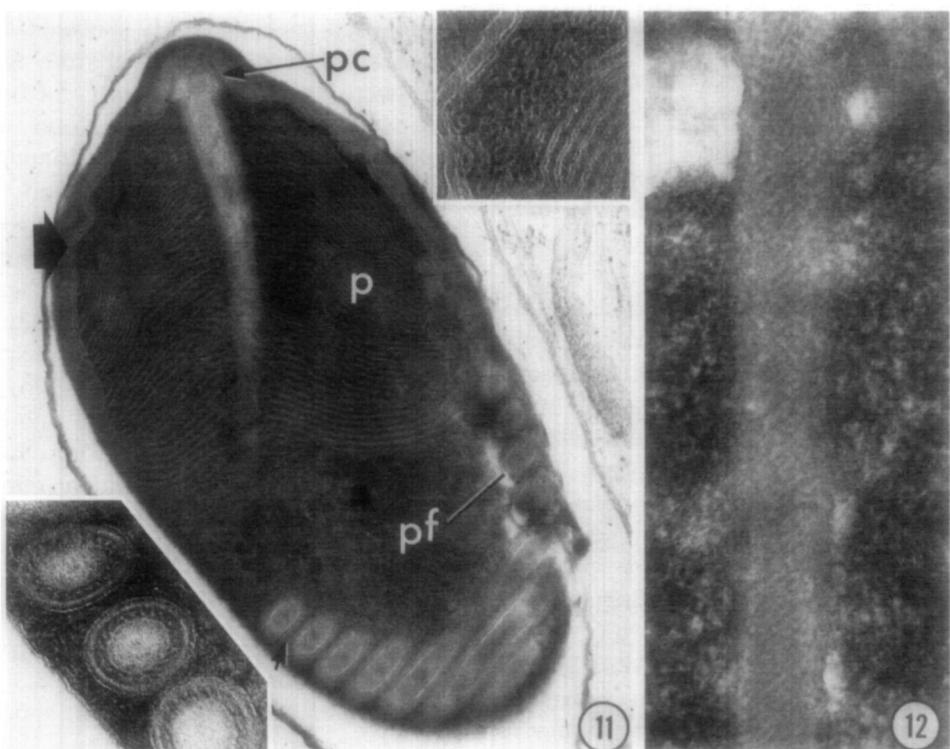
The earliest stage of the life cycle recognized was a dividing schizont (Fig. 1). It was not possible to distinguish between non-

dividing schizonts and early sporonts. Both stages were rare, as only mature infections were studied. Sporonts (schizonts?) were seen with 2, 4, 8, 16, and more nuclei (Figs. 2–6). Large, multinucleate sporonts were occasionally seen, apparently dividing (Fig. 7), and were possibly undergoing a type of plasmotomy similar to that reported for species of *Pleistophora* by Canning et al. (1964). When the sporont is mature, the cytoplasmic cleavage takes place and results in a pansporoblast containing 16 or more almost spherical sporoblasts (Fig. 8). These form spores within the pansporoblast which remains intact (Figs. 8, 10). At no stage in the life cycle were paired nuclei ("diplokarya") recognized. In stained smears, the following measurements were recorded: binucleate schizonts (sporont?), 3.2 μm in diameter; sporonts with 4 nuclei, 8.3 μm ; sporonts with 8 nuclei, 10.1 μm ; sporonts with 16 nuclei, 16.7 μm ; pansporoblasts, 21.7 μm ; and spores, 4.7 \times 2.2 μm .

The numbers of spores in pansporoblasts were very variable; 16 and 32 were the commonest numbers but up to 39 spores have been counted, and pansporoblasts with large but undetermined numbers of spores were seen. In fresh preparations, the pansporoblast measured about 25 μm or larger, while the spore size was 4.9 to 6.7 \times 2.7 to 3.4 μm . Macrospores, measuring about 7.7 \times 4.0 μm , were occasionally seen. The long polar filament was easily evaginated, and the filaments ranged in length from 110 to 193 μm (Fig. 9).

Because of the taxonomic value of the polar filament (Burges et al., 1974), ultrastructural studies of the mature spore were carried out. Details of the polar cap, polaroplast, and coiled polar filament were seen but, after several attempts, glutaralde-

Figs. 1–10. The life cycle of *Pleistophora oncoperae* sp.n.; air-dried, methanol-fixed, and Giemsa-stained. (1), Dividing schizont. $\times 1800$. (2), Binucleate schizont (sporont?). $\times 1800$. (3), Sporont with 4 nuclei. $\times 1800$. (4), Sporont with 8 nuclei. $\times 1800$. (5), Sporont with 16 nuclei. $\times 1800$. (6), Sporont with more than 16 nuclei. $\times 1800$. (7) Multinuclear sporont showing cytoplasmic cleavage. $\times 1800$. (8), Pansporoblast containing developing sporoblasts. $\times 1800$. (9), Spore with extruded polar filament (phase). $\times 1000$. (10), Mature spores within a pansporoblast (phase). $\times 2300$.



hyde fixation was found to be inadequate for the nuclear material (Fig. 11). In cross section, the polar filament appeared almost circular with an outer narrow electron-lucent layer enclosing three more layers of contrasting electron density and, finally, an inner layer consisting of about 20 distinct, longitudinal ridges (Fig. 11). These ridges were arranged spirally at an angle of 35° to the longitudinal axis of the polar filament and were about 8 nm wide (Fig. 12). The central core of the polar filament was generally electron lucent and appeared empty (Figs. 11, 12). The total diameter of the polar filament was about 150 nm while the central core was about 90 nm in diameter. No differences in this basic structure were seen throughout the entire length of the polar filament. Similar ridges have been described by Vavra (1972) from *Nosema whitei* and by Lom and Corliss (1967) from *Pleistophora hyphessobryconis*. The "spiral fibrils" reported by Sprague and Vernick (1969) are probably similar structures.

In longitudinal sections of the spore, the polar filament is seen as a single row of coils or with the final two to five coils forming a double row inside the others. The numbers of turns were found to be quite variable, ranging from 13 to 20, though in the majority of spores 14 coils were seen. The anterior angle of tilt (Burges et al., 1974) was measured for a number of spores, and the lowest value was 53.5°.

The anterior half of the spore was occupied by the polaroplast (Fig. 11). The most anterior part of the polaroplast consisted of a large number of tubules or sacs (Fig. 11, inset). However, most of the polaroplast consisted of a large number of interconnect-

ing lamellae which in cross section circled the polar filament (Fig. 13) in parallel bands about 25 nm apart and in longitudinal section appeared parallel to the horizontal axis of the spore (Fig. 11). At the polar cap the two outer membranes separate (Fig. 11); one is apparently joined with the inner spore wall and the other forms an outer membrane or "sheath" (Sprague and Vernick, 1969) around the polar filament. No connection between the posterior end of the polaroplast and other parts of the spore could be detected.

In an effort to improve the fixation, spores which had been stored at -20°C were fixed in potassium permanganate (Gassouma and Ellis, 1973). The treatment gave satisfactory fixation of the polar filament, but the nuclear material was not well defined and the polaroplast appeared highly vacuolated. The subsequent discovery that the polar filament would no longer evaginate suggested that this disruption was caused by freezing and that the spores were non-viable. Previous reports on the ability of microsporidian spores to withstand storage at -20°C are contradictory (Bailey, 1972).

Pathology

In older larvae, the infection was easily detected by the abnormal white coloration of the infected muscle. Infections were generally confined to the fifth and sixth abdominal segments; however, in most severe cases other segments were infected as well as the fat body and rectal muscles (Fig. 14). Mature larvae fed actively and developed normally to produce infected adults devoid of external symptoms. In contrast, the infection was fatal to young

FIG. 11. Electron micrograph of a thin section through the side of a mature spore showing polaroplast (p), polar cap (pc), and polar filament (pf) with the ridges in cross section (arrow). Glutaraldehyde fixation. $\times 28,000$. Details of polaroplast are shown in top right inset ($\times 100,000$) and of the polar filament of another spore in the lower left inset ($\times 72,000$).

FIG. 12. Longitudinal section through polar filament at the end adjacent to the polar cap showing ridges as diagonal banding. Permanganate fixation. $\times 77,000$.

FIG. 13. Electron micrograph of a thin section across the upper half of a mature spore showing polar filament (pf) encircled by the lamellae of the polaroplast (p). Glutaraldehyde fixation. $\times 55,000$.

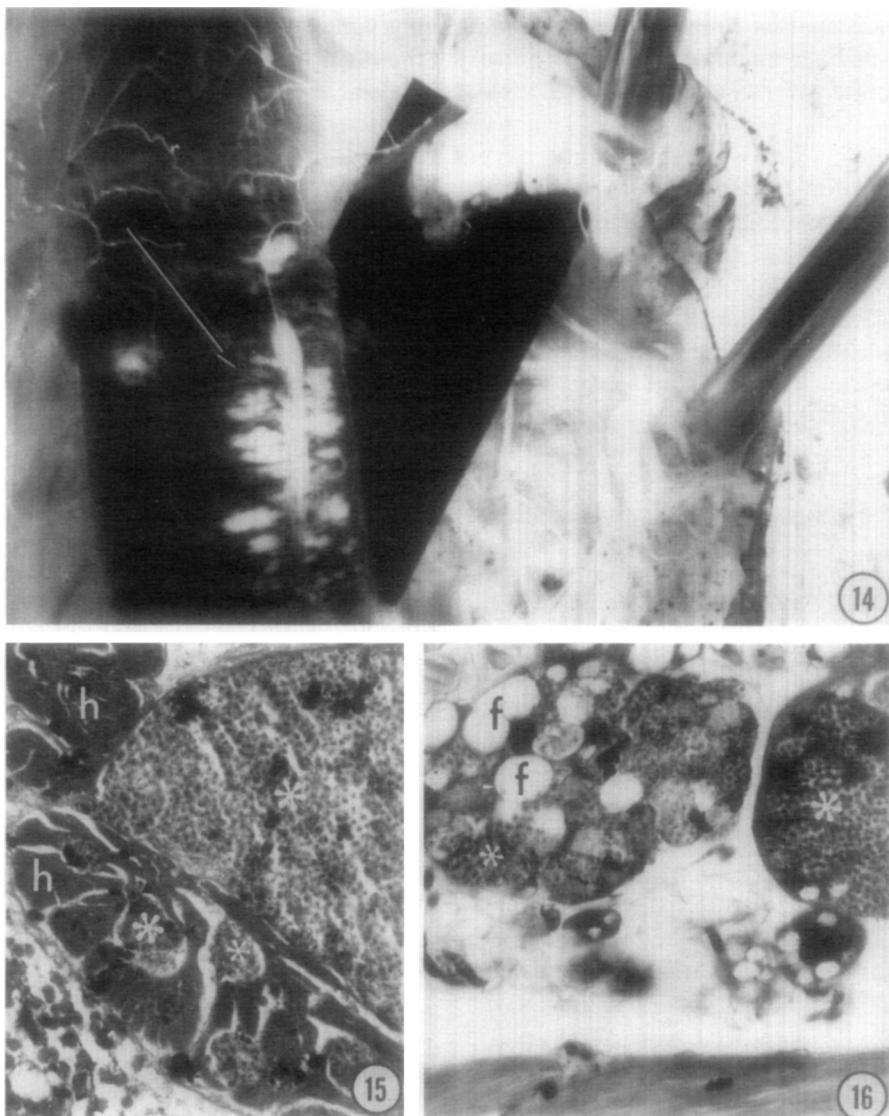


FIG. 14. A larva of *Oncopera alboguttata* dissected to show the white areas of the muscles (arrow) which were infected by *Pleistophora oncoperae* sp.n. Note that a piece of black card has been inserted beneath the rectum.

FIG. 15. Section through muscle of *Oncopera alboguttata* showing healthy areas (h) and areas infected by *Pleistophora oncoperae* sp.n. (*). $\times 350$.

FIG. 16. Section through fat body of *Oncopera alboguttata* showing fat globules (f) and spores of *Pleistophora oncoperae* sp.n. (*). $\times 350$.

larvae which had probably been infected via the egg (Milner, 1977).

Histological sections confirmed that the muscle was the main site of infection (Fig. 15). Infected fat body (Fig. 16) and connective tissue were also detected.

SYSTEMATICS

Host and site. All stages of *Oncopera alboguttata* and *Oncopera rufobrunnea*. Main site of infection is muscle but also fat body and connective tissue.

TABLE I
SPECIES OF *PLEISTOPHORA* RECORDED FROM LEPIDOPTERA

<i>Pleistophora</i> species	Host	Locality	Main tissue infected	Spore size (mm)	Reference
<i>P. aporiae</i>	<i>Aporia crataegi</i>	Czechoslovakia	Midgut epithelium	2.0 × 1.5 (4.5 × 2.5)	Weber (1956)
<i>P. bilbiani</i>	<i>Antherea pernyi</i>	Czechoslovakia	Midgut epithelium	2.0 × 1.5	Weber (1963)
<i>P. californica</i>	<i>Gnorimoschema operculella</i>	United States	Fat body, Malpighian tubules	2 × 1	Steinhaus and Hughes (1949)
<i>P. neustriæ</i>	<i>Malacosoma neustriæ</i>	Germany	Fat body, midgut	3.0 × 1.8	Günther (1958)
<i>P. oncopera</i>	<i>Oncopera alboguttata</i> <i>O. rufobrunnea</i>	Australia	Muscle	5.9 × 3.1	This paper
<i>P. operophtherae</i>	<i>Operophtera brumata</i>	United Kingdom	Silk gland	2.3–3.0 × 1.0–1.5	Canning (1960)
<i>P. pandemis</i>	<i>Pandemis corylana</i>	Czechoslovakia	Midgut epithelium	3.0 × 2.0	Weber (1957)
<i>P. reciprocaria</i>	<i>Ascotis selenaria</i> <i>reciprocaria</i>	South Africa	Fat body, Malpighian tubules	6.0 × 4.5	Buitendag (1965)
<i>P. schubergi</i>	<i>Porthetria dispar</i> <i>Euproctis chrysorrhœa</i>	Germany	Midgut epithelium	2.0–2.5 × 3.0–4.5	Zwölfer (1927)

Morphology. Fresh material. Pansporoblasts measure about 25 µm and contain 16, 32, or more spores. Mean spore size ± standard error was $5.9 \pm 0.089 \times 3.1 \pm 0.035 \mu\text{m}$ ($n = 40$) with an occasional macrospore measuring up to $7.7 \times 4.0 \mu\text{m}$. Polar filament measures $158 \pm 8.3 \mu\text{m}$ (range = 110 to 193 µm, $n = 10$). The anterior angle of tilt of the polar filament was 53.5°, and the number of coils usually 14 (range, 13 to 20).

Type locality. Ebor, New South Wales, Australia.

Type slides. Slides will be deposited with Dr. Reutzler, International Protozoan Type Slide Collection, Smithsonian Institution, Washington, D.C.

Derivation of name. A *Pleistophora* sp. from two species of *Oncopera*.

Differentiating characters. On the basis of spore size and the main tissue infected, this species can be distinguished from all other *Pleistophora* spp. described from Lepidoptera (Table 1). The species most similar in spore size, *P. reciprocaria*, has a much shorter polar filament (up to 84 µm compared with 110 to 193 µm for *P. oncoperæ*).

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