

## *Tuzetia boeckella* sp. nov. (Protozoa: Microsporida), a Parasite of *Boeckella triarticulata* (Copepoda: Calanoidea) in Australia

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A hitherto undescribed microsporidian has been found in the Australian freshwater copepod, *Boeckella triarticulata*, collected from Lake Burley Griffin, Canberra. We name this protozoan *Tuzetia boeckella* n.sp. and describe it in this paper. Large numbers of spores were found in the muscle of both sexes and all stages of the animals. The pyriform spores measured  $5.1 \times 2.7 \mu\text{m}$  with the extruded polar filament measuring  $102 \mu\text{m}$ . Ultrastructural studies revealed the presence of a pansporoblastic membrane around each spore. The polar filament was arranged in a single row of 13–14 turns and decreased in diameter toward the posterior end. Few details of the life cycle were elucidated; however, evidence is presented for each sporont forming eight spores. Differentiating characters to distinguish this species from the six other known members of the genus are given.

### INTRODUCTION

Copepods of the genus *Boeckella* (Copepoda: Calanoida) are common and plentiful in standing fresh water in nontropical Australia and New Zealand. The type species, *B. triarticulata* (Thomson), is the most common and widely distributed of the Australasian species (Bayly, 1964).

*B. triarticulata* usually appears to be translucent and straw colored; however, some opaque, salmon-orange-colored animals were netted from Lake Burley Griffin in Canberra, Australia, during February 1979 and found to contain vast numbers of microsporidian spores.

Sprague (1977a) lists some 23 species of Microsporida from copepods. It seems that no microsporidians have been reported from *Boeckella*. Recent studies, notably by Maurand and colleagues in France, have indicated that certain *Nosema*-like Microsporida from copepods and black flies (Dip-

tera: Simuliidae) have uninuclear spores each enveloped by a pansporoblastic membrane. A new genus *Tuzetia* was created for these species (Maurand et al., 1971). The type species *T. infirma* was originally described as *Nosema infirmum* by Kudo (1921) and is parasitic in *Cyclops albidus* in the United States and *Macrocylops fuscus* in France.

### MATERIALS AND METHODS

*Field sampling.* Copepods were hand netted from the surface 0.5 m of Lake Burley Griffin at irregular intervals during 1979–1980. Infected individuals showed a characteristic salmon pink or orange color and were easily separated from the normal translucent copepods using a blunt Pasteur pipette.

*Light microscopy.* Infected copepods were smeared onto clean coverslips, allowed to dry, and fixed with methanol. The wet smears were then stained in a 10% Giemsa (Gurrs R66) buffered at pH 7.4, de-

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hydrated in acetone, and mounted in neutral mounting medium (Gurrs).

Measurements of spores were made from fresh smears using a micrometer eyepiece. Polar filaments were evaginated using the wet-dry-wet method of Kramer (1960). Measurements were made from photographs using a calibrated map measurer.

*Electron microscopy.* Some animals were fixed for electron microscopy in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, containing 1% glucose, for 4 hr at room temperature. Others were fixed overnight in 4% glutaraldehyde in Na-cacodylate buffer, pH 7.4, containing 7% glucose. Both types of specimen were then washed in buffer, postfixed in 2% osmium tetroxide, and embedded in Spurrs medium (Polysciences). Sections were cut with a diamond knife and stained with Toluidine blue (thick sections) or with uranyl acetate and lead citrate (thin sections). The thin sections were viewed with a Hitachi H500 or a JEM 100C electron microscope.

## RESULTS

### *Field Observations*

Infected animals were netted in Lake Burley Griffin throughout the year. Usually less than 0.5% of the population was obviously infected, i.e., orange colored. The behavior and vigor of infected animals did not appear to differ from that of normal individuals. In some preliminary experiments, normal and infected animals survived well in laboratory cultures. However, although the infection was observed in immature copepods and adults of both sexes, infected animals were never found carrying eggs or sperm.

Thick sections from the Spurr's embedded material revealed that most of the cells of the copepod were infected (Fig. 1), and contained so many spores that the tissues involved were unrecognizable, though muscle was probably the main tissues attacked. Gut tissues and the epidermal cells were generally free of infection.

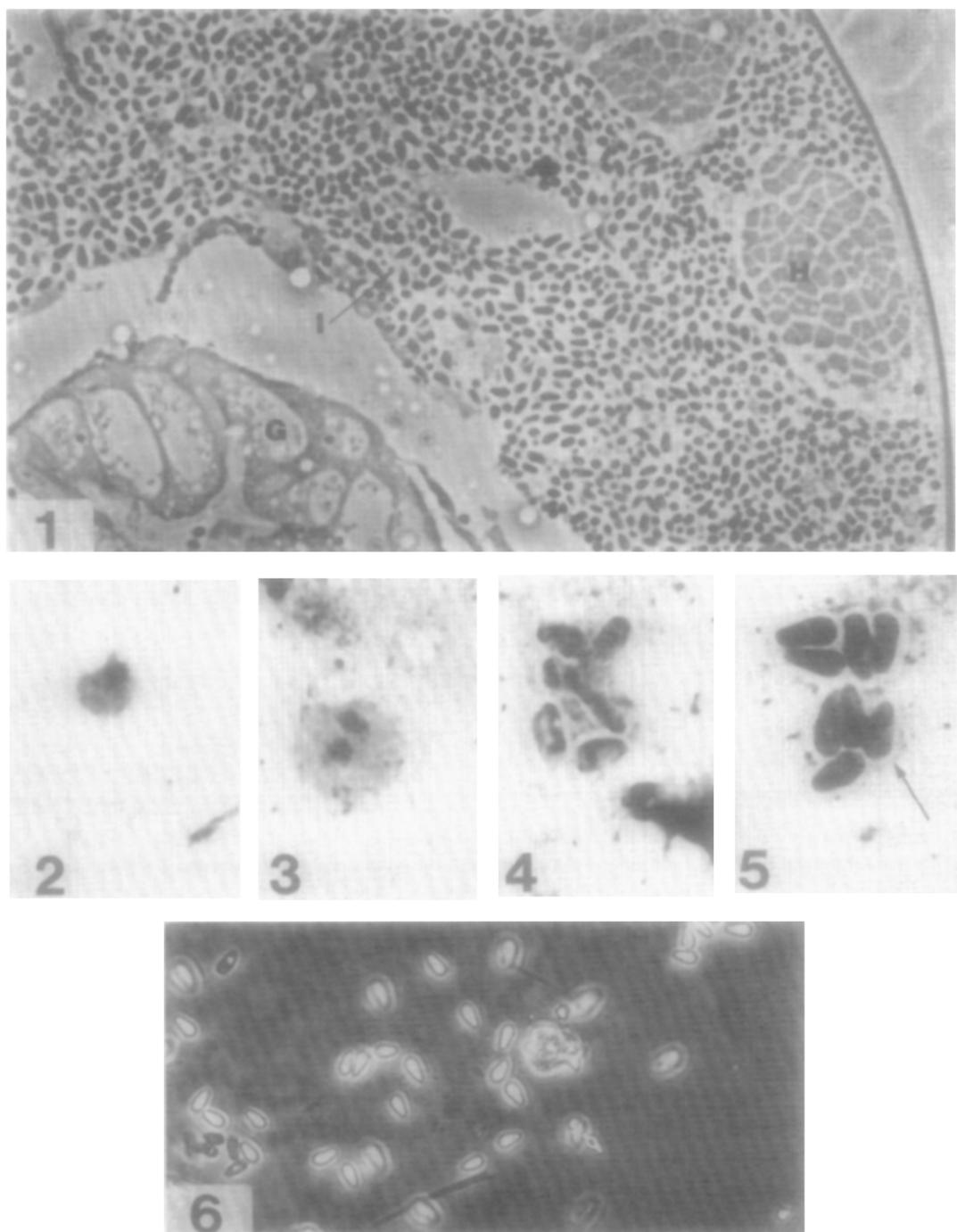
### *Developmental Stages*

Only severely infected animals were examined so we have no information on the early stages of infection. However, from a study of the various stages seen and by analogy with other species Microsporida, the following development sequence is suggested.

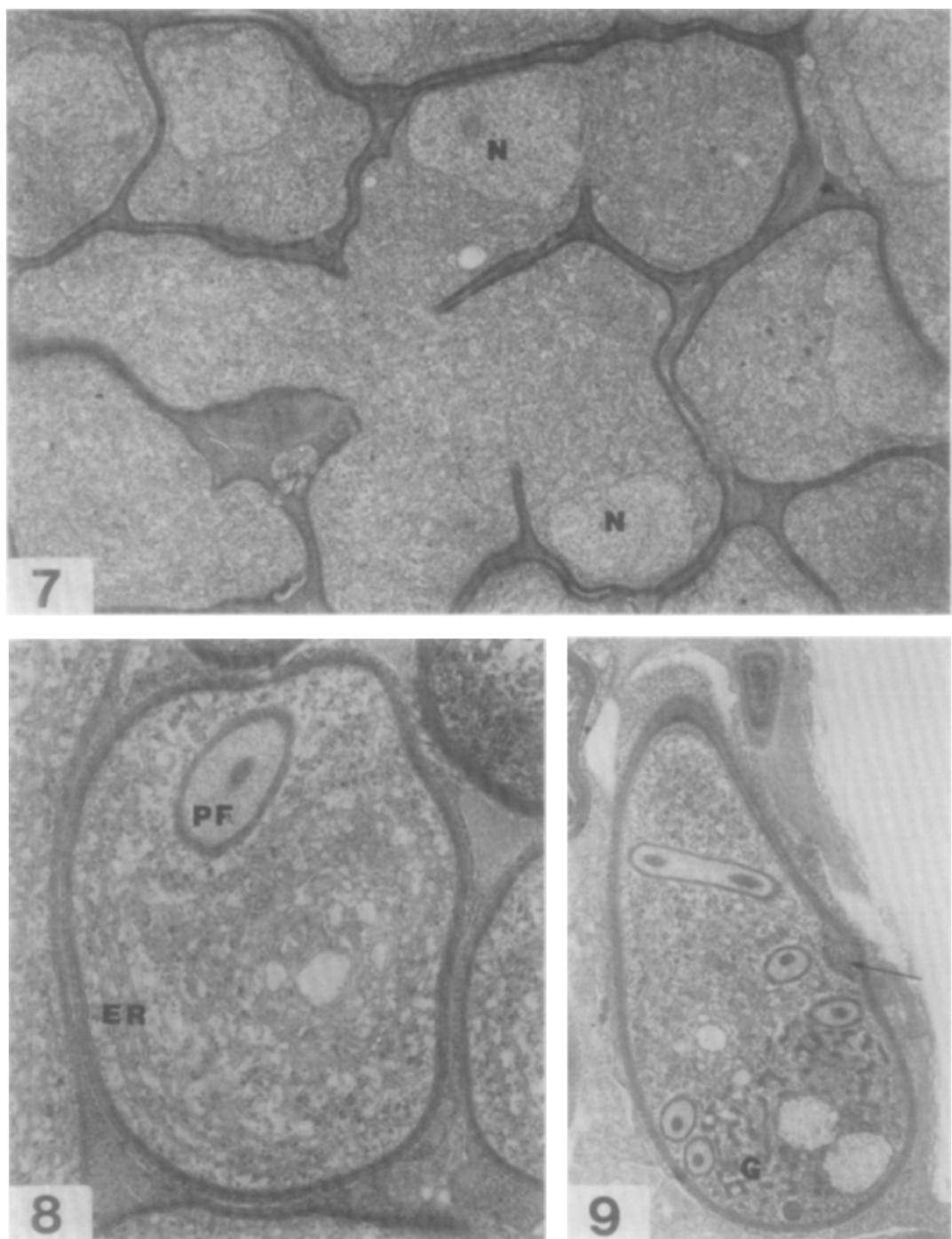
The earliest stages seen with the light microscope, were uni- and binuclear cells interpreted as being sporonts (Figs. 2, 3); paired nuclei ("diplokarya") were not seen probably because the early vegetative stages were not observed. Sporonts became multinuclear and the sporoblasts were individually budded off as each nucleus migrated to the cell surface (Fig. 7). The sporoblasts then developed into spores in a loose cluster (Figs. 5, 6).

With the electron microscope, a single large nucleus was seen in each lobe of the dividing sporont (Fig. 7), while the cytoplasm was densely filled with ribosomes, fragmented endoplasmic reticulum, and a few scattered vacuoles. The sporont wall was thin, probably composed of a double unit membrane. No mitochondria, Golgi bodies, or developing polar filament could be seen.

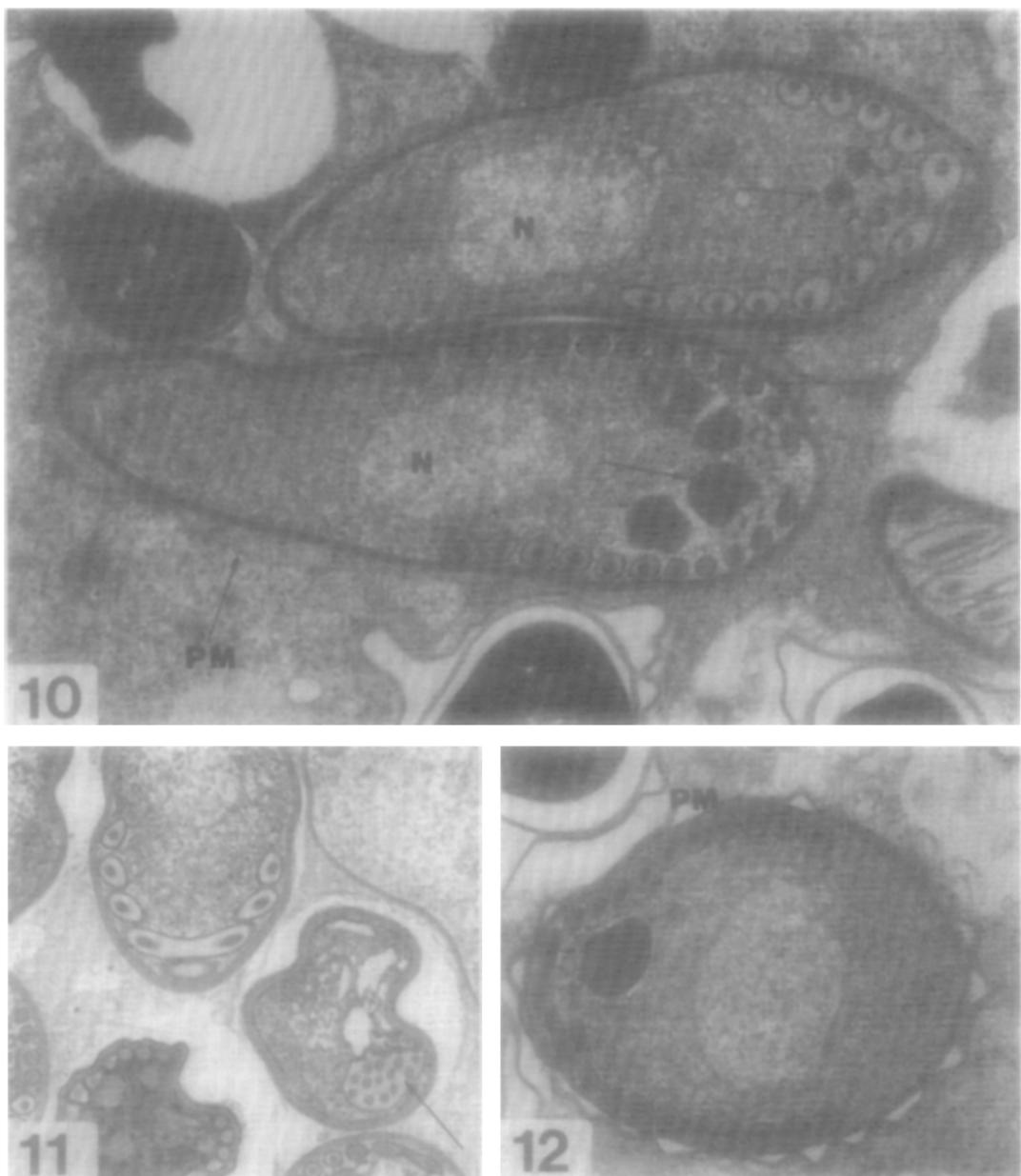
As with most microsporidia, the development of the sporoblasts into spores is a complex process which proceeds very quickly, and so few intermediate stages can be recognized. Sporoblast development was almost synchronous within a cluster of spore-forming cells. The first stage seen was a dividing sporont (Fig. 7). Each independent sporoblast then develops a thick, complex cell wall, elongate plates of endoplasmic reticulum, and the first signs of polar filament formation (Figs. 8, 9). The mass of tubules invaginated in from the cell wall, as seen in Figure 9, is interpreted as a scindosome (Vavra, 1977). The polar filament was seen arising from an area at the posterior end of the sporoblast (Fig. 9). A number of vacuoles could be seen, and around each, three or four densely stained bodies coalesced. The coils of the polar fil-



Figs. 1-6. Light micrographs of *Tuzetia boeckella* sp. n. Fig. 1. Thin section ( $1 \mu\text{m}$ ) of infected *Boeckella triarticulata* showing healthy gut tissue (G), heavily infected muscle (I), and healthy muscle (H)  $\times 800$ . Fig. 2. Uninuclear stage (sporont?). Methanol:Giemsa preparation.  $\times 1500$ . Fig. 3. Binuclear stage (sporont?). Methanol:Giemsa preparation.  $\times 1500$ . Fig. 4. Division of sporont into at least six sporoblasts. Methanol:Giemsa preparation.  $\times 1500$ . Fig. 5. Group of eight sporoblasts in a common pansporoblastic membrane (arrow). Methanol:Giemsa preparation.  $\times 1500$ . Fig. 6. Fresh spores of *T. boeckella*.  $\times 700$ .



Figs. 7-9. Electron micrographs of the developmental stages of *Tuzetia boeckella* sp. n. Fig. 7. Dividing a multinuclear sporont. (N) nucleus.  $\times 15,400$ . Fig. 8. Sporoblast showing the complex cell wall, endoplasmic reticulum (ER), and developing polar filament (PF).  $\times 28,000$ . Fig. 9. Sporoblast showing the polar filament developing in a Golgi-like area (G) a complex cell wall and its associated scindosome (arrow).  $\times 18,000$ .



FIGS. 10–12. Electron micrographs of developing sporoblasts of *Tuzetia boeckella* sp. n. Fig. 10. Section through two sporoblasts each showing densely stained posterosomes (arrows), a nucleus (N), and the early stages of polar filament formation. The first appearance of the pansporoblastic membrane can be seen (PM).  $\times 22,000$ . Fig. 11. Sporoblast with a group of 13 polar filament coils being formed within the same membrane (arrow).  $\times 13,000$ . Fig. 12. A more mature sporoblast showing the completely separated pansporoblastic membrane (PM), other feature as in Fig. 10.  $\times 18,000$ .

ament were formed from the posterior end and, as they were formed, seemed to move anteriorly along the spore. Thus a single peripheral row of coils was formed (Fig. 10). Also at this time a number of very

densely stained bodies were formed at the posterior end; these are sometimes known as posterosomes (Vavra, 1977) and their function is unknown. Occasionally a number of coils were seen being formed



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FIG. 13. Longitudinal section through a mature spore of *Tuzetia boeckella* sp. n. The various features seen include the pansporoblastic membrane (PM) inner (IW) and outer (OW) spore walls, polar cap (PC), polaroplast (P), and the arrays of ribosomes (R) at the posterior end.  $\times 43,000$ .

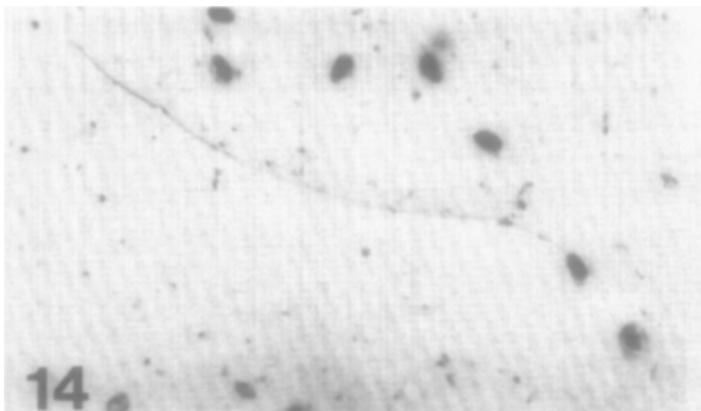


FIG. 14. Polar filament of *Tuzetia boeckella* sp. n.  $\times 700$ .

within a single membrane (Fig. 11). The highly laminate cell wall of the sporoblast can also be seen in this figure. The pansporoblast membrane, which surrounded each mature spore, was first clearly recognizable after polar filament formation but before the polaroplast was formed (Fig. 11). No stages could be seen between this and the almost mature spore complete with polaroplast (Fig. 13).

Determining the number of spores formed from each sporont has proved difficult in *T. boeckella*. In the absence of a true pansporoblast membrane around a group of developing spores, it is only possible to estimate the number of nuclei in a sporont or the number of sporoblasts in a loose cluster. In smears, viewed with the light microscope, developing spores were commonly seen in groups of 6 to 8 (Figs. 4, 5); similarly electron microscope observations indicated that at least five sporoblasts were budded off from a single sporont (Fig. 7). Larger groups of spores were also seen but were thought to have arisen from more than one sporont.

#### *Spores*

The spores are pyriform and measured  $5.09 \pm 0.04$  by  $2.74 \pm 0.03 \mu\text{m}$  ( $n = 50$ ) (Fig. 6). The polar filament measured  $102 \pm 4.23 \mu\text{m}$  ( $n = 8$ ) (Fig. 14).

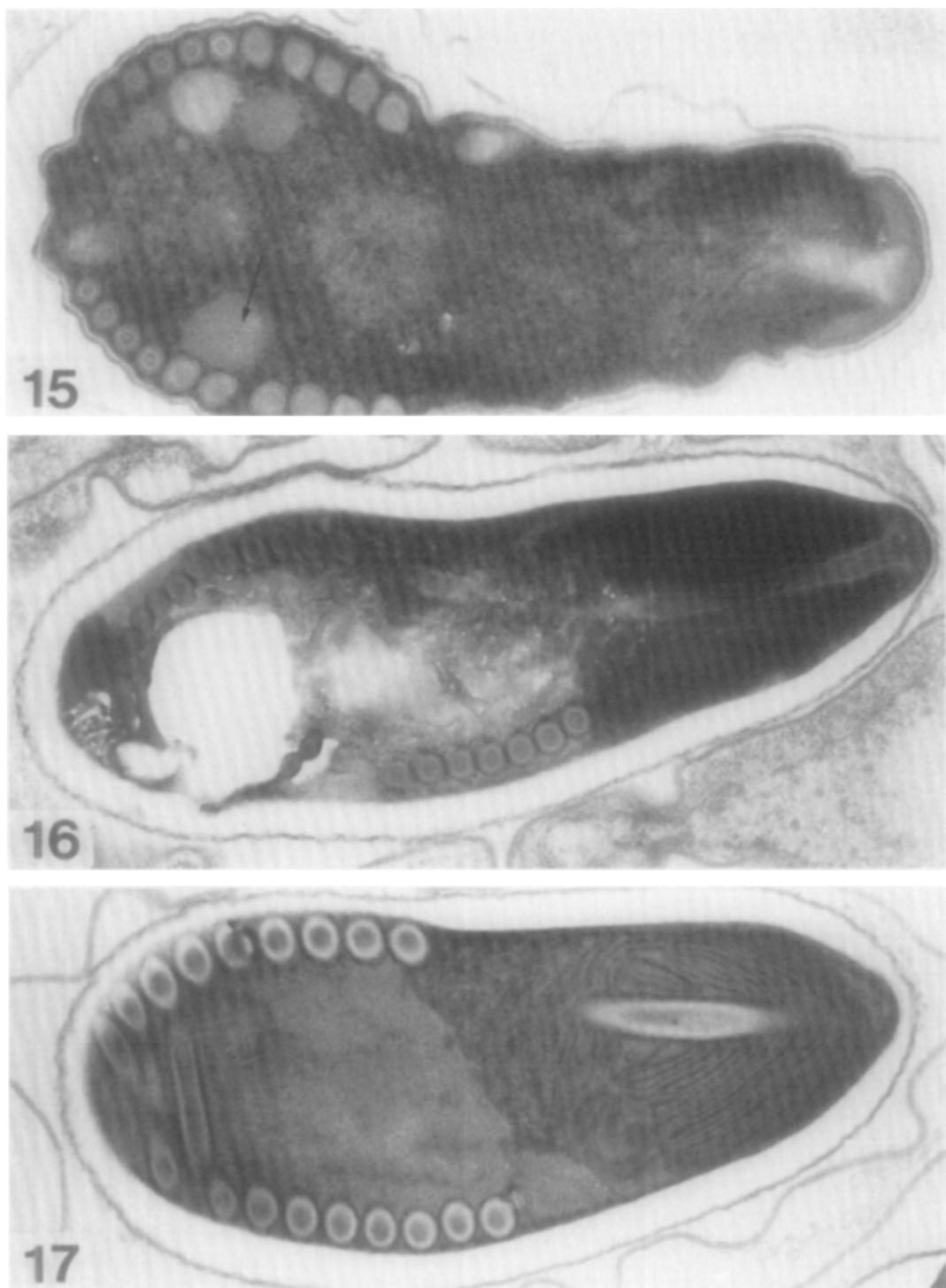
Thin sections of the mature spore dem-

onstrated that the polar filament was arranged in a single row of coils with 13 or 14 turns. In cross section, the filament was broader in the anterior five or six coils and then narrower in the posterior coils (Figs. 15, 16). A number of membrane-bounded posterior bodies could be seen (Fig. 15), these stained less intensely than the posterosomes seen in the sporoblasts and consequently may be distinct. Their function is also unknown. Each spore contained a single nucleus which often dropped out in thin sections (Fig. 16), a prominent polar cap, and a well-developed regular polaroplast. Crystalline arrays, possibly of ribosomes, were clearly visible in some spores (Fig. 17). Each spore was enclosed within a thin pansporoblast wall, an outer spore wall, and an inner spore wall (Fig. 13).

#### SYSTEMATICS

*Host and site.* Both sexes of the copepod, *Boeckella triarticulata*, infection mainly in the muscle though other tissues may also be infected. Gut tissue not infected.

*Morphology.* Vegetative stages unknown. Sporonts with one to eight nuclei unpaired. Sporonts produce about eight sporoblasts which each develop a pansporoblast membrane. The pyriform, unicellular spores measure  $5.09 \pm 0.04 \times 2.74 \pm 0.03 \mu\text{m}$  ( $n = 50$ ). The polar filament



Figs. 15–17. Sections through mature spores of *Tuzetia boeckella* sp. n., showing in particular the narrowing of the polar filament at the posterior end (Figs. 15, 16), the posterior bodies (arrow) (Fig. 15), and the crystalline array possibly of ribosomes (Fig. 17). Fig. 15.  $\times 31,000$ . Fig. 16.  $\times 32,000$ . Fig. 17.  $\times 34,000$ .

TABLE I  
SUMMARY OF THE CHARACTERISTICS OF ALL KNOWN SPECIES OF *Tuzetia*

Species of <i>Tuzetia</i>	Host	Host group	Locality	Main tissues
1. <i>T. infirma</i> (syn. <i>Nosema infirmum</i> )	<i>Cyclops albidus</i>	Copepod	USA	Fat body, etc.
	<i>Macrocylops fuscans</i>	Copepod	France	Ovaries only
2. <i>T. entericola</i>	<i>Rhithrogena semicolorata</i>	Ephemeroptera	Roumania	Gut
3. <i>T. lipotropha</i>	<i>Rhithrogena semicolorata</i>	Ephemeroptera	Roumania	Fat body
4. <i>T. schneideri</i> (syn. <i>Nosema schneideri</i> )	<i>Ephemera vulgula</i>	Ephemeroptera	France	Gut
5. <i>T. debaisieuxi</i> (syn. <i>Pleistophora debaisieuxi</i> )	<i>Baetis rhodani</i>	Ephemeroptera	Brazil	Gut
	<i>Simulium maculata</i>	Diptera	Czecho-slovakia	Fat body
6. <i>Tuzetia</i> sp.	<i>Simulium ornatum</i>	Diptera	France	Fat body
	<i>Macrocylops fuscus</i>	Copepod	France	?
7. <i>T. boeckella</i>	<i>Boeckella triarticulata</i>	Copepod	Australia	Muscle, fat body

is coiled in a single row of 13 to 14 turns and is  $102.5 \pm 4.2 \mu\text{m}$  long ( $n = 8$ ). The polar filament is thinner in the posterior half. Posterior bodies prominent in the immature spores and similar less densely stained bodies seen in mature spores.

*Type locality.* Lake Burley Griffin, Canberra, Australia.

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

*Differentiating characters.* Host species, spore size, number of turns of polar filament, number of sporoblasts formed per sporont serve to distinguish this species from all other known *Tuzetia* spp. (Table 1).

*High classification.* (Based on Sprague, 1977b):

Phylum : Protozoa

Order : Microsporida

Suborder : Pansporoblastina

Family : Tuzetiidae

## DISCUSSION

The genus *Tuzetia* was described by Maurand *et al.* (1971) for the species previously known as *Nosema infirmum* Kudo, 1921. The genus is defined by having a pansporoblast membrane which "divides simultaneously with its contents, enclosing each of several sporoblasts within its sacchet" (Sprague, 1977b). Species of the genus also have uninuclear spores and possess paired nuclei ("diplokarya") only in the early vegetative stages (Loubès and Maurand, 1976). All the species described to date (Table 1) have as their hosts aquatic invertebrates (copepods, mayflies, or blackflies).

*T. boeckella* is typical of the genus and closely resembles the type species *T. infirma*. The two species are clearly distinguished on the basis of spore size and shape: the spores of *T. infirma* are larger and are pointed at the posterior end. Also, according to Maurand *et al.* (1971), *T. in-*

TABLE 1—Continued

Species of <i>Tuzetia</i>	No. of spores per sporont	Spore size ( $\mu\text{m}$ )	Polar filament length ( $\mu\text{m}$ )	No. of turns of polar filament	Author (date)
1. <i>T. infirma</i> (syn. <i>Nosema infirmum</i> )	?	5.6–6.4 × 3.0	90–115	?	Kudo (1921)
	5–6	?	?	?	Maurand <i>et al.</i> (1971)
2. <i>T. entericola</i>	?	6–7 long	?	?	Codreanu and Codreanu-Balcescu (1975)
3. <i>T. lipotrophia</i>	?	6 long	150	?	Codreanu and Codreanu-Balcescu (1975)
4. <i>T. schneideri</i> (syn. <i>Nosema schneideri</i> )	?	4 × 2	90	?	Codreanu-Balcescu and Codreanu (1976)
	?	?	?	?	Codreanu-Balcescu and Codreanu (1976)
5. <i>T. debaisieuxi</i> (syn. <i>Pleistophora debaisieuxi</i> )	?	6.8 × 3.5	20–30	?	Jirovec (1943)
	20–30	?	?	?	Loubès and Maurand (1976)
6. <i>Tuzetia</i> sp.	3–6	5 × 2.5	?	?	Maurand <i>et al.</i> (1972)
7. <i>T. boeckella</i>	8	5.1 × 2.7	102	13–14	This paper

*firma* develops only in the ovaries of the female copepod, while *T. boeckella* infects both sexes and develops mainly in the muscle. In this respect the species described by Maurand *et al.* differs from that originally described by Kudo (1921) in that both sexes are infected and that the fat body, muscle, and reproductive organs are all attacked.

All other named species of *Tuzetia* have been recorded from insects, and of these only *T. debaisieuxi* has been adequately described. This species differs from *T. boeckella* in spore size, polar filament length, and in the number of spores formed from each sporont (Table 1).

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