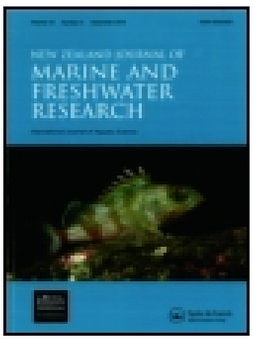

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Variations in the spores of *Myxidium zealandicum* Hine, 1975 (Protozoa: Myxosporidea)

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ABSTRACT

Myxidium zealandicum Hine, 1975 was recorded from several tissues in New Zealand freshwater eels. The gills were the most frequent site of infection, but spores also matured on the gill arch, in the skin, and occasionally in the urinary and swim bladders. Thus *M. zealandicum* is a histozoic species that may have evolved from a coelozoic form.

Variation in spore size (8.0–11.5 μm) was found in relation to site of infection; variation in the number and arrangement of valve striations was also observed. Deposition of fibroblasts occurred at all sites of sporogony, except among the goblet cells of the epidermis.

The small size (6.0–10.5 μm) of unstriated spores occurring with normal spores in the gills of eels from Lake Otomangakau appeared to be related to the size of the cysts in which they occurred, and to the host *Anguilla dieffenbachii*.

INTRODUCTION

Myxidium zealandicum was originally described from the gills of *Anguilla australis* Richardson, 1848 and *A. dieffenbachii* Gray, 1842 (Hine 1975). However, morphologically indistinguishable spores were occasionally found in the gall, swim, and urinary bladders of these eels. McCraren *et al.* (1975) reported spores of *Henneguya* sp. in the gills, barbels, adipose fins, skin, gall bladder, mandibular teeth, sub-cutaneous tissues, sclera and muscles of the eye of channel catfish, and showed variation in tissue response depending on the site of infection. More recently Komourdjian *et al.* (1977) reported *M. zealandicum* from the gills and kidneys of *Anguilla rostrata* in Canada and noted variation in host response in relation to site of infection, and morphological variation in *M. zealandicum* spores.

MATERIALS AND METHODS

Eels were examined alive after being caught in fyke nets set overnight or by electric fishing. After examination of the skin, the gills were excised, each gill arch examined individually, and air dried smears made of the contents of a few cysts, especially any small cysts that may have contained *Myxidium acinum* Hine, 1975. Internally the body cavity and viscera were examined, the urinary bladder excised and opened, the gut removed and cut longitudinally, and air dried smears made of all cysts. Liver imprints, gall and urinary bladder smears were also taken from 205 of the eels examined. Smears and imprints were stained with Giemsa's stain, initially examined at ×480 for the presence of spores, and further examined and measured at ×1 600 using a hairline micrometer on a Zeiss Universal microscope. Material for transmission electron microscopy was fixed in Palades fixative, stained with uranyl nitrate and lead citrate, and examined on a Zeiss EM9S electron microscope. For observations on striations, spores were fixeed in 3% glutaraldehyde, dehydrated in ethanol, dried by critical point freezing, coated with gold, and examined on Cambridge Stereoscan S600 and Mark 2A microscopes. Elements in the spore surface were determined by an energy dispersive analysis of X-rays (EDAX) system.

The sampling areas used in this study are listed in Table 1.

RESULTS

SITES OF INFECTION

Cysts containing *M. zealandicum* spores were found in the gill filaments, on the gill arch, in the skin, in the walls of the swim and urinary bladders, and on the serosal surface of the stomach. Free spores were also found in the liver, gall bladder, and lumen of the urinary bladder. The prevalence of *M. zealandicum* at these sites in 155 eels from Lake Ellesmere between October 1974 and August 1975, is given in Table 2. Although absent from gall bladder smears, the parasite occurred in less than 1% of bile smears examined in a previous study (Hine 1975) and the bile of 1 of 16 eels examined from the Waimeha Stream. The gills were the most common site of infection, followed by the urinary and swim bladders (Table 2). Infection by free spores or cysts elsewhere in the body occurred independently of gill infections.

Cysts in the gills or gill arch, swim and urinary bladder walls, and serosal wall of the stomach, were surrounded by a layer of fibroblasts of varying thickness laid down by the host. Eels examined from an

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TABLE 1—Locations from which eels were sampled in the present study (LF (Long-fin) = *Anguilla dieffenbachii;* SF (Short-fin) = *A. australis*).

Area	Map Reference	No. Examined LF	No. Examined SF	Date
Waikato River – Huntly	N56/668756	0	18	25 Sep. 1975
Lake Otomangakau	N112/118911	41	2	Nov. 1975 – Nov. 1976
Waimeha Stream	N157 & Pt156/571731	5	11	17 Apr. 1975
Burlings Stream	N165/697278	19	15	17 Jun. 1974
Makara Stream	N164/282276	4	26	Oct. 1975 – May 1976
South Branch	S76/940646	157	130	Feb. 1975 – Jun. 1976
Lake Ellesmere	S93/767238	41	281	Oct. 1974 – Jun. 1976
Lake Mahinapua	S57/459466	7	21	28 Apr. 1976
Hou Hou Creek	S50/51/546583	15	4	28 Apr. 1976

eel farm in July 1974 and Lake Ellesmere in February 1975 had *M. zealandicum* cysts less than 3 mm in diameter in the spongy corium of the dermis along the lateral line. Although this was more commonly observed in the cultured eels, infections in wild eels were accompanied by inflammation and bleeding from the congested vascularised spongy corium. Corial cysts were surrounded by fibroblasts (Fig. 1; A) whereas 'cysts' less than 1 mm in diameter between the goblet cells of the epidermis of eels in Makara Stream had no well-defined wall (Fig. 1; B) and could be readily scraped from the skin without opening lesions. There was no indication that underlying cysts shed these spore-like cysts, but rather the organised clustering of spores between the epidermal goblet cells suggested that this was the site of sporogony and there was no resultant host response.

SPORE SIZE AND STRUCTURE

Gills: Variations in spore size are shown in Fig. 2, and spore structure in Table 3. The size distribution most commonly found in spores from the gills is shown in Fig. 2 (centre column). Although spore size ranged from 8.0–11.5 μm, a peak in spore size at 9.5–10.0 μm was usually observed; spores from Lake Mahinapua eel gills showed a peak at 10.5–11.0 μm (Fig. 2F). This small size range and the distinct peak in gill spore size varied little throughout the country, except for samples taken at Lake Otomangakau (Fig. 2H).

Scanning electron microscope (SEM) observations on the striations of gill spores showed that the number of striations and their arrangement varied (Figs. 3 & 4). Usually 12–14 striations occurred on each valve (Fig. 3 A–C), but this number varied from 11 to 20 (Fig. 3F). One pattern of striations was found to occur on several spores (Fig. 4A), but most gill spores showed a wide variation in surface sculpturing (Fig. 4B–D). Occasionally valves of unequal size were also observed, distorting the plane in which the sutural ridge occurred (Fig. 3F).

Infections in the gills of Lake Otomangakau eels differed from gill infections elsewhere in three ways:

(1) Cysts were larger than those from other areas. Cysts of 6.0 mm in diameter have been recorded (Hine 1975) but they seldom exceeded 2 mm in diameter, whereas in Lake Otomangakau cysts less than 3 mm in diameter were rare and most were 3–9 mm in diameter.

(2) Cysts varied in shape from spherical to cigar-shaped, the latter extending the whole length of the primary gill filament. The cigar-shaped cysts were not observed in eels from other parts of the country.

(3) Spore measurements from smears of large cysts showed two distinct size-frequency modes and considerable variation in size from 6.0–10.5 μm (Fig. 2; H). The larger spores corresponded to the size frequency observed in fully developed spores from other areas (Fig. 2; D–G). The smaller spores contributing to the other mode were fully formed and had distinct polar capsules, sporoplasm and capsulogenic nuclei. Sub-

TABLE 2—Prevalence of *Myxidium zealandicum* cysts and free spores in eels *Anguilla dieffenbachii* (long-fin) and *A. australis* (short-fin), Lake Ellesmere October 1974 – August 1975.

Site	*A. dieffenbachii* No.	*A. dieffenbachii* (%)	*A. australis* No.	*A. australis* (%)	Total No.	Total (%)
Gills	6	(35.3)	26	(18.8)	32	(20.6)
Gill arch	0	(0.0)	3	(2.2)	3	(1.9)
Skin (spongy corium)	1	(5.9)	0	(0.0)	1	(0.6)
Swim bladder	2	(11.8)	0	(0.0)	2	(1.3)
Urinary bladder	1	(5.9)	4	(2.9)	5	(3.2)
Stomach wall	0	(0.0)	1	(0.7)	1	(0.6)
Liver	0	(0.0)	1	(0.7)	1	(0.6)
Gall bladder	0	(0.0)	0	(0.0)	0	(0.0)
Total infested	10	(58.8)	35	(25.4)	45	(29.0)
Total examined	17	(111.0)	138	(89.0)	155	(100.0)

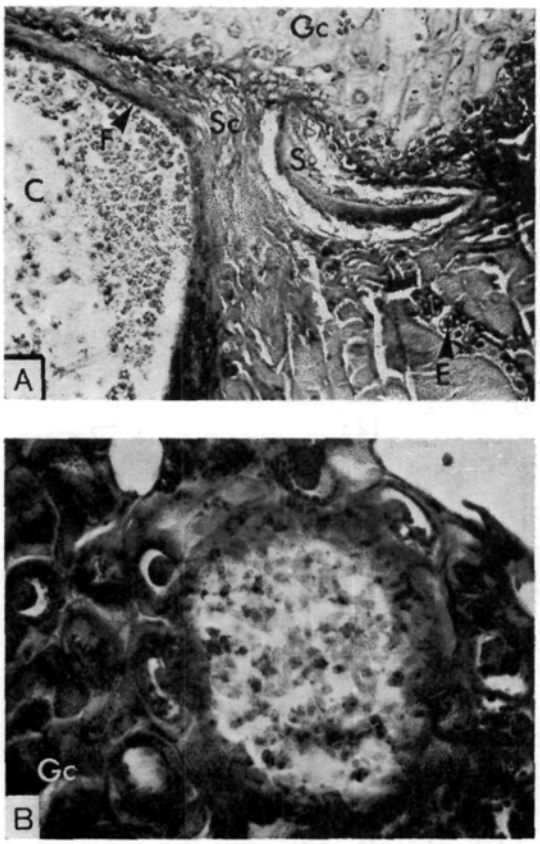

FIG. 1—*Myxidium zealandicum* in the skin of freshwater eels: A = In the spongy corium of the dermis (in an eel from an eel farm, Auckland); B = Among the goblet cells of the epidermis (in an eel from Makara Stream, Wellington); C = cyst; E = erythrocyte; F = fibroblasts; Gc = Goblet cells; S = scale; Sc = spongy corium.

sequent SEM studies, however, showed that these smaller spores lacked any surface striations (Fig. 3C & D), and their shape was altered during preparation for scanning electron microscopy suggesting they were more pliable than mature spores. The number and arrangement of striations on the latter were similar to those of normal gill spores. Mature spores had silicon, zinc, and magnesium in their surfaces, while unstriated spores had only silicon and zinc. Under light microscopy the polar capsules of these small spores appeared slightly granular, and treatment with 1% NaOH did not initiate filament extrusion, whereas this occurred in mature spores.

Sites Other Than Gills: Spores from cysts on the gill arch, skin, and from some cysts in the urinary bladder closely resembled those from gill cysts in their complete development and morphology (Table 3). Surface striations on these spores showed similar variations to those observed in spores from the gills but were larger (Figs 2, 3A–D, 4). However, in size range and frequency distribution these spores were larger than those from the gills (Fig. 2; A–C, I).

At the other extreme, spores from cysts in the serosal wall of the stomach showed almost no internal differentiation (Table 3) and the thin-walled non-striated valves readily collapsed when smears dried. Because of the lack of morphological features it is only assumed these are the spores of *M. zealandicum*. The size-frequency distribution and shape of the spores are very similar to cysts in the wall of the swim bladder (Fig. 2; K & L) which can be identified as *M. zealandicum* because of the rare occurrence of polar capsules, a sporoplasm, and capsulogenic nuclei.

Some urinary bladder smears comprised a mixture of immature spores similar to those taken from cysts on the swim bladder wall and mature spores (Table 3; Fig. 2; J). Similarly, limited observations on spores in gall bladder smears and liver imprints indicated a mixture of mature and immature spores (Table 3), but insufficient numbers of spores on individual slides precluded comparative measurements.

DISCUSSION AND CONCLUSIONS

Apparently, the gills are the primary and normal site of infection for *Myxidium zealandicum* as frequency of infection is highest in the gills, full development occurs there, and there is a uniformity of development and size in gill spores. Despite this uniformity of size and internal structure, variation does occur in the number and arrangement of surface striations, and in the orientation of the sutural ridge. Similarly, Komourdjian *et al.* (1977) studied *M. zealandicum* from the gills and kidneys of *Anguilla rostrata* in Canada and observed "considerable variation in spore striation pattern". They also noted that more than one striation pattern was associated with the same striation number.

The surface of the gill arch and skin, both histozoic sites, are also suitable for complete development of the spores. The urinary bladder may be a site of complete *M. zealandicum* spore development, but it is not known whether the mature spores encountered derived from the urinary bladder region or were being passed out from cysts in the kidneys, uriniferous tubules and ureters. The presence of cysts in the kidneys of *A. rostrata* (Komourdjian *et al.* 1977, Hulbert *et al.* 1977) might indicate that the kidney is a suitable site for sporogony, but no kidney cysts were observed in the present study. Fully developed spores were rarely observed from swim bladder cysts, the only other coelozoic site studied in detail.

Spores in liver imprints were not associated with any particular cell type, but McArthur (1977) found *Myxobolus* spores being ingested by macrophages in

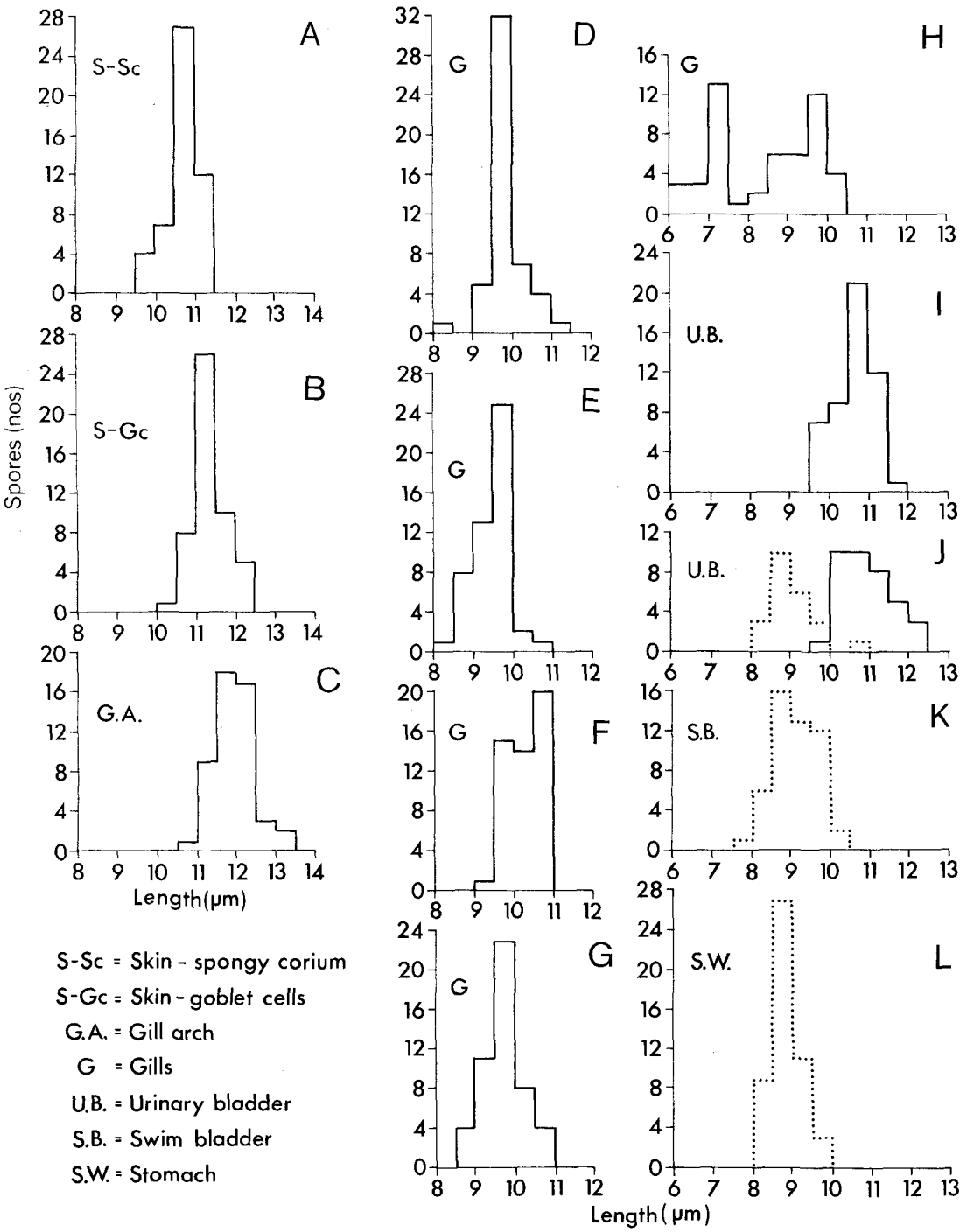

FIG. 2—Length frequencies (μm) of spores of *Myxidium zealandicum* based on measurements of 50 spores in one eel from each site: A = Eel Farm, Auckland; B = Makara Stream; C = Lake Ellesmere; D = Waikato River; E = Lake Ellesmere; F = Lake Mahinapua; G = Hou Hou Creek; H = Lake Otomangakau; H & J = South Branch, Waimakariri River; K = Lake Mahinapua; L = Lake Ellesmere; dotted line = immature spores.

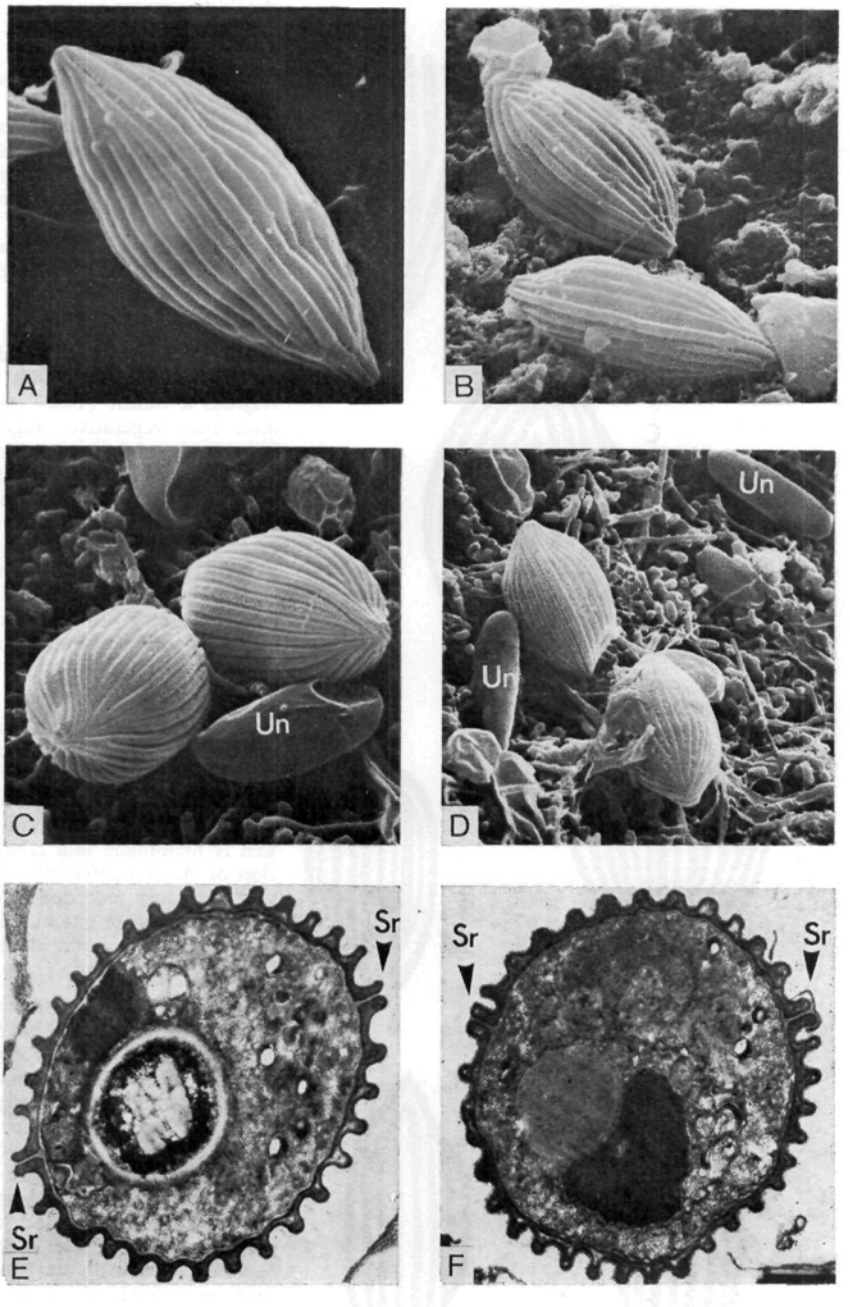

FIG. 3—Electron micrographs of spores of *Myxidium zealandicum* in eels from: A = Burlings Stream (gills); B = Makara Stream (epidermis of the skin); C = Lake Otomangakau (gills); D = Lake Otomangakau (gills); E = section through spore showing 14 striations per valve; F = section through spore showing unequal valves (E & F = Lake Otomangakau, gills). Sr = sutural ridge; Un = unstriated spores.

liver imprints from New Zealand anguillids, and concluded they were circulating spores filtered out by the reticuloendothelial system. *Myxidium* cysts were never observed in the liver and therefore these mature spores may have been introduced by the blood system.

M. zealandicum is, therefore, a histozoic species which may produce spores in a coelozoic site. As the genus is normally coelozoic, and *M. zealandicum* may occasionally be coelozoic, this histozoic species may have evolved, or be evolving, from a coelozoic form.

Komourdjian *et al.* (1977) showed that sporogony and full development of *M. zealandicum* occurred in the gills and kidneys of *A. rostrata.* From differences in histopathological response they hypothesised that

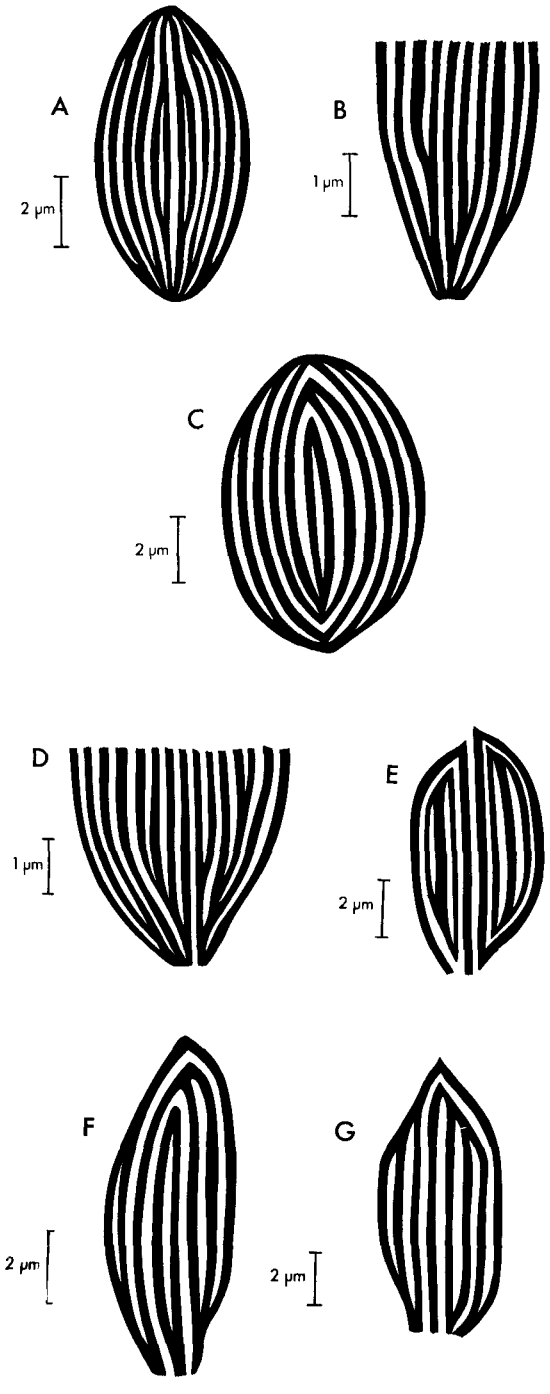

FIG. 4—Variations in sculpturing on the surface of spores of *Myxidium zealandicum* in eels from: A = Burlings Stream (gills); B–D = Lake Otomangakau (gills); E–G = Makara Stream (epidermis of the skin).

the kidney may be an older site for sporogony than the gill, and that if this is so, the relationship to *A. rostrata* may be older than that to *A. australis* and *A. dieffenbachii*. However, this seems unlikely as the coelozoic habit is more primitive than the histozoic habit, but no coelozoic spores were recorded from *A. rostrata*. Also, the gills were the most common site of infection in Canadian and New Zealand eels, and possibly this is the normal site of development in all three eel species. Furthermore, consideration of the total parasite fauna suggests eels of the genus *Anguilla* originated from the South Pacific (Manter 1967).

Sporogony at all sites, except between the epidermal goblet cells, resulted in the formation of fibroblasts around the spores thickening the cyst wall. Nigrelli & Smith (1938) reported for *Myxobolus lintoni*, that vegetative stages, spores, and fibroblasts formed the stroma of tumour-like growths on *Cyprinodon variegatus*. McCraren *et al.* (1975) reported *Henneguya* spores from infection of a variety of channel catfish tissues and showed variation in tissue response depending on infection site. Komourdjian *et al.* (1977) observed that the host response to gill infections with *M. zealandicum* is more pronounced than to kidney infections, and that gill infection may be sufficiently severe to cause loss of physiological function in the gills.

Whereas spores of *M. zealandicum* from serosal and swim bladder cysts were considered immature because of their lack of development, the size and morphological features of the small spores in Lake Otomangakau gill cysts suggests that development was abnormal rather than retarded. Lake Otomangakau is man-made and contains a land locked population of *A. dieffenbachii* and a few *A. australis*. Infection was only recorded from *A. dieffenbachii* as few *A. australis* were examined, but large cysts (> 3 mm diameter) have been observed only on *A. dieffenbachii* in other areas.

The size of the cysts may affect development as the spores of *M. zealandicum* were originally described as 6.4–10.5 μm long (Hine 1975), but mature gill spores in the present study were 8.0–11.5 μm long. Examination of original material shows two distinct spore sizes similar to those from Lake Otomangakau which were 6.0–10.5 μm long. These spores derived from a large cyst in the gills of *A. dieffenbachii* from Burlings Stream, Wairarapa. A large cyst was chosen as it yielded the most spores. Thus this phenomenon was seen in large cysts from *A. dieffenbachii* in two widely separated areas and may, therefore, be related to cyst size, and this in turn to host species.

Myxosporideans may show morphological variation within a species, but the existence of strains has never been conclusively proven and indeed McCraren *et al.* (1975) referred to 'forms' of infection by *Henneguya* in catfish. The morphology of the mature spores in eel gills in Lake Otomangakau is similar to those taken from gills elsewhere and suggests that they may be conspecific.

TABLE 3—Occurrence of morphological features in spores and cysts, and the presence of a host response to *Myxidium zealandicum* (P.C. = polar capsules; + = present; − = absent; ± = may be present or absent).

Site	Striations	P.C.	Sporoplasm	Capsulogenic Nuclei	Cyst	Host response
M. zealandicum						
Gills	+	+	+	+	+	+
Gill arch	+	+	+	+	+	+
Epidermis (goblet cells)	+	+	+	+	+	−
Dermis (spongy corium)	+	+	+	+	+	+
Urinary	±	±	±	±	±	±
Swim bladder	−	±	±	±	+	+
Gall bladder	±	+	±	±	−	−
Liver	±	+	±	−	−	−
Outside stomach wall	−	−	−	−	+	+
M. serum (all sites)	+	+	+	+	+	+

Although *M. zealandicum* occurs in a variety of tissues in Lake Ellesmere eels (Table 2), including the spongy corium of the dermis along the lateral line, no spore groups have been found between the goblet cells of the epidermis. Conversely, spore groups occurred among the epidermal goblet cells of 7 of about 150 eels examined at different times from the Makara Stream but were not found in any other part of these eels. These spore groups have not been observed in eels from other locations. The spores in these spore groups are also morphologically indistinguishable from those on the gills (Fig. 3A & B). Physiologically different strains may occur, but there is no morphological evidence of this.

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