

PSEUDOKLOSSIA SEMILUNA N. SP. (APICOMPLEXA: AGGREGATIDAE): A COCCIDIAN PARASITE OF THE KIDNEY OF BLUE MUSSELS, SPECIES OF *MYTILUS*, FROM BRITISH COLUMBIA, CANADA

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Summary :

Three of 91 mussels, taken from Pacific coastal waters in Nanaimo, British Columbia, were infected with a new species of coccidian parasite. Gamogonic and sporogonic development were observed in renal tubular epithelial cells. Mature macrogametocytes were crescent-shaped. Oocysts sporulated within the host. Mature oocysts were spherical, mean 23.9 µm [range 22-25 µm] with approximately 24 ellipsoidal sporocysts [approximately 6 x 3 µm], each of which contained two sporozoites. Ultrastructural features of immature and mature macrogametocytes are described. Although found in all five populations of mussels from various locations in British Columbia, prevalence of infection was usually less than 16%, intensity of infection was usually light (less than 50 coccidia per histological section of kidney tissue), and evidence of associated pathology was not observed.

KEY WORDS : bivalve, coccidia, macrogamete, oocyst, sporocyst.

RÉSUMÉ : *Pseudoklossia semiluna* n. sp. (APICOMPLEXA: AGGREGATIDAE): COCCIDIE RÉNALE DES MOULES BLEUES, ESPÈCES DE *Mytilus*, EN COLOMBIE BRITANNIQUE (CANADA)

Sur un groupe de 91 moules récoltées sur la côte Pacifique, à Nanaimo (Colombie Britannique), trois étaient infectées par une nouvelle espèce de coccidie. La gamogonie et la sporogonie s'effectuent dans les cellules épithéliales des tubules rénaux. Les macrogamétocytes mûrs sont en forme de croissant. Les oocystes sporulent dans l'hôte. Les oocystes mûrs sont sphériques, mesurent 23,9 µm (22-25 µm) de diamètre et ont 24 sporocystes ellipsoïdaux, d'environ 6 x 3 mm, contenant chacun deux sporozoites. Description et interprétation des caractéristiques ultrastructurales des macrogamétocytes mûrs et immatures. L'infection existe dans les cinq populations de moules prospectées en Colombie britannique, mais la prévalence est habituellement inférieure à 16%. La plupart du temps, l'infection est légère (moins de 50 coccidies par coupe histologique du rein) et n'entraîne pas de lésions notables.

MOTS CLÉS : moule, coccidie, macrogamétocytes, oocystes, sporocystes.

INTRODUCTION

Few species of coccidian parasites have been described from bivalves, and their taxonomy and life cycles are not well understood. Since Léger's (1897) first description of *Hyaklossia pelseneeri* from the kidneys of *Donax* sp. and *Tellina* sp. from coastal France, almost a dozen similar parasites have been described, mainly from the kidneys of European and North American marine molluscs (reviewed by Desser & Bower, 1997). On the basis of multisporocystic oocysts observed in the tissues of their molluscan hosts, seven of these parasites were designated species of *Pseudoklossia*. Because the majority of these species exhibited merogonic development, Desser & Bower (1997) transferred four of them from

the family Aggregatidae Labbé, 1899 to the family Eimeriidae Minchen, 1903. These four species were moved to the new genus, *Margolisella*, which was established to accommodate a new species, *M. kabatai*, a parasite in the kidneys of native littleneck clams, *Protothaca staminea*, from British Columbia, Canada. The two remaining named species of renal coccidiids, meronts of which were not observed, were retained in the genus *Pseudoklossia* (see Desser & Bower, 1997).

Unidentified species of *Pseudoklossia* have been reported in the kidney cells of blue mussels, *Mytilus edulis*, from the east coast of the United States (Farley, 1988) and from *M. edulis* and *Mytilus galloprovincialis* in Galicia, Spain (Bower & Figueras, 1989; Robledo *et al.*, 1994). Bower (1992) & Bower *et al.* (1994) briefly described an unidentified coccidian parasite with crescent-shaped gametocytes and multisporocystic oocysts in the cytoplasm of renal epithelial cells of the blue mussel, *Mytilus* sp. from British Columbia. In this study, we describe and illustrate a new species of *Pseudoklossia* from the kidneys of blue mussels belonging to the *Mytilus edulis/galloprovincialis/trossulus* species complex from British Columbia.

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MATERIALS AND METHODS

During November 1995, the kidneys of 91 *Mytilus* sp. from Pacific coastal waters in Nanaimo, British Columbia, were examined for coccidian parasites. The mussels were maintained in 50 L fibre-glass tanks supplied with flow-through ambient sea water at 8° C, and were dissected, examined and processed within two weeks of their collection. Each mussel was shucked and the kidneys were excised. Renal tissue was pressed between a glass slide and coverslip, and examined for parasites with a compound microscope. Infected renal tissue was fixed in Davidson's solution and processed for routine histological examination. Sections, 5 µm in thickness, were stained with Harris modified haematoxylin and 0.5% alcoholic eosin. Ten fresh and ten fixed, sporulated oocysts, and ten fresh crescent-shaped gametocytes were measured with an ocular micrometer. Various stages of fresh and fixed parasites were photographed with a Zeiss photomicroscope equipped with differential interference contrast (DIC) optics using Kodak Technical Pan film. For electron microscopy, pieces of infected kidney were fixed in cacodylate buffered 2.5% glutaraldehyde, postfixed in cacodylate buffered 2.0% osmium tetroxide, dehydrated in ethanol, and infiltrated and embedded in Spurr's resin (Desser *et al.*, 1983). Ultra-thin sections were examined using a Hitachi H7000 transmission electron microscope.

In order to confirm the lack of merogonic development in mussels from British Columbia, archived histological sections (stained with Harris modified haematoxylin and 0.5% alcoholic eosin) that contained sections through the kidneys of 473 mussels were examined for the presence of coccidia. The sections were derived from 95 mussels that were preserved immediately after collection from Departure Bay on August 1985 to October 1986 (68), Sooke Harbour in October 1982 (seven), Booker Lagoon (five) and Indian Arm (five) in August 1987, and Becher Bay in August 1989 (ten). The remaining 378 mussels were obtained from Departure Bay in early November 1986 and held in laboratory tanks supplied with flow through ambient sea water for 21 to 175 days before being preserved.

RESULTS

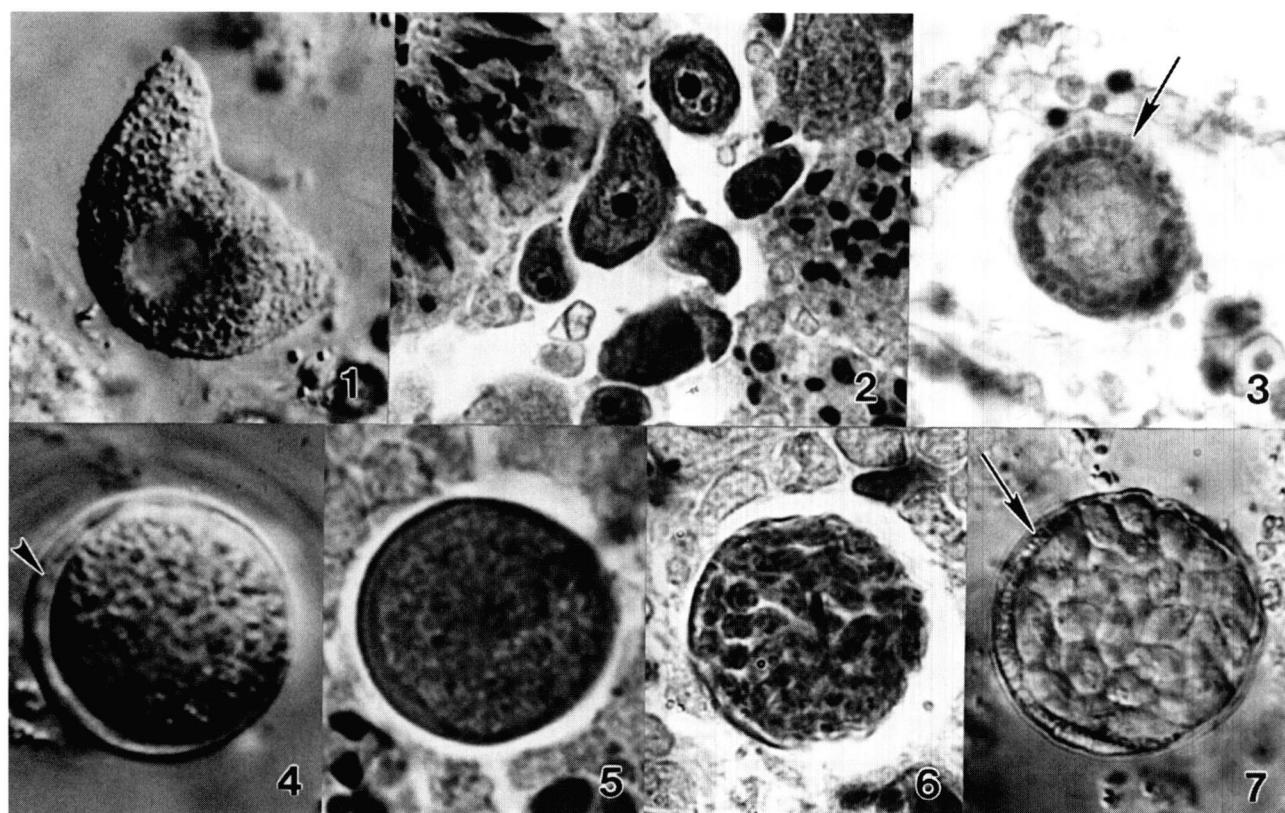
Eimeriorin parasites were found in the kidneys of three of the 91 mussels (3.3%) examined. The parasites, which consisted mainly of gametocytes in various stages of development and occasionally, unsporulated and sporulated oocysts, were observed in both the renal tubular epithelium and the lumen of the tubules.

Squash preparations of fresh infected kidney contained large crescent-shaped gametocytes (Fig. 1), which were readily distinguished from the surrounding host cells. Developing macrogametocytes were seen in the lumen of infected tubules in histological sections (Fig. 2). Gametocytes were spherical to ellipsoidal, depending on the plane of section. The largest macrogametocytes were crescent-shaped and fresh specimens measured 30.8 × 20.8 µm (30-32 × 18-26 µm). Microgametes were observed budding from the peripheral cytoplasm of a spherical microgametocyte (Fig. 3), which measured about 20 µm.

Unsporulated oocysts were spherical in shape and were surrounded by a characteristic wall of uneven thickness (Figs. 4 and 5). Striations were apparent in the thickened portion of the oocyst wall of fresh specimens examined by DIC microscopy (Fig. 7). Sporulated oocysts contained about 24 closely packed ellipsoidal sporocysts, which measured about 6 × 3 µm, each containing two sporozoites (Figs. 6 and 7). Fresh sporulated oocysts measured 23.9 µm (22-25 µm) whereas fixed specimens measured 19.7 µm (19-21 µm).

Electron microscopy revealed that young macrogametocytes were generally spherical to ovoid with a dense, irregular boundary layer. The nucleus was large and vesicular with a prominent nucleolus (Fig. 8). The cytoplasm of immature macrogametocytes contained abundant lipid inclusions and amylopectin (Fig. 9). An extensive network of cisternae of granular endoplasmic reticulum (ER) occurred in the peripheral cytoplasm which also contained numerous mitochondria and Golgi apparatus, and some dense-walled spherical bodies. Deep invaginations were observed in several maturing macrogametocytes, several of which appeared to be folded sharply upon themselves (Fig. 10). The cytoplasmic components of mature macrogametocytes differed considerably from those of earlier stages (Figs. 10 and 11). Lipid inclusions and amylopectin were less abundant and the ER cisternae, prevalent in immature gametocytes, were no longer evident. The cytoplasm contained many vesicular bodies, two types of which were distinctive, and will be referred to as Types I and II. Type I vesicles were spherical and had a loosely granular matrix often containing amorphous dense inclusions (Fig. 11). Small, slender projections lined the inner surface of the limiting membranes and extended a short distance into the vesicular matrix. When sectioned near their edge, the matrix of Type I vesicles appeared to be filled with uniformly arranged dense punctate bodies (Fig. 11). Other vesicles of similar size and appearance, but without the slender projections, were presumably earlier stages of development of the Type I vesicles.

The cytoplasm of mature macrogametocytes contained loose aggregates of electron-dense material which



Figures 1-7. – Photomicrographs of the endogenous stages of *Pseudoklossia semiluna*.

Fig. 1 : Differential interference contrast (DIC) photomicrograph of a fresh crescent-shaped macrogametocyte $\times 1,310$. Figs. 2, 3, 5, 6 : Histological sections of renal tissue containing various stages of development (stained with haematoxylin and eosin). Fig. 2 : Several macrogametocytes in the tubular lumen $\times 660$. Fig. 3 : Microgametocyte with peripherally arranged, budding microgametes (arrow) $\times 1,330$. Fig. 4 : DIC photomicrograph of a fresh unsporulated oocyst. Note the thickened portion (arrow) of the oocyst wall $\times 1,520$. Fig. 5 : Unsporulated oocyst (section) $\times 1,520$. Fig. 6 : Sporulated oocyst with closely-spaced sporocysts (section) $\times 1,520$. Fig. 7 : DIC photomicrograph of a sporulated oocyst. Note striations (arrows) in the thickened portion of the oocyst wall $\times 1,520$.

were seen free in the cytoplasm and also within vesicles (designated as Type II) (Fig. 11). Larger vesicles of variable shape containing filamentous material were also apparent.

The oocyst wall was composed of two distinct layers in the thinner region, with an additional layer interposed between them in the thickened region (Fig. 12). The boundary of the oocyst cytoplasm was lined by an uneven layer of dense material, and was separated by a narrow space from the inner smooth surface of the dense oocyst wall. The interposed, membrane-bounded layer contained agglomerations of electron-dense bodies and small vesicular bodies which lay in direct contact with the smooth inner surface of the dense oocyst wall (Fig. 12).

Fifty six of the 473 mussels in archived histological sections were infected with *Pseudoklossia semiluna*. None of the 1684 *P. semiluna* observed in the 56 infected mussels were undergoing merogonic development. The prevalence of infection was highest (33%) in the sample of 18 mussels collected from Departure Bay on 27 October 1986. In all other samples, the prevalence

of infection was 16% or less. The intensity of infection was also low, with only six of the 56 infected mussels having more than 50 *P. semiluna* present in one histological section through the kidney tissues. In all cases, the morphology of the kidney was similar to that of uninfected mussels (except for the infected epithelial cells which were usually hypertrophied to accommodate the relatively large parasite). Also, there was little to no evidence of an accumulation of haemocytes in response to the infection.

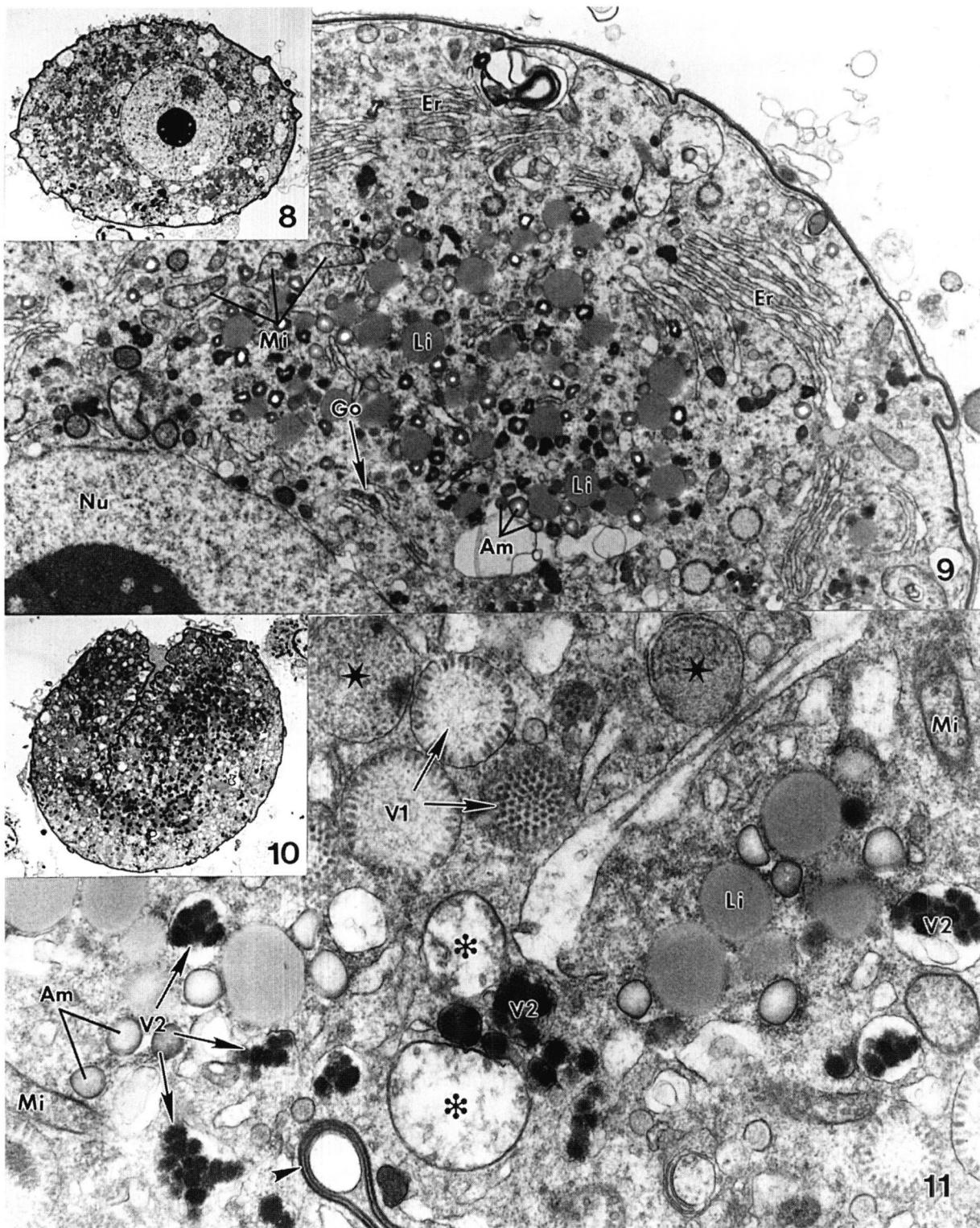
TAXONOMIC SUMMARY

PSEUDOKLOSSIA SEMILUNA N. SP.

Suborder: Eimeriorina Léger, 1911.

Family: Aggregatidae Labbé, 1899.

Gamogonic and sporogonic development in renal tissue; merogony absent. Mature macrogametocytes usually crescent-shaped, 30.8×20.8 ($30-32 \times 18-26$) μm . Fresh sporulated oocysts spherical with uneven wall, $22.9 \mu\text{m}$ ($22-25 \mu\text{m}$); fixed specimens $19.7 \mu\text{m}$ ($19-20 \mu\text{m}$); with approximately 24 ellipsoidal sporocysts ($6 \times 3 \mu\text{m}$), each containing two sporozoites.



Figures 8-11. – Electron micrographs of immature and mature macrogametocytes.

Fig. 8 : Immature gametocyte with a vesicular nucleus containing a prominent nucleolus $\times 1,600$. Fig. 9 : Cytoplasmic components of an immature gametocyte include abundant cisternae of granular endoplasmic reticulum (Er), Golgi apparatus (Go), lipid inclusions (Li), amylopectin (Am), and mitochondria (Mi). Nu-nucleus $\times 9,450$. Fig. 10 : Mature, sharply reflexed macrogametocytes possibly assuming the spherical shape of an oocyst $\times 1,500$. Fig. 11 : The cytoplasm of mature macrogametocytes contains many vesicular bodies. Regularly arranged fine projections line the inner margin of Type I vesicles (V1). The punctate appearance of the projections lining the inner membrane of a Type I vesicle is apparent in a specimen sectioned through its edge (V1). Dense granular inclusions occur within Type II vesicles (V2). Vesicles (stars) similar to Type I but without the fine projections, and other vesicles (asterisks) containing flocculent material are also seen. Note the bilaminar appearance of an invaginated portion of the boundary layer (large arrowhead) $\times 23,800$.

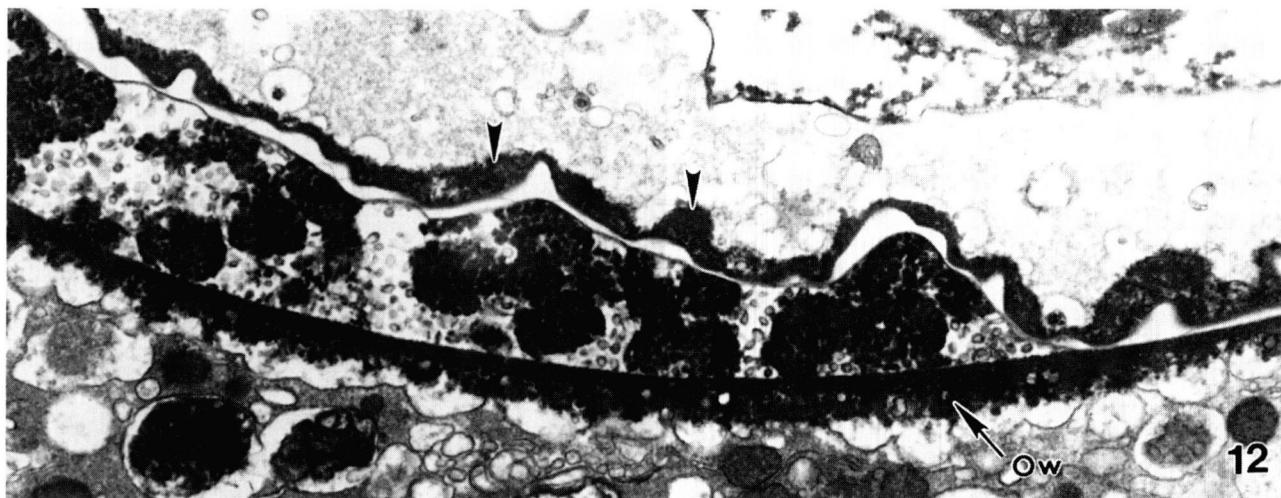


Fig. 12. – Transitional zone in oocyst wall from thinner to thickened region. In this region, the oocyst cytoplasm, which is lined by dense material (arrowheads), is retracted from the wall (Ow) and in the interposing, thickened area is a membrane delimited and composed of agglomerations of electron-dense bodies and small vesicular structures of relatively uniform size. This interposed layer lies in contact with the smooth inner surface of the electron-dense wall of oocyst, whose outer surface is irregular in appearance $\times 15,000$

Type Host: *Mytilus edulis/galloprovincialis/trossulus* species complex (Mollusca: Bivalvia: Mytilidae).

Type Locality: Departure Bay, adjacent to the Pacific Biological Station, Department of Fisheries and Oceans, Nanaimo, British Columbia (geographical quadrilateral co-ordinates of 49° N, 123° W).

Habitat: epithelium of renal tubule.

Etymology: the specific name refers to the often crescent or half-moon shape of mature macrogametocytes.

Type Material: histological sections containing various stages of *P. semiluna* in *Mytilus edulis* have been submitted to the Canadian Museum of Nature Parasite Collection, Ottawa, Ontario, Canada. Catalogue number CMNPA1997-0050.

DISCUSSION

Léger & Duboscq (1915) established the genus *Pseudoklossia* to accommodate the type species *P. glomerata*, which they observed in the kidney of two species of marine bivalves of the genus *Tapes*. According to the criteria of these authors, species of *Pseudoklossia* have zero to many sporozoysts, each containing two sporozoites, do not exhibit merogony in the host in which sporogony occurs, and possibly undergo heteroxenous development. The genus *Pseudoklossia* was included in the eimeriorin family Aggregatidae Labbé, 1899, several members of which have heteroxenous life cycles with merogonic development occurring in one host, and gamogonic and sporogonic development in another.

Although merogonic development was not seen in mussels infected with *P. semiluna*, asexual replication

may occur in a second host. It is also possible that oocysts are infective to mussels, and that sporozoites give rise directly to gametocytes in the renal tubular cells.

The crescent-shaped gametocytes and asymmetric configuration of the oocyst wall of *P. semiluna* appear to be unique features among the species of *Pseudoklossia* described thus far. The sharply reflexed macrogametocytes, seen by electron microscopy, suggest that in the later stages of their maturation, the crescent-shaped gametocyte «round up» and assume a more typical spherical shape. Electron microscopy also provided some information on the synthesis of material for the oocyst, and possibly the sporocyst walls. The cytoplasm of young macrogametocytes contained considerable synthetic machinery, including numerous mitochondria, Golgi apparatus, abundant lipid and amylopectin inclusions, and an extensive network of granular ER. The contents of Types I and II vesicles, seen in the cytoplasm of mature macrogametocytes, appeared to contribute to the formation of the oocyst wall. The vesicles were probably analogous to the wall-forming bodies seen in macrogametocytes of other coccidian species (Hammond & Long, 1973). Unfortunately, the mechanism of oocyst and sporocyst wall formation could not be elucidated because of the paucity of oocysts in the electron microscopic material and also the poor fixation of the few specimens observed. A low prevalence (1% or less) of *Pseudoklossia* sp. has been reported in *M. edulis* and *M. galloprovincialis* from either sides of the Atlantic Ocean (Farley, 1988 & Robledo *et al.*, 1994, respectively). The mussel host of *P. semiluna* in the Pacific is similar to the mussel host of *Pseudoklossia* sp. in the Atlantic. On the Pacific

coast of Canada the specific identity of the blue mussels is confused. The two blue mussel sibling species, *M. edulis* and *M. galloprovincialis*, have been introduced to the Pacific coast of North America and are morphologically similar to the native species *Mytilus trossulus*. In Departure Bay (Nanaimo), where most of the mussels for the species description of *P. semiluna* were obtained, at least 5% of the mussels were found to have a minimum of one allele specific for either *M. edulis* or *M. galloprovincialis* (Heath *et al.*, 1995). The genetic identity of each host specimen for *P. semiluna* is not known, and it is possible that this parasite occurs in blue mussels other than *M. trossulus*. Thus, *P. semiluna* is described as infecting mussels of the *Mytilus edulis/galloprovincialis/trossulus* species complex. It would be of value to examine the *Pseudoklossia* sp. that occurs in *Mytilus* spp. in the Atlantic Ocean to ascertain the taxonomic relationship to *P. semiluna*.

Compared to those species infecting vertebrate hosts, the coccidian parasites of invertebrates are poorly documented and understood. Elucidation of the life cycles of monoxenous coccidia of molluscs will be difficult, and the discovery of alternate hosts of heteroxenous coccidian species of molluscs especially challenging.

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