Restoration of the genus *Coelosporidium* and a description of *Coelosporidium oithonae* n. sp. from the body cavity of a marine copepod, *Oithona rigida*

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MS received 4 November 1975; after revision 2 February 1976

**ABSTRACT**

A haplosporidian parasite, *Coelosporidium oithonae* n.sp. parasitic in the body cavity of the marine copepod, *Oithona rigida*, is described. It is suggested that both the genera, *Coelosporidium* and *Nephridiophaga* be retained, the former to include parasites of the body cavity and the latter to include those which have part intracellular and part haemo-coelic development.

1. **INTRODUCTION**

During 1971–72 while examining the marine planktonic copepod, *Oithona rigida* we came across a small percentage of hosts (2–3%) infected with a haplosporidian parasite which is placed in the genus *Coelosporidium* because the entire development of the parasite takes place in the body cavity of the host. It is described as new as it differs from the only other species, *C. schmackeriae* described earlier from copepods in several respects.

2. **MATERIALS AND METHODS**

The infected hosts could be distinguished from healthy ones by their bluish black colour and sluggish movements. The parasites were studied by testing out infected hosts on a slide and examining them in the body fluid of the host supplemented with a drop of ringer's or normal saline when necessary. Smears were prepared on a slide, wet fixed in methyl alcohol and stained either in Giemsa or fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin. Smears were also fixed in Carnoy's fluid and stained according to Feulgen's or PAS technique. Material for sectioning was fixed in alcoholic Bouin's fluid at 60°C for 1 hr and for 24 hrs at room temperature. Sections were cut at 8μm thickness and were stained with Heidenhain's iron haematoxylin or by Feulgen's technique.
Figures 1-12. 1. Sagittal section of an entire copepod showing the distribution of the parasite in the cephalothoracic region. 2. An early plasmodium showing two nuclei. 3. A later stage showing 8 nuclei. 4. A fully grown plasmodium showing about 200 nuclei. 5. A plasmodium showing the differentiation of uninucleate bodies in the plasmodium. 6, 7, and 8 Smaller plasmodia which are the result of the larger plasmodia undergoing plasmotomy. 9. A cyst showing numerous spores. 10. A single spore stained with iron haematoxylin. 11. A spore stained by the Giemsa's method. 12 A spore stained according to the Feulgen's technique.

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3. OBSERVATIONS ON THE PARASITE

The earliest stage of the parasite observed was rounded or oval measuring 3.0-3.5 μm in diameter (figure 2). The cytoplasm is coarsely alveolated and there are no inclusions. The nuclei are vesicular with a deeply staining centrally placed endosome. No nuclear membrane was observed. Stages showing a variable number of nuclei have been observed and the largest plasmodium was irregular in outline measuring 25.4 x 12.5 μm and contained about 200 nuclei (figure 4). The cytoplasm and the nuclear characteristics remain the same as in the earlier stages. Even in very heavily infected hosts the infection was restricted to the anterior part of the cephalothorax. The occurrence side by side of large and small plasmodia shows that plasmotomy occurs. The smaller plasmodia may repeat the process of division, ultimately forming uninucleate bodies or they may once again grow up to form larger plasmodia. In some of the larger plasmodia the cytoplasm appeared to break up into several uninucleate bodies by developing a more densely stained portion of cytoplasm around each nucleus (figure 5). Some binucleate bodies measuring 4.8 x 3.5 μm were observed along with uninucleate bodies in the same area. Whether the binucleate bodies are the result of fusion of two uninucleate bodies or only stages preceding the formation of uninucleate bodies, a situation similar to what has been encountered in another haplosporidian, Coelosporidium schmackeriae is not clear.

The uninucleate bodies are oval in shape with one end more pointed than the other and measure 4.8 x 1.6 μm. They have a well defined rigid wall and the cytoplasm is hyaline. The nuclei are vesicular and the endosome is centrally placed and deeply stained. A nuclear membrane has not been observed and there is a clear halo around each nucleus. These are probably the spores. The spores in varying numbers are surrounded by a thin, tough and transparent wall forming cysts. The cysts are spherical in shape and vary from 16.0-27.0 μm in diameter. The size of the cyst appeared to depend on the size of the plasmodium from which it has been differentiated. The number of spores depend upon the size of the cyst.

4. DISCUSSION

The genus Coelosporidium Mesnil and Marchoux2 was created for a sporozoan parasite Coelosporidium chydomicola described from the body cavity of a cladoceran, Chydorus sphariclus which they considered intermediate between Amoebidium and Sarcosporidium. Lutz and Splendore8 described a sporozoan parasite from the Malpighian tubules of the cockroach
Periplaneta americana and believing it to be a microsporidian named it Nosema periplanetae. Pernin assuming that the parasite which he studied from Periplaneta (= Blatta) orientalis was the same microsporidian described from *P. americana* by Lutz and Splendore classified it as *Plistophora periplanetae*. Crawley considered this parasite as a haplosporidian and described it as *C. blatellae*. Leger described from the Malpighian tubules of the tenebrionid beetle a sporogenous parasite whose life-history resembled that of haplosporidians of cockroaches. He considered it a mycetozoan, and after studying Crawley's *Coelosporidium* from the Malpighian tubules of the cockroach *Blatta germanica* decided that it as well as a sporogenous parasite of the tubules of *Forficula auricularia* should be transferred to the new mycetozoan genus *Peltomyces*. The spores of all the three species differ in size according to him. As pointed out by Woolever in heavy infections of *Nephridiophaga blatellae* the extremely elongate and somewhat branched plasmodia resemble a portion of the capillitium and this may have misled Leger. Schwarzewsky renamed this haplosporidian *Coelosporidium periplanetae*. Crawley and Pernin examined sectioned material of this parasite and did not observe any intracellular stages in the development of the parasite. Ivanić using the same parasite employing smear method and whole mounts of tubules also did not mention the presence of any intracellular stages. Debaisieux however, reported the occurrence of intracellular stages in the development of the parasite but did not illustrate them.

Ivanić created the genus and type species *Nephridiophaga apis* for a haplosporidian parasite of the epithelium of the Malpighian tubules of the honey bee, *Apis mellifica*. He stated that one of the important characters of this genus is its intracellular development. He also observed a very close similarity between the life-history of this form and that of *Phistophora periplanetae* and accepting the suggestion of Debaisieux regarding the presence of intracellular stages in the life-history of *Coelosporidium periplanetae* (rejecting Crawley's and Pernin's statement of absence of intracellular stages in the sectioned material observed by them and ignoring his own work where he did not find any intracellular stages) transferred *Coelosporidium periplanetae* to the new genus created by him under the name *Nephridiophaga periplanetae*.

Sprague was not in favour of this transfer and in his opinion the intracellular stages in neither of the parasites have been conclusively demonstrated for the smear method alone on which Ivanić based his observations and which does not seem adequate. Sprague examined sectioned material of *Coelosporidium periplanetae* and did not observe any intracellular stages.
Caullery\textsuperscript{14} elevated the order Haplosporidia to the rank of a class Haplosporea Caullery, 1953 containing one order with a single family including 5 genera (\textit{Haplosporidium}, \textit{Urosporidium}, \textit{Anurosporidium}, \textit{Nephridiophaga} and \textit{Physcosporidium}). At the same time he rejected about 30 genera, most of which he considered to be fungi.

Ganapati and Narasimhamurti\textsuperscript{15} suggested the necessity of retaining both \textit{Coelosporidium} and \textit{Nephridiophaga} as distinct genera. Sprague\textsuperscript{16} discussing the recent problems of taxonomy and morphology of Haplosporidia accepted the suggestion of Caullery\textsuperscript{14} of raising the Haplosporidia to the rank of a class with one order, but he divided the single order into into two families, Haplosporididae Caullery and Mesnil, 1905 and Nephridiophagidae. The former family included 3 genera, \textit{Haplosporidium} Luhe, 1900; \textit{Minchinia} Labbe, 1896; and \textit{Urosporidium} Caullery and Mesnil, 1905. The latter family included 2 genera \textit{Nephridiophaga} Ivanic, 1937 and \textit{Physcosporidium}, Awerinzew, 1925. There was however no mention of the genus \textit{Coelosporidium}. The authors feel that the present parasite from the marine copepod, \textit{Oithona rigida} resembles the one described previously from another marine copepod, \textit{Schmackeria serricaudata} and as such the present form is included in the same genus under the name \textit{Coelosporidium oithonae}.

\textbf{ACKNOWLEDGEMENTS}

We are thankful to Prof. K. Hanumantha Rao, Head of the Department of Zoology for the facilities provided to carry out this work. Our thanks are also due to Prof. P. N. Ganapati, Emeritus Professor, for critically going through the manuscript. One of us (C. K.) is thankful to the CSIR, New Delhi, for placement in the scientists pool during the tenure of which this work has been carried out.

\textbf{REFERENCES}