First record of a *Paramarteilia*-like parasite in a decapod crustacean

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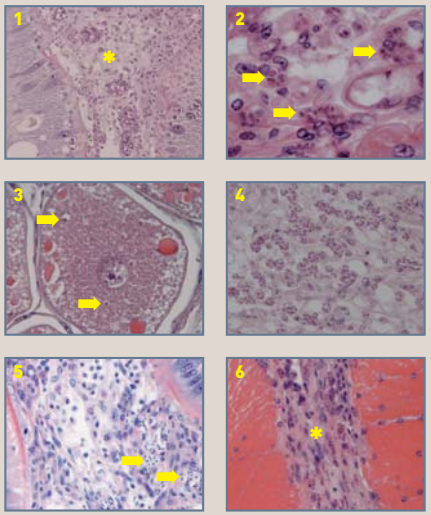
European edible crab (Cancer pagurus)

Introduction

Members of the paramyxean genera *Marteilia* and *Marteilioides* are protistan parasites of marine bivalve molluscs and some species, such as *M. refringens* and *M. sydneyi*, are important pathogens affecting cultured molluscs worldwide. The phylum also contains the genera *Paramyxa* and *Paramyxoides* from polychaetes and *Paramarteilia*, which contains a single species (*Paramarteilia orchestiae*), described from an amphipod crustacean. Here we report the first description of infection with a *Paramarteilia*-like species in the European edible crab (*Cancer pagurus*). A total of four crabs, three from Weymouth and another from Guernsey (all English Channel, UK) were infected with the parasite (up to 3.3% prevalence).



Figures 1 to 6: Histopathology of Paramarteilia-like infection of C. pagurus. (1) Infected haemocytes, fixed phagocytes and connective tissue cells within the sinusoids of the hepatopancreas (asterisk); (2) Higher power image of sinusoid showing infected host cells (arrows); (3) Infection of vitellogenic oocyte (arrows); (4) Infected spongy cells in the pericardium of the heart; (5) Co-infection with a yeast-like organism (arrows); (6) Infection of skeletal muscle. Parasites are most visible in the region of the sarcoplasmic membrane (asterisk).

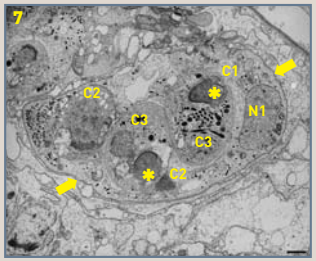


Figure 7: Transmission electron micrograph showing details of the typical structure of the larger stages seen in histological sections (Figure 2). The primary cell (C1) delimited by a plasmalemma (arrows) contains a prominent nucleus (N1) and two secondary cells (C2), one of which contains two enclosed tertiary (sporal) cells (C3). Each of these enclose a single quaternary (sporoplasm) cell (asterisk). The cytoplasm of the primary cell and tertiary cells contain numerous conspicuous electron-dense haplosporosomes (see figure 14). Scale bar = 1um.

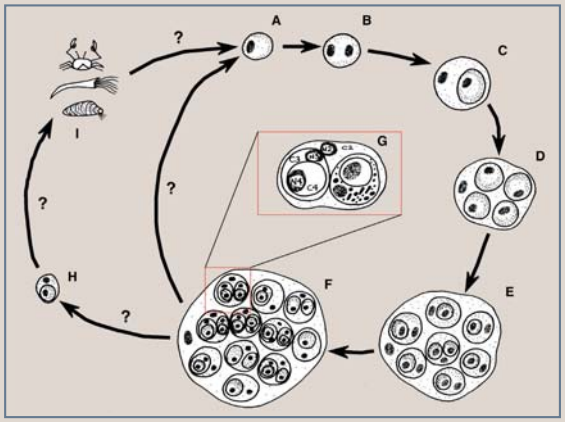
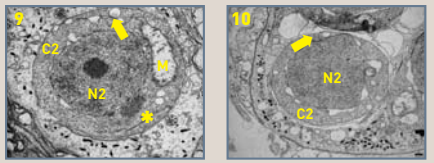
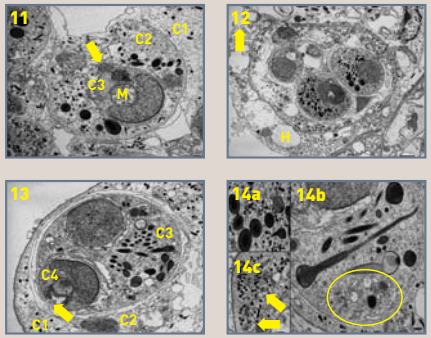


Figure 8: Hypothetical lifecycle of Paramarteilia sp. from edible crabs (not to scale). Following entry by the parasite into the crab, the primary cell containing a single nucleus (A), undergoes nuclear division (B) followed by production of a secondary cell within the primary cell by endogenous budding (C), as typified by Figure 9. Further divisions produce multiple secondary cells within the primary cell (D), which can be seen in Figure 10. Endogenous budding of the secondary cells followed by division produces two tertiary (early sporal) cells per secondary cell (E) as in figure 7. The final stage in the crab (F) is formed by the endogenous budding of the tertiary (sporal) cells and division of secondary and tertiary cells to give rise to a primary cell containing up to 12 secondary cells, which are shown in detail in G. In this stage, the secondary cell (C2) cytoplasm contains a single nucleus (N2) and two tertiary cells (C3), both of which contain a nucleus (N3) and haplosporosomes (shown in the right spore only for clarity). The sporoplasm (C4) containing a single nucleus (N4) is also visible in each sporal cell.
The remainder of the lifecycle after this point is speculative and may cycle within the host via an autoinfection route back to point A or may involve an unidentified intermediate host (I), such as another crustacean, polychaete or mollusc.



Figures 9 and 10: Secondary cells (C2) within the primary cell of Paramartelia sp. Scale bars = 200 nm. (9) Note the large nucleus (N2) and prominent nucleolus. A single mitochondrion (M) and sparse strands of endoplasmic reticulum can be seen (arrow). Cytoplasmic microtubules are also present (asterisk). (10) Secondary cell in the process of division. Note the presence of centriole in close proximity to an indentation of the nucleus (arrow). Nuclear pores are also clearly seen.



Figures 11 to 14: (11) Secondary cell (C2) containing a tertiary (sporal) cell (C3) with a pseudopodium extending into the cytoplasm of the C2 (arrow). C3 is characterised by the presence of a large nucleus with a peripheral region in close contact with the plasmalemma and partially surrounding a single mitochondrion (M) with inconspicuous cristae. Small rod – shaped cytoplasmic haplosporosomes are present in both the primary cell (C1) and C2 where they enlarge at one end and lengthen (see figure 14). Bar = 200nm. (12) Parasite within host haemocyte (H). There is no evidence of degeneration of the parasite which contains a series of enclosed cells, whereas the host cell is vacuolated with a ruptured plasma membrane (arrow). Bar = 1µm. (13) Detail of a C3 with a quaternary (sporoplasm) cell (C4) apparently ingesting the plasma membranes of C2 and C3 (arrow). Possibly a mechanism for the release of C4 into the cytoplasm of C1 for further development. Bar = 200nm. (14) Detail of haplosporosomes genesis and pleomorphic structure. (14a) Transverse section through haplosporosomes of a C2 showing a clear cortex and medulla in both immature and fully formed (electron dense) forms. Bar = 200nm (14b). Apparent formation of haplosporosomes within C1 cytoplasmic vacuoles (arrows). Bar = 200nm. (14c) Longitudinal sections through immature and mature haplosporosomes showing these to be bound by a double membrane containing finely fibrous material which becomes electron dense in mature haplosporosomes. A possible region for haplosporosome formation is circled. This region is characterised by the presence of vacuoles of various sizes, several of which contain flocculent or electron-dense material. Bar = 200nm.

Discussion

This presentation reports the discovery of only the second member of the genus *Paramartelia* and the first from a decapod crustacean. The infection appeared to be rare but provoked a significant host response. The parasite was intracellular in several cell types, including connective tissue and ova (Figures 2 & 3). External signs of infection were not apparent. The discovery of this parasite has provided an excellent opportunity to advance knowledge on morphology and development of this little known group and provides comparative data with *Marteilia* species, which infect bivalve molluscs (see Figure 15). Some of these, such as *Marteilia refringens* are significant pathogens of cultured molluscs. The results of the current study have revealed the majority of developmental stages previously reported for *Paramarteilia orchestiae*, including bi-cellular spores (Ginsburger-Vogel & Desportes, 1979), confirming the proposed placement within the genus *Paramarteilia* rather than *Marteilia*, which has tri-cellular spores. In addition, details of the formation of the enigmatic haplosporosomes within vacuolar structures of primary and sporal cells have been detected (see Figure 14). The function of these organelles remains unknown. Further studies are required to establish the full developmental sequence of the parasite (see Figure 8) from *C. pagurus* and the relationship of the species described here with *P. orchestiae*. The impact of infections in *C. pagurus* populations also requires further investigation.

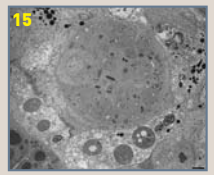


Figure 15: Marteilia refringens from a bivalve mollusc

References

Ginsburger-Vogel, T. & Desportes, I. (1979) Etude ultrastructurale de la sporulation de *Paramarteilia orchestiae* gen. n., sp. n., parasite de l'amphipode *Orchestiae gammarellus* (Pallas). The Journal of Protozoology, (26), 3, 390-403.

Acknowledgements

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