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Observations on two infectious agents found within the rootlets of the parasitic barnacle, *Sacculina carcini*

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Two types of infectious agent within rootlet cells of the parasitic barnacle, *Sacculina carcini* have been recognized by transmission electron microscopy. The rootlets were dissected from the common shore crab, *Carcinus maenas*, collected from two locales—Plymouth and Pwllheli. Yeast cells were identified within cells of *S. carcini* rootlets from crabs collected at both locations and an iridovirus was also found, but only in rootlets from Plymouth crabs. These infectious agents were never found co-occurring in the rootlets from Plymouth crabs. Both agents, when present in rootlets, were also present in the respective host crab tissues. It is therefore concluded that *S. carcini* rootlets are susceptible to invasion from natural infectious agents of the host crab.

Sacculina carcini Thompson is a kentrogonid rhizocephalan which parasitizes certain brachyuran hosts, amongst them the common shore crab, *Carcinus maenas* (Linnaeus). This parasite, although occurring in discrete crab populations, is widely distributed around the British coasts and also the coasts of north-west Europe (Høeg & Lützen, 1985). *Sacculina carcini* has a ramifying system of nutrient-absorbing rootlets spread amongst the tissues in the haemolymph spaces of the host crab. The rootlet system connects by a narrow stalk through the integument of the host's ventral abdomen to the external reproductive sac, the externa. Transverse sections of rootlets viewed in a transmission electron microscope (TEM) have revealed a circular profile with two different cell-types present, a single layer of flattened epidermal cells at the periphery forming the cortex, surrounding a central area of axial cells—the medulla; rootlets can have a central non-cellular area within the medulla (Payen et al., 1981; Russell, 1998). The outer surface of a rootlet is a continuous thin cuticle secreted by the underlying epidermal cells; this cuticle is not moulted.

Parasitized crabs collected from two widely separated locations—Plymouth (MBA animal supply: crabs collected within the River Tamar Estuary) and within the harbour at Pwllheli, North Wales, have been used in electron microscope studies of *S. carcini* rootlets carried out at Menai Bridge (see Russell, 1998). During these studies two types of infectious agent were observed in the rootlets and are reported upon here.

A small number of the Plymouth crabs acquired and dissected in October/November contained abnormally bright white *S. carcini* rootlets; normal rootlet coloration is grey or yellow. These white rootlets, together with surrounding crab tissues, were dissected out, fixed in 2% osmium tetroxide in 0.1 M phosphate buffer at 2°C for 1.5 h, dehydrated up a series of ethanols before embedding in Araldite, then sectioned for TEM investigation. The ultrathin sections on copper grids were stained with uranyl acetate and lead citrate before viewing in a GEC/AEI Corinth 500 transmission electron microscope at 60 Kv accelerating voltage.

Viral particles (virions) occurred in high densities within the epidermal and axial cells of such rootlets (Figure 1A), as well as in the surrounding haemolymph and within cells of the host crab. These latter observations give a strong indication that the virion is an infection of the host crab, which can somehow be transferred into the parasite rootlets. Within the rootlet cells the virions occurred free within the cytoplasm, but some were found aggregated within membrane-bound vesicles (Figure 1B). At high magnification the virion exhibited an hexagonal (icosahedral) profile with internally a dense nucleocapsid core containing the genetic material, surrounded by a more electron-lucent capsid (Figure 1C). Virion diameter measured $\sim 0.15\,\mu$m, categorising such particles as iridoviruses (Carey et al., 1978). Similar iridoviruses have been reported in insects (Kelly & Robertson, 1973; Carey et al., 1978), crustaceans including isopods (Frederici, 1980), branchiopods (Frederici & Hazard, 1975) and cirripedes (Leibovitz & Koulish, 1989). Iridoviruses were not found within any of the *S. carcini* rootlets from crabs taken at Pwllheli.

On dissecting parasitized crabs from Pwllheli and Plymouth at all times of the year several contained the unusual bright white *S. carcini* rootlets. However, when such rootlets were made into squash preparations in seawater on slides sausage-shaped cylindrical cells, $12.5 \pm 0.2\,\mu$m long and $3.5 \pm 0.5\,\mu$m wide, were released into the surrounding medium upon mechanical damage to the rootlets. These yeast cells were non-motile and at the light microscope level showed a number of characteristic dark granules concentrated at each pole (Figure 1D).

Transmission electron microscope examination of the yeast cells revealed an electron-dense cytoplasm in which a central nucleus and several smaller bodies—possibly mitochondria—were just visible (Figure 1E). Lipid globules (0.5–0.75 μm in diameter) at each pole (corresponding to the dark granules with the light microscope) were the consistent prominent cytoplasmic feature. Outside of the plasma membrane was a relatively thick ($\sim 0.08\,\mu$m) cell coat (Figure 1F).

Light microscope examination of smears of crab haemolymph taken from parasitized and unparasitized animals also showed the presence of the yeast cells, which again points to the fact that the yeast naturally infects the crab. Somehow the yeast is able to traverse the cuticle of *S. carcini* rootlets and multiply within rootlet cells causing severe cytoplasmic disruption, undoubtedly leading to the eventual demise of the rootlet.

Delage (1884) and Bang (1983) have both described the occurrence of small rod-shaped 'parasites' (10×3–$4\,\mu$m) in the rootlets of *S. carcini* from Roscoff, France. The latter author considered these parasites to be microsporidians (subkingdom Protozoa,

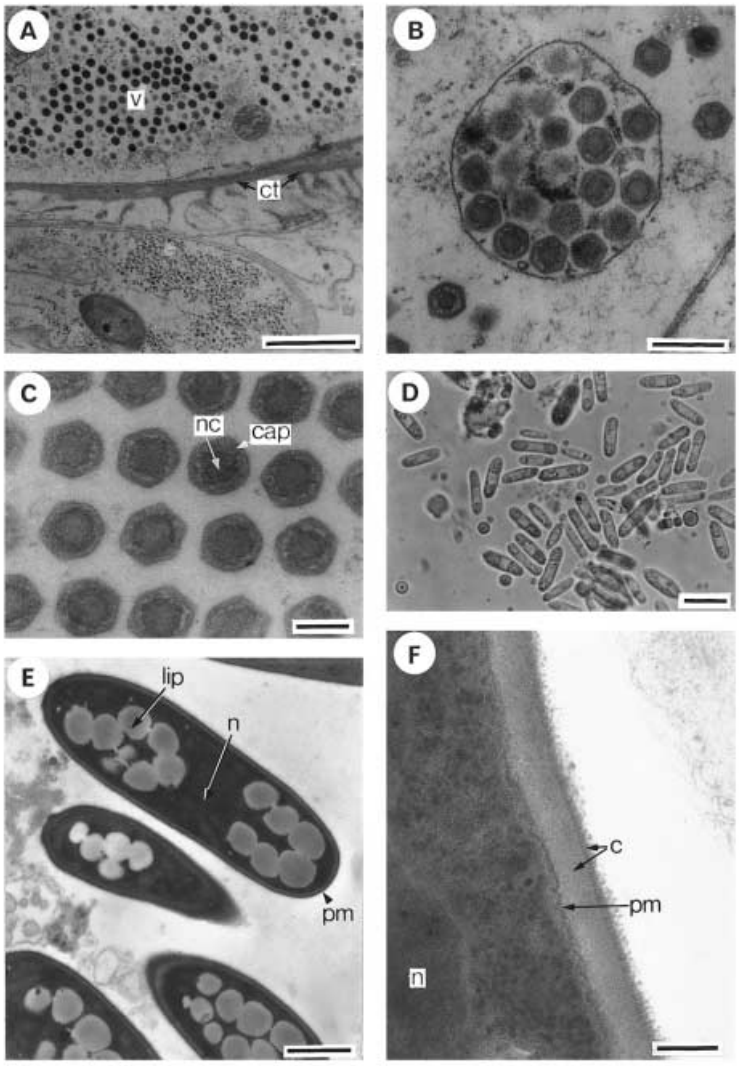

Figure 1. (A) *Sacculina carcini* rootlet. Transmission electron microscope (TEM) micrograph showing the presence of virions (v) within the cytoplasm of an epidermal cell. The rootlet cuticle (ct) is also clearly seen. (B) Electron micrograph showing virions free in the cytoplasm and enclosed by a membrane within a rootlet axial cell. (C) High power micrograph illustrating the hexagonal form of the virions. Each virion comprises an electron-dense nucleocapsid core (nc) surrounded by the more electron-lucent capsid (cap). (D) Light microscope photograph showing live yeast cells released from *S. carcini* rootlets in a squash preparation. (E) TEM micrograph showing the cytoplasmic features within the yeast cells. Note the characteristic lipid globules (lip) concentrated at each pole, the central nucleus (n) and plasma membrane with external coat (pm). (F) Higher power micrograph of the plasma membrane (pm) and external coat (c) of a yeast cell. The nucleus (n) of the cell can also be seen. Scale bars: A, B, E & F, 1 μm; C, 0.15 μm; D, 10 μm.

phylum Microspora, class Microsporididea). However, the present ultrastructural observations show a more yeast-like arrangement of organelles. Morphologically there is great similarity to the cells of *Myrmecomyces annellisae* (Deuteromycotina: Hyphomycetes), an endoparasitic yeast found in the haemolymph of fire ants, *Solenopsis* sp. (Jouvenaz & Kimbrough, 1991).

Sacculina carcini is a most successful parasite of *C. maenas*, with its rootlets able to evade the immune response of the host. Nevertheless these rootlets have been shown to be vulnerable to invasion by at least two types of infectious agent suffered by the crab host.

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