Hematodinium australis n. sp., a parasitic dinoflagellate of the sand crab Portunus pelagicus from Moreton Bay, Australia

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ABSTRACT. A new species of parasitic dinoflagellate is described from the portunid crab Portunus pelagicus. The dinoflagellate is a member of the genus Hematodinium which formerly consisted of a single species, H. perezi. Members of the genus have been reported in crabs and lobsters from Europe and North America, where in some circumstances they cause significant mortalities to host populations. The new species is the first member of the family Syndinidae to be fully described from Australia. The new species differs from other forms of Hematodinium primarily by the size of the trophont (vegetative stage), the ovoid plasmodium, and the small beaded form of condensed chromatin in the nucleus. Infection experiments indicated that the parasite may be transmitted within and between the host species. In addition, the pre-patent period of the new form was at least 16 d which is much greater than that reported from other forms.

KEY WORDS: Hematodinium australis · Portunus pelagicus · Parasitic dinoflagellate

INTRODUCTION

Hematodinium spp. are parasitic dinoflagellates that infect decapod crustaceans. They invade and proliferate in the hemolymph of crabs and lobsters and frequently result in host mortality. The type species, Hematodinium perezi, was originally described from the portunid crabs Carcinus maenas and Liocarcinus (= Portunus) depurator from France (Chatton & Poisson 1931). The type species has recently been reported in Cancer pagurus (Latrouite et al. 1988) and Liocarcinus puber (Wilhelm & Boulo 1988). The same or a related species has since been identified from a wide range of host species from several geographic regions. On the eastern seaboard of the USA, a related parasitoid infects the sand crab Portunus pelagicus (Shields 1992), the mud crab Scylla serrata (Hudson & Lester 1994) and a coral crab, Trapezia aerolata (Hudson et al. 1993). On the western coast of Scotland, Hematodinium sp. infects the Norway lobster Nephrops norvegicus (Field et al. 1992). Similar parasites have also been reported from gammarid amphipods (Johnson 1986).

The taxonomy of the genus Hematodinium in the family Syndinidae needs better definition. The type species was found in only 3 of 3500 crabs (Chatton & Poisson 1931). Further, electron microscope (EM) studies of the type species are nonexistent. While more recent studies have provided excellent EM descriptions of Hematodinium spp., they have not assigned specific names to the parasite(s). Other problems make a taxonomic study difficult. For example, the dinospore, which may have taxonomic value, has not been observed in the type species; it has, however, been observed in the dinoflagellate that infects Tanner crabs.

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Transmission experiments were initiated with apparently uninfected mature and juvenile crabs (Table 1). The hemolymph from crabs was examined for the presence of dinoflagellate parasites. Those crabs having no parasites detected were used as sham controls for the experiments. To obtain the parasite, infected crabs were bled and parasite numbers (cell counts) were determined using a hemocytometer. Mature uninfected crabs received 0.1 ml while uninfect juvenile crabs received 0.05 ml of undiluted infected hemolymph. For Trial 1, infected hemolymph from a mud crab containing 2 x 10⁵ vegetative Hematodinium cells ml⁻¹ was used. Two adult mud crabs and 1 adult and 1 juvenile sand crab were injected with infected hemolymph. One adult mud crab was injected with 0.1 ml of uninfected mud crab hemolymph as a sham control. For Trial 2, infected hemolymph from a sand crab containing 1 x 10⁶ vegetative Hematodinium cells ml⁻¹ was used. Three adult mud crabs and 3 juvenile sand crabs were injected with the infected hemolymph. One trial with a new species of Hematodinium based upon (1) the appearance of the plasmodium, (2) the appearance of the chromatin, (3) various life history parameters, (4) the geographic location and (6) the host, Portunus pelagicus. We then present aspects of the gross and microscopic appearance, ultrastructure and transmission of the new species of Hematodinium from P. pelagicus and a Hematodinium sp. from Scylla serrata. While there are reports of syndinids from Australia (Kimmerer & McKinnon 1990, Shields 1992, Hudson et al. 1993), none of the parasites have been described. The new species is the first member of the genus to be described from the southern hemisphere and the first to be named in over 50 yr. This species represents the dinoflagellate observed by Shields (1992) and Hudson et al. (1993).

MATERIALS AND METHODS

During February to May 1992, sand crabs were collected by trawl in 5 to 7 m of water from the Bramble Bay subestuary of Moreton Bay (27° 18' S, 153° 06' E), Australia, as described in Shields (1992). Mud crabs were caught by crab pot in 2 to 5 m of water from the Albert-Logan subestuary of Moreton Bay (27° 36' S, 153° 20' E) during May 1991 to February 1992. Mud crabs were restrained for transportation and handling by immobilizing their very large chelae with twine. All of the crabs were transported to the laboratory, and kept in aquaria at 21 °C prior to dissection. They were anaesthetized by refrigeration at 4 °C until they became torpid (45 to 60 min), their blood was then drawn and the crabs were dissected. The sex, carapace width and moult stage of the crabs were determined during the examination.

Hemolymph was extracted by puncturing the axilla of the 5th pereopod with a clean pipette for each crab. Several drops of blood were withdrawn and immediately examined in a wet smear. Blood smears were made by drying the blood film on a methanol-cleaned slide, followed by fixation in methanol for 30 s, then staining with Giemsa for 10 min (Humason 1979). Infected and uninfected gill and muscle tissue was fixed for light microscopy in Davidson's fixative (AFA) for 24 h, then transferred into 70% alcohol before being prepared for histological examination. Sections were cut at 7 μm and stained with Harris' hematoxylin and eosin (H&E).

<table>
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<tr>
<th>Source of Hematodinium</th>
<th>Experimental transmission</th>
<th>Survival post-injection (d)</th>
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<td><strong>Trial 1</strong></td>
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<tr>
<td>Injected with</td>
<td>0/2 S. serrata (A)</td>
<td>114–193</td>
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<td>Hematodinium sp.</td>
<td>0/1 P. pelagicus (A)</td>
<td>79</td>
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<tr>
<td>from S. serrata</td>
<td>0/1 P. pelagicus (J)</td>
<td>266</td>
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<td><strong>Trial 2</strong></td>
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<td>Injected with</td>
<td>1/3 S. serrata (A)</td>
<td>16⁴–234 (T)</td>
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<td>H. australis</td>
<td>2/3 P. pelagicus (J)</td>
<td>16⁴–234 (T)</td>
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<td><strong>Trial 3</strong></td>
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<td>Fed H. australis</td>
<td>0/5 S. serrata (A)</td>
<td>21–234 (T)</td>
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<td>infected tissue</td>
<td>0/2 S. serrata (J)</td>
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<td>from P. pelagicus</td>
<td>0/2 P. pelagicus (J)</td>
<td>67–234 (T)</td>
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*All 3 crabs experimentally infected with H. australis died 16 d post-injection*
adult mud crab and 1 juvenile sand crab were injected with 0.1 ml and 0.05 ml of uninfected hemolymph, respectively, as sham controls. Crabs were inoculated in the right axilla of the 5th walking leg and later bled from the left axilla. For Trial 1, all crabs were maintained in separate tanks and were part of a large recirculating system maintained at 21°C with a salinity of 35 ppt. For Trials 2 and 3, crabs were maintained in separate tanks in a recirculating system at 28°C with a salinity of 35 ppt. Mature crabs were bled weekly for the first month and every 2 wk thereafter. Juveniles were only examined if they appeared moribund or dead. Crabs were fed prawns, fish, molluscs and pelleted food (Aquafeed-Barramundi Starter).

RESULTS

General observations

The sternae and ventral surfaces of sand crabs that were heavily infected with *Hematodinium* often had a chalky, white appearance. These crabs were moribund when first caught and died within 24 h after capture. Heavily infected mud crabs showed no external signs of the disease and survived in the laboratory for 72 h before examination. Examination of the poorly clotting, cloudy hemolymph from both the sand and mud crabs revealed the presence of nonmotile, circular or dividing cells with vacuoles and refractive granules (Fig. 1). Crab hemocytes were rare or nonexistent in heavy infections. The nuclei of the parasite possessed condensed chromatin that occurred as distinct strands when stained.

The internal organs of infected hosts appeared milky and the hepatopancreas and gills were cream in colour as opposed to yellow and translucent yellow, respectively. Examination of the various organs revealed that the parasite had invaded all of the hemal spaces. The gills were not grossly damaged or distorted but numerous parasite cells were found, with few if any hemocytes present (Fig. 2). The separation of the muscle bundles (Fig. 3) was due to the parasite and not a fixation artefact as healthy muscle bundles remained closely packed after fixation. In the hepatopancreas, increased vacuolation of the tubule epithelial cells indicated degeneration with rupturing or replacement of interstitial connective tissue by numerous parasites (Fig. 4). Dinoflagellates filled hemal spaces and ruptured or replaced interstitial connective tissue of the ovary of a heavily infected sand crab but the ova were not invaded by the parasite (Fig. 5). No host cell response was observed.

Transmission

In Trial 1, none of the 4 crabs injected with the dinoflagellate from the infected mud crab *Scylla serrata* acquired the infection. Trichocysts were absent in the dinoflagellates from the infected mud crab. Two adult mud crabs survived 114 and 193 d, while 1 adult and 1 juvenile sand crab *Portunus pelagicus* survived 79 and 266 d, respectively. In Trial 2, 1 of 3 adult mud crabs and 2 of 3 juvenile sand crabs that were injected with the dinoflagellate from the infected sand crab acquired the infection. Trichocysts were present in the dinoflagellates from the infected sand crab. All of the infected crabs died 16 d after inoculation, and they all possessed bacteria in their hemolymph. Dinoflagellates from the experimentally infected crabs possessed trichocysts. No mortalities occurred in the crabs in the sham control. In Trial 3, no infections were achieved in the 7 mud crabs and 2 juvenile sand crabs that were fed infected sand crab muscle (Table 1).

Description

*Hematodinium australis* n. sp. (Figs. 6 to 9)

**Trophont** (vegetative stage) (Figs. 6 to 8, *Portunus pelagicus*): Spherical. Amphiosmal alveoli present in the pellicle, no thecal plates or microtubules observed. Cytoplasm with vacuoles empty, or with either membranous debris or lipid-like material. Mitochondria with tubular cristae present. Trichocysts present in trophonts from *P. pelagicus* (N = 50). **Early vegetative stage**: 7.9 to 8.9 μm in diameter, average diameter 8.3 μm (Fig. 6). Average diameter of nucleus 7.7 μm (N = 10, EM preparation). Nucleoplasmin irregularly dispersed; chromatin uncondensed. **Late vegetative stage**: 9.9 to 11.9 μm in diameter, average diameter 10.9 μm (Figs. 7 & 8). Average diameter of nucleus 8.7 μm (N = 10, EM preparation). Nucleoplasmin irregularly dispersed; chromatin distinctly condensed in small beads. Double membrane surrounding nucleus. Nucleolus usually present. Vacuoles larger than those present in early trophont.

**Plasmodium** (Fig. 9): Ovoid. Similar to late vegetative stage but larger with 2 to 5 nuclei present, 20.1 to 47.5 μm (N = 10, EM preparation). Nucleoplasmin irregularly dispersed; chromatin distinctly condensed in small beads. Trichocysts present.
Figs. 1 to 5. Light micrographs of dinoflagellates from *Portunus pelagicus*. Fig. 1. Wet hemolymph smear of unicellular dinoflagellates; note refractile granules in trophons. Scale bar = 20 μm. Fig. 2. Longitudinal section of gill (G) showing numerous dinoflagellates (H) occupying hemal spaces. Hematoxylin and eosin (H&E). Scale bar = 40 μm. Fig. 3. Section of muscle (M) showing separation (arrows) of muscle bundles by dinoflagellates (H). H&E Scale bar = 100 μm. Fig. 4. Transverse section of hepato-pancreatic tubule showing increased vacuolation of B cells (B) and replacement of interstitial connective tissue by numerous dinoflagellates (H). H&E. Scale bar = 100 μm. Fig. 5. Section of ovary showing replacement of interstitial connective tissue by numerous dinoflagellates (H), however no invasion of ova (O) occurred. H&E. Scale bar = 100 μm.
Details

Type host and site of infection: Portunus pelagicus (L.) in hemolymph and hemal spaces of internal organs.

Type locality: Australia, Queensland, Brisbane, Moreton Bay, 153°06’ E, 27°18’ S.

Hapantotype: Material from Portunus pelagicus is from 21 March 1992, with 1 slide and 1 EM block deposited with the Invertebrate Section of the Queensland Museum, Accession Numbers G 211359.

Etymology: The specific name, australis, is derived from austral and refers to the southern continent, Australia, the region where the parasite was discovered.

Additional material: Australia, Queensland, Brisbane, Moreton Bay, 27°18’ S, 153°06’ E; ex Portunus pelagicus, in hemolymph and hemal spaces of internal organs; Invertebrate Section, Queensland Museum, Accession Number GL 13049 (Shields 1992).

Diagnosis

The generic characters of Hematodinium are the amphiesmal structure of the pellicle, the condensed and beaded chromatin that forms V-shaped configurations of the chromosomes, the plasmodal nature of the meront, the continuous state of mitotic activity in the nucleus and the type of mitosis exhibited (dinomitosis) (Chatton & Poisson 1931, Newman & Johnson 1975, Cachon & Cachon 1987). H. australis n. sp. can be distinguished from H. perezi by its larger vegetative stage, its ovoid as opposed to vermiform plasmodium, its austral geographic location and by its different host species (Table 2). The trophont of H. australis differs from the Hematodinium sp. from the Tanner crab Chionoecetes bairdi (Meyers et al. 1987) in that it is smaller, possesses trichocysts and has the small form of beaded chromatin (see below). H. australis differs from the Hematodinium sp. from the Norway lobster Nephrops norvegicus (Field et al. 1992) in that the trophont stage is larger, it possesses the small form of beaded chromatin and it does not appear to have the vermiform plasmodium that is present in the heart of the lobster.

Remarks

A comparison of the vegetative stages of the members of the genus Hematodinium shows that there are marked differences in the sizes of the trophonts (Table 2). Although there is some variability in the size of the trophonts of the parasites there is little overlap in size between forms. Newman & Johnson (1975) believed that their parasite from the western Atlantic corresponded to H. perezi sensu Chatton & Poisson (1931), from the eastern Atlantic. MacLean & Ruddell (1978), however, found that the size of the trophont in 3 different host species from the western Atlantic was greater than that described by Newman & Johnson (1975). Meyers et al. (1987) and Eaton et al. (1991) also found that Hematodinium sp. from Alaska was considerably larger than H. perezi sensu Chatton & Poisson (1931).

Eaton et al. (1991) observed various stages of the vegetative cells of Hematodinium sp. from the Tanner crab Chionoecetes bairdi. Their early type 1 cell corresponds to our early vegetative cell while their type 2 cell corresponds to our late vegetative stage. However their later type 1 plasmodial stage and the early prespore stage differ considerably from the binucleate or plasmodial stage observed in H. australis.

The morphology of the plasmodial stage of Hematodinium spp. appears to be a useful taxonomic character. The plasmodium observed in H. perezi from Callinectes sapidus, Carcinus maenas and Liocarcinus depurator is vermiform and motile, but in Hematodinium spp. from Chionoecetes bairdi, Portunus pelagicus and Scylla serrata it is round and stationary. In Nephrops norvegicus, the plasmodium of the parasite is round except when attached to host tissue (Table 2).

A characteristic feature of dinoflagellates is their condensed and beaded chromatin (Cachon 1987). In Hematodinium spp. the chromatin pattern appears to vary between species. The large form of chromatin was observed in Hematodinium sp. from Chionoecetes bairdi and in Hematodinium sp. from Nephrops norvegicus (Meyers et al. 1987, Field et al. 1992). The small form of chromat was observed in H. perezi sensu Newman & Johnson (1975) and in H. australis. The difference between the small and large forms of chromatin is probably not the result of processing for transmission electron microscopy (TEM) as poorly fixed specimens of Hematodinium sp. ex Trapezia areolata possessed the small chromat form (Hudson et al. 1993). Other parasitic dinoflagellates such as Syndinium sp. (Hollande 1974, Ris & Kubai 1974) and Haplozoon axiothellae (Siebert & West 1974) also have chromat that is condensed into small beads. The genus Hematodinium is different from all other parasitic genera of syndinids in that the chromatin remains condensed through all of the stages, except for the very early vegetative stage (Cachon & Cachon 1987; Fig. 6).

The presence or absence of trichocysts varies in Hematodinium spp. MacLean & Ruddell (1978) did not mention the presence of trichocysts in material from Cancer spp. and Ovalipes ocellatus, and none were observed by Meyers et al. (1987) in the vegetative stage from Chionocectes spp. Meyers et al. (1987) did however find trichocysts in the dinospores. Other workers have observed trichocysts in the vegetative stages...
from various hosts (Table 2). Trichocysts were observed in the vegetative stage of the parasite of the sand crab *Portunus pelagicus*. The *Hematodinium* sp. observed in the mud crab *Scylla serrata* is similar in size, morphology and chromatin form (Figs. 10 & 11) to *H. australis* except that it lacked trichocysts. Trichocysts were only observed in *Hematodinium* sp. from *S. serrata* experimentally infected with *H. australis* from *P. pelagicus*.

**DISCUSSION**

The biology of *Hematodinium* spp. and other Syndinida has not been well studied. Indeed, while there are over 140 described syndinid species, their complete life cycles remain largely unknown (Drebes 1984). A long latency period punctuated by shorter, intense periods of spore release and host mortality seems typical of the *Hematodinium* spp. (Love et al. 1993). Variations in the life cycle of *Hematodinium* spp. may, however, occur primarily in the timing of transmission and developmental pattern of different species in various hosts.

Outbreaks of *Hematodinium perezi* have been reported in *Callinectes sapidus* from the southeastern USA in which up to 30% of the crabs were infected (Newman & Johnson 1975). In areas such as Alaska, northern France, and Scotland, the prevalence of *Hematodinium* can be high, reaching 70 to 95% of the host population (Meyers et al. 1987, Wilhelm & Boulo 1988, Field et al. 1992). We speculate that these high
levels of infection in crabs and lobsters in Alaska and Scotland, respectively, may be the result of environmental factors associated with poor water circulation in the fjords and firths. Similar patterns of infection have been noted for rhizocephalans on golden king crabs Lithodes aequispina (Sloan 1984, 1985), and nemerteans on red king crabs Paralithodes camtschaticus (Kuris et al. 1991, Kuris & Lafferty 1992). Infection levels of rhizocephalans and nemerteans were lower in oceanic regimes than those in enclosed coastal waters (Kurochkin & Rodin 1970 cited in Sloan 1984, Kuris et al. 1991). Kuris & Lafferty (1992) suggest that poor circulation coupled with high larval retention may drive infections to high levels in the enclosed systems. As there appears to be a relationship between prevalence of infection and moulting (Meyers et al. 1990, Eaton et al. 1991, Field et al. 1992), poor circulation, high retention of vegetative cells and/or dinospores coupled with moulting could lead to high levels of infection of Hematodinium spp.

Hematodinium australis had low prevalences of infection of 0.9 to 4.0% in the sand crab Portunus pelagicus and 1.5% prevalence in Hematodinium sp. in the mud crab Scylla serrata in Moreton Bay (Shields 1992, unpubl. data, Hudson & Lester 1994). These low prevalences may be due, in part, to the high flush rate of Moreton Bay. Even though Moreton Bay is an enclosed system, the flush rate of water is high, as approximately an equal volume of Moreton Bay water is exchanged with outside oceanic water every tidal flush (Newell 1971). The prevalence of Hematodinium sp. was also low, below 5%, in 3 species of crabs from
Hudson & Shields: *Hematodinium australis* n. sp., a parasite of sand crab

Hematodinium spp. occur in late summer in the Tanner crab *Chionoecetes bairdi* in Alaska (Eaton et al. 1991, Love et al. 1993), in winter in *Liocarcinus puber* in France (Latrouite et al. 1988, Wilhelm & Boulo 1988), and in late winter in the Norway lobster *Nephrops norvegicus* (Field et al. 1992). In the American blue crab *Callinectes sapidus*, the parasite is absent from winter to early spring and peaks in prevalence during the early fall (Newman & Johnson 1975). There is an apparent absence of seasonality for *H. australis* but the low prevalence of infection may mask any seasonal patterns (Hudson & Shields unpubl. data). The roles of temperature, salinity and other factors in the onset and cycle of the disease need further investigation.

We were able to transmit by injection vegetative stages of *Hematodinium australis* between individuals of the same and different host species. Trichocysts were present in *H. australis* cells obtained from experimentally infected mud crab *Scylla serrata*, indicating that the infection was transmitted from the infected sand crab *Portunus pelagicus*. *H. australis* possesses trichocysts whereas *Hematodinium* sp. from naturally infected *S. serrata* do not. Although Meyers et al. (1987) were able to transmit by injection the cultured vegetative stage of a *Hematodinium* sp. isolated from Tanner crabs *Chionoecetes bairdi* to other *C. bairdi*, they could not transmit the *Hematodinium* sp. from Tanner crabs *C. bairdi* to the red king crab *Paralithodes camtschatica*.

Cannibalism has been suggested as a possible route of transmission of *Hematodinium* spp. That is because (1) crabs are known cannibals, (2) the vegetative stage can survive in seawater and presumably dead flesh for several days (Meyers et al. 1987), and (3) because of the apparent lag between newly infected crabs (April–June) and crabs infected with the presumptive infectious stage, the dinospore (June–August) (Meyers et al. 1987). Transmission of other protozoan parasites is known to occur via cannibalism (*Ameson michaelis*; Overstreet 1978), yet attempts to transmit *Hematodinium* spp. via cannibalism have so far been unsuccessful (present study). This may be due, in part, to the long pre-patent period of new infections (55+ d; Meyers et al. 1987). The pre-patent period of *H. australis* in *Portunus pelagicus* appears to be much less (16+ d) than that of *Hematodinium* sp. in the Tanner crab *Chionoecetes bairdi*, and may be the result of its warmer, subtropical habitat.

As vegetative *Hematodinium* cells can remain viable for up to 5 d in seawater (Meyers et al. 1987), they could be eaten by smaller crustaceans, which act as an intermediate host, where they undergo sporulation. Other crustaceans, such as amphipods, of which most are carnivores and/or scavengers (Schram 1986), could act as reservoirs of the parasite. *Hematodinium*-like
dinoflagellates have been observed in gammarid amphipods by Johnson (1986), who found that 2 types of Hematodinium-like dinoflagellates developed from a few single cells or small plasmidia. In contrast, Syndinium sp., a parasitic dinoflagellate that attacks copepods, develops from a massive primary plasmodium (Johnson 1986). Gammarid amphipods occurred in approximately 20% of the foreguts of intertidal Portunus pelagicus from Moreton Bay (Williams 1982). Amphipods also occurred in 15.8%, 10.3% and 2.1% of Cancer irroratus, C. borealis and Ovalipes ocellatus, respectively, from the New York Bight (Stehlik 1993). It may be possible that crabs acquire the infection by ingesting infected amphipods. If sporulation occurs in the amphipods, direct exposure of the dinospores or meronts to the gut of the sand crab would be possible.

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