RESEARCH NOTES

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Three New Crustacean Hosts for the Parasitic Dinoflagellate *Hematodinium perezi* (Dinoflagellata: Syndinidae)

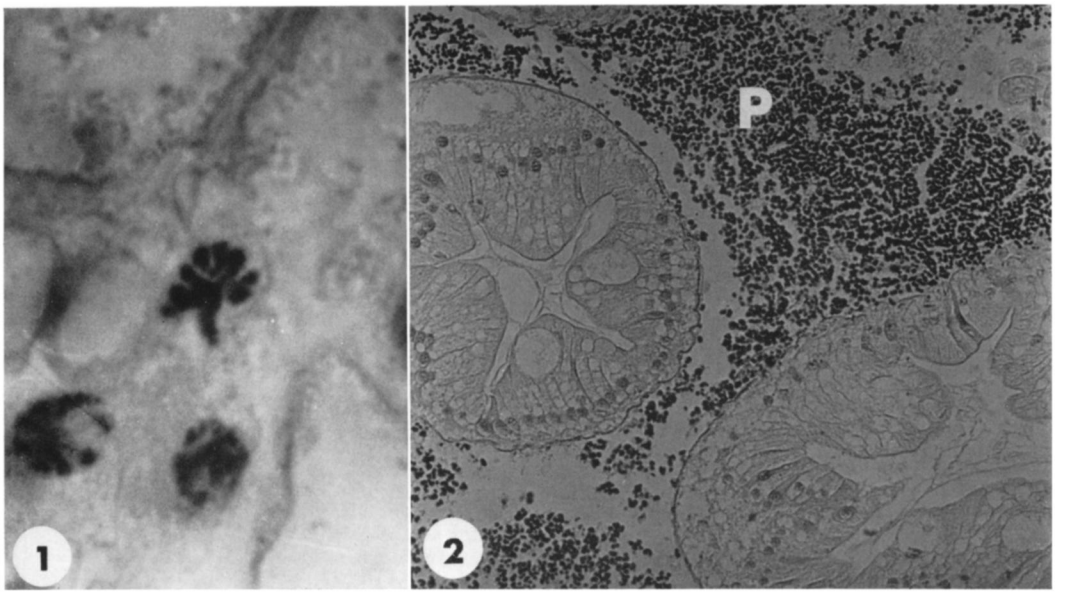
Hematodinium perezi Chatton and Poisson 1931 originally was described as a rare and unusual parasite of the decapod crustaceans *Carcinus maenas* and *Portunus* (*Macropipus*) *depurator*. More recently, *H. perezi* commonly was detected in the hemolymph of the blue crab, *Callinectes sapidus*, collected at certain seasons of the year from the southeastern United States (Newman and Johnson, 1975, J Parasitol **61:** 554–557). The present note extends the reported range to include the cancer crabs, *Cancer irroratus* and *Cancer borealis*, and the portunid crab, *Ovalipes ocellatus*, and, in addition, extends the geographical range to include the inshore and offshore waters of the Mid-Atlantic Bight.

Crabs were collected by otter trawls, small biological trawls, or dredges from several sites in the Mid-Atlantic Bight, including the New York Bight Apex (depth 15 to 30 m) and the continental shelf and slope waters off the New Jersey coast (21 to 400 m depth). Both rock crabs, *Cancer irroratus*, and lady crabs, *Ovalipes ocellatus*, were taken from the New York Bight Apex near Ambrose Light during all seasons 1973–1977. Rock crabs and Jonah crabs, *Cancer borealis*, were taken from the continental shelf and slope waters off the New Jersey coast during five benthic trawl cruises from November 1975 to April 1977. All crabs were examined grossly for evidence of disease or abnormalities, measured for carapace width, and dissected. Various tissues were preserved for histopathology, either in Davidson's or Dietrich's fixing solutions, embedded in paraffin, and sectioned at 5 to 6 μm. Sections were stained with hematoxylin-eosin or treated by the Feulgen reaction.

One male of 155 (0.6%) *O. ocellatus* and two male *C. irroratus* of 518 (0.4%) from the New York Bight Apex were found to be parasitized. From the continental shelf and slope waters off Delaware, one (unsexed) of 180 (0.6%) rock crabs and five (two females, three unsexed) of 125 (4.0%) *C. borealis* were also infected.

Grossly, with the exception of two rock crabs from the New York Bight, which had a pink coloration to the tissues, infected crabs were unremarkable. Histologically, the hemal spaces of infected crabs were found to contain small (diameter 9 to 14 μm) rounded cells which resembled crab hemocytes and occasionally appeared in the form of multinucleate masses. The nuclei of these cells were quite atypical for hemocytes and appeared to be synchronously in a late prophase or metaphase configuration in which five V-shaped chromosomes were invariably present (Fig. 1). The cells were identified by Dr. Phyllis Johnson and Mr. Martin Newman (both of National Marine Fisheries Service, Oxford, Md.) as *Hematodinium perezi*. No overt pathology was associated with light *H. perezi* infections. In heavily infected individuals, hemal spaces in all tissues sampled were filled with parasites and relatively few normal hemocytes were observed (Fig. 2).

We cannot draw conclusions concerning the host-parasite relationship of *H. perezi* because of the relatively few infected animals found. Yet, the infection rate for *C. borealis* was high in comparison to that of the other species examined (4.0% vs. $\leqslant$0.6%) and provided us with additional information. (1) Infected Jonah crabs were taken during all seasons of the year, in contrast to the seasonal occurrence of the parasite in blue crabs reported by Newman and Johnson (1975, loc. cit.). Four of the five infected *C. borealis* were taken from a depth of 400 m, which may indicate that seasonal influences apply principally in the shallower nearshore stations. (2) *Hematodinium* was found only in *C. borealis* measuring 4.5 cm and over, although 60% of the specimens examined were smaller. Also, the largest infected crab measured 7.0 cm. This apparent relationship between the size of the host and presence of the parasite may be explained if crabs less than 4.5 cm and over 7.0 cm carapace width are not infected by *H. perezi*, or the parasite remains quiescent in the host at this



FIGURES 1, 2. *Hematodinium perezi*, a parasite of crabs. **1.** In *Ovalipes ocellatus*. Note characteristic V-shaped chromosomes of the dinoflagellate. Feulgen. × 1,600. **2.** Hepatopancreas from *Cancer irroratus* showing heavy infection. The parasites (P) completely fill the hemal spaces to the exclusion of normal hemocytes. Feulgen. × 160.

point. Alternatively, active *H. perezi* infections may inhibit ecdysis of the crabs, or, as suggested by Chatton and Poisson (1931, C R Soc Biol **105:** 553–557), *H. perezi* kills its host.

A milky or opalescent appearance of the hemolymph and tissues is characteristic of crabs heavily parasitized by *H. perezi* (Chatton and Poisson, 1931, loc. cit.; Newman and Johnson, 1975, loc. cit.). The first observed *H. perezi* infection in our study was a heavily parasitized rock crab from the New York Bight, which had a pink opalescent appearance of the tissues. Although over 3,000 rock crabs were examined grossly, we observed the unusual pink color only in the two infected rock crabs from the New York Bight. This pink coloration may prove to be yet another useful indicator of this disease in *C. irroratus*. Jepps (1936–37, Q J Microsc Sci **79:** 589–662) mentioned that calanoid copepods infected with plasmodial parasitic dinoflagellates often were pink to deep red. The causes of discoloration in the diseased host are unknown.

The finding of *H. perezi* as an enigmatic parasite of various decapod crustaceans in North America may lead to important new observations on the biology, life cycle, and systematics of its poorly understood host-parasite relationship. Dinospores, which are important for the identification of other genera of parasitic dinoflagellates, have not been reported for the genus *Hematodinium* and this has contributed to the confusion concerning the taxonomic status of this organism (Chatton and Poisson, 1931, loc. cit.; Cheng, 1973, *in* General Parasitology, p. 159, Academic Press; Grasse, 1952, Traité de Zoologie, Tome 1, fasc 1, p. 358, 376, Masson et Cie).

The present note reports new hosts for the parasitic dinoflagellate, *Hematodinium perezi*, and, with previous reports, demonstrates its wide host affinity and its apparent infrequent occurrence.

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An Easily Prepared Defined Medium for Cultivation of *Leishmania donovani* Promastigotes

The following defined medium, HOSMEM-II (Table I), is a modification of the semi-defined medium HOSMEM that was previously described in this journal (Berens et al., 1976, J Parasitol **62:** 360–365). The 10% fetal calf serum component of HOSMEM has been replaced with a purine base, vitamin, albumin mixture (Solution A, Table II) based on the defined media REI (Steiger et al., 1976, J Parasitol **62:** 1010–1011) and HX25 (Cross et al., 1973, Parasitology **67:** 315–331). The advantages of HOSMEM-II are that it is defined, supports continuous growth after repeated subcultures, and, compared to REI, very easy to prepare. Growth characteristics of the organisms and culture techniques essentially are identical to those reported for HOSMEM. Cultures, *L. donovani* Malakal area Sudan strain (1S), can be initiated in HOSMEM-II by transfer of organisms grown in HOSMEM to a final concentration of 10^5 organisms/ml. A typical culture reaches stationary phase in 6 days with a final cell density of 3×10^7 cells/ml when grown at 26 C. In addition, since this medium is based on the components of Eagle's minimal essential medium, nutritional experiments can be easily done by dividing the F-14, essential amino acids, and nonessential amino acids into their individual components using Gibco's "Select Amine Kit."

TABLE I. *Composition of HOSMEM-II.*

Components*	Amount per liter
Minimal essential medium (MEM) (Eagle) for suspension culture: F-14 powder	10.58 g
$NaHCO_3$	1.00 g
MEM amino acids (50×)	10.00 ml
MEM nonessential amino acids (100×)	10.00 ml
Na pyruvate (1 g/100 ml stock)	11.00 ml
MOPS (30 mM)	6.28 g
Glucose	2.00 g
Solution A	10.00 ml
Biotin	0.10 mg
p-Amino benzoic acid	1.00 mg

The above components are dissolved in 800 ml double glass distilled H_2O pH adjusted to 7.2 with 5 N NaOH, and the volume adjusted to 1 liter. The medium is sterilized by pressure passage through a 0.22 μ filter and stored frozen at –20 C. Before use, 2.5 ml of Solution B (Table 2)/liter medium is added.

* The components were obtained from the following sources: MEM components—Gibco, Grand Island, New York; fatty acid free bovine albumin fraction V—Miles Laboratories, Elkhart, Indiana; all other chemicals—Sigma, St. Louis, Missouri.

TABLE II. *Composition of solutions.*

Components	Amount per 100 ml
Solution A	
Hypoxanthine	150 mg
Ascorbic acid	2 mg
Vitamin B-12	2 mg
Bovine albumin fraction IV-fatty acid free	150 mg
Double distilled H_2O	91 ml
Thioctic acid*	4 mg
Menadione*	4 mg
Retinol acetate*	4 mg
Solution B	
Mixture of 250 mg hemin and 500 mg folic acid dissolved in 50 ml 0.05 N NaOH, made up to 100 ml with double distilled H_2O, pH adjusted to 8.0 with 1N HCl and sterilized by autoclaving. Stored at –20 C.	

* These three compounds dissolved in 1 ml 95% ETOH added to rapidly stirring mixture of other compounds. Final volume adjusted to 100 ml. Stored frozen at –20 C.

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