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Description of *Anophryoides haemophila* n. sp. (Scuticociliatida: Orchitophryidae), a pathogen of American lobsters *Homarus americanus*

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ABSTRACT: The etiological agent of 'bumper car' disease in lobsters *Homarus americanus* is described as a new species of ciliate, *Anophryoides haemophila* (Scuticociliatida: Orchitophryidae). *A. haemophila* n. sp. is distinguished from other species in the genus by the curved rectangular oral polykinetid 2, a somatic kinety range of 16 to 18, and its relatively small size. The parasite is easy to maintain *in vivo* and *in vitro* for extended periods at 2 to 5°C. Apparently the ciliate can be a significant impediment to the economic viability of coldwater lobster impoundments in eastern North America. However, factors inducing epizootics of 'bumper car' disease are unknown.

KEY WORDS: *Anophryoides haemophila* · American lobster · *Homarus americanus* · Scuticociliatida

INTRODUCTION

For economic efficiency, crustaceans are highly confined in postcapture and aquaculture situations. In 1993, the lobster fishery had landed values of Can$294 million in Canada (Fisheries and Oceans Canada 1994), and of Can$213 million in the United States (U.S. Department of Commerce 1994). Although difficult to determine, postharvest losses are estimated at 10 to 15% annually (Can$50 to 75 million). After capture, numerous problems can be associated with intensive and extensive holding systems for lobsters and other crustaceans (Overstreet 1986). In particular, lobster impoundments and other holding facilities can facilitate development of epizootics with subsequent loss of valuable re-sources. Recently, ciliate parasites have gained recognition as etiologic agents of important diseases of crustaceans (Morado & Small 1995).

'Bumper car' disease, caused by an *Anophrys*-like ciliate, was first documented from eastern Canada as a pathogen of captive adult and third-stage larval lobsters *Homarus americanus* held in a flowthrough system at 0 to 10°C (Aiken et al. 1973). 'Bumper car' refers to the high activity and rapid motility of the ciliates *in vivo* and *in vitro* at cold temperatures. During winter 1971–72, prevalence (via haemolymph examination) of the ciliate was 20% in market size lobsters from New Brunswick; these lobsters all died within 6 wk. During winter 1972–73, infected adult lobsters from Nova Scotia and Prince Edward Island also died. Additionally, third-stage larval lobsters infected with ciliates died. The 'bumper car' disease problem was apparent from November through March or April in these tank-held, laboratory stocks of lobsters (Aiken et al. 1973). Subsequently, the ciliates were observed during winter months in wild stocks of lobster at cold (0 to 5°C) temperatures (Aiken & Waddy 1986). Details of the numbers of lobsters involved (and mortality rates) in the above studies were not provided (Aiken et al. 1973, Aiken & Waddy 1986).

More recently, a ciliate mistakenly identified as *Mugardia* sp. (see Morado & Small 1994, 1995) caused

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significant mortalities in lobster impoundments of eastern North America (Loughlin & Bayer 1991, Sherburne & Bean 1991, Loughlin et al. 1993). Epizootics of 'bumper car' disease were documented in winter and spring in Maine (1990, 1991, 1992) and New Brunswick (1989, 1992, 1993), with mortalities up to 25% (Sherburne & Bean 1991, Loughlin et al. 1993, 1994). Additionally, affected lobsters have reduced muscle mass, poorer meat quality and unsavoury flavour (Loughlin et al. 1993). Survivability during shipping can be highly reduced (Sherburne & Bean 1991). Clinically, 'bumper car' disease is difficult to detect; lobsters can be weak and lethargic, have a 'spread-eagle' posture, and in tidal pounds, have a heavier than normal algal growth on the shell (Loughlin et al. 1993). Anecdotally, 'bumper car' disease caused severe losses in some Nova Scotia lobster pounds in 1991 and 1994. Although the prevalence (via haemolymph examination) of ciliates was zero (n = 787) in wild-caught Maine lobsters examined from August to December 1992 (Loughlin et al. 1994), our recent (unpublished) observations utilizing a monoclonal antibody directed to the ciliate in a highly sensitive and specific indirect fluorescent antibody test indicate that the parasite occurs in a minimum of 17.8% (41/230) of lobsters from the south shore of Prince Edward Island (fall 1994, spring 1995). Epizootiological factors which determine when ciliate infections progress to 'bumper car' disease in lobsters are unknown.

Our study herein describes this ciliate, *Anophryoides haemophila* n. sp., from haemolymph of lobsters and *in vitro* cultures. The parasite was first established from an infected lobster from a Maine lobster pound and transferred from lobster to lobster by intrahaemocoelic inoculation. Ciliates were also maintained *in vitro* in artificial seawater with frozen lobster muscle, at 5°C. *A. haemophila* n. sp., the causative agent of 'bumper car' disease, is easy to maintain *in vitro* and *in vivo* in lobsters. The model of 'bumper car' disease should facilitate study of health and infectious disease processes in lobsters, utilizing the holistic approach recently suggested by Stewart (1993).

MATERIALS AND METHODS

Source and maintenance of ciliates. Lobsters were collected from impoundments in eastern Maine, USA, during winter months; 0.3 ml of haemolymph was removed from a ventral sinus with a 26-gauge needle on a tuberculin syringe and examined immediately with light microscopy. Lobsters infected with *Anophryoides haemophila* n. sp. were transported to the University of Maine, Orono, Maine, for further processing. One ml of infected haemolymph was put into 50 ml of chilled seawater in a 250 ml tissue culture flask containing 0.1 g of lobster muscle. Flasks were maintained at 0 to 2°C.

In March 1993, under permit from Agriculture Canada, culture flasks containing numerous ciliates of *A. haemophila* n. sp. were transported on ice to the Atlantic Veterinary College (AVC), University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada. At AVC, ciliates were transferred in both 25 cm^2 (with 15 ml of sterile artificial seawater) and 75 cm^2 (50 ml of sterile artificial seawater) tissue culture flasks (Corning Inc., Corning, New York 14831, USA). The seawater medium comprised Instant Ocean (Aquarium Systems, Mentor, Ohio 440060, USA) at a salinity of 30 ppt, filter-sterilized with a 0.22 µm filter (Corning). Lobster muscle, stored frozen, was thawed and added to cultures, as food for ciliates. Ciliates were washed by centrifugation several times to reduce bacterial contamination prior to inoculation of flasks and subpassaged (1 ml of infected culture medium into 50 ml of fresh medium prepared as above), every 4 to 6 wk. Other cultures were initiated with single ciliates which had been isolated by repeated serial dilution from flask cultures. Cultures were maintained at 5.0 ± 0.1°C.

Source and maintenance of lobsters. 'Canner' lobsters (carapace length 65.1 to 80.9 mm, weight 190 to 400 g) were purchased during the spring lobster season in Lobster Fishing Area 26A, Prince Edward Island, and transported to the Aquatic Animal Facility, AVC. Lobsters were held in a 6-tank, 4700 l capacity, saltwater recirculation system that was equipped with both particle and biological filtration. Artificial salt water was prepared from Instant Ocean. Each tank housed 20 lobsters (all claws banded) in individual compartments in 2 stacked trays. Water quality was monitored weekly for unionized ammonia (to be <0.01 mg l^{-1}), nitrate ion levels (to be <20.0 mg l^{-1}), nitrite ion (to be <0.1 mg l^{-1}), pH (range 7.9 to 8.4), and salinity (range 29 to 30 ppt), and continuously for temperature (2 ± 1°C). Lobsters were fed weekly, one blue mussel *Mytilus edulis* with a cracked shell per lobster, and the system was monitored daily for ill or dead lobsters. Lobsters were maintained in accordance with Guidelines of the Canadian Council on Animal Care, on a 12 h light:12 h dark photoperiod.

Observation and staining of ciliates. Live ciliates from both lobster haemolymph and cultures were studied with phase-contrast and differential interference contrast microscopy. Smears of ciliates were made either with fresh haemolymph of experimentally infected lobsters or with ciliates grown *in vitro*. The latter were washed by centrifugation (150× *g* for 5 min), mixed with mucus from mussel gill (to ensure adhesion

to glass coverslips) and smeared on clean glass cover slips. All smears were fixed immediately by floating cover slips on fresh Bouin fixative in a Petri dish. Protargol staining (protargol-silver protein) (Roboz Surgical Instrument Co., Inc., Rockville, Maryland 20850, USA) followed the method of Lee et al. (1985); subsequently, cover slips were mounted on glass slides with Flo-texx mounting medium (Lerner Laboratories, Pittsburgh, Pennsylvania 15275, USA). Measurements were made from fixed ciliates utilizing an eyepiece micrometer. Descriptive terminology for the parasite followed Corliss & Lom (1985) and Small & Lynn (1985).

RESULTS

Anophryoides haemophila n. sp. (Fig. 1)

Diagnosis. Oral polykinetid 1 about 4 µm in length, triangular, composed of at least 3 kinetosomes at posterior margin and a single kinetosome at anterior. Oral polykinetid 2 about 5 µm long, curved rectangular, usually composed of 4 files of kinetosomes. Oral polykinetid 3 triangular to square on anterior wall of oral cavity. Paroral extends from anterior end of oral polykinetid 2 to the level of oral polykinetid 3 and then curves along the right side of the oral cavity depression.

Type location. Gulf of Maine, Maine, USA. 44° 33′ N, 68° 25′ W.

Type specimen. A slide containing the holotype (USNM 47825) from lobster haemolymph was deposited in the Ciliate Type Slide Collection, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA.

Etymology. The species name refers to the apparent preference this ciliate has for lobster haemolymph and haemocytes.

Description. *Anophryoides haemophila* n. sp. is an elongate ovoid scuticociliate (Figs. 2 & 3). Body length 34.8 ± 3.2 µm (25.5 to 41.0, n = 32) from lobster haemolymph; body length 25.5 ± 2.5 µm (21.8 to 31.9 µm, n = 33) in culture. Body width 22.6 ± 3.1 µm (14.6 to 27.3 µm, n = 32) from lobster haemolymph; body width 14.2 ± 2.5 µm (7.3 to 19.1 µm, n = 33) in culture.

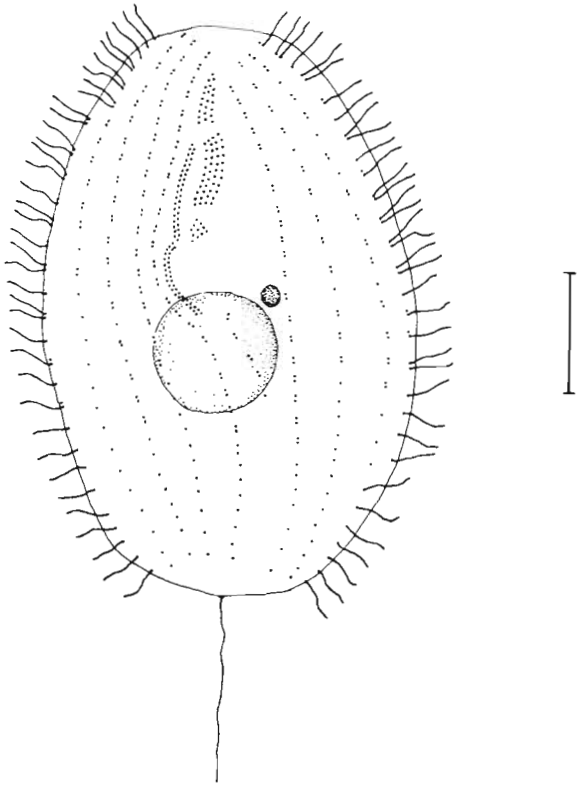

Fig. 1. *Anophryoides haemophila* n. sp. Schematic drawing based on protargol-stained specimens. Note that the more elongate, pointed shape of the living cells (cf. Fig. 2) is altered by Bouin fixation and protargol staining to the more blunted form illustrated here (cf. Fig. 3 & 4). Scale bar = 5.0 µm

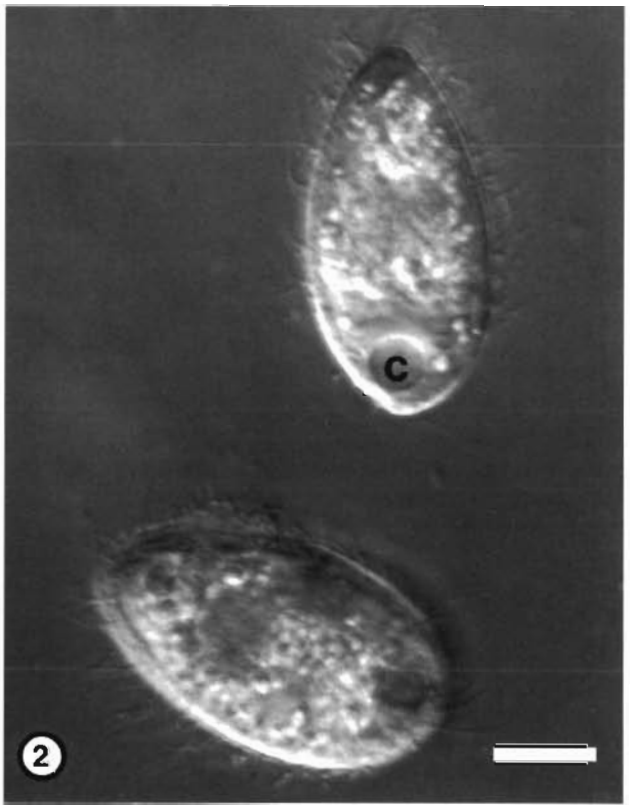

Fig. 2. *Anophryoides haemophila* n. sp. Live specimens, in haemolymph of experimentally infected lobster. C: contractile vacuole. Differential interference contrast microscopy. Scale bar = 10.0 µm

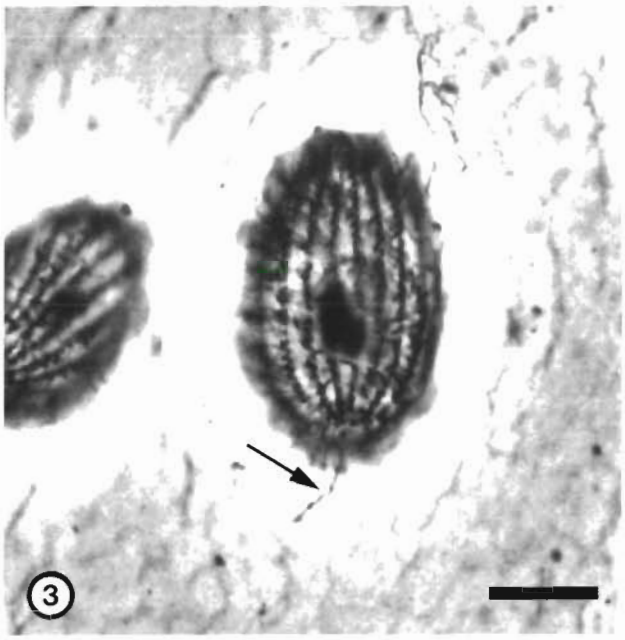

Fig. 3. *Anophryoides haemophila* n. sp. Overview of Bouin-fixed specimen, in haemolymph of experimentally infected lobster. Protargol stain. Note caudal cilium (arrow). Scale bar = 10.0 µm

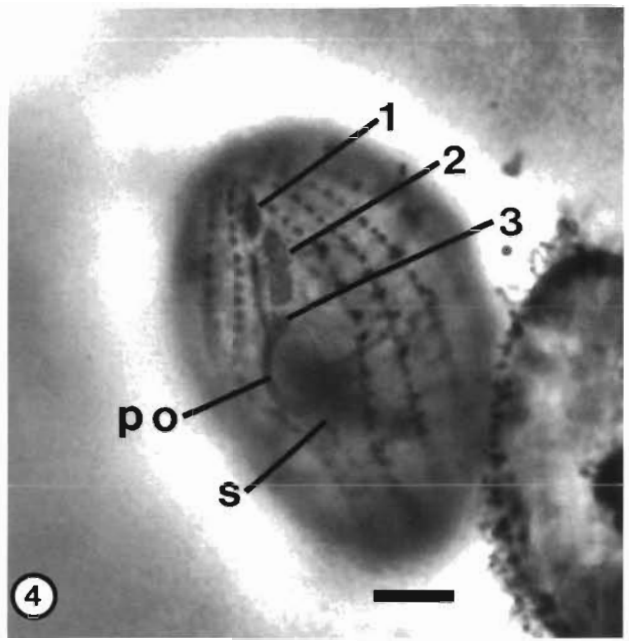

Fig. 4. *Anophryoides haemophila* n. sp. Details of oral ciliature of Bouin-fixed specimen, in haemolymph of experimentally infected lobster. Protargol stain. Scale bar = 5.0 µm. 1: oral polykinetid 1; 2: oral polykinetid 2; 3: oral polykinetid 3; po: paroral; s: scutica

The oral region is elongate and restricted to the anterior half (Fig. 4). Posterior to oral polykinetid 2, the oral region forms an oral cavity depression at the bottom of which is the cytostome. Oral region length 18.1 ± 1.4 µm (15.5 to 21.8 µm, n = 30) from lobster haemolymph. Oral polykinetid 1 is triangular, composed of at least 3 kinetosomes at its posterior margin and tapering to a single kinetosome at its anterior end. Oral polykinetid 1 length 3.8 ± 0.4 µm (3.2 to 4.6 µm, n = 23) from lobster haemolymph. Oral polykinetid 2 is curved rectangular, usually composed of 4 files of kinetosomes. Oral polykinetid 2 length 5.2 ± 0.6 µm (4.6 to 6.4 µm, n = 23) from lobster haemolymph. Oral polykinetid 3 is triangular to square and is placed on the anterior wall of the oral cavity depression. The paroral kinety is composed of dikinetids that zig-zag along their length, beginning near the anterior end of oral polykinetid 2. The paroral extends from the anterior end of oral polykinetid 2 to the level of oral polykinetid 3 and then forms a curve along the right side of the oral cavity depression. The scutica forms a short file, usually of dikinetids, that extends between somatic kineties 1 and n.

Somatic kineties, 16 to 18 (n = 48), extend from the anterior pole to just anterior to the posterior pole. The anterior half of each somatic kinety is composed of dikinetids that commonly have both kinetosomes ciliated. A prominent caudal cilium, about 15 µm long, arises from the posterior pole. There is a single contractile vacuole that opens through 1 or 2 contractile vacuole pores, located at the posterior end of somatic kineties 1, 2 or 3.

The macronucleus is almost spheroid. Macronuclear length 8.4 ± 1.3 µm (6.4 to 11.8 µm, n = 32) from lobster haemolymph; macronuclear length 6.8 ± 1.1 µm (5.5 to 9.1 µm, n = 31) in culture. Macronuclear width 6.4 ± 1.1 µm (4.6 to 9.1 µm, n = 32) from lobster haemolymph; macronuclear width 5.5 ± 0.8 µm (4.6 to 7.3 µm, n = 27). Spheroid micronucleus, single, usually closely adjacent to the macronucleus. Micronuclear diameter 1.76 ± 0.16 µm (1.37 to 1.82, n = 22) from lobster haemolymph.

DISCUSSION

Anophryoides was established as a genus by de Puytorac & Grolière (1979) for a ciliate originally described by Mugard (1949). *Anophryoides haemophila* n. sp. is placed in the genus *Anophryoides* primarily because the paroral begins near the anterior end of oral polykinetid 2 and oral polykinetids 1 and 2 are of similar length (Small & Lynn 1985). Morado & Small (1994) have provided a description of paranophryid ciliate genera with their description of *Mesanophrys pugettensis*, a ciliate that causes mortality in

Cancer magister, and reviewed the distinguishing characteristics of the different genera.

Prior to 1985, *Anophryoides* was a monotypic genus. At this time, Small & Lynn (1985) briefly described *A. soldoi* and provided *A. puytoraci* as a new name for *A. salmacida* de Puytorac & Grolière 1979. Subsequently, Strüder & Wilbert (1992) transferred *Paranophrys carnivora* Czapik & Wilbert, 1986, to the genus *Anophryoides*. With the addition of *A. haemophila* n. sp., there are now 4 species in the genus, as we now regard *A. puytoraci* to be a junior synonym of *A. salmacida*. *Anophryoides* is a bacterivorous species that can be maintained in an axenic medium (E. B. Small pers. comm.). However, *A. salmacida* and *A. carnivora* are both reported to be histophages that were fed in culture on tissues of invertebrates (Czapik & Wilbert 1986) or vertebrates (de Puytorac & Grolière 1979). These authors did not indicate what these species prefer as food in nature. *A. haemophila* n. sp. can be grown on autoclaved lobster tissue. However, this is apparently a minimal medium, since its cell size (see above) and growth rate (unpubl. obs.) are increased when the parasite infects the lobster host.

Anophryoides soldoi differs from the other 3 species in having its contractile vacuole just posterior to the paraoral rather than near the posterior end of the cell. *A. haemophila* n. sp. is distinguished from *A. salmacida* and *A. carnivora* primarily by the length of the oral polykinetids, which are smaller: oral polykinetid 1 about 4 µm compared to 6 to 8 µm and oral polykinetid 2 about 5 µm compared to 9 to 11 µm (see Strüder & Wilbert 1992). It also has fewer somatic kineties, 16 to 18 compared to between 21 and 22. *A. haemophila* n. sp. is shorter than *A. salmacida* (25.5 to 41.0 µm vs 55 to 85 µm), but overlaps in cell length with *A. carnivora* (25.5 to 41.0 µm vs 35 to 56 µm). Thus, it can be distinguished from *A. salmacida* by its shorter cell length, shorter oral polykinetids, and smaller number of somatic kineties, and from *A. carnivora* by the latter 2 features. On these grounds, this new species is justified.

Currently, we do not know whether *Anophryoides haemophila* n. sp. shows host specificity only for the American lobster *Homarus americanus*. The parasite does retain its infectivity and pathogenicity to lobsters although maintained for long periods (>12 mo) *in vitro* (unpubl. obs.). Overall, use of *A. haemophila* n. sp. as a model of 'bumper car' disease should facilitate elucidation of health and infectious disease processes in lobsters and related crustaceans.

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