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Pathogenic Amoebas from Brackish and Ocean Sediments, with a Description of *Acanthamoeba hatchetti*, n. sp.

Abstract. Acanthamoeba culbertsoni *was isolated from a sewage-spoil dump site near Ambrose Light, New York Bight. A second species,* Acanthamoeba hatchetti, *n. sp., was isolated from Brewerton Channel, Baltimore Harbor, Maryland. Both species killed laboratory mice after infection by the intranasal route.*

Small filose amoebas belonging to the family Acanthamoebidae Sawyer & Griffin, 1975 (*1*), have been studied extensively since the first species was discovered by Castellani in 1930. Culbertson *et al.* (*2*) found that at least one species of *Acanthamoeba* caused severe pathology and death in laboratory animals, and they clearly demonstrated the potentially pathogenic role of so-called free-living amoebas. Singh and Das (*3*) named the pathogenic species *Hartmannella culbertsoni* and, at the same time, described the pathogenicity of *Hartmannella rhysodes* in mice. Most investigators agree that the two species placed in the genus *Hartmannella* by Singh and Das (*3*) belong to the genus *Acanthamoeba* Volkonsky, 1931. Culbertson *et al.* (*4*) further reported than some less virulent strains of *Acanthamoeba* may produce chronic granulomatous responses of long duration in experimental animals. Subsequently, Jones *et al.* (*5*) described two cases of corneal ulceration in humans caused by *Acanthamoeba polyphaga* and one case of *Acanthamoeba* sp. uveitis, which resulted in the death of a patient. Nagington *et al.* (*6*) also described two cases of corneal involvement in England. Thus, *A. polyphaga*, and possibly other species of *Acanthamoeba* which do not cause death after nasal instillation into experimental animals, may be involved in chronic disease of the human eye. The ubiquitous distribution of *Acanthamoeba* in nature and the ability of certain species to survive and grow in oceanic seawater (*7*, *8*) suggest that their role in diseases of humans and animals is just beginning to be understood and documented.

The study reported here concerns the isolation of *Acanthamoeba* from oceanic and brackish-water bottom sediments and the subsequent identification of two species that killed experimental mice after infection by the nasal route. Ocean sediment from a sewage-spoil dump site in the New York Bight apex near Ambrose Light was collected with a Smith-McIntyre grab, and additional samples were similarly taken from 50 stations which ranged from 10 to 90 miles offshore from Lewes, Delaware. Sediments from Baltimore Harbor were collected by scuba diving in the Brewerton and Craighead shipping channels. All sediment samples were placed in sterile plastic dishes and sealed with waterproof tape at the time of collection. Cultures were prepared by transferring small amounts of sediment on the tip of a bacteriological loop to the bottom of glass milk-dilution bottles previously coated on one surface with 8 ml of agar media streaked with *Aerobacter* (=*Klebsiella*) *aerogenes*. Culture media were made with 1 percent Bacto-agar containing 0.01 percent malt extract and 0.01 percent yeast extract dissolved in distilled water, brackish water [salinity, 5 parts per thousand (ppt)], or oceanic seawater (30 ppt). Initial cultures were prepared in bottles containing agar media prepared in low-salinity water (5 ppt) and incubated in an upright position at 40°C, as described by Griffin (*9*). When all experiments at 40°C were completed, a second series of cultures were prepared and incubated at room temperature (22° to 25°C) to test for amoebas which did not grow at the higher temperature. Pure cultures of amoebas were established by placing a single cyst of each isolate on agar medium and making subcultures at monthly intervals. Salinity tolerance by each strain was tested by maintaining cultures on media prepared with distilled water or with oceanic seawater of 30 ppt salinity. Mouse pathogenicity tests were conducted by inoculating approximately 10,000 amoebas intranasally into groups of white mice weighing either 10 to 12 g or 15 to 20 g (*10*). Mice were observed daily to record signs of disease and the day of death. Brain and lung tissues were taken from dead or dying mice for histological examination and for further culture studies on agar media. Living amoebas and cysts were measured with an ocular micrometer and photographed with phase contrast optics.

Amoebas that grew at 40°C were isolated from one of two samples of sewage sediment collected in the New York Bight apex and from four of six samples of bottom sediment collected in Baltimore shipping channels. None of the 50 samples taken 10 to 90 miles offshore yielded amoebas after incubation at 40°C, but one *Acanthamoeba* species grew from sediment collected 20 miles offshore and incubated at room temperature. The New York Bight strain was identified as *Acanthamoeba culbertsoni*, a recognized pathogen of experimentally infected laboratory animals (Fig. 1A). The Baltimore isolates were identified as

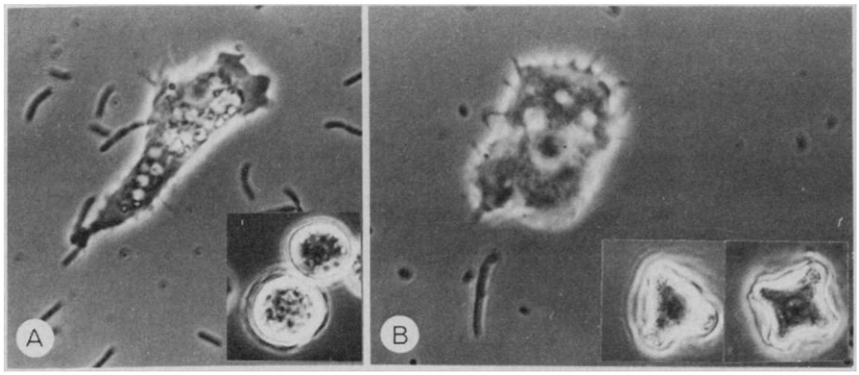

Fig. 1. Two species of *Acanthamoeba* that are pathogenic to white mice weighing 10 to 12 g (phase contrast photograph, × 1400). (A) *Acanthamoeba culbertsoni* trophozoite (insert: cysts with spherical endocyst). (B) *Acanthamoeba hatchetti*, n. sp., trophozoite (insert: cysts with three- to four-pointed endocysts).

Schizopyrenus russelli, *Acanthamoeba polyphaga*, *Acanthamoeba castellanii*, and *Acanthamoeba hatchetti*, n. sp. (Fig. 1B), to be described here. Amoebas from sediment taken 20 miles offshore were identified as *A. polyphaga* and were not tested in mice. Growth studies with the amoebas showed that all of the species grew on agar media made with distilled water, brackish water, and full-strength seawater. Growth was luxuriant on all test media, but consistently larger populations of amoebas were produced on brackish-water media of 5 ppt salinity. Comparative measurements of five species of *Acanthamoeba* are presented in Table 1.

Table 1. Comparative measurements of five species of *Acanthamoeba* grown on distilled water–agar media; 25 specimens of each species were measured.

Species	Length (μm)		Width (μm)		Cyst diameter (μm)	
	Range	Mean	Range	Mean	Range	Mean
A. culbertsoni	29.9–46.0	37.0	13.8–27.6	17.6	13.8–19.6	16.7
A. rhysodes (HN-3)	21.6–35.9	28.5	10.1–21.6	17.8	16.1–18.4	16.7
A. castellanii	18.4–30.0	24.0	9.2–13.8	10.4	13.8–20.7	17.3
A. polyphaga	23.0–34.5	26.9	9.2–16.1	13.1	11.5–15.0	13.0
A. hatchetti, n. sp.	18.4–25.3	23.6	11.5–20.7	16.0	11.5–16.1	13.1

Inoculation of mice weighing 10 to 12 g with *A. culbertsoni* and *A. hatchetti* consistently caused death; inoculated mice that weighed 15 to 20 g showed symptoms of the disease, but most of them survived for 30 days or more. The olfactory lobes and the superficial regions of the cerebral cortex of dead mice showed extensive inflammatory reaction and wide areas of necrotic tissue. Amoebas were seen interspersed within degenerated brain tissue. Lung tissue from dead mice showed extensive inflammatory reaction, but amoebas were not observed. Amoebas from infected brain tissue were successfully reestablished after incubation on agar media. Intranasal inoculation of *S. russelli*, *A. polyphaga*, and *A. castellanii* did not cause symp-symptoms of disease or death in test animals.

Acanthamoeba hatchetti, n. sp., is described as follows: *Acanthamoeba hatchetti* (Acanthamoebidae Sawyer & Griffin, 1975). Small filose amoebas with features typical of the genus *Acanthamoeba*. Locomotive form elongate to broadly triangular, 18.4 to 25.3 μm long (mean, 23.6 μm) and 11.5 to 20.7 μm wide (mean, 16.0 μm) when grown on distilled water and agar. Feeding form variable in shape and size; refractile, and without wide, clear, hyaline anterior zone of protoplasm. Cysts small and refractile with thick ectocyst and the distinctly three- or four-pointed endocyst which gives a triangular shape to most cysts; diameter, 11.5 to 16.1 μm (mean, 13.1 μm) on distilled water and agar. Division by mesomitosis with disappearance of nuclear membrane and nucleolus during late prophase. Growth and survival normal on distilled water, brackish water, and full-strength seawater media. Amoebas pathogenic to mice when inoculated by the intranasal route. Habitat: known only from brackish-water sediments collected from Brewerton Channel, Baltimore, Maryland. The new species is named in honor of the late Dr. Stephen P. Hatchett, director of Research Grants, National Institutes of Health, Bethesda, Maryland.

Our studies on brackish and oceanic sediments have extended the known range of pathogenic *A. culbertsoni* to include the nearshore waters of the Atlantic Ocean and have established a new species, *A. hatchetti*, as a pathogen from brackish-water sediment. The isolation of both pathogenic species was facilitated by employing the methods of Griffin (*9*), which eliminated species that did not grow at 40°C. The selective growth of freshwater or soil amoebas was also facilitated by using low-salinity media to inhibit marine species, which require a moderate to high salt concentration. Low-salinity media and incubation at 40°C probably inhibited the growth of most amoeba species and selectively allowed *Acanthamoeba* and *Schizopyrenus* to compete successfully for bacterial food organisms on the agar plates. These studies suggest that species of *Acanthamoeba*, commonly found in soil and freshwater, are distributed in brackish and oceanic sediments by freshwater runoff from streams and rivers, and by the disposal of sewage and dredge spoils at sea. Singh and Das (*3*) isolated *A. culbertsoni* and *A. rhysodes* from five samples of sewage examined in India and demonstrated the pathogenicity of both species by inducing death in experimental mice; Griffin (*9*) described the growth of both species after incubation at 40°C.

The current practice of dredging shipping channels and depositing the spoils in secondary locations and the practice of dumping sewage spoils at sea may be mechanisms by which species of free-living freshwater or soil amoebas are distributed in coastal environments. Thermal pollution of nearshore waters may be another mechanism that favors the growth of heat-tolerant amoebas. Studies by De Jonckheere *et al.* (*11*) have shown that species of *Naegleria* are recoverable from freshwater thermal effluents. The role of *Acanthamoeba* in marine waters has received very limited attention. Cannon *et al.* (*12*), however, reported the occurrence of *Acanthamoeba glebae* in association with marine algae; amoebas fed extensively on filamentous blue-greens and acted as a reservoir for strain LPP-1 cyanophage. The authors suggested that infected *A. glebae* may have a role in the transport of phage virus to areas where algae were previously uninfected. The importance of amoeba-virus interactions in the marine environment merits further study.

Nagington *et al.* (*6*) described the clinical manifestations of eye infections caused by *Acanthamoeba* in England and suggested that such infections may be more prevalent than was previously recognized. Our incomplete state of knowledge concerning human infections by *Acanthamoeba* and the increasing number of reports of the isolation of *A. culbertsoni* and *A. polyphaga* from natural sources suggest that caution should be exercised in the dumping of sewage and dredge spoils in previously unaffected areas.

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