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Marine Amoebae in Waters of Chincoteague Bay, Virginia: Ecological Significance of "Old" and "New" Species.

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ABSTRACT

Surface waters from Chincoteague Bay, Virginia, were sampled for marine amoebae in 1971-72. Seawater cultures yielded four new genera and 20 new species in addition to l6 genera and l5 species of previously described marine amoebae. Among the 16 "older" genera, six were described during 1874-l912, six during 1921-31, and four during 1950-72. The description of 20 new species in 1975 indicated that marine amoebae had not received adequate attention as important components of marine ecosystems. Several of the "new" species are now recognized as indicators of healthy or stressed environments. Similarly, the "old" genus *Paramoeba* Schaudinn, 1896 now includes a parasitic species that causes fatal disease in blue crabs, *Callinectes sapidus*, and *Acanthamoeba* Volkonsky, 1931 includes several that may cause meningitis or blindness in humans. Coastal waters of Virginia are known to harbor free-living pathogenic amoebae that deserve further ecological and taxonomic study, especially in marshlands, tidal creeks, and rivers.

Key Words:protozoa, marine amoebae, Chincoteague Bay

INTRODUCTION

Naked marine amoebae are unicellular animals (Protozoa) classified taxonomically in the same group (Sarcodina) as the radiolaria, heliozoa, and foraminifera. In contrast to most of the other groups of protozoa, amoebae are devoid of visible scales or tests that provide fixed or persistent morphological features that aid in their identification. Most amoebae have distinct shapes while in locomotion and possess distinct types of pseudopods that assist in placing them in broad taxonomic groupings at least to the family level. Although many species of freshwater and soil amoebae have cyst stages that are useful in identifying them, marine species rarely, if ever, undergo encystment. Identifications to species often require studies on stained specimens to determine types of nuclear division, culture in dilute seawater media to determine salinity tolerance, and/or studies with the electron microscope.

Prior to the 1900's there were approximately 12 well-described species of marine amoebae and 11 others placed within rather loosely defined genera. Schaeffer (1926) published the first monograph to place a diverse group of freshwater and marine species into well-described genera and families. His descriptions were based on patterns of locomotion, types of pseudopods, crystalline inclusions, food habits, *etc.* He also conducted salinity tolerance tests and found that none of the

marine species could be cultured continuously in freshwater. Bovee (1950, 1953) extended the work of Schaeffer (1926) and established many new genera and species. Bovee (1953) published an excellent account of the various morphological features useful for identifying free-living amoebae. Bovee and Sawyer (1979) and Page (1983) have published keys to the identification of marine amoebae.

Studies on marine amoebae presented here were carried out by culturing species from the surface waters of Chincoteague Bay, Virginia, near Franklin City during the period January 1971 - January 1972. The discovery of four new genera and 20 new species in Virginia waters (Sawyer, 1975a,b,c,) brought the total of well-described marine species to approximately 84 (Bovee and Sawyer, 1979). The project was designed to provide an historical database on the diversity of amoebae readily cultured from reasonably clean or unpolluted coastal waters. Results of the project were published to provide a basis for future studies on environmental quality as land and water use continue to impact on the Bay.

METHODS

Water from Chincoteague Bay was sampled by floating sterile 1 liter bottles on the surface until they became half-filled and began to sink. String attached to the bottle necks was used to hold them in an upright position and prevent them from sinking below the surface. Samples were filtered through 1.2 μ filters under weak vacuum until a thin layer of water remained on the surface. Filters were inverted and floated overnight on the surface of filtered water placed in sterile 60 mm plastic culture dishes. Filters were removed and each dish examined with an inverted Leitz microscope to note the presence of amoebae, ciliates, diatoms, *etc.* Water from each dish was removed with a sterile fine-tipped pipette leaving approximately 1 ml in the dish, and placed in sterile test-tubes. Water remaining in each dish was used to flush the bottom and 2 -3 drops were placed on agar plates streaked with bacteria (*Klebsiella aerogenes*). Original cultures were re-established by returning the water from the test-tubes to the appropriate plate. Agar media were prepared by melting 15g Difco agar, 0.1g yeast extract, and 0.1g malt extract in seawater (20-24 o/oo) sterilized by autoclaving. Culture methods, staining procedures, and methods used for identifications are as published earlier (Sawyer, 1975a; Bovee and Sawyer, 1979; Sawyer and Bodammer, 1982).

RESULTS

Microscopic studies on 185 strains of marine amoebae showed that 30 of them, culturable on semi-moist agar plates, and five that would grow only with a seawater overlay, were distinct species assignable to 20 genera (Sawyer, 1973) Four genera and 15 species (20 strains), did not have the features of any adequately described species and were described as new to science (Sawyer, 1975a,b,c). Seven of the recognized genera (Table 1), *i.e*, *Hyalodiscus*, *Saccamoeba*, *Thecamoeba*, *Paramoeba*, *Vahlkampfia*, *Pelomyxa*, and *Acanthamoeba,* were described prior to Schaeffer's 1926 monograph. Five others (Table 1) were described by Schaeffer, *i.e.*, *Mayorella*, *Unda*, *Gibbodiscus*, *Vexillifera*, *Flabellula*; two by Bovee, *Vannella*, *Triaenamoeba*, and two by Page,*Platyamoeba*, and *Rhizamoeba*. The four newly described genera were *Clydonella*, *Lingulamoeba*, *Boveela*, and *Stygamoeba* (Table 1). All of the amoebae were recovered from waters that did not exceed 2m deep. Among the 20 new species, eight were 20 μ or less in greatest dimension, seven

TABLE 1. Distribution of marine amoebae in coastal waters of the northeastern United States (partial listing).

Genus	Chincoteague[1] Bay	Chincoteague[2] Bay	Sandy Hook[2] New Jersey	WoodsHole[2] Massachusetts
Flabellula	+	+	+	+
Platyamoeba	+	+	+	+
Thecamoeba	+	+	+	+
Pelomyxa	+	+	+	-
Clydonella	+	+	-	+
Mayorella	+	+	-	+
Paramoeba	+	-	-	-
Boveela	+	-	-	-
Stygamoeba	+	-	-	-
Vannella	+	+	-	+
Acanthamoeba	+	+	-	-
Lingulamoeba	+	-	-	+
Triaenamoeba	+	-	-	-
Vexillifera	+	-	-	-
Gibbodiscus	+	-	-	-
Vahlkampfia	+	+	+	+
Unda	+	-	-	+
Saccamoeba	+	+	+	+
Hyalodiscus	+	-	-	-
Rhizamoeba	+	-	-	-
Genera present[3]	20/20	10/20	6/20	10/20

[1]From Sawyer, 1975a,b,c
[2]Bovee, E.C. (Personal communication)
[3]Numerator = no. genera identified per location/Denominator = Total no. genera identified.

measured over 20 but less than 30 μ, 11 measured between 30 and 40μ, and only one *Mayorella corlissi*, measured up to 100 μ.

DISCUSSION

The Chincoteague Bay studies yielded the first comprehensive account of marine amoebae in Virginia coastal waters. The discovery of 20 new species illustrated the very limited knowledge on species diversity among naked sarcodines in estuarine waters of the northeastern U.S. Recent interest in amoebae as important components of marine waters and sediments (Sawyer, 1980) have shown that several genera, including *Paramoeba*, *Platyamoeba*, and *Clydonella* probably are cosmopolitan in saline waters. Species of *Acanthamoeba* are also common in coastal and ocean sediments contaminated with sewage wastes (Sawyer, 1980; O'Malley *et al.*, 1982; Sawyer *et al.*, 1982). Taxonomic studies have shown that careful microscopic studies on seemingly unimportant or transient features of marine amoebae are often the definitive characteristics for identifying these protozoa (Schaeffer, 1926; Bovee, 1953; Bovee and Sawyer, 1979; Page, 1983).

Follow-up studies in Chincoteague Bay should benefit from the historical findings reported here and possibly provide useful information on changes in species diversity that may have occurred during the past two decades.

Significance of "Old" Genera & Species - The genus *Hyalodiscus* Hertwig & Lesser, 1874 has not been studied adequately since its original description. Although valid, the genus would require complete revision should the type species be found in future studies. *Thecamoeba* Fromentel, 1874 is a well-described genus and now accommodates a variety of freshwater and marine species that are readily identified with existing keys. *Paramoeba* Schaudinn, 1896 was the only genus to be proposed for an exclusively marine species prior to 1900. Species assigned to *Paramoeba* may bear superficial resemblance to species of *Mayorella* Schaeffer, 1926 and *Vexillifera* Schaeffer, 1926, but differ by having a stainable inclusion near the nucleus. The inclusion has been called a secondary body, Nebenkörper, amphosome, parasome, *etc*. The genus *Paramoeba* includes *P. perniciosa* Sprague, Beckett, & Sawyer, 1969, parasitic in the blue crab, *Callinectes sapidus*. Sawyer (1976) reported a parasitic amoeba from histologic sections of the American lobster, *Homarus americanus*, and the rock crab, *Cancer irroratus*, that was identical to *P. perniciosa* as seen in sections of the blue crab. The genus *Vahlkampfia* Chatton & LaLung-Bonnaire, 1912 was established to accommodate an amoeba isolated from a diarrheic stool of a hospitalized patient. The genus now includes a variety of soil, freshwater, and marine amoebae. All species within this genus have a mitotic pattern where the nuclear membrane and nucleolus persist throughout the division process. All of the "old" genera accommodate species of amoebae that are important in the marine food web feeding upon bacteria, other protozoa, diatoms, etc. Some species that are recognized as obligate or opportunistic pathogens are important in the health of plant and animal life in nature and in aquaculture systems.

Significance of "New" Genera & Species - Most of the marine amoebae described since 1926 were studied from pure clonal cultures thereby eliminating descriptions that may have been based on mixed populations. Many of the species recognized prior to 1926 have been re-described from clonal cultures using phase-contrast microscopy, staining techniques, and scanning and transmission electron microscopy (Page, 1983). Recent interest in marine pollution, nutrient recycling in marine food webs, taxonomy, systematics, and diseases of marine animals have shown that amoebae, in addition to well-known flagellates and ciliates, are more important than previously recognized. Most of the amoebae that are frequently cultured from surface waters feed almost exclusively on bacteria, and others probably on microalgae and flagellates. *Platyamoeba* Page, 1969, *Clydonella* Sawyer,1975, *Vannella* Bovee, 1965, and several small species of *Paramoeba* Schaudinn, 1896, are common in marine surface waters and feed extensively on bacteria. Munson (1988) routinely found *Paramoeba*, *Platyamoeba*, *Clydonella*, *Vannella*, and *Hartmannella* in surface waters 10 and 33 km. off of Sapelo Island, Georgia. Larger amoebae, e.g., *Mayorella*, *Vexillifera*, *Thecamoeba*, *Hyalodiscus*, and *Unda* have been found primarily in shallow coastal waters and marshes where they may feed upon algae and diatoms, as well as bacteria. The relative ease of identifying most of the "new" species was estimated by Bovee (personal communication). He studied amoebae from surface water, tidal pools, and salt marshes at

Chincoteague Bay, Sandy Hook, New Jersey, and Woods Hole, Massachusetts. Bovee found 11 of the "new" species in Chincoteague waters, two at Sandy Hook, and seven at Woods Hole. He considered the Chincoteague ecosystem to be relatively clean, Woods Hole moderately polluted, and Sandy Hook as highly polluted. Bovee's unpublished findings agreed with his estimates of ecosystem quality, *i. e.*, 13 genera and 27 species from clean waters, 10 genera and 27 species from moderately polluted waters, and seven genera and 19 species from highly polluted waters. The distribution of genera shown in Table I suggests that new studies are desirable to support the generally accepted belief that protozoan diversity attains a maximum only in clean unpolluted ecosystems. Previous studies (Sawyer, 1980) indicated that small bactivorous species adapt to changes in water quality, however, very little is known about factors that influence the presence or absence of larger shoreline or salt marsh species.

Progress in Understanding the Pathogenic Potential of Marine Amoebae - Chatton (1910) published one of the earliest reports of marine amoebae (*Vahlkampfia*) as being associated with fish mortalities. He noted mortalities in aquaria fish and found massive numbers of amoebae (*Vahlkampfia mucicola*) covering the gills and probably causing respiratory stress. Hogue (1914) found two new species of *Flabellulla* in the gut contents of oysters, *Crassostrea virginica* (originally placed in the genus *Vahlkampfia*). Later, *Thecamoeba hoffmani* was described from gills of fingerling fish (Sawyer *et al.*, 1975) and *Pseudovahlkampfia emersoni* was described from the gills of rock crabs, *C. irroratus*, and the intestinal contents of blue crabs, *C. sapidus* (Sawyer, 1980). *Paramoeba pemaquidensis* Page, 1970, a well-known free-living amoeba, was recently reported from the gills of dead or dying Coho salmon, *Oncorhynchus kisutch* reared in aquaculture facilities (Kent, *et al.*, 1988). Thus, marine amoebae, as well as other groups of protozoa, may be involved with stress in marine animals to a greater extent than presently appreciated. Sick or dying animals usually are dissected and their tissues examined for microorganisms in histologic sections. Gill-fouling microorganisms are often lost or reduced in numbers subsequent to fixation, processing, sectioning, and staining. Mortalities among marine fish and crustacea, regardless of the cause, are difficult to assess since weakened or stressed animals are readily consumed by predators.

Salinity tests showed that only one strain of euryhaline amoeba was recovered from surface waters of the Bay. The amoeba, although not identified to species, formed resistant wrinkled cysts characteristic of the genus *Acanthamoeba*. Several species belonging to this genus are known to grow at mammalian body temperatures and cause fatal amoebic meningitis in humans and experimental animals (Martinez, 1985). Other species of *Acanthamoeba* that do not grow at elevated temperatures have been cultured and identified from patients with eye disease (Morbidity and Mortality Weekly Report, 1986). Several years after the Chincoteague Bay studies, it was learned that both pathogenic and non-pathogenic species of *Acanthamoeba* may be cultured from sewage-contaminated marine sediments when distilled water or low salinity seawater (3-5 o/oo) are used in preparing the agar media (Sawyer *et al.*, 1977; Sawyer *et al.*, 1982; Daggett *et al.*, 1982). Today it is recognized that certain cyst-forming soil amoebae (*Acanthamoeba*, *Hartmannella*, *Vahlkampfia*), although

not classified as marine amoebae, serve as excellent indicators of sewage or thermal pollution in fresh, brackish, and marine ecosystems.

CONCLUSIONS

Modern taxonomic keys to marine amoebae by Bovee and Sawyer (1979) list approximately 34 genera and 84 species, and by Page (1983) , 31 genera and 81 species Further studies undoubtedly will add many other new species to this growing list and contribute to redescriptions of some that are of uncertain taxonomic status. Page (1983) lists 14 species assigned within the genera *Amoeba* or *Amiba*, described between 1841-1963 (10 prior to 1900) that require further taxonomic study. Among the 84 species listed by Bovee and Sawyer (1979), 20 were first isolated from Chincoteague Bay, inland near Franklin City. Further studies on open waters, vegetated shorelines, and bottom sediments would be likely to yield other new species, especially larger ones that feed on diatoms,algae, blue green algae, and other microorganisms. Other studies on sewage-associated bacteria and freshwater or soil (anthropomorphic) cyst-forming amoebae could provide new information on the present status of sewage pollution resulting from increasing recreational, residential, and commercial land and water use. Sawyer and Munson (1988) made a preliminary study on cyst-forming amoebae in shoreline and inland sediments at Sapelo Island, Georgia and identified 10 species of *Acanthamoeba*, including several that are well-known pathogens. Historical data on species diversity among marine amoebae during 1971-1972 should provide a useful historical record for developing a water quality model for the shoreline of Chincoteague Bay as it undergoes further development.

LITERATURE CITED

Bovee, E. C. 1950. Some observations, based on the use of the phase contrast microscope, concerning the morphology of certain free-living amoebas. Ph. D. Thesis, Univ. Calif., Los Angeles. 407 pp.

_____. 1953. Morphological identification of free-living Amoebida. Proc. Iowa Acad. Sci. 60:599-615.

_____., and T. K. Sawyer. 1979. Marine Flora and Fauna of the Northeastern United States. Protozoa: Sarcodina: Amoebae. U.S. Dept. Commerce, Natl. Mar. Fish. Service Circ. 419. 56 pp.

Chatton, E. 1909. Une Amibe, *Amoeba mucicola*, n.sp., parasite des labres, associée à une trichodine. C.r. Séanc. Soc. Biol., Paris. 67:690-692.

Daggett, P. -M., T. A. Nerad, and T. K. Sawyer. 1982. Distribution and possible interrelationships of pathogenic and nonpathogenic *Acanthamoeba* from aquatic environments. Microbial Ecol. 8:371-386.

Hogue, M . J. 1914. Studies on the life history of an amoeba of the limax group, *Vahlkampfia calkensi*. Arch. Protistenk. 35:154-163.

Kent, M. L., T. K. Sawyer, and R. P. Hedrick. 1988. *Paramoeba pemaquidensis* (Sarcomastigophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in seawater. Diseases of Aquatic Organisms. 5:163-169.

Martinez, A. J. 1985. Free-living Amebas: Natural History, Prevention, Diagnosis, Pathology, and Treatment of Disease. CRC Press, Inc., Boca Raton, Florida. 156pp.

Morbidity and Mortality Weekly Report. 1986. *Acanthamoeba* keratitis associated with contact lenses - United States. Center for Disease Control, U. S. Public Health Service. 35:405-408.

Munson, D. A. 1988. A comparison of marine amoebae from surface waters of a tidal creek on and two neritic sites off Sapelo Island, Georgia. Trans. Amer. Microscop. Soc. 107:102.

O'Malley, M. L., D. W. Lear, W. N. Adams, J. Gaines, T. K. Sawyer, and E. J. Lewis. 1982. Microbial contamination of continental shelf sediments by wastewater. J. Water Pollut. Control Fed. 54:1311-1317.

Page, F. C. 1983. *Marine Gymnamoebae*. Inst. Terrestrial Ecol. Cambridge , England. 54 pp.

Sawyer, T. K. 1970. The influence of seawater media on growth and encystment of *Acanthamoeba polyphaga*. Proc. Helminthol. Soc. Wash. 37:182-188.

_____. 1971. *Acanthamoeba griffini*, a new species of marine amoeba. J. Protozool. 18:650 - 654.

_____. 1973. An analysis of the principal genera of amoebae in surface waters of Chincoteague Bay, Virginia with descriptions of species. Ph.D. Dissertation, Univ. of Md. 154 pp.

_____. 1975a. Marine amoebae from surface waters of Chincoteague Bay, Virginia: Two new genera and nine new species within the families Mayorellidae, Flabellulidae, and Stereomyxidae. Trans. Am. Microscop. Soc. 94:71-92.

_____. 1975b. Marine amoebae from surface waters of Chincoteague Bay, Virginia: One new genus and eleven new species within the families Thecamoebidae and Hyalodiscidae. Trans. Amer. Microscop. Soc. 94:305-323.

_____. 1975c. *Clydonella*, N. G. (Amoebida: Thecamoebidae), proposed to provide an appropriate generic home for Schaeffer's marine species of *Rugipes*, *C. vivax* (Schaeffer, 1926) N. Comb. Trans. Amer. Microscop. Soc. 94:395-400.

_____. 1976. Two new crustacean hosts for the parasitic amoeba, *Paramoeba perniciosa*. Trans. Amer. Microscop. Soc. 95:271.

_____. 1980. Marine amoebae from clean and stressed bottom sediments of the Atlantic Ocean and Gulf of Mexico. J. Protozool. 27:13-32.

_____. and S. M. Bodammer. 1982. Marine amoebae (Protozoa: Sarcodina) as indicators of healthy or impacted sediments in the New York Bight apex, 338-352: *In*: Wastes in the Ocean: Industrial and Sewage Wastes in the Ocean., I. W. Duedall, B. H. Ketchum, P. K. Park,and B. H. Kester (eds.). Volume 1: John Wiley & Sons, Inc.

_____., J. G. Hnath, and J. F. Conrad. 1975. *Thecamoeba hoffmani*, sp. n. (Amoebida:Thecamoebidae) from gills of fingerling salmonid fish. J. Parasitol. 60:677-682.

_____., E.J. Lewis, M. Galasso, D. W. Lear, M. L. O'Malley, W. M. Adams, and J. Gaines. 1982. Pathogenic amoebae in ocean sediments near wastewater sludge disposal sites. J. Water Pollut. Control Fed. 54:1318-1323.

_____., G. S. Visvesvara, and B. A Hartke. 1977. Pathogenic amoebas from brackish and ocean sediments with a description of *Acanthamoeba hatchetti*, n.sp. Science. 196:1324-1325.

_____., and D. A. Munson. 1988. Potentially-pathogenic amoebae (Acanthamoebidae) from Sapelo Island, Georgia, sediments. Trans. Amer. Microscop. Soc. 107:102-103.

Schaeffer, A. A. 1926. Taxonomy of the Amoebas. Carnegie Instit. Wash. Publ. No. 345, 116 p.

Sprague, V., R. L. Beckett, and T. K. Sawyer. 1969. A new species of *Paramoeba* (Amoebida: Paramoebidae) parasitic in the blue crab, *Callinectes sapidus*. J. Invert. Pathol. 14:167-174.