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Morphological Identification of Free-Living Amoebida

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By Eugene Cleveland Bovee

Introduction

One of the knottiest problems in zoology is the specific identification of naked, free-living amoebas of the order Amoebida. Usual methods of taxonomic identification, i.e. easily distinguishable morphological contours of sufficient permancy seem to be lacking in them.

Schaeffer (1926), amongst others, points out that such a creature as "the amoeba," does not exist. He speculates, perhaps rightly, that the naked, free-living amoebas are of polyphyletic origin, and that to attempt to classify them phylogentically is virtually impossible. The supposed "simplicity" of amoebas, because of their resemblance to the "basic cell," has given credence to the belief that there is little morphological diversity amongst them. Some have therefore concluded that naught but the nucleus is of taxonomic value (Hartman and Naegler 1908; Calkins 1919; Doflein 1928).

If "the amoeba" is so phylogentically ancient as is supposed, its opportunities for mutative variance are certainly at least as great as those of annelids, molluscs, arthropods, or vertebrates, even if the genetic vagaries due to sexual reproduction (not definitely shown for amoebas) be admitted. Hence, citing Schaeffer (1926) once more, research done on, or reference to "the amoeba" or "an amoeba" is of no more scientific worth than labors practiced upon "an insect", or "a vertebrate".

It remains of paramount importance, then to be able to identify an amoeba by means of morphological features. For some of the larger free-living amoebas these are fairly well-known, but their validity is debated by zoologists who love an amoeba under the name by which they have learned to speak of it, and will not admit of reclassification, regardless of evidence of probable relationship otherwise. Such a wrangle has developed around the easily recognizable, but dubiously named *Amoeba proteus* Pallas; and about *Chaos carolinensis* (*Chaos chaos Linnaeus; Pelomyxa carolinensis* Wilson). (Schaeffer 1926; Mast and Johnson 1934; Wilber 1947; King and Jahn, 1948; Kudo 1952).

Schaeffer (1926) proposed a classification based on the larger of the naked, free-living amoebas. He employed the morphological patterns of these animals as they appeared in "normal" existence, eschewing "changes" produced experimentally (Verworn, 1894). In using the "natural system" he picked up where earlier taxonomists, whose specific identifications of amoebas are most often considered valid, left off. (F. E. Schultz 1875; Leidy 1879; Gruber 1885; Penard 1902; Cash and Hopkinson 1905; Cash & Wailes 1915).

Although Schaeffer's system has been soundly berated (Mast & Johnson 1934; Kudo 1946; Wilber 1947), the new species he has described are easily recognizable and their names have been widely accepted both in the classroom and in the research laboratory, particularly *Metachaos* (Amoeba) *dubia, Flabellula mira,* and *Mayorella bigemma.*

Schaeffer, by his own admission (1926), had not provided for the classification of small amoebas, parasitic nor soil amoebas. Attacks on his system have come often from specialists on those forms (Doflein, 1928; Calkins, 1941), with, perhaps, some justification.

Discussion

Studies made on free-living, naked amoebas over the past five years, employing the phase microscope, have revealed Schaeffer's system to be usable for small species, as well as larger ones, particularly those living in fresh, brackish and sea water.

An extension of Schaeffer's general system is herein attempted, pointing out morphological patterns and structures and internal bodies by which these amoebas may be singled out.

Activity Cycles

Many free-living aquatic amoebas have characteristic daily activity cycles. (Bovee, 1951) Parasitic amoebas and soil amoebas do not seem to possess them, perhaps because they spend their trophic existence in darkness. Whether these activity cycles are true diurnal rhythms remains to be investigated.

Amoebas having activity cycles seem not to have them affected by light or temperature artificially applied during darkness (Bovee, 1951).

These cycles regularly show rest or inactivity from early evening to early morning, slowly increased activity, reaching a peak from mid-day to early afternoon. Activity then lessens until, by early evening, resting patterns are again resumed.

Most oft-observed patterns of recurrent nature are:

(1) Inactive (a) on the substrate and (b) afloat;
(2) in slow movement without locomotion;
(3) in slow locomotion;
(4) in moderate locomotion;
(5) in rapid locomotion.

Size

Each amoeba appears to have a definite upper limit to its size, as well as lower limits. Penard (1902) observed a direct relationship between the ratio of nuclear surface area and cytoplasmic volume, and Warren (1949) has used cytoplasmic and nuclear volume ratios to divide the species *Chaos diffluens* (Amoeba proteus) into 3 separate species.

Size in any species may be sufficient to distinguish it from another larger, (or smaller) morphologically similar species.

Shape

The contours of any amoeba are never exactly the same during any two moments of its life because of its activity and growth. This is equally true of all other living things, but the changes in contour are less apparent, and an impression of morphological stability is lent to most animals, which does not seem to be given the amoebas.

However, during the activity cycles of most amoebas, at least one, and often several, morphological patterns and contours appear by which the amoeba may be taxonomically placed.

Inactive forms on the substrate for the family *Chaidae* Poche are usually smoothly to rugously spherical; the *Mayorellidae* Schaeffer papillately, knobbily or smoothly spherical; the *Hyalodiscidae* Poche smoothly or wrinkled and irregularly spherical; the *Thecamoebidae* Schaeffer, spherical with knobby or wrinkled surface, or smoothly spherical with thick, clear ectoplasm.

Floating stages of *Chaidae* are spherical, or stellate with indeterminate, finger-like, granule or crystal-filled pseudopods of varying lengths. *Mayorellidae* extend rigid, clear, conical pseudopods of definitive length and number, or with granule filled, indeterminate pseudopods bearing a clear, conical tip of determinate length. *Hyalodiscidae* are spherical, or with palp-like, clear pseudopods bearing several, small, conical pseudopods in clusters at their tips. *Thecamoebidae* are spherical, resembling the inactive stages on the substrate.

Slowly active stages without locomotion in *Chaidae* are irregularly flattened with granule-filled, digitate pseudopods at any point on the periphery, and in variable numbers, or in some genera with slowly-formed, eruptive waves. *Mayorellidae* form pairs of clear, conical pseudopods partly connected by a clear wave between each pair, one to several pairs being present, the pairs sometimes extended upwards on a clear, stalk-like pseudopod above the body; or there is present a clear wave extended along the substrate and bearing dentate pseudopods. *Hyalodiscidae* form angular, clear waves, bearing one to several dentate pseudopods, or bear surrounding clear wave with many, dentate pseudopods. *Thecamoebidae* pro-

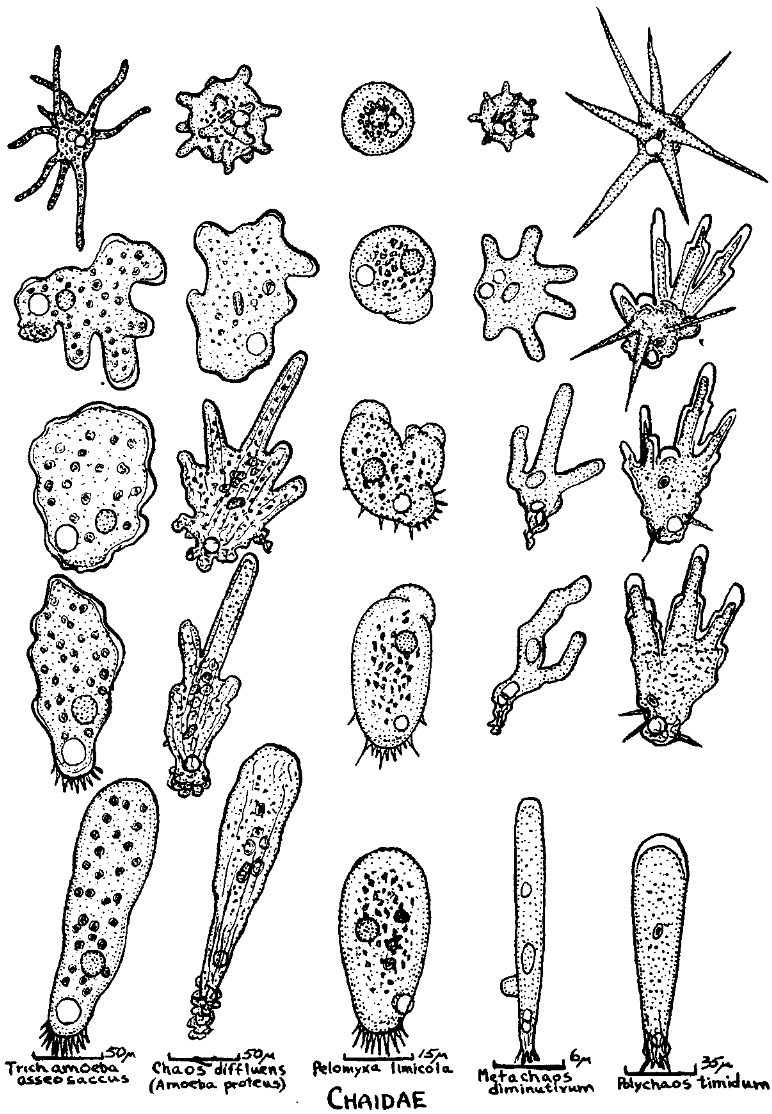

PLATE I

ject a clear wave about the same breadth as the body, having an oval to irregular border.

In slow locomotion *Chaidae* are irregularly palmate with digitate pseudopods, or egg-shaped with anterior, eruptive waves. *Mayorellidae* are spatulate to fan-shaped, with a clear, anterior wave of greater or lesser extent, usually with conical, clear pseudopods at the periphery. *Hyalodiscidae* are fan-shaped with a broad, clear wave anteriorly and extending laterally. *Thecamoebidae* are ovate to broad, with a clear anterior wave, and wrinkles in the dorsal ectoplasm.

In moderate locomotion *Chaidae* are roughly triangular with several digitate pseudopods, or are ovate, forming anterior eruptive waves. *Mayorellidae* are spatulate to fan-shaped, the body somewhat more elongated than in slow locomotion. *Hyalodiscidae,* fan-shaped with clear anterolateral wave. *Thecamoebidae,* ovate with longitudinal dorsal ridges in the ectoplasm and clear anterior wave.

In rapid locomotion *Chaidae* are clavate, with rear end wrinkled or bearing a cluster of uroidal filaments. *Mayorellidae* are clavate, spatulate or fan-shaped, pseudopods appearing singly, in pairs, or a broad wave maintained anteriorly. *Hyalodiscidae,* broadly fan-shaped, or broadly ovate, with or without a broad, clear, anterolateral wave. *Thecamoebidae,* ovate with narrow, clear anterior wave and longitudinal ridges in the dorsal ectoplasm.

Locomotor Organelles.

The typical locomotor organelle of the amoebas is the pseudopod. There are, however, various types of pseudopods. Free-living, naked amoebas of the order Amoebida have a type of more or less blunt, unbranched pseudopod known as a lobopod. (Jahn and Jahn, 1949).

These lobopodia have been used by Schaeffer (1926) to distinguish the families which he designated in his taxonomic system.

Indeterminate Pseudopods

Pseudopodia which are continuously formed, cylindrical tubes through which the amoeba flows, absorbing the tube posteriorly to produce more inner sol. They are granule filled, have a clear, thin ectoplasm, and a somewhat thicker, clear, advancing tip. One to several present; one selected as the principle avenue of progress, the remainder retracted. Particularly characteristic of the *Chaidae,* they have a hemispherical tip. They appear as waving pseudopods in some *Mayorellidae,* bearing a conical, clear tip. (Bovee, 1952).

Eruptive Waves

Hemispherical bubbles of endoplasm which burst through pinpoint holes in the surface membrane at the antero-lateral margin of the amoeba. Characteristic of some *Chaidae*: according to Wilber, (1946) the indeterminate pseudopod may arise as a rapidly formed series of these eruptive waves. Soil amoebas and parasitic amoebas also form eruptive waves.

Determinate Pseudopods

Clear and conical, tapering to a hemispherical tip; extended to a definite, limited length for each species. Often present at the lateral borders of (and partly connected by) a flat, protoplasmic, clear wave. May project above, or forward through, or forward from, the clear, anterior wave, or from the dorsal surface. Characteristic of *Mayorellidae*: They appear also as dentate pseudopods at the border of anterior waves of *Hyalodiscidae*.

Indeterminate-determinate Pseudopods

Some *Mayorellidae* extend a clear, determinate pseudopod, followed by and attached to an indeterminate, granule-filled pseudopod, which is usually waved about and retracted, but many, in at least one species, drop forward to become the main body mass in rapid locomotion.

Clear Anterior Waves

Margins of clear protoplasm extended anteriorly, or anterolaterally by *Mayorellidae, Hyalodiscidae,* and *Thecamoebidae*. Although continuously created anew, its contours are often so well maintained that outline, width, and extent of the wave become diagnostic. Some soil amoebas and parasitic amoebas from similar clear, anterior waves through the rapid coalescence of eruptive waves at their anterior margins.

Waving Pseudopods

Either determinate or combined *determinate-indeterminate* structures, extended from dorsal or lateral surfaces. Found principally in the *Mayorellidae*. The determinate type waves in most instances only during retraction (Bovee, 1951 b.). The determinate-indeterminate combination waves actively, but only within the indeterminate portion.

An exceptional type of waving, determinate pseudopod is found in one Mayorellid which is often confused with the mastigamoebas. This one, *Flagellipodium piscanavigans* (Bovee, 1950), forms a slender, vibratile, determinate pseudopod which can be moved about by its base along the body surface and is vibrated and retracted at will. During rapid locomotion it is held horizontally and rigidly anterior. The author chooses to call it a *flagellipod.*

Numbers of Pseudopods and Waves

The number of pseudopods rarely at any one time is definite for any one species. Still, each species ordinarily has approximately the same number present during a characteristic pattern of activity. Acquaintance with a variety of amoebas renders number of pseudopods semi-diagnostic. *Chaidae* rarely have more than two of the same size, except when afloat, or more than a single eruptive wave in formation at the same time.

Mayorellidae often form pseudopods in pairs, frequently partly connected by a clear wave.

Uroidal Structures

Schaeffer (1918) coined the word "uroid" for the tuft of slender posterior filaments which appear on some amoebas of the family *Chaidae* during rapid locomotion. Bovee (1950) indicates their formation from adhering, and stretched ectoplasm as the amoeba passes over the point of adherence. Many have noted that the filaments are present for long periods of time as a ball-like tuft at the extreme posterior of some amoebas (Leidy, 1879; Penard, 1902; Wallich, 1863; Schaeffer, 1926; Radir, 1927; Bovee, 1951).

True uroidal structures do not appear in most amoebas, although the posterior ends of most in rapid locomotion are somewhat wrinkled or mammillary. Trailing pseudopods occassionally lend a uroid-like appearance to some *Mayorellidae.*

Permanent Posterior Gel Disc

Some *Chaidae,* notably those which form eruptive waves, have a permanent concavo-convex gel disc at the posterior end (Leidy, 1879; Radir, 1927; Bovee, 1951). The uroid forms at its ventro-exterior periphery, or at its surface. It has not been identified as present in *Mayorellidae, Hyalodiscidae* or *Thecamoebidae.*

Ectoplasmic Markings

Most naked, free-living amoebas show some wrinkling of ectoplasm due to contractions of the posterior end. The latter often, in the *Chaidae* without the posterior gel disc, is mammillary in appearance. Longitudinal ridges appear on the pseudopods of some *Chaidae,* as well.

In the *Mayorellidae* wrinkles are noticeable in larger species, but there is no definite pattern to the folds.

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Some *Hyalodiscidae* show granules embedded in the ectoplasm, giving a rough-surfaced appearance to the body mass.

Striations or ridges in the ectoplasm are most prominent in the *Thecamoebidae,* often in parallel, longitudinal ridges. The number of ridges is frequently characteristic of the species.

Endoplasm

The nature of the endoplasm is of accessory value. In *Chaidae,* plasmasol is usually fluid, and plasmagel firm, body contours being often tube like. In the *Mayorellidae* the gel is often not so tense anteriorly lending a more spatulate appearance. *Hyalodiscidae* have a firm gel which gives the body mass an ovate shape. *Thecamoebidae* have thick ectoplasm; thin, highly contractile endo-

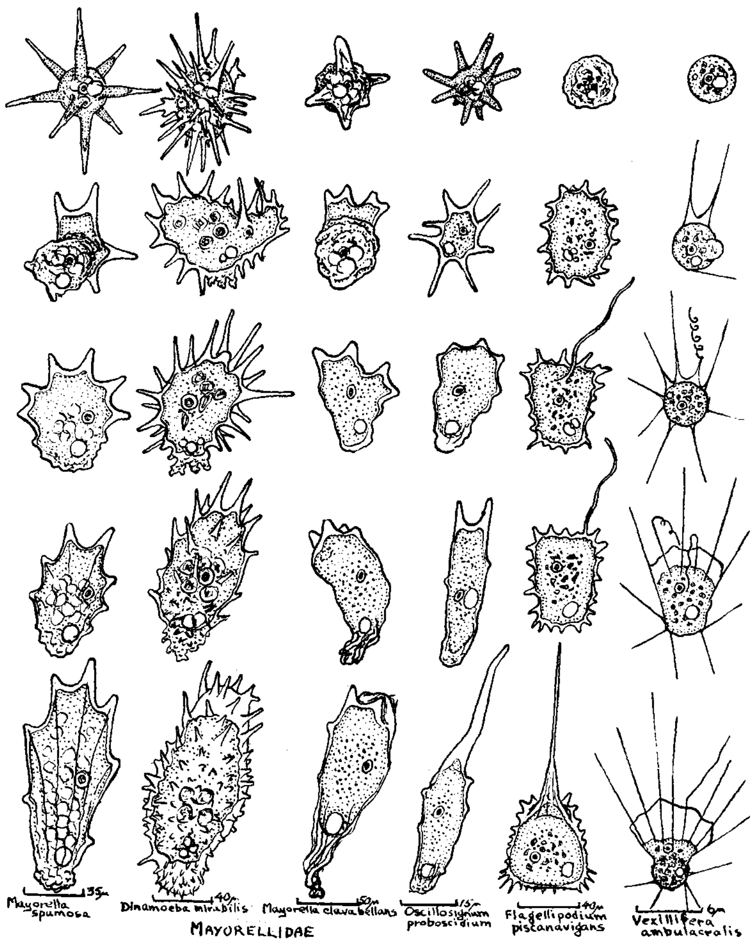


PLATE II

plasmic gel; and a fluid endoplasmic sol; resulting in ovately flattened contours.

Inclusions

Visible structures in the endoplasm are widely used in the taxonomy of the amoebas. Of them, the nucleus is considered by some (Calkins, 1913, 1919; Hartmann and Naegler, 1908; Doflein 1916, 1928) to be of paramount importance. The contractile vacuole in freshwater amoebas is often of value. Crystals and granules appear to be characteristic enough of some freshwater amoebas to be of some taxonomic use. Vacuoles containing food, crystals, food reserves, gas, or foreign bodies are also sometimes characteristic of certain amoebas.

Nucleus

The size of the nucleus, its shape, color, surface contours, endosomal granules, and volumetric (Warren, 1949) or surface area ratio (Penard, 1902) to cytoplasmic volume have been used by taxonomists as diagnostic. Some, mostly parasitologists, consider the nucleus the principal, usable taxonomic indicator.

Chaidae often have a firm, clear, spherical or discoid nucleus of relatively large size, difficult to see and having endosomal granules evenly distributed against the nuclear membrane. *Mayorellidae* have a firm to plastic, spherical or ovate nucleus, with a distinct central endosome, or cluster of endosomes. *Hyalodiscidae* have a nucleus similar to that of the *Mayorellidae,* but with a less optically distinct nuclear membrane. The nucleus of *Thecamoebidae* also resembles that of *Mayorellidae,* generally more plastic and having a slightly larger endosome in proportion.

Multinuclearity in amoebas seems associated with large size and volume of cytoplasm (Penard, 1902). Giant amoebas most often are the possessors of many nuclei. Smaller amoebas are almost always uninucleate. It is doubtful that multinuclearity can be used for other than specific identification. However, most known, multinucleate amoebas show other features that place them in the *Chaidae.*

Position and movements of the nucleus within circulating sol currents may be clues also to the identity. The nucleus is most often located in the middle of the body mass; a little behind the center, usually, in *Chaidae,* and forward, slightly, in most *Mayorellidae, Hyalodiscidae* and *Thecamobidae.*

Contractile Vacuoles

These water-bailing devices are present only in freshwater forms. Sea water varieties are not obliged to handle osmotic differences requiring them. A few species which can live in both fresh and sea water, such as *Flabellula mira* Schaeffer, possess the vacuole in fresh water only.

The size, numbers, rate of activity, circulatory movements in the endoplasm, manner of formation, and locus and manner of discharge are of value in specific identification.

Gas Vacuoles

Mayorellidae have these structures when afloat. Gas vacuoles are usually larger than the contractile vacuole, several in number, and disappear when the amoeba descends to the substrate. Usually centrally located, clustered about the nucleus, they are not discharged through the membrane, but shrink and disappear (Bovee, 1950).

Some *Mayorellas* intermittently have numerous smaller vacuoles which give the internal plasmasol a frothy appearance. They may appear and disappear in the same organism in a few minutes. Their cause and purpose are unknown, but they so commonly appear in one species, (*Mayorella spumosa*) that they are responsible for its specific name.

Inclusion Vacuoles

In some amoebas, and in particular many *Mayorellidae,* inclusions such as crystals, food reserves, and granules are often individually enclosed in vacuoles. In some cases they assist in species identification.

Pseudovacuoles

In amoebas which employ pseudopods to support the body mass, usually during "ambulatory locomotion" while the body progresses over them, the bases of pseudopods appear as indistinct "vacuoles" seen through the body mass. They are most often apparent in the *Mayorellidae.*

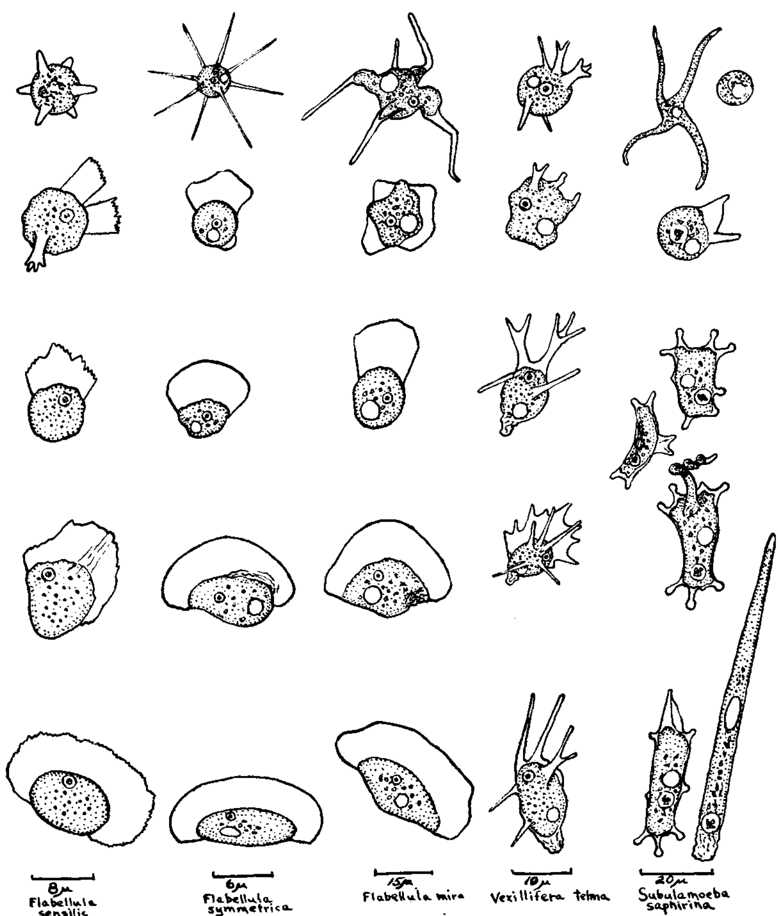
Crystals

Larger freshwater *Mayorellidae* and *Chaidae* may have crystals of definite shape, often abundant. The shapes of them may assist in specific identification. Attempts to classify them according to geologic systems place them mostly in the orthorhombic, tetragonal or hexagonal systems (Schaeffer, 1926).

The origins of the crystals are obscure. Schaeffer (1926) believes them to be stored excretions. Mast and Doyle (1935b) believe them to be crystals of magnesium salts in some amoebas, and amino-acid crystals in others.

Granules

Both freshwater and seawater amoebas have very numerous small granules in the endoplasm in addition to the identifiable crystals. Those of freshwater amoebas are usually the larger and more refractile, and sometimes lend a distinctive color to the endoplasm. What they are structurally or chemically is not known, except that some appear to be mitochondria or fat globules (Mast and Doyle, 1935 a & b).



MAYORELLIDAE

PLATE III

Food Reserves

In some larger amoebas, particularly *Chaidae,* highly refractile spheres are present. These are apparently glycogen reserves (Hayes, 1924; Leiner, 1924) or lipoprotein (Mast and Doyle, 1935a). Since they are present only in well-fed organisms, they are of only accessory significance.

Food Habits

Larger *Chaidae* which form no uroid are principally carnivores, and other protozoa may often be seen in their food vacuoles. Large *Mayorellidae* feed principally on algal filaments, diatoms and desmids. Smaller *Mayorellidae* feed on bacteria. *Hyalodiscidae* and *Thecamoebidae* feed on bacteria as well, for the most part.

Food preferences, if known, may aid identification; so may the manner of food capture. Large *Chaidae* secure food by extending a pseudopod around the organism. Large *Mayorellidae* form a food cup of several determinate pseudopods, grasping food like a hand. Bacteria feeders usually move over the food and pull it up into a food vacuole formed ventrally behind the anterior, clear wave.

Symbionts and Parasites

These are of doubtful value. Some large *Chaidae* which form eruptive waves often contain many bacilli, which may indicate their identity when they are not moving.

A few large *Mayorellas* harbor symbiotic algae (Doflein, 1907), but Schaeffer (1918) claims to have seen the same amoeba more frequently without symbionts. Hence, they are of doubtful taxonomic value.

Externally attached objects are usually of no taxonomic value. "Cils" cited by Leidy (1879) on Dinamoebid Mayorellidae are perhaps parasitic, since the same species is frequently seen without them. The same can be said of the fungal filaments of his Ouramoebid Chaidae.

Division Stages

Few amoebas have been investigated in division (Doflein, 1907; Chalkley and Daniel, 1933). Where such are known, the division stages may assist identification. Chatton (1910), for soil amoebae, found such variation in division patterns that he believed them of little taxonomic value.

Encysted Stages

Encysted stages are known for few free-living amoebas. In parasitic forms cyst characters have long been used as a principal means of identification. It is assumed by most zoologists that free-living amoebas do encyst. When such stages are found, they may prove of use in taxonomy.

Pure Lines

Pedigreed cultures may serve as a means to determine relationships and subspecies. Warren (1949), Doflein (1907), Dobell and Jepps (1918) and Kudo (1950) have employed pure lines in their taxonomic studies.

Physiological Relationships

Investigations in enzymology lend some support to morphological relationships within the *Chaidae* (Holter and Doyle 1938; Holter and Kopac, 1937; Andersen and Holter, 1949). These are too laborious for the usual investigator, and provide only additional evidence for classification.

Changes of Direction of Progress

Each species of free-living, naked amoeba appears to have its own peculiar method of changing direction. *Chaidae* generally accomplish it by extending a pseudopod, flowing through it, and retracting others. Locus and manner of formation is often of significance. Most *Mayorellidae* which form pairs of conical pseudopods anteriorly usually stop, flatten, extend a new pair of pseudopods laterally and pass between them. *Mayorellidae* which form fan-shaped anterior waves usually gelate and constrict a portion of the wave, anchoring it and using it as a pivot point about which to turn, each species having a characteristic manner of accomplishing the process. *Hyalodiscidae* employ a similar procedure. *Thecamoebidae* usually constrict unilaterally and progress "around the curve" thus formed. (Bovee, 1950).

Descent to the Substrate from Afloat.

Many amoebas have each a characteristic way of descending from afloat. Radiate forms usually make contact with a pseudopod, anchor it, and settle, flowing through the pseudopod or absorbing it, particularly the larger *Chaidae* and *Mayorellidae.* Many others settle, flatten, and begin extending their characteristic pseudopods. (Bovee, 1950).

Defensive patterns

When physically disturbed, amoebas tend to adopt a defensive pattern, contracting on the substrate or going afloat. Many *Mayorellidae* contract, keeping general contours of their locomotive pattern, form internal gas vacuoles, and float, descending later. *Hyalo-*

discidae and *Thecamoebidae* contract into spherical or wrinkled forms on the substrate. *Mayorellidae* which form fan-shaped waves may go afloat in radiate form when disturbed. *Chaidae* tend to contract into spherical or wrinkled masses on the substrate.

Keys

There is no generally accepted key to the free-living, naked amoebas. The need for such has been suggested at intervals. Hertwig & Lesser (1874) and Penard (1902) wrote of the need for it, but did not attempt the task. Some have erected such systems, but no system is generally accepted (Frenzel, 1897; Maggi, 1876;

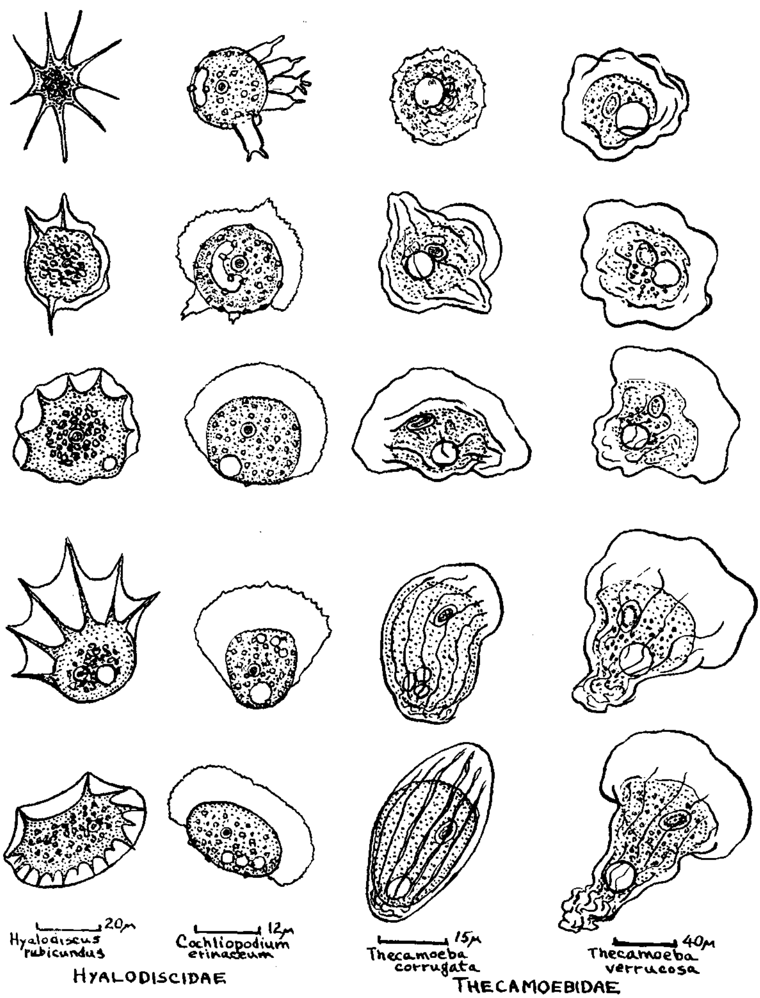


PLATE IV

Calkins, 1913, 1919; Doflein, 1916, 1928; Poche, 1913; Cockerell, 1920; Kudo, 1947, 1952; Jahn and Jahn, 1949).

The need is apparent, and the task should sometime be done. The author has begun some preliminary work on such a key, but finds such a mass of material to consider that it will be some considerable time before a publishable key will be developed. Schaeffer's classification is used herein, although it is admittedly very generalized and in need of reinvestigation and revision.

Reference for generic and specific identifications should be made to the works of Cash (1905, 1915), Leidy (1879), Penard (1902), Schaeffer (1926), Doflein (1928), Kudo (1947), and Jahn and Jahn (1949) and to articles by individual observers.

Summary

1. A need for more accurate methods of identification, of free-living, naked Amoebida is expressed.

2. A variety of characteristics of these amoebas which have proved taxonomically useful are discussed. The major categories covered are, with reference to principal families:

a. *Size and shape* during regularly recurrent patterns within a daily activity cycle; stressing inactive stages, slow movement without locomotion, moderate locomotion, and rapid locomotion.
b. *Locomotor Organelles,* including indeterminate pseudopods, eruptive waves, determinate pseudopods, combined indeterminate and determinate pseudopods, clear anterior waves, waving pseudopods, numbers of pseudopods and waves.
c. *Uroidal Structures.*
d. *Permanent Posterior Gel Discs.*
e. *Ectoplasmic Markings.*
f. *Endoplasm.*
g. *Inclusions;* particularly the nucleus and numbers of nuclei, contractile vacuoles, gas vacuoles, inclusion vacuoles, pseudovacuoles, crystals, granules, and food reserves.
h. *Food Habits.*
i. *Symbionts and Parasites.*
j. *Division Stages.*
k. *Encysted Stages.*
l. *Pure Lines.*
m. *Physiological Relationships.*
n. *Changes of Direction of Progress.*
o. *Descent to the Substrate from Afloat.*
p. *Defensive Patterns.*

3. The need for a revised key to the free-living, naked amoebas is presented, with a list of the authors who have attempted such revisions in the past.

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