

Two New Amoebae, *Striamoeba sparolata* n. sp. and *Flamella tiara* n. sp., from Fresh Water¹

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ABSTRACT. Two new amoebae are described from phase contrast observations made on specimens collected from Florida and Ohio, USA. Ohio specimens occurred among Aufwuchs on the midland painted turtle, *Chrysemys picta marginata*, and the common snapping turtle, *Chelydra serpentina serpentina*, while Florida specimens were found among the bottom detritus in muddy ponds. *Striamoeba sparolata* n. sp. (Thecamoebidae) is spearhead-shaped, 35-45 μm by 12.5-22.5 μm at the broadest point when in motion, with a clear bulbous anterior end and smooth uroid. There are usually two indistinct dorsal ridges, a spherical nucleus, 2-4 μm in diameter with a variable number of parietal nucleolar pieces and many endoplasmic light-refractile granules. No pseudopods are formed during locomotion. *Flamella tiara* n. sp. (Flabellulidae) exhibits continuous changes in shape while in rapid motion but is more or less broadly arcate in steady slow progress. This amoeba is 30-40 μm wide, 12-25 μm long, with many conical, papulate subpseudopodia extending from the body surface and leading edge. Adhesive, pseudo-uroidal filaments are trailed. An indistinct, spherical nucleus, about 3.5 μm , is present. The endoplasm is finely granular with no crystals. These two rarely encountered species occur as small populations and the present description will enable recognition by future researchers.

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INTRODUCTION

Since amoebae may often be found in very limited numbers or have a tendency to be highly localized in their distribution, the discovery of previously undescribed species of amoebae has been infrequent. Examples of valid taxonomic determinations in spite of their sporadic occurrence or small numbers are known in the literature. For example, *Chaos illinoisense* was discovered in a strip mine pond in Illinois, cultured, and described by Kudo (1950). Except for an unpublished record of Bovee from the Suwanee River in Florida, *C. illinoisense* has not, to our knowledge, been reported from field collections since. Similar circumstances led to the descriptions of *Dinamoeba horrida*, *Metachaos rarum*, and *Mayorella augusta*. Each was described from a single specimen collected once by Schaeffer (1926). The accuracy of his description of *M. augusta* was verified from specimens taken from two marshy sites in Florida and one in Virginia by Bovee (unpublished data).

The history of the two species described in this paper is similar. Over a period of more than 40 years, data were collected for a number of amoebae with isolated distributions or limited numbers of specimens by Bovee. Recently, Fishbeck sent data and photomicrographs of two different amoebae for identification to Bovee. They corresponded to two previously recorded sets of data in the notes of Bovee and led to the description of the two new species in this paper. Such seldom observed amoebae need to be described so that a record is available for future investigators who may occasionally collect them.

MATERIALS AND METHODS

Representatives of the first species of amoeba described here were collected from the trickling filter system of the sewage disposal plant at the University of Florida, Gainesville, on 27 January 1957 and were observed as single specimens on 2, 17 and 24 February 1957 (ECB). This species was again recently found in Aufwuchs growing on the carapace of nine individual specimens of the midland painted turtle, *Chrysemys picta marginata* Agassiz, collected in a privately owned lake near Ravenna, OH, on 24 May, 24 July, and 14 September 1990, and 16 May and 10 September 1991; amoebae have also been observed in collections taken from two common snapping turtles, *Chelydra serpentina serpentina* (Linnaeus), on 25 May and 14 September 1990 (DWF).

The second amoeba was discovered in a sample dredged from a mud bottom pond near the biological station of Florida State University at Alligator Harbor on 18 April 1958, and two specimens were observed on 21 and 22 April 1958 (ECB). More recently it was collected from Aufwuchs growing on two different specimens of the midland painted turtle captured in the same privately owned lake near Ravenna, OH, on 24 July 1990 and 16 May 1991, and on a single snapping turtle taken on 14 September 1990 (DWF).

Attempts by Bovee to culture both species were unsuccessful in the 1950s. An attempt by Fishbeck to culture the first amoeba using a GSA medium recently (August 1992) likewise was unsuccessful.

Sealed wet-mounts were observed with phase contrast microscopes at magnifications of 100 to 600x. Measurements were made with calibrated ocular micrometers. Photomicrographs were made at a magnification of 400x by the first author with an Olympus 35mm camera (Model C-35AD-4).

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RESULTS

A diagnosis of the two amoebae follows.

Striamoeba sparolata n. sp.

Size: At rest or afloat, 12-18 μm diameter; during initial locomotion, 18-22 μm long x 12-20 μm wide; locomotive, 35-45 μm long x 12.5-22.5 μm at the greatest breadth. Shape: Spheroidal afloat or at rest; spearhead-shaped when active. Ectoplasm: Clear, thin around cell mass in inactive state; in the locomotive state forms a clear rounded anterior bulge continuous with thin clear lateral margins and bulbous smooth uroid (Figs. 1, 2).

Usually, two narrow, low-relief, ephemeral longitudinal ridges were present on the upper surface (Figs. 1c-g, 2). Occasionally, a third median, incomplete ridge makes a fleeting appearance. Endoplasm: Clear, as an ovate mass within the ectoplasm; contains the nucleus, water-expelling vesicles (1 or 2), food vesicles, and many miniscule, light-refractile granules. Pseudopods: None except for an occasional antero-lateral ectoplasmic bulge that is the incipient avenue of change in direction. Nucleus: Vesicular, single, round, more or less central in the body, 2-4 μm with a variable number of parietal nucleolar pieces present (Figs. 1c-g, 2). Water expelling vesicle: Formed in the endoplasm, usually somewhat forward of the nucleus; drifts to rear enlarging to 3.5-4.0 μm at expulsion, which occurs either lateral or ventral to the uroid with temporary enlargement of uroid as the vesicle bulges within it. Light-refractile granules: Many, about 0.5 μm , shape indistinct; also many barely resolvable granules. Cysts: Not observed. Reproduction: Not observed. Locomotion: Slow, about the length of the body, 30-40 $\mu\text{m}/\text{min}$ in steady progress. Feeding: Probable bacterivore, however feeding not observed. Habitat: Among the organic detritus in ponds or Aufwuchs growing on turtle carapaces.

Flamella tiara n. sp.

Size: Dimensions variable owing to very rapid changes of shape during active locomotion; during rapid locomotion, 21-42.5 μm wide, 12-25 μm long. Shape: General locomotive shape while moving is arcate, however peripheral contours change continuously, especially during rapid locomotion (Figs. 4, 5). Ectoplasm: Thin, clear over body surface, forms subpseudopodia and leading margin of body (Figs. 3g-j, 4, 5); posterior edge often adheres to slide and stretches briefly as ragged, trailing pseudo-uroidal filaments during locomotion (Figs. 3g,h, 4, 5). Endoplasm: Clear, faintly granular, some light-refractile granules (Figs. 4, 5). Pseudopods: Many short, clear, papulate, rounded subpseudopodia; 3-5 μm long on upper surface; those along the leading edge 3-6 μm long, formed singly or as pairs, occasionally fusing into the clear, narrow leading border during locomotion (Figs. 4, 5). Nucleus: Single pale with indistinct outline, about 3.5 μm in diameter, in a thin clear perinuclear vesicle, with no distinct nucleolus; usually near the center of body mass. Water expelling vesicle: Usually single, slowly formed, about 5 μm in diameter at expulsion in those from Aufwuchs. Crystals: None large enough to determine shape, if present. Other inclusions: Some food vesicles containing bacteria in Aufwuchs specimens. Cysts: Not

observed. Reproduction: Not observed. Locomotion: Variable, about 40 to 50 $\mu\text{m}/\text{min}$, rarely to 70 $\mu\text{m}/\text{min}$, owing to rapid changes of shape. Feeding: Probable bacterivore, however feeding not observed. Habitat: Among detritus on bottom of muddy ponds or associated with Aufwuchs growing on turtle carapaces.

DISCUSSION

Schaeffer redefined the genus *Thecamoeba* Fromentel as "amoebas with a pellicle," having: a) the upper surface thrown into ridges and folds during locomotion, b) the anterior end consisting of clear protoplasm, and c) the posterior half to two-thirds of the body consisting of granular protoplasm.

The larger species in the genus with a more rugose upper surface and broader anterior, i.e., the "verrucosid" species, were separated from the smaller ovate to elliptical species with more-or-less parallel dorsal ridges, and the genus *Striamoeba* was proposed for these smaller forms (Jahn et al. 1974). Further refinement of Schaeffer's group was accomplished by establishment of the genus *Dermamoeba* for amoebae with no or a few, short, dorsal ridges extending somewhat forward from a morulate uroid (Page and Blakey 1979).

The body length of *Striamoeba sparolata*, being well under 100 μm , fits into the size range of the genus *Striamoeba* as defined (Jahn et al. 1974, Bovee 1985). This amoeba also exhibits lateral, low-relief ridges that extend forward about two-thirds to three-fourths of the length of the body, similar in extent to those associated with the genus *Striamoeba* (Jahn et al. 1974). In addition, a third, transient and incomplete mid-body ridge appears very occasionally in *S. sparolata*. The smooth, bulbous uroid contrasts with the characteristic morulate uroid of *Dermamoeba*. A suite of characters including small size, ovate shape, low-relief ridges, and prominent smooth uroid favors placing this amoeba in the genus *Striamoeba*.

Two amoebae bearing the trivial name, "lanceolata," *Amoeba lanceolata* Pateff and *Thecamoeba lanceolata*, described by Lepsi in 1957 and considered by him to be conspecific with Pateff's *A. lanceolata*, superficially resemble *Striamoeba sparolata* (Lepsi 1957, 1960; Pateff 1924). If we assume that the amoebae described by Pateff and Lepsi are identical, except for the presence of low-relief longitudinal ridges and a vesicular nucleus, *S. sparolata* exhibits several other features that are characteristically very different (Table 1). *T. lanceolata* is both longer and wider than *S. sparolata* and is a much faster amoeba (3-5 times as fast). A bulbous uroid, characteristic of *S. sparolata*, is missing in *T. lanceolata*, and Pateff states that *A. lanceolata* always has a pleated posterior end and illustrates it as such (p. 10). Contrary to observations for *T. lanceolata* (Lepsi 1957), pseudopodia are never observed in *S. sparolata* and, at most, a transient bulge, indicating a change of direction, is seen. Lepsi (1957) reported that *Thecamoeba lanceolata* assumes a triangular shape, however, this has never been observed in *Striamoeba sparolata*. Nuclear visibility in *T. lanceolata* is apparently poor (Lepsi 1957), but in *S. sparolata* the nucleus is very conspicuous (Fig. 2). The anterior end is also described as being narrower than the posterior end

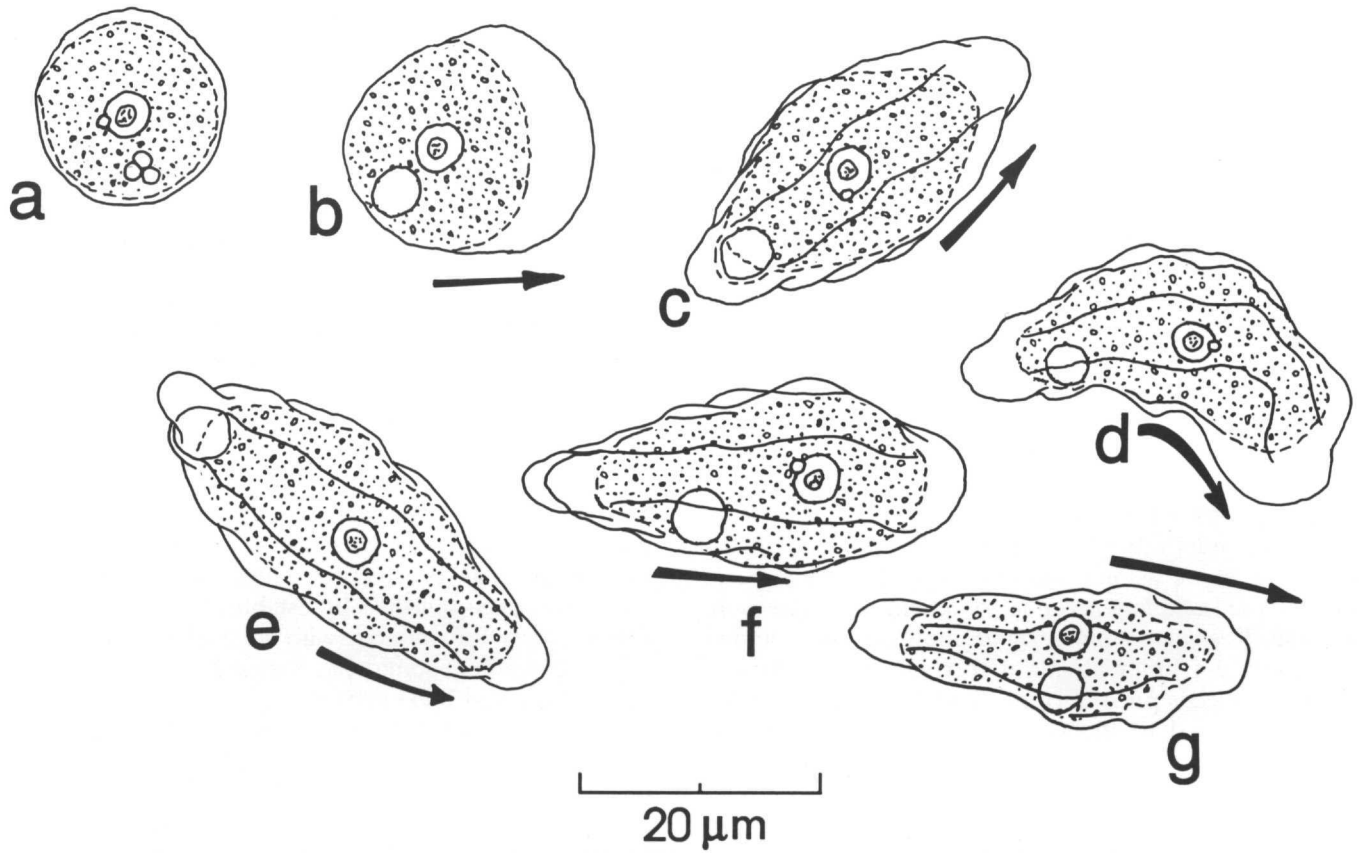


FIGURE 1. *Striomoeba sparolata*. a: Inactive form. b: Initiation of locomotion. c, e, f, g: Locomotory forms illustrating the relative position of the water expelling vesicle during active movement. d: Body shape during a directional change to the right.



FIGURE 2. *Striomoeba sparolata*. Locomotory form. Note longitudinal fold, nucleus with parietal nucleolar pieces, bulbous uroid and clear anterior and lateral ectoplasm.

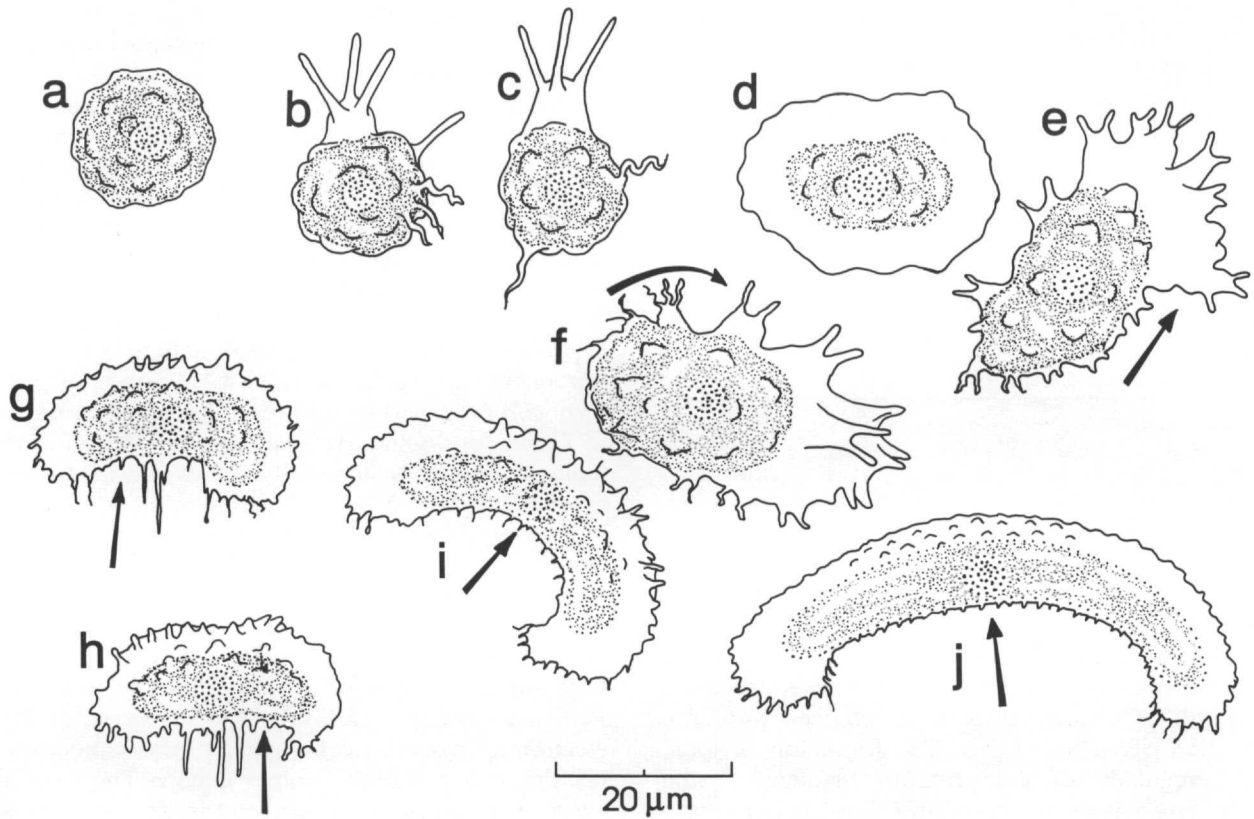
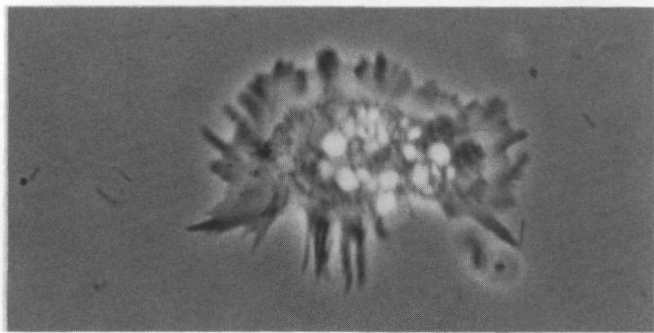
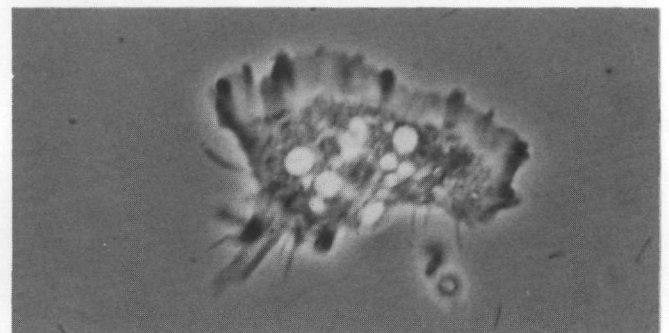


FIGURE 3. *Flamella tiara*. a: Inactive form. b-f: Initiation of locomotory stages. g, h: Body conformation during rapid locomotion. i, j: Arcate form assumed during slow locomotion.



20 μm

FIGURE 4. *Flamella tiara*. Body form during rapid locomotion. Note trailing filaments. Those at the rear corners become trailing filaments. Note numerous subpseudopodia.



20 μm

FIGURE 5. *Flamella tiara*. Body form of the same amoeba (Fig. 4) approximately 10 seconds later. Note shape change and subpseudopodia, some of which are paired.

in *T. lanceolata*, but in *S. sparolata* the anterior end is broader than the posterior (Figs. 1, 2).

The genus *Flamella* includes amoebae that are irregularly ovate with a thin sheet of clear protoplasm extending along the advancing edge with conical subpseudopodia projecting from the body mass and with some subpseudopodia fusing with the margin. The irregu-

larly shaped body mass is granular. In addition, rapid movement is characteristic of the genus (Schaeffer 1926). *Flamella tiara* exhibits this combination of characters.

Other traits of *Flamella tiara* distinguish it from the two related species described in the genus (Table 2). In contrast, *F. tiara* is considerably shorter than either while its width overlaps somewhat with the lower part of the

TABLE 1
Comparative characteristics of
Striamoeba sparolata and *Thecamoeba lanceolata*.

Characteristics	<i>Striamoeba sparolata</i>	<i>Thecamoeba lanceolata</i>
Length*	35-40	60
Width*	12.5-22.5	30
Pseudopodium Formation	none	2-3
Uroid	smooth, bulbous	none or pleated
Locomotory Speed**	30-40	120-150

* μm ** $\mu\text{m}/\text{min}$

ranges of both species. This is deceptive, however, because the width of active forms varies with speed in *Flamella tiara* and *F. citrensis*. During both slow and rapid locomotion the body width of *F. citrensis* exceeds that of *F. tiara* by 10-20 μm . Either the speed does not affect body width in *F. magnifica* or differences were not observed by Schaeffer (1926). The formation of long, ragged, irregularly shaped posterior filaments during rapid movement is very common in *F. tiara*, in contrast to the appearance of small numbers of fine short filaments in *F. citrensis* and none in *F. magnifica*. Habitats of the three are varied. The formation of a water expelling vesicle obviously depends upon occurrence in fresh water.

Although *F. tiara* gives the impression of moving rapidly, when compared to *F. citrensis*, its progress is sloth-like. Even the slowest speed described for *F. magnifica* is only 10 μm below the occasional 70 $\mu\text{m}/\text{min}$ surges observed in *F. tiara* (Table 2).

Schaeffer could not distinguish a nucleus in living amoebae by direct light microscopy and only as fragments of "chromatin" (methylene blue stain) in fixed specimens.

The inability to clearly observe the nucleus in *F. magnifica* seems to be a characteristic of the genus *Flamella* since that is true for *F. tiara* and was also reported for *F. citrensis* (Bovee 1956).

The two amoebae presently under discussion are apparently rarely encountered and occur in small numbers. *Striamoeba sparolata* has been observed by the authors in Florida and Ohio with an intervening time span of 33 years. Prior to this, it may have been included in Leidy's (1879) composite of "verrucosid" amoebae (Plate III) and mentioned by Penard (1902). A similar species may have been observed by Pateff (1924) and Lepsi (1957, 1960). The time involved here between these infrequent sightings spans at least 111 years (1879-1990). *Flamella tiara* was encountered by the authors, again in Florida and Ohio, 32 years apart and again in very limited numbers. Neither of these two species responded to standard culture methods, suggesting that their ecological requirements may be more stringent than most amoebae. The encounter of small populations of rare species warrants adequate and appropriate descriptions with temporary generic assignment to facilitate efforts of future workers.

If sufficient numbers of *Striamoeba sparolata* and *Flamella tiara* become available to observe each with electron microscopic techniques, it may be necessary to reassign either or both to other genera. The most likely alternative genus for *Striamoeba* is *Thecamoeba* since they appear to be closely related genera in the same family with a number of shared characteristics, e.g., a thick pellicle, dorsal ridges, ovate shape (Bovee 1985, Page and Siemensma 1991). A key characteristic used by Bovee (1985) to separate the two genera is size (*Striamoeba* <100 μm , *Thecamoeba* >100 μm). Page (1988) considers the separation on the basis of size to be inadequate and prefers *Thecamoeba*.

On the other hand, *F. tiara* exhibits characteristics that define the genus *Flamella*, e.g., a body shape consisting of an irregular oblong granular mass that constantly changes with a thin sheet of hyaloplasm projecting

TABLE 2

Contrasting traits among three species of the genus *Flamella*.

Traits	<i>F. tiara</i>	<i>F. magnifica</i>	<i>F. citrensis</i>
Length*	12-25	30-60	30-50
Width*	21-42.5	30-60	35-55
Water Expelling Vesicle	single	none	single
Trailing Filaments	many, long, ragged	none described	few, short, fine
Locomotory Speed**			
Slow	40-50	60-180	60-120
Fast	70		180-240
Habitat	fresh water pond detritus, turtle Aufwuchs	marine	citrus waste water

* μm ** $\mu\text{m}/\text{min}$

anteriorly, numerous slim, irregular subpseudopodia that may fuse with the anterior hyaloplasm, and rapid locomotion. The nucleus in *F. tiara* is very difficult to detect clearly in the granular endoplasm as was also true of *F. magnifica* (Schaeffer 1926) and *F. citrensis* (Bovee 1956). An alternate genus for *F. tiara* might be *Paraflabellula*. Shared characteristics include rapid changes in body shape and a similarity in formation of ragged, adherent, trailing filaments. Although there is an almost continuous variety of shapes in *F. tiara*, a consistent body form (Figs. 3-5), is almost always maintained by this amoeba. The triangular body shape, apparently so characteristic of *Paraflabellula reniformis* (Schmoller 1964) Page and Willumsen 1983 and *P. kudoii* (Singh and Hanumaiah 1979) Page and Willumsen 1983 is almost never seen. As *F. tiara* slows down, it is likely to assume the tiara-like form (Fig. 3i,j).

Recognition and acceptance of some of the newly proposed taxa in Schaeffer's 1926 monograph were slow. More recent research has broadened the scope of his taxa, generally leading to current acceptance of the validity of many of the families, genera, and species described in his classic work (Jahn et al. 1974, Bovee and Sawyer 1979, Bovee 1985, Page 1988, Page and Siemensma 1991).

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