

## Recent Observations on Neck Extensions in Folliculinids (Protozoa)<sup>1</sup>

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DESPITE species variations, the process of folliculinid lorica formation is fundamentally similar (Penard, 1919; Andrews, 1923; Fauré-Fremiet, 1932; Dewey, 1939; and Das, 1947). In all a motile, nonfeeding stage becomes attached, secretes a sac and neck, and gradually metamorphoses into a sessile feeding stage characterized by peristomal lobes.

Although in certain well-established colonies restrictive attachment areas may modify sac length, breadth, and height, modifications in neck length and number of spiral whorls seem not to be thus affected (Matthews, 1963). Despite the fact that certain folliculinids with poorly developed necks (*Ascobius simplex* and *Folliculina boltoni*) seem not adversely affected, nevertheless it is commonly assumed that well-developed necks and neck extensions afford some survival value; i.e., the entrance of predators and detritus is lessened. Although this is an engaging conjecture, actually long necks and neck extensions afford little advantage. Rather, such folliculinids, responding to current disturbance, contract their peristomal lobes, whereas short-neck forms, not so affected, continue to feed. Since our knowledge of folliculinids is too meager to warrant conclusions as to why extensions are made, our attention for the present might best be focused on the stage (or stages) of the life-cycle responsible for their formation. The purpose of this paper is to place in question the limited alternatives of existing theories, and to rekindle interest in a question unsolved since 1923.

As previously stated, on completion of a lorica a nonfeeding swimmer usually metamorphoses into a feeding sessile organism characterized by peristomal lobes. It is generally

assumed that, in nature, this organism responsible for the lorica remains for some time its occupant. However, once the terminal lip is completed, a disturbed organism may sever its body-attachment point and, without developing peristomal lobes or actually living in its lorica, may vacate it and subsequently begin the process anew.

Usually, however, on completion of the terminal lip the organism withdraws into its sac and, following a rest period, metamorphoses into a sessile feeding stage. Under laboratory conditions, this stage may last from one to several days. This period is followed by one of two possible courses: either metamorphosis results in a motile stage which vacates the original lorica; or, following binary fission, a distal portion metamorphoses into a motile stage whereas a proximal portion metamorphoses into a sessile stage which, for some time, occupies the original lorica.

Thus, subsequent neck extensions might be the result of (1) the stage that secreted the original lorica, (2) the stage remaining in the original lorica following binary fission, (3) the stage leaving the original lorica following binary fission, or (4) a "new" swimmer (or swimmers) entering another lorica. Although most investigators agree on the general process of lorica formation, few agree on the stage of the life cycle responsible for neck extensions. And, despite the above possibilities, the formation of neck extensions is today explained in the light of limited alternatives: either they are the result of the sessile stage which secreted the original lorica, or they are the result of a "new" swimmer which enters an empty lorica.

In a personal communication E. A. Andrews (1952) states:

No one has seen extensions actually in the process of making, but Hadzi [1951] gives some pages of argument that they are made

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by swimmers locating in empty tests, while I maintain it is less improbable to imagine the adult can attempt a secondary imperfect neck or even a third while dwelling in the old test.

Following Andrew's suggestion, glass plates to which were attached *Metafolliculina andrewsi*, *M. nordgardi*, *Parafolliculina violaceae*, and *Lagotia viridis* were brought into the laboratory and the following neck-extension possibilities were studied:

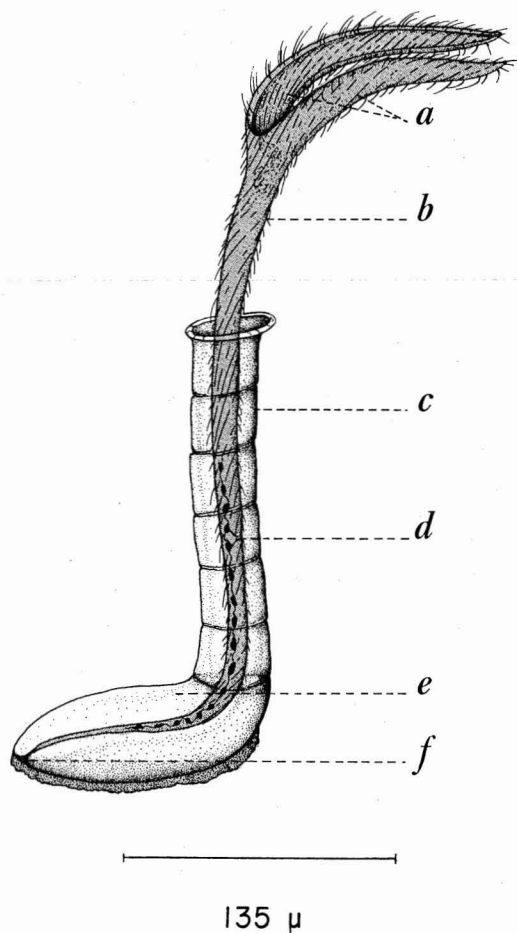


FIG. 1. *Metafolliculina andrewsi* as viewed from the right side showing: *a*, peristomal lobes; *b*, extended body; *c*, neck without extensions; *d*, nucleus; *e*, sac; and *f*, body attachment point.

#### I. Neck Extensions by the Stage that secreted the Lorica.

Although living *M. andrewsi* and *M. nordgardi* (Matthews, 1963) with and without extensions were present, these were ignored for the moment because, even if subsequent neck extensions were formed, the possibility remained that these could be the product of some stage other than that which secreted the lorica; for example, a new swimmer (or swimmers) that had entered an empty lorica. To exclude this possibility the aquarium in which these plates were held was completely covered with black paper except for one small area in which unetched glass slides were placed. By this method the entire lorica-forming process of *M. andrewsi* was observed. Thus it was made certain that the folliculinid occupying a particular lorica was indeed its original builder. Metamorphosis of these original lorica builders into swimmers was frequently observed and, as each swimmer vacated its lorica, a small, but easily distinguishable body attachment point (Fig. 5*d*) was left in the proximal region of the empty sac. Likewise, binary fission and the subsequent metamorphoses into sessile and motile stages was observed. In such instances the original body attachment point appeared unaltered either as to size, shape, position, or number.

In approximately 25 *M. andrewsi*, only one instance of a neck extension was observed. This particular folliculinid was brought to my attention by my inability to bring into sharp focus the region just distal to the lip. When first observed at 8:25 AM the organism, with a single point attachment, lay contracted in the proximal end of its sac. Slowly it relaxed and extended its peristomal lobes above the cloud-like, viscous mass which surrounded the lip, only to contract again into the sac. The relaxation of the body and the freeing of the peristomal lobes above the distal opening of the neck had been observed frequently in other specimens of *M. andrewsi*. In such instances, as the body relaxed the spirally twisted peristomal lobes were carried aloft where their pectinellae burst into a "running flame" of activity resembling the spiral ignition of a gas stove burner. In the present specimen such was not the case. As the body relaxed (Fig. 2*g*) and the peristomal lobes (*a*,

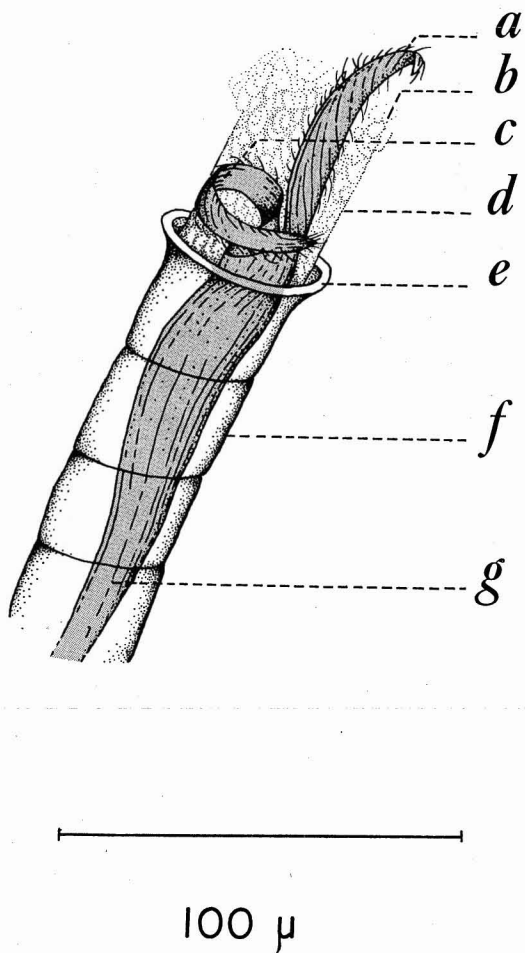


FIG. 2. *Metafolliculina andrewsi* showing: *a*, slight, distal fold of right peristomal lobe; *b*, indistinct, distal region of neck extension; *c*, left peristomal lobe curved in a semicircle at right angles to main, longitudinal axis of neck; *d*, distinct, proximal region of neck extension; *e*, lip of neck; *f*, neck; and *g*, portion of body.

*c*) were carried aloft, sometimes the right lobe (*a*) but as frequently the left (*c*) formed a semicircle at right angles to the longitudinal axis of the neck (*f*) and, in this position, was finally carried above the lip (*e*). The other lobe, which was slightly folded near its distal end (*a*), appeared as if to tap or "feel" an indiscernible neck boundary. Not once, even when both peristomal lobes were free, was any activity of the pectinellae detected. Relaxation and con-

traction of the body continued without interruption throughout the day. Because a slight body secretion followed each contraction, it appeared that the peristomal lobes, functioning like a plasterer's trowel, merely carried this material aloft and spread it rather than produced it themselves. Gradually, the proximal portion of the extension (*d*) darkened somewhat and became clearly discernible, but the distal portion (*b*) became only vaguely so. At 4:00 PM the extension, still indistinct distally, measured approximately 66 $\mu$ . The organism now lay in the proximal portion of the sac. After perhaps 2 hours of "inactivity," this sessile stage metamorphosed into a motile swimmer which, following three or four unsuccessful attempts, finally swam free of the original lorica leaving, as usual, the distinct green area in the proximal region of the sac which marked the old body attachment point. Unlike other observed swimmers, this one "crawled" slowly along the surface of the submerged glass plate. Its vermiform body, only slightly attenuated posteriorly, measured 415 $\mu$  long but only 33 $\mu$  wide. The following morning this swimmer was found dead not far from the lorica whose neck had been extended. As far as was discernible, it had made no attempt to secrete a new lorica. The extension (Fig. 3) had darkened throughout its entire length but unfortunately was frayed distally (*a*) and devoid of lip (*a*) and spiral whorls (*b*).

Were this the only case in point, one might accept for all neck extensions Andrews' (1923: 242) statement:

While the original [lorica] is made by an animal without lobes which then transforms into the lobed form, it seems probable that the extensions are added by the animal when with quite different anatomy at the anterior end. . . . If true that the perfect form can secrete spiral tube and terminal lip without the usual special neck and mushroom shape it would seem to follow that it is not so much one specialized part of the body that makes the form of the dwelling as it is temporary contractions and secretions that may be active in very different parts of the body, since the area of secretion that must have been active in the secondary tube and

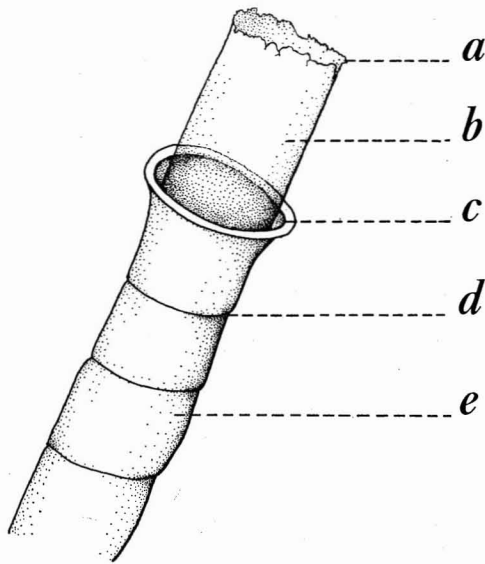


FIG. 3. *Metafolliculina andrewsi* showing: *a*, frayed, distal end of neck extension; *b*, neck extension without spiral whorls; *c*, lip of neck; *d*, spiral whorl; and *e*, neck.

lip-making is very far removed from the area originally active in the making of the first tube [neck], . . . [since] the arms are made from the region very far from the anterior end of the larval swimmer.

While my single observation proves that a neck extension, even though imperfect, can be the product of the sessile stage it does not exclude other extension possibilities.

## II. Neck Extensions by the Stage Remaining in the Lorica following Binary Fission.

As previously stated, the proximal portion following binary fission metamorphoses into a sessile stage, characterized by peristomal lobes.

This stage persists under laboratory conditions from one to several days. This metamorphoses back into a swimmer but there is every reason to assume that, after a period of rest, it might undergo binary fission or indeed produce, as previously described, an imperfect neck extension. However, this is mere conjecture.

## III. Neck Extension by the Stage Leaving the Lorica following Binary Fission.

Although this stage might possibly produce a neck extension, it was never observed to make one. Swimmers were often observed which seemed to experience considerable difficulty in leaving the lorica, but finally all were able to do so.

## IV. Neck Extensions by a New Swimmer (or Swimmers) entering Another Lorica.

*Lagotia viridis* (Fig. 4), with well-developed sac (*g*) and neck (*e*), was observed trapped in its lorica by a swimmer whose attachment point (*b*) was located either on or just below the well-developed lip (*c*). While at first sight this stage of neck extension might seem to fall under III (the Stage Leaving the Lorica following Binary Fission) such was not the case. As far as is known, *L. viridis* does not make neck extensions. Although it undergoes binary fission, the distal portion metamorphoses into a swimmer which leaves the old lorica to start the process anew. Moreover, when first observed the anterior or pectinellae end of the swimmer pointed down into the neck and only later contracted and formed a living plug which completely closed the neck (*e*). During this time the entrapped folliculinid lay motionless at the proximal end of the sac. As in *M. andrewsi*, a viscous secretion appeared just above the lip (*c*). There was no apparent movement either of the swimmer or of its fused cilia. Whereas the folliculinid in the sac was a light bottle green, the swimmer attached near the lip was a deep violet. During the next 2 hours this became lighter until finally it approximated the green of the folliculinid in the sac. Slowly there emerged what at first was mistaken for a proboscis. This came not from the very top of the old lorica but rather more from the side and extended (as illustrated) to the top of the old sac. Not once, however,

were observed the counter clockwise movements so characteristic of normal neck formation. This was understandable because, although it resembled a próboscis, it already possessed peristomal lobes (*d*).

Believing the process completed, I made a small aperture (*b*) in the lorica (*g*) in order to determine if the entrapped folliculinid would attempt to free itself. Rather more quickly than expected, it metamorphosed into a small but otherwise normal folliculinid which almost immediately gained access to the outside by means of this aperture (*b*). There was no "feeling around" inside the sac for the location of this opening: rather, the animal went directly to and through it.

These two folliculinids shared the same lorica from March 22 to March 24. During this time the folliculinid attached to the sac continued to use the aperture made for it. It would relax and extend its body and peristomal lobes (*f*) high above the dorsal surface of the old sac but made no effort to secrete a new neck. The folliculinid attached near the old lip (*c*) was at no time

as active as the one in the old sac. Its peristomal lobes (*d*) were never held aloft and the beating of their pectinellae was never observed. The following morning (March 25) both folliculinids had vacated the old lorica. Whether or not they metamorphosed into swimmers was not observed. However, the swimmer that had attached itself near the old lip had secreted a strange type of neck extension (*a*). This consisted of a hemispherical cap whose opening was placed at right angles to the main or longitudinal axis of the original neck.

Although it is difficult to distinguish one species of swimmer from another, the fact that this swimmer was at first a deep violet and that *Parafolliculina violaceae* were present on the original plate suggests that in this instance the neck extension may possibly have been the result of another species!

Because under laboratory conditions organisms frequently respond abnormally, modified experiments were performed in the organism's natural habitat. Empty loricae of *M. andrewsi* without extensions were stained with acidulated borax-carminé, washed thoroughly, and the glass plates were returned to the anchorage lagoon at Coconut Island. At the same time, other empty unstained lorica without extensions were marked and these glass plates were returned to the organisms' natural habitat. Although both stained and unstained loricae were observed over a period of 2 months, neither possessed new occupants nor extensions. And although the discouraging results of these experiments throw some doubt on the possibility that neck extensions are formed by new swimmers entering old loricae, other naturally occurring examples point very strongly to this possibility.

In *M. andrewsi* the condition illustrated in Figure 5 is frequently encountered. Two body attachment points (*d, e*) are clearly discernible. Although Penard (1919:317) incorrectly assumes that longitudinal fission occurs in *Folliculina boltoni* (see his Fig. 17), he correctly points out that only a single body attachment point persists, that of the original occupant. If, as suggested by Andrews, neck extensions are the result of the original occupant of the lorica, then only a single attachment point should be present. It is absurd to assign two attachment

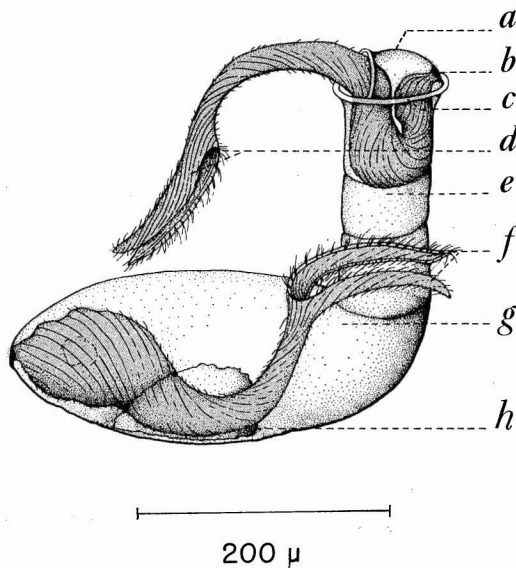


FIG. 4. *Lagotia viridis* as viewed from the right side showing: *a*, hemispherical, caplike neck extension; *b*, body attachment point; *c*, lip; *d*, peristomal lobes of new occupant; *e*, neck; *f*, peristomal lobes of original occupant; *g*, sac; and *h*, aperture cut in sac.

points (*d*, *e*) to a folliculinid now known to undergo only binary fission. It is equally absurd to assume that the present body attachment point (*e*) permits a better peristome exit. In light of possibility I (Neck Extensions by the Stage that Secreted the Lorica), two attachment points and two neck extensions might be explained as follows: the builder of the original lorica, once the neck (*c*) was completed, contracted into its sac but, after a period of rest, instead of metamorphosing into a swimmer, relaxed and, while in the lobed stage, secreted the first imperfect neck extension (*b*). It then withdrew and metamorphosed into a swimmer which vacated the lorica. The present body attachment point (*e*) is that of a new swimmer which entered, became attached, and, while in the motile stage, secreted the second extension (*a*). This may explain in part why spiral whorls are absent in the first extension (*b*) and why

they are present in the second (*a*). This might also account for the fact that the diameter of the second extension is approximately half that of the first. While these possibilities are not conclusive, other examples suggest that neck extensions may have multiple origins.

Frequently *M. Nordgardi* (Fig. 6A) is observed in which the body attachment point (*d*) is far removed from the base of the lorica (*e*). In such instances either the body has freed itself from its original attachment point (*e*) and become reattached (*d*), or another swimmer has entered the old lorica and established itself. Because many *M. nordgardi* with and without extensions (Fig. 6B-C) possess loricae whose lengths exceed that illustrated in Figure 6A and yet experience no difficulty in extending their peristomal lobes, it seems rather unlikely that reattachment in the shorter form was the result of necessity. Moreover, if *d* (Fig. 6A) was the original body attachment point there is no way, based on our present knowledge, to explain the formation of that portion of the lorica between *d* and *e*.

If one assumes that the lorica illustrated in Fig. 6A is not the product of its present occupant, how does one explain the formation of the extensions illustrated in Fig. 6C, since only one body attachment point (*e*) is present? If one rejects Andrews' theory that the present occupant (Fig. 6C) is responsible for the lorica (*d-e*) and its extensions (*c-d* and *b-c*) then one must extend Hadzi's theory to include the possibility that that portion of the lorica between *c-d* may have been secreted by a second swimmer, and that portion between *b-c* by a third. As improbable as this may at first appear, there is some evidence at *e* (Fig. 6C) to support this view. Although it is possible that none of the original attachment-point material remains (Fig. 6A-e), occasionally (Fig. 6C-e) material accumulates whose texture and staining affinity appear identical with those of the present body attachment material.

Surely, for those examples in which swimmers have entered old lorica and built extensions, Hadzi is correct in limiting the count of the spiral whorls to those of the original lorica and excluding the number of whorls added by new swimmers. However, in cases in which the

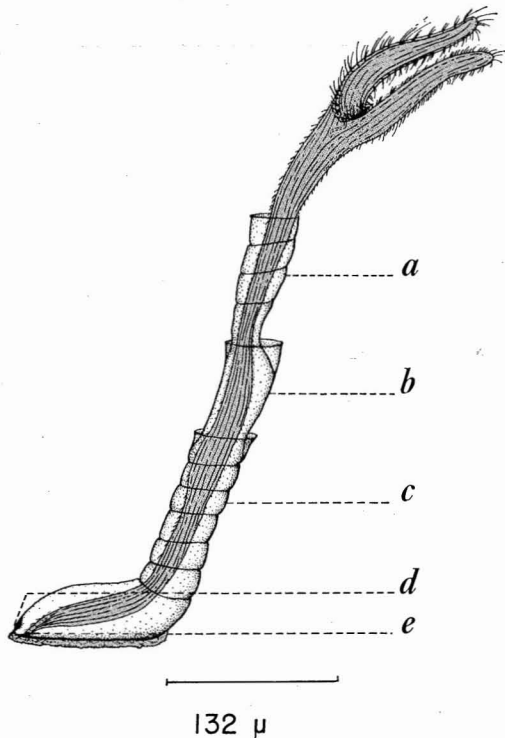


FIG. 5. *Metafolliculina andrewsi* as viewed from the right side showing: *a*, second neck extension; *b*, first neck extension; *c*, neck; *d*, original body attachment point; and *e*, present body attachment point.

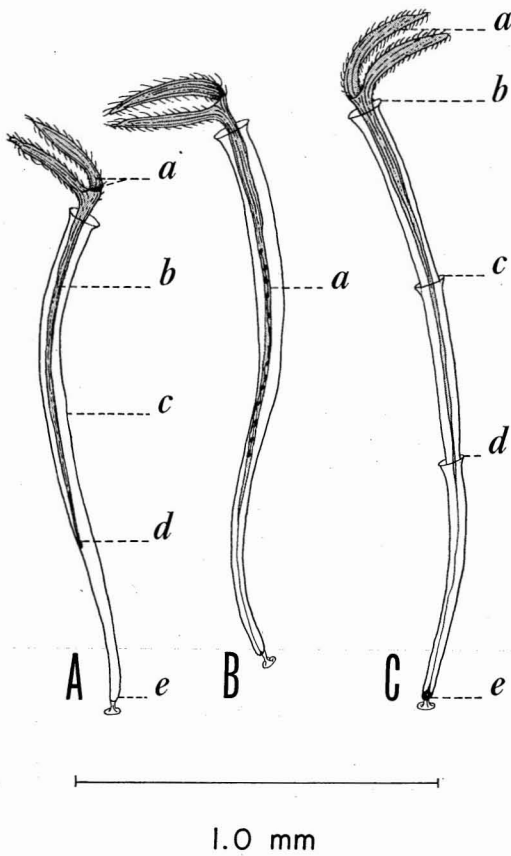


FIG. 6. *Metafolliculina nordgardii* showing: A. a, peristomal lobes; b, body; c, lorica; d, body attachment point; and e, base of lorica. B. a, nucleus. C. a, peristomal lobes; b-c, second extension; c-d, first extension; and e, present body attachment point.

builder of the original lorica also adds extensions this method of counting may or may not result in a correct whorl number.

Although these possibilities of accounting for neck extension place in question the limited alternatives of older theories, the process must be observed in many species before these possibilities can be accepted unequivocally.

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