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A SCANNING ELECTRON MICROSCOPIC STUDY OF THE MORPHOLOGY OF *SCYPHIDIA PHYSARUM* LACHMANN, 1856 (CILIOPHORA: PERITRICHIDA)

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Abstract

The morphology of *Scyphidia physarum* was investigated by scanning electron microscopy (SEM). Both contracted and uncontracted zooids were examined. The pellicular striations encircled the body at mean intervals of 1.1 µm. Pores were randomly distributed over the body surface with a mean density of 21 pores per 100 µm². The telotroch band was represented by three closely spaced striations. The adoral ciliature consisted of a single row of cilia in the haplokinety and three rows in the polykinety. The two sets of cilia were separated by a pellicular ridge. At the end of each row of cilia was a short cilium followed by a series of barren kinetosomes. There were two rows of parasomal sacs associated with the polykinety, one with the inner row of kinetosomes the other with the outer row.


Introduction

The potential importance of scanning electron microscopy (SEM) in the study of peritrich ciliates has been highlighted by several authors (Small and Ranganathan, 1970; Zagon, 1971; Finley et al. 1972; Carey and Warren, 1983). Peritrichs possess various surface structures of taxonomic importance such as pores and striations which have traditionally been studied by the examination of silver-stained specimens in the light microscope. The higher resolution offered by the SEM enables these and other structures to be studied in greater detail. Furthermore, due to the innate contractility of peritrichs, there have been few studies of uncontracted specimens. However, various rapid-fixation and relaxation techniques are now available enabling zooids to be examined by SEM in an uncontracted condition (Carey and Warren, 1983).

In this paper, the morphology of the stalkless sessile peritrich *Scyphidia physarum* is investigated. *Scyphidia physarum* lives as an epizoite on the head-foot and antennae of freshwater snails. It was first described by Lachmann (1856) and has been subsequently reported by several authors. The literature for *S. physarum* was reviewed by Foissner and Schiffmann (1979) who also provided a biometric analysis based on observations of silver-stained specimens.

*Scyphidia physarum* has not previously been examined by SEM, although other closely related species have (Small and Ranganathan, 1970; Fish and Goodwin, 1976; Pickering et al. 1985). In the present study, the taxonomically important surface structures of *S. physarum* are examined and some peristomial features are described for the first time. The observations made by SEM are discussed in light of previous ultrastructural and silver-stain studies.

Materials and Methods

*Scyphidia physarum* was found on the freshwater snails *Physa fontinalis* and *Lymnaea peregra* (Mollusca: Gastropoda) collected from the River Crane, Middlesex, UK (national grid reference TQ732128). Mollusc tissue bearing *S. physarum* zooids was removed and fixed in Párducz's solution (Párducz, 1967), comprising a mixture of six parts 2% OsO₄ and one part saturated HgCl₂, at
10°C for 30 minutes. Specimens were dehydrated to 100% ethanol either via a graded series of alcohols with 15 minutes in each solution, or using the continuous dehydra-
tion apparatus described by Roberts and Warren (1987). Dehydrated specimens were transferred via four steps to 100% acetone and then critical point dried from CO₂.
Dried specimens were mounted on a stub, coated with gold/palladium for 3 x 30 seconds and viewed in an Hitachi 800 scanning electron microscope operated at 8 kV.

Results

General morphology

*Scyphidia physarum* was found in large numbers as an epizoite on the head-foot and antennae of the freshwater snails *Physa fontinalis* and *Lymnaea peregra*. Each individual or zooid was attached to its host via a special organelle at the posterior end of the cell called the scopula. The zooid was roughly cylindrical in shape, 60 - 80 µm long x 20 - 35 µm wide (Figure 1a). There was a slight constriction just above the scopular region marking the site of the telotroch band (also called the trochal band). The diameter of the peristomial lip was slightly greater than the maximum body width. There was a single contractile vacuole situated in the peristomial region. The macronucleus was large, ovoid and lay in the anterior part of the zooid. Food vacuoles were large, spherical and often numerous.

Under light microscopy, the pellicular striations were conspicuous with concave ribbing between the striations.

SEM observations

Contracted, partially contracted and uncontracted zooids were observed among the specimens prepared for SEM (Figure 2). In contracted and partially contracted zooids the cilia were withdrawn into the peristome and enclosed by the peristomial lip. In the uncontracted zooids, the cilia were fully extended and wound counterclockwise around the peristome towards the buccal cavity. In all cases, the striations, pores and telotroch band were clearly visible on the zooid surface.

Striations (Figures 1b, 3 and 4). The pellicular striations appeared as transverse ridges encircling the body. The majority of striations were equally spaced and ran parallel to one another. The mean distance between neighbouring striations was 1.1 µm. Occasional abnormalities were observed with striations bifurcating or terminating (Figure 1b). The mean number of striations per zooid was about 55 although this was often difficult to determine due to the masking of some striations, particularly those at either end of the zooid.

Pores (Figures 3, 5 and 6). Pellicular pores were observed over the entire zooid surface with the exception of the peristomial disc and telotroch band (where they were absent) and the scopula (which was not examined). The pores were circular in shape and approximately 0.1 µm in diameter. The distribution of pores appeared to be random with some laying alongside the striations, either on the posterior or anterior side, while others lay in the space between the striations (Figure 3). On the peristomial lip, the majority of the pores were situated along the anterior side of the striations (Figures 5 and 6). There were between 19 and 23 (mean 21) pores per 100 µm² on the body surface.

Telotroch band (Figure 4). The telotroch band, the site of the aboral ciliary wreath in the motile telotroch, was situated near the posterior end of the zooid just above the scopular region. The telotroch band was represented by three closely spaced striations. The distance between the striations was 0.20 - 0.25 µm. Pellicular pores were absent from the telotroch band.

Peristome (Figures 7 - 10). The peristome was situated at the apical end of the cell and comprised a disc bounded by a peristomial lip and rows of oral cilia which wound counterclockwise around the disc before passing into the buccal cavity. Figure 7 shows the peristome of an uncontracted zooid; the disc was roughly circular in shape when viewed from above, 12 - 15 µm in diameter and was without pellicular pores and striations. The cilia were arranged in rows; there was a single outer row (the haplokinety) and a treble inner row (the polykinety). The haplokinety and polykinety were separated by a pellicular ridge or comb (Figure 8). At the end of each ciliary row was a short cilium followed by a series of barren kineto­somes (Figures 8, 9 and 10). The barren kinetosomes of the polykinety were arranged in a ladder-like series of short, obliquely set rows of three, while those of the haplokinety were arranged in what appeared to be a row of single kinetosomes. The two sets of kinetosomes were separated by a continuation of the pellicular ridge which divided the ciliary rows. The number of barren kineto­somes (or sets of three in the case of the polykinety) was found to vary over a range 6 to 30 among the zooids examined. Parasomal sacs were associated with the barren kinetosomes and cilia of the polykinety (Figures 9 and 10). They appeared as circular pores about 0.1 µm in diameter. The parasomal sacs were arranged in two rows, one alongside the inner row of kinetosomes of the polykinety the other alongside the outer row. Parasomal sacs were not observed in association with either the middle row of the polykinety nor with the haplokinety.

Discussion

SEM examination of the pellicle of *S. physarum* revealed that it is furnished with striations and pores, the general pattern of which is consistent with that reported in SEM by Small and Ranganathan (1970) and by Foissner and Schiffmann (1979) from their study of silver-stained specimens. The striations were widely spaced ("Weitstreifensystem" or WST - Foissner and Schiffmann, 1974, 1979). The average distance between neighbouring striations in these specimens fixed for SEM was 1.1 µm; this fell within the range of 0.9 - 1.3 µm reported previously for silvery-stained cells (Foissner and Schiffmann, 1979). The occasional abnormalities such as bifurcations and terminations, which occurred among the striations, are consistent with those reported for *S. physarum* (Foissner and Schiffmann, 1979) and for *Scyphidia* sp. (Small and Ranganathan, 1970). Similar variations in the pattern of striations were observed by Zagon (1971) for the colonial peritrich *Carchesium polyspinum*.

The number and distribution of pellicular pores revealed in this study confirms those previously reported for *S. physarum* (Foissner and Schiffmann, 1979). Pores were
SEM study of *Scyphidia physarum*

situat-ed both alongside and between the striations although none were found on the striations themselves. This is in contrast to the findings of Finley et al. (1972) and Small and Ranganathan (1970) who observed pores both on (*"marginal") and between (*"basal") the striations of a variety of peritrichs. The number of pores per 100 µm² on the zooid (19 - 23, mean 21) fell within the range 18 - 25 (mean 21) reported by Foissner and Schifffmann (1979).

The present investigation revealed that the telotroch band of *S. physarum* was represented by three closely spaced ridges whereas according to Foissner and Schifffmann’s (1979) silver-stain study, the telotroch band consists of only two argentophilic lines. Previous SEM studies have revealed that the telotroch bands of a variety of peritrichs may be represented by either two or three ridges (Zagon, 1971; Carey and Warren, 1983).

The peristome of *Scyphidia* has never previously been described in detail for uncontracted specimens. Cells that are impregnated with silver or processed for ultrastructural investigation almost invariably contract (Fish and Goodwin, 1976; Foissner and Schifffmann, 1979). By employing the relaxation and rapid-fixation technique described by Carey and Warren (1983), Pickering et al. (1985) were able to obtain uncontracted specimens of *Scyphidia* sp. for SEM examination. In the present study, rapid-fixation alone produced a sufficient number of uncontracted specimens to facilitate a detailed study of the peristome. The oral ciliature of peritrichs is divided into the haplokinety and the polykinety (Chatton, 1936). Silver-stain and ultrastructural studies have revealed that the haplokinety consists of a staggered double row of kinetosomes with those of the outer row only bearing cilia, the inner row being barren (Fauré-Fremiet et al., 1962; Bradbury, 1965; Lom and Corliss, 1968; Hausmann and Hausmann, 1981; Walker et al., 1986). The present study confirms that there is a single row of cilia in the haplokinety of *S. physarum*.

The number of ciliary rows in the peritrich polykinety may not be consistent throughout the group. Noland and Finley (1931), Fauré-Fremiet et al. (1962), and Lom (1964), for example, reported the presence of only two rows of cilia in the peritrichs, whereas Chatton (1936), Zagon (1971), Hausmann and Hausmann (1981), and Walker et al. (1986) reported three. The present study confirms that there are three rows of cilia in the polykinety of *S. physarum*. This is consistent with the findings of Lom and Corliss (1968) from their ultrastructural studies of *Scyphidia ubiquita* and *S. inclinans*, and with Pickering et al. (1985) from their study of *Scyphidia* sp. from the brown trout *Salmo trutta*. The ciliary rows of both the haplokinety and polykinety were composed of sets of individual cilia working independently; none of the cilia were fused with neighbouring cilia at any point along their length. This is in contrast to the findings of Finley et al. (1972) who, from their SEM study of the mobiline *Telo-trochidium*, concluded that the adoral ciliature of peritrichs is composed of semi-membranes with each cillum fused distally with neighbouring cillum of the same row. The pellicular ridge separating the haplokinety from the polykinety is probably equivalent to the ‘comb’ in *S. ubiquita* (Lom and Corliss, 1968). The comb is formed by tapering ele-

vations protruding from oral ribs (Noirot-Timothee and Lom, 1965; Rosenberg and Grim, 1966; Lom and Corliss, 1968).

At the end of each ciliary row in both the haplokinety and polykinety was a short cillum followed by a series of barren kinetosomes. The number of barren kinetosomes varied from zooid to zooid. A possible interpretation for these observations is that, following morphogenesis and cell division, the adoral cilia are formed in sequence with those at the end distal to the buccal cavity developing last. If this is the case, the short cillum at the end of each ciliary row may represent an early stage in its development prior to attaining its full size. Furthermore, the variable number of barren kinetosomes in different zooids may be indicative of the maturity of each zooid, mature zooids having fewer barren kinetosomes than immature zooids since more of their cilia are fully developed. Further studies are required in order to confirm this interpretation of the data.

Two rows of parasomal sacs were found associated with the barren kinetosomes and cilia of the polykinety of *S. physarum*. Their arrangement, one row associated with the inner set of kinetosomes and the other with the outer set, is consistent with those previously reported for *Opisthoheta henneguyi* (Rosenberg and Grim, 1966) and *Carchesium polyplum* (Zagon, 1970, 1971). Although no parasomal sacs were observed in association with the haplokinety of *S. physarum* this could have been due to masking of the relevant area by nearby structures such as the pellicular ridge (comb) and the peristomial lip. The presence of haplokinetal parasomal sacs has been revealed by transmission electron microscopy (TEM) in both *Opisthoheta* (Rosenberg and Grim, 1966) and *Carchesium* (Zagon, 1971).

In conclusion, the present study has confirmed many of the data reported previously from silver-stain and ultrastructural studies, e.g., the number and arrangement of pellicular pores and ridges. However, some findings, such as the structure of the telotroch band, are not consistent with those of previous studies, while others, such as the barren kinetosomes and development of the adoral ciliature, are reported for the first time. It is therefore concluded that no single technique can provide all the data required for a complete understanding of peritrich morphology and in order to achieve this goal, a range of techniques including silver staining, TEM and SEM should be employed.

Acknowledgements

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References

Figure 1. Scyphidia physarum. (a) Light micrograph of detached zooid, Nomarski optics; bar = 20 µm; (b) SEM of a contracted zooid showing the arrangement of striations with some bifurcating or terminating (arrow); bar = 5 µm.

Figure 2. Group of individuals attached to tissue of host Physa fontinalis, showing contracted, partly contracted and uncontracted zooids; bar = 20 µm.

Figure 3. Zooid surface showing pellicular pores (P) and striations (S); bar = 2 µm.

Figure 4. Telotroch band (TB). Note the three closely spaced striations and the absence of pellicular pores; bar = 1 µm.

Figure 5 (facing page). Partly contracted peristome (PL = peristomial lip); bar = 5 µm.

Figure 6 (facing page). Peristomial lip of partly contracted peristome. Note that the pellicular pores (arrow) are located almost exclusively on the anterior side of the striation; bar = 1 µm.

Figure 7 (facing page). Uncontracted peristome showing the rows of adoral cilia winding counter-clockwise around the disc; bar = 5 µm.

Figures 8-10 (facing page). Details of peristome. Figure 8 showing the cilia of the haplokinety (H) and polykinety (Po) separated by the pellicular ridge or comb (C). Note the short cillum (arrow) and barren kinetosomes (BK) at the end of each row; bar = 1 µm. Figure 9 showing the barren kinetosomes of the haplokinety (H) and polykinety (Po), and the parasomal sacs (PS); bar = 0.5 µm. Figure 10 showing variation in the number of barren kinetosomes; bar = 1 µm.


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SEM study of *Scyphidia physarum*
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Discussion with Reviewers

D.H. Lynn: Can the author tell whether the "striations" are helically coiled around the body?

Author: Each striation appears to encircle the body in a plane that is normal to the longitudinal axis of the cell. There is no indication that the striations are helically coiled around the body.

D.H. Lynn: How does the author explain the differences in observations of pore distributions between his results and those of Finley et al. (1972) and Small and Ranganathan (1970)?

Author: Neither Finley et al. (1972) nor Small and Ranganathan (1970) were able to resolve pellicular structures as clearly and at such high magnifications as those illustrated here. Therefore, it is possible that the 'marginal' pores, i.e. pores on the striations, of the previous studies may in fact be 'basal' pores which lie in very close proximity to the striations. Furthermore, neither Finley et al. (1972) nor Small and Ranganathan (1970) presented data for *Scyphidia physarum*.

D.H. Lynn: How does the author explain why he did not observe the "fusion" of oral cilia observed by Finley et al. (1972)?

Author: Fusion or partial fusion of the oral cilia of pertrichs has, to the best of author's knowledge, yet to be confirmed (see Carey and Warren, 1983). It is possible that the ciliary fusion observed by Finley et al. (1972) was an artefact.

D.H. Lynn: The author proposes an interesting hypothesis to explain the variation in numbers of non-ciliated kinetosomes in the haplokinetin and polykinetin. How might this hypothesis be tested?

Author: In order to test the hypothesis *Scyphidia physarum* could be cultured in the laboratory and observed for telotroch production. It may be possible to synchronize cell division and telotroch formation by chemical or physical shock treatment. Samples of cells may be fixed at various times following settling by the telotrochs on their substrata. Each sample may be examined by SEM to reveal numbers of barren kinetosomes. Correlations may then be sought between these numbers and the time interval following settling.