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Ultrastructure of the Endosymbiont *Symbiodinium microadriaticum* from a Sea Anemone

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Abstract. During the present study, the sea anemone *Anemonia sulcata* (Pennant) was collected from the Alexandria coast on the Mediterranean Sea. Numerous symbiont vacuoles were observed in the endoderm of sea anemone organisms. These endosymbionts were identified as *Symbiodinium microadriaticum Freudenthal* and may be considered as a first record for the Egyptian fauna. Scanning and transmission electron microscopy studies were used to describe the ultrastructure of these symbiotic dinoflagellates. The ecological importance and symbiotic relationship of these zooxanthellae to the sea anemone are discussed.

Introduction

Symbiotic dinoflagellates termed zooxanthellae are found in a variety of marine organisms, notably radioalarians, foraminiferans and ciliates among protozoans. Moreover, many invertebrate hosts (especially scleractinian corals, sea anemones, hydrozoans, Jellyfishes, acoel flatworms and bivalve molluscs) are also symbiotic with zooxanthellae. Among zooxanthellate anemones, the intertidal actinian *Anemonia sulcata* (Pennant) is common along the east and west coasts of Alexandria on the Mediterranean Sea [1 and 2]. This anemone appears to prefer well illuminated habitats in shallow rocky shores. It extends its green and brown snaky tentacles fully in sun light as well as in artificial light. It seems that the symbiotic zooxanthellae are important for the activity and flourishing of the anemone host. The structure, taxonomy, phylogeny and symbiotic association of these endosymbiotic zooxanthellae were studied by several

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authors [3-7; 8, pp.723-731; 9-14]. In the Egyptian fauna it is surprising that very little is known about these marine microorganisms. The present study deals with their fine-structure and identification.

Materials and Methods

Specimens of the sea anemone *A. sulcata* were collected from the Alexandria coast of the Mediterranean sea as shown in the map of Egypt (Fig. 1). They were maintained in natural sea water aquaria in the laboratory. For transmission electron microscopy (TEM), excised tentacles were fixed in 3% glutaraldehyde in phosphate buffered salt solution (0.25M) at pH 7.3 for 12 hours at 4°C. They were post-fixed in 1% osmium tetroxide in the same buffer for 2-5 hours. They were stained in uranyl acetate and lead citrate and examined with Philips transmission electron microscope model 201 at the Electron Microscopy Center, Ain Shams University.

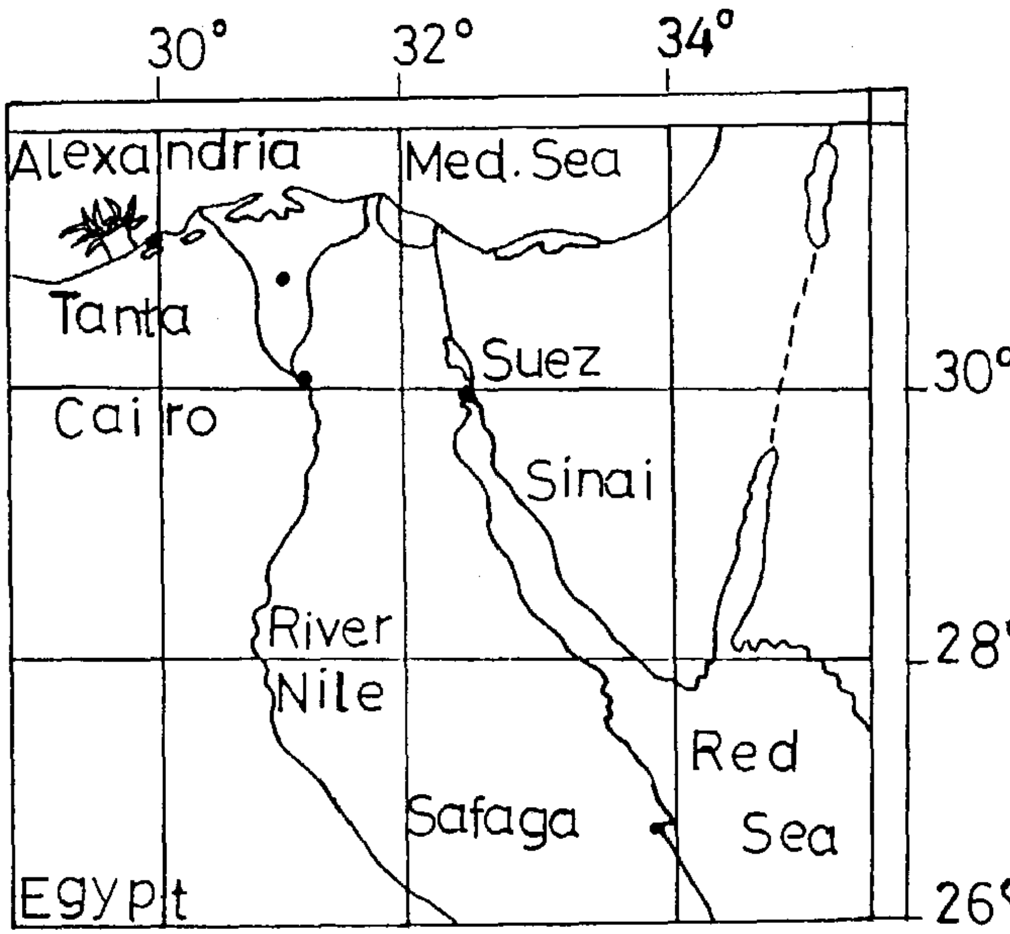

Fig. 1. A map of Egypt showing the geographical position of Alexandria, the site of collection of sea anemons used in the present study.

For scanning electron microscopy (SEM), anemone tissues were fixed as mentioned above and dehydrated in a graded series of ethanol solutions (50%, 70%, 80%, 90% and two changes of absolute ethanol) for 30 min at each step. The samples were mounted on aluminium stubs, critical-point dried with liquid CO_2 followed by sputter-coating with gold-palladium. They were examined with an Amray scanning electron microscope model 1200 B at the Electron Microscopy Center, Tanta University.

Results and Discussion

Transmission electron microscopy

The endoderm of *A. sulcata* contains symbiotic dinoflagellate cells as spherical or oval cysts within vacuoles (Fig. 2). These endosymbiotic microorganisms range from 9.5 to 11.2 μm in diameter. Transmission electron microscopy shows that the endodermal epithelial cells are heavily vacuolated with numerous intercellular spaces. The zooxanthellae are separated from the endodermal epithelial cells of their host by a periplast or zooxanthella membrane (about 0.48 μm in thickness). The interior of each zooxanthella is lined by a large multilobed chloroplast at the periphery (Fig. 3). Their lamellae appear as bands traversing their entire length. The width of the chloroplast lamellae ranges from 1.7 to 2.4 μm.

In these symbiotic zooxanthellaė, the pyrenoid is spherical or club-shaped. It is usually attached to the inner surface of the chloroplast by a short stem and is surrounded by a starch sheath (Fig. 4). The nucleus of these microorganisms is oval (4-5.6 μm in diameter) and contains a small nucleous. The chromosomes (Fig. 3) are elongated in shape and randomly distributed in the nucleoplasm. The zooxanthella cell also contains mitochondria, accumulation bodies and vacuoles containing crystals (Figs. 3 & 5). The mitochondrion is oval (1.6 μm in diameter) with tubular or microvilli-like cristae and is found in different positions within the cell. The accumulation bodies occupy a large portion of the cell's interior. Vacuoles containing crystals are observed at the periphery of the cell and are easily recognized by the rectangular shape crystals in a large vacuole (Fig. 5). These crystals are similar in shape to the calcium oxalate crystals described by Taylor [6].

Identification of zooxanthellae

The ultrastructure of these symbotic dinoflagellates indicates that they belong to *Symbiodinium microadriaticum* [4]. Recently, a classification of living dinoflagellates has been presented by [13]. The dinoflagellates belong to different genera, families and orders. Among the genera, *Symbiodinium* is common and includes several species. The genus *Symbiodinium* was placed in the Family Symbiodiniaceae and order Suessiales [13].

Symbiodinium microadriaticum, the symbiont from *A. sulcata*, was reported in other

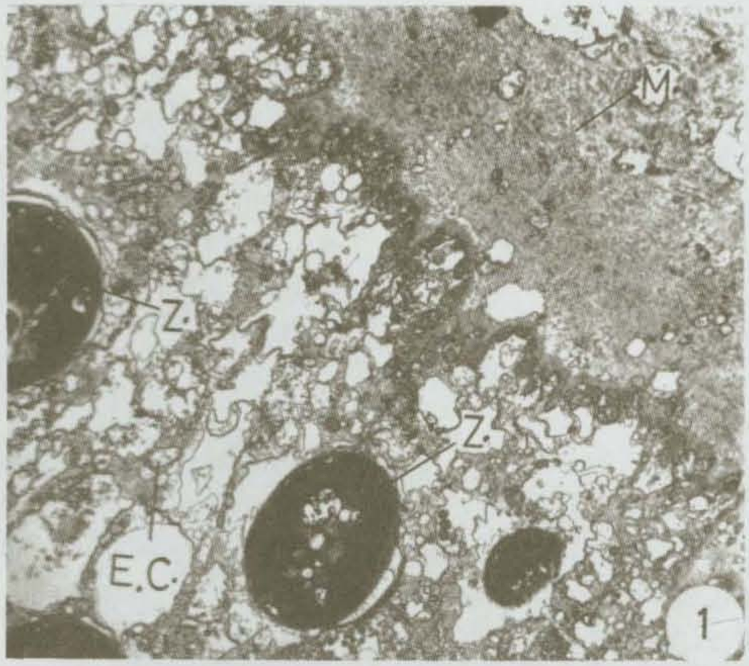

Fig. 2. Transmission electron micrograph of endodermal cells of the sea anemone anemonia sulcata showing the symbiotic zooxanthellae *Symbiodinium microadriaticum* (x3,600). E.C., endoderm cells; M., mesoglea; Z., zooxanthella.

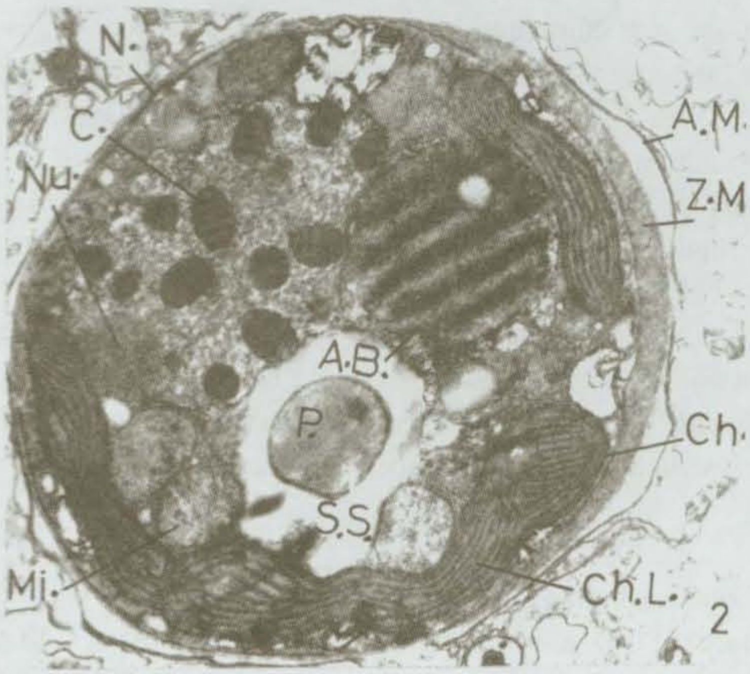

Fig. 3. Transmission electron micrograph of a symbiotic zooxanthella showing the appearance of condensed chromosomes in the nucleus and multilobed chloroplast (X13,000). A.B., accumulation body; A.M., anemone membrane; C., chromosomes; Ch., chloroplast; Ch.L., chloroplast lamellae; Mi., mitochondrion; N., nucleus; Nu., nucleolus; p., pyrenoid; S.S., starch sheath; Z.M., zooxanthella membrane.

Fig. 4. Transmission electron micrograph of a zooxanthella in the endoderm of the anemone tentacle showing a pyrenoid (stored carbohydrate) projecting into a starch sheath and chloroplast lamellae at the periphery (X13,000). A.M., anemone membrane; Ch.L., chloroplast lamellae; E.C., endoderm cells; P., pyrenoid; S.S., starch sheath; Z., zooxanthella.

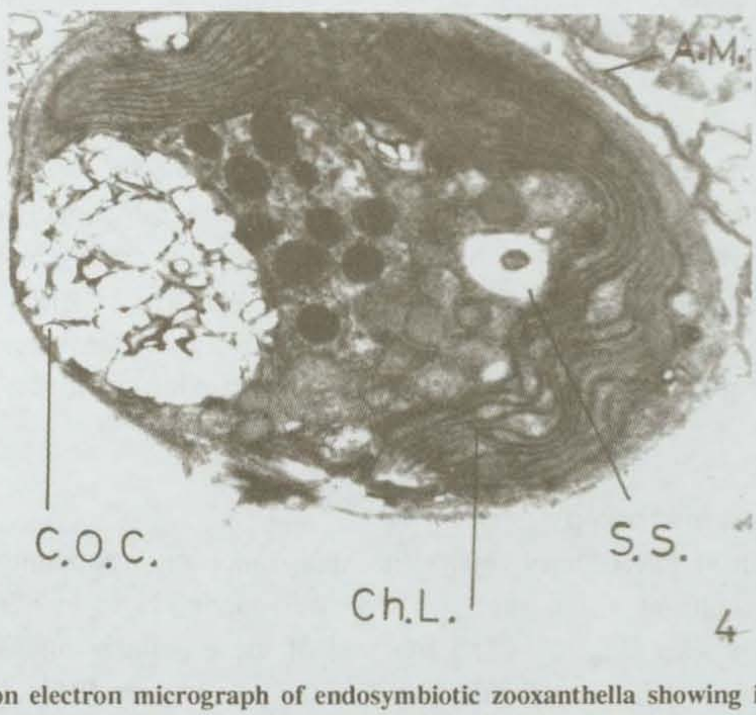

Fig. 5. Transmission electron micrograph of endosymbiotic zooxanthella showing increase of vacuole containing crystals (calcium oxalate crystals) and decrease of pyrenoid inside starch sheath (X13,000). A.M., anemone membrane; Ch.L., chloroplast lamellae; C.O.C., calcium oxalate crystals; S.S., starch sheath.

anemones such as *Aiptasia pallida* [9], jellyfishes as *Cassiopeia xamachana* and *C. frondosa* [15,16] and some nudibranchs [17]. Furthermore, other species of the genus *Symbiodinium* were recorded in symbiotic onidarians. The tropical sea anemone *Aiptasia pulchella* harbours zooxanthellae belonging to *Symbiodinium pulchrorum* [14]. The dinoflagellate *Symbiodinium cariborum* is the symbiont from the sea anemone *Condylactis gigantea* [12]. *Symbiodinium meandrinae* is associated with the scleractinian coral *Meandrina meandrites* [14]. The Hawaiian stony coral *Montipora verrucosa* harbors Symbiodinium-like dinoflagellates [18].

Moreover, other genera of dinoflagellates were identified in symbiotic onidarians. The dinoflagellate *Gloeodinium viscum* is associated with the hydrocoral *Millepora dichotoma* from the Gulf of Aqaba. In addition, *Scrippsiella velellae* is the symbiont from the hydrozoan chondorophore *Velella velella* from the Pacific [12].

Degenerate zooxanthellae

The ultrastructure of some zooxanthellae cells, inhabiting the tentacles of the sea anemone *A. sulcata*, showed several signs of degeneration as shown in Figs. 2 and 4. These signs appeared in the thickening of the outer periplast, increase in the amount of stored crystals, enlargement of the accumulation bodies and decrease in the stored carbohydrate (starch). It has been stated [6,19] that the waste products of the algae are aggregated in the accumulation bodies and calcium oxalate vacuoles: In addition, these degenerate symbionts of tentacles continue to accumulate wastes and finally reach a phase of aging leading to death. These degenerate cells are transported to the anemone mesenteries for excretion [6].

On the other hand, Steen and Muscatine [20] observed the discharge of degenerate symbionts from the coelenteron of the sea anemone *Aiptasia pulchella* as individual cells or as pellets of cells in mucous substance. Some of these zooxanthellae were in various stages of disintegration. Similarly, our observations during rearing of the sea anemones in the aquarium suggest that discharged zooxanthellae are expelled from the mouth opening through a mucous material. In fact, it seems that the mesenteries of the sea anemone *A. sulcata* perform an excretory function, in addition to the known digestive function.

Scanning electron microscopy

Scanning electron microscopy shows the appearance of a zooxanthella among long finger like evaginations (kinocilia). These microappendages cover the ectodermal surface of the tentacles (Fig. 6). The function of these cellular microappendages is to create endogenous water currents in and out of the coelenteron [21]. It is probable that they aid in the movement of materials (e.g. mucus and symbionts) across the epithelial surface. Furthermore, a scanning electron microscopic examination demonstrates that the

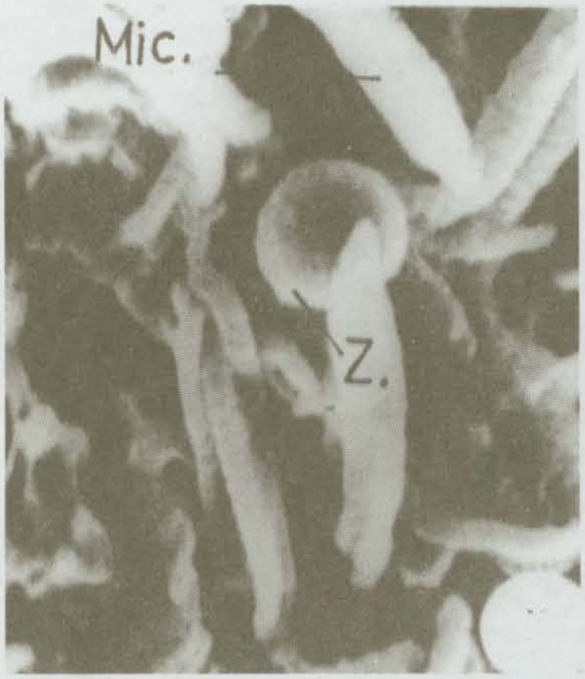

Fig. 6. Scanning electron micrograph showing a zooxanthella among cellular microappendages (kinocilia) on the ectodermal surface of the tentacle of A. sulcata (X5,000). Mic., microappendages; Z., zooxanthella.

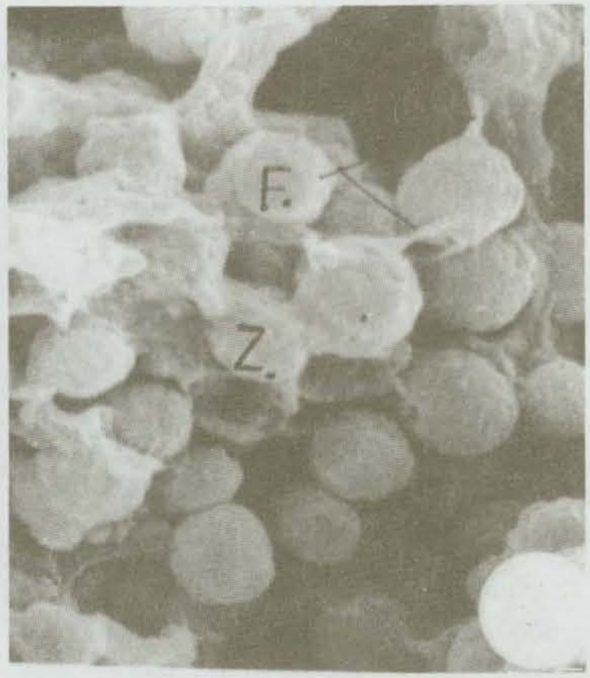

Fig. 7. Scanning electron micrograph showing the abundance of zooxanthelae within endodermal cells of tentacles (X3,000), F., flagella; Z., zooxanthella.

Fig. 8. Scanning electron micrograph showing enlarged zooxanthella (X9,000), Z., zooxanthella.

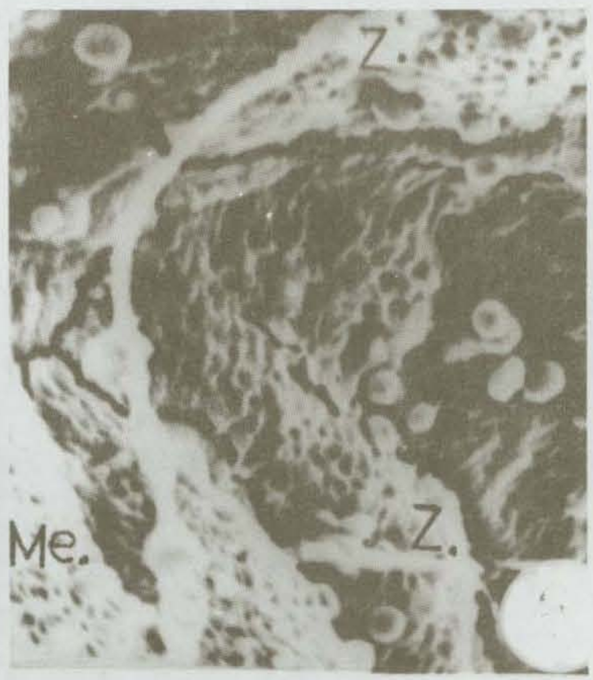

Fig. 9. Scanning electron micrograph showing the presence of some zooxanthelae on the outer surface of mesenteries (X1,000), Me., mesenteries; Z., zooxanthella.

zooxanthellae reach the endodermal cells of tentacles in high numbers and some of them showing flagella (Figs. 7 and 8). Also, other symbionts are detected on the surface of the anemone mesenteries (Fig. 9).

Symbiotic significance

The sea anemone *A. sulcata* collected from Egyptian water was never found without its algal partner *Symbiodinium microadriaticum* which may suggest that the symbiosis here is obligatory and not facultative. The significance of this relationship between zooxanthellae and onidarians, especially sea anemones, was mentioned by several authors [6, 10 and 22]. The partnership involves transfer to the anemone host of energy, organic carbon, oxygen and probably organic nitrogen and even phosphorus and sulphur compounds in products released by the symbionts. The symbiont may gain carbon dioxide, inorganic nitrogen, phosphate and sulphur compounds, shelter and sometimes vitamins from the host [23]. Zooxanthellae excrete glycerol, glucose and organic acids, among other compounds, and up to 80% of the carbon fixed by zooxanthellae may be released as maltose with smaller amounts of alanine and glycollic acid [24]. However Muscantine [5] stated that the algal pyhotosynthetic product, glycerol, is easily transported outside zooxanthellae to the benefit of the host. In spite of this, the anemone cannot replace its main habit of nourishment as a carnivorous animal.

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التركيب الدقيق للمتكافل الداخلي سيمبيودنيوم ميكروأدرياتيكوم في أحد شقائق النعمان البحرية

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(استلم في ١٥ ربيع ثان ١٤١٥هـ؛ قبل للنشر في ٢٥ ذو الحجة ١٤١٦هـ)

ملخص البحث. تمّ في هذه الدراسة تجميع عيّنات شقائق النعمان التي تتبع النوع «أنيمونيا سولكاتا» من شواطىء الاسكندرية على البحر المتوسط. أظهرت الدراسة بالميكروسكوب الإلكتروني النافذ (المتخلل) وجود العديد من الفجوات التكافلية التي تحتوي على كائنات وحيدة الخلية في طبقة الإندودرم لشقائق النعمان. وتم تعريف هذه الكائنات التكافلية والتي تنتمي إلى النوع سيمبيودنيوم ميكروأدرياتيكوم، ويعتبر ذلك إضافة جديدة إلى الفونة المصرية. كما تمّ وصف التركيب الدقيق لهذه الكائنات باستخدام الميكروسكوب الإلكتروني بنوعيه السطحي والنافذ، ويناقش البحث الأهمية البيئية والتكافلية لهذه الزوجسنثيللى.