

Symbiotic algae in milioline foraminifera: CO₂ uptake and shell adaptations

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Experiments with living *Amphisorus hemprichii* demonstrated algal symbiont uptake of ¹⁴CO₂ directly through the thin lateral test walls of this imperforate, porcellaneous species. An identical uptake mechanism is suggested for *Sorites* and *Marginopora* because of their similar thin lateral walls. *Peneroplis*, *Spirolina*, *Cyclorbiculina*, and *Triloculina rupertiana* show structural adaptations towards thin test walls by development of furrows and pits. These features may also enhance CO₂ diffusion through the test walls for use by their symbiotic algae.

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Intracellular symbiotic algae within host foraminifera require CO₂ when actively photosynthesizing. A symbiont-foraminifer relationship, as seen in the perforate calcitic *Amphistegina lobifera*, can recycle as much as 50% of the metabolic carbon (Muller 1978). Accordingly, the symbionts need additional CO₂ for their photosynthesis besides that originating from the metabolism of the foraminifer. A recent work by Leutenegger and Hansen (in press) demonstrated that the pores in certain (and probably all) perforate foraminifera serve the purpose of oxygen uptake from the surroundings and, in forms with symbiotic algae, the inward passage of CO₂ when the symbionts are active.

This naturally raises the question of how imperforate foraminifera with symbiotic algae get the additional supply of CO₂ for their symbionts. The question is particularly relevant in very large porcellaneous forms with obligate symbiosis. If the supply of CO₂ has to pass through the aperture, the resultant diffusional distances of up to 10 mm or more seem unlikely. By contrast, direct uptake of CO₂ through the lateral test walls appears to be a more attractive hypothesis.

This work is an attempt to demonstrate, using labeled carbon, that CO₂ can pass through the walls of *Amphisorus hemprichii*, and, using SEM and TEM micrographs, the possible route of passage of the CO₂.

Materials and methods

Living specimens of *Amphisorus hemprichii* were collected from shallow water close to the Marine Biology Station, The Hebrew University, Elat, Israel.

For the labeled carbon uptake experiments, living specimens were mounted according to the modified technique of Berthold (1976) described by Leutenegger and Hansen (in press). The specimens were mounted on pieces of aluminum foil so that the central part of the specimens were exposed to seawater containing ¹⁴C added to the medium as Ba¹⁴CO₃ with NH₄Cl. Activity of the seawater was approximately 0.7 μCi ¹⁴C/ml. 10 specimens were illuminated with a microscope lamp for 150 minutes. After incubation, the specimens were washed and fixed in 96% ethanol, decalcified in dilute HCl, washed and dehydrated on polished brass studs. The studs and a photographic plate were dried in high vacuum for two days. Then the studs were placed on the photo plate with the dried remains of the foraminifera in direct contact with the photographic emulsion. The photo plate was thus exposed for two weeks under high vacuum.

Fractured specimens of various symbiont-bearing, porcellaneous, imperforate foraminiferal species were examined in the scanning electron microscope. Living specimens

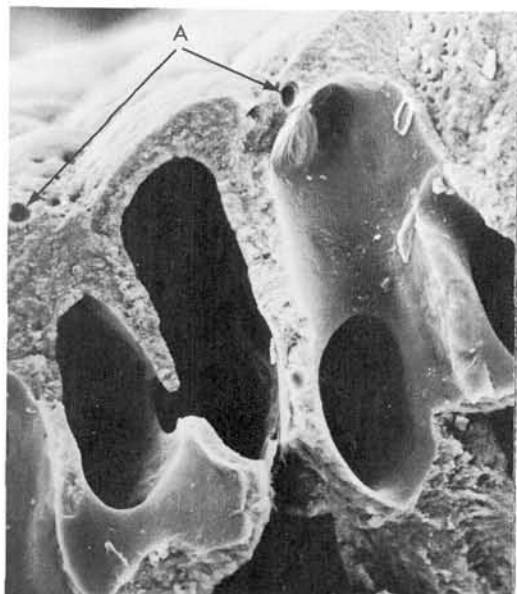


Fig. 1. *Amphisorus hemprichii* (Ehrenberg). Nabek, Gulf of Elat. Detail of fractured empty shell. A = algal boring. 482 x.

were studied in the TEM using the conventional techniques of double fixation, ultrasectioning and double stain with heavy metals. The specimens were photographed using a Cambridge Mk.IIa or 180 scanning electron microscope and a Hitachi HU 11C transmission electron microscope housed in the Geological Institute of the University of Copenhagen.

Observations

The living *Amphisorus hemprichii* incubated in the light exposed the film substantially, illustrating that the symbionts were actively photosynthesizing and incorporation the labeled CO_2 although the apertures of the hosts were not in contact with the culture medium.

The structure of the shell of *A. hemprichii* is well known as belonging to the porcellaneous category. On SEM micrographs of fractured specimens, the thickness of the lateral walls could be determined to be about $7 \mu\text{m}$ (fig. 1–2). When viewed under the light microscope, the lateral walls of the chamberlets appear thin and transparent (almost hyaline). Furthermore, the early portion of the shell of *Amphisorus* has a irregularly pitted surface (fig. 3).

Ultrasections of fixed, decalcified living specimens in the TEM showed much organic material between the calcitic elements of the wall (fig. 4). A more dense organic part with a thickness of about $2 \mu\text{m}$ was found on the inside. Numerous symbiotic algae were found close to the inner surface of the shell. The algae have been described previously by Leutenegger (1977).

Discussion and conclusions

From the experiments with labeled CO_2 , it can be concluded that uptake of CO_2 from the surrounding seawater takes place through the thin lateral chamber walls in *Amphisorus hemprichii*. Because CO_2 for the symbionts can pass through the very thin, pitted walls of *Amphisorus*, it is relevant to study wall thickness in other large, imperforate, symbiont-carrying, porcellaneous species.

In their overall morphology (disc-shaped shells with annular subdivided chambers), *Sorties orbiculus* and *Marginopora vertebralis* are similar to *Amphisorus*. The lateral walls of *Sorties* are about $3 \mu\text{m}$ thick (fig. 5) with a somewhat irregularly pitted surface (fig. 6). The early portion of *Marginopora* (fig. 7–9) shows this pittedness



Fig. 2. Detail of fig. 1 showing a thickness of $7.5 \mu\text{m}$ of the lateral wall. 4255 x.

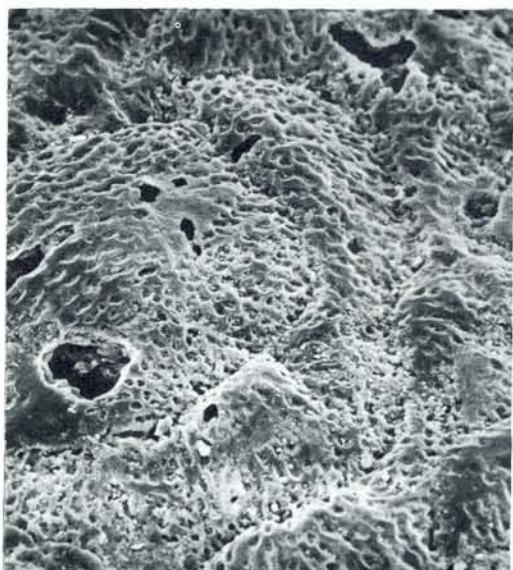


Fig. 3. *A. hemprichii* (Ehrenberg). Detail showing densely pitted lateral walls of the early shell portion of a dead and somewhat worn specimen. 396 x.

while the lateral chamber walls of the later portion is rather smooth. The thickness of the lateral walls is about 6 μm . Thus, both species have lateral walls with thickness and pittedness like that found in *Amphisorus*. Because only living speci-

mens of *A. hemprichii* were available, no experiments involving *Sorites* and *Marginopora* were possible. Nevertheless, the authors are convinced that these two forms can also take up CO_2 through their thin lateral walls.

For additional morphological comparisons, the lateral walls of several other symbiont-bearing, imperforate, porcellaneous species were examined using the TEM. *Peneroplis* (fig. 10–12) has lateral walls ornamented by ribs oriented in the direction of coiling. Each rib has a width of about 12 μm ; furrows between the ribs are about 4 μm wide. The wall thickness in the furrows is further thinned by the presence of pits (fig. 13). *Spirolina* (figs. 14–15) has a somewhat similar wall morphology, but the pits are larger and less numerous. The distance from the bottom of a pit to the inner surface of the wall is about 2 μm (figs. 16–17). *Cyclorbiculina compressa* (figs. 18–19) has walls that are densely pitted with wall thickness at a pit of about 3 μm .

Triloculina rupertiana is a porcellaneous, imperforate, (supposedly) symbiont-bearing species which is not closely related to the other species examined. Yet the wall construction of *T. rupertiana* (figs 20–22) is strongly reminiscent of that of *Spirolina*. The wall thickness measured from the bottom of a pit to the shell inside is about

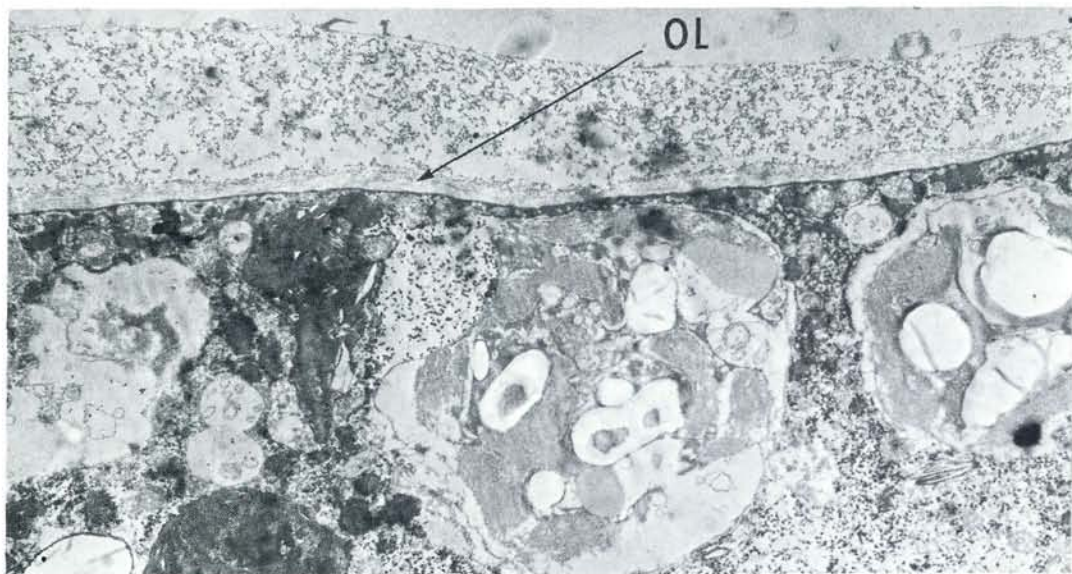


Fig. 4. *A. hemprichii* (Ehrenberg). Off the Marine Biology Station, Elat. Detail of ultrasection of fixed, decalcified living specimen showing position of symbiotic algae relative to the organic portion of the wall. Note a more dense organic layer close to the inner surface (OL). 6.750 x.

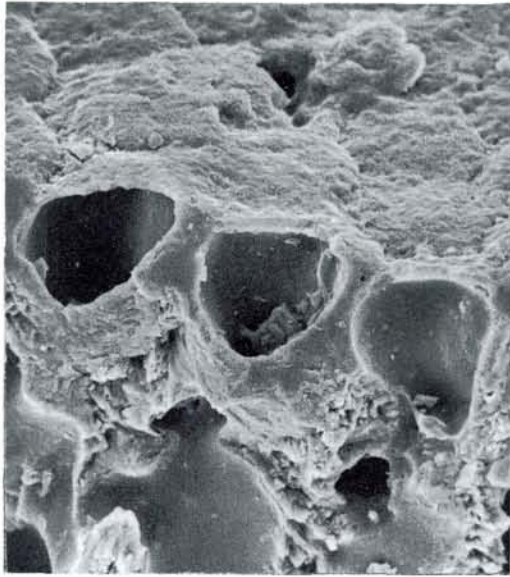


Fig. 5. *Sorites orbiculus* (Ehrenberg). Off Doelah, Kei Islands. Fractured specimen showing thickness of the lateral wall of about 3 μ m. 442 x.



Fig. 7. *Marginopora vertebralis* (Batsch). Off Toeal, Kei Islands. Pitted surface of lateral walls of the early portion of the shell. 833 x.

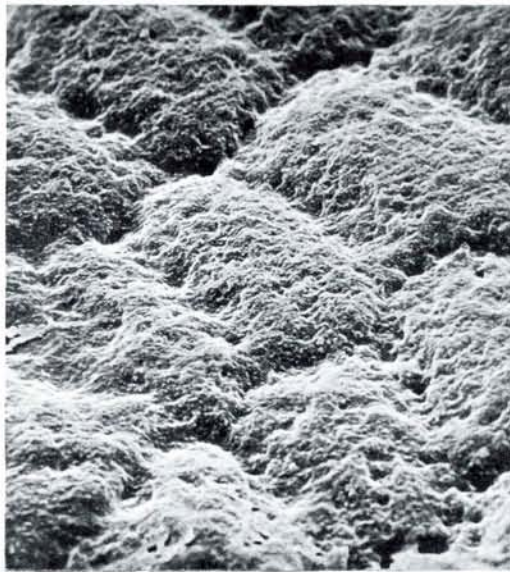


Fig. 6. *S. orbiculus* (Ehrenberg). Irregular, slightly pitted surface of lateral chamber walls. 797 x.

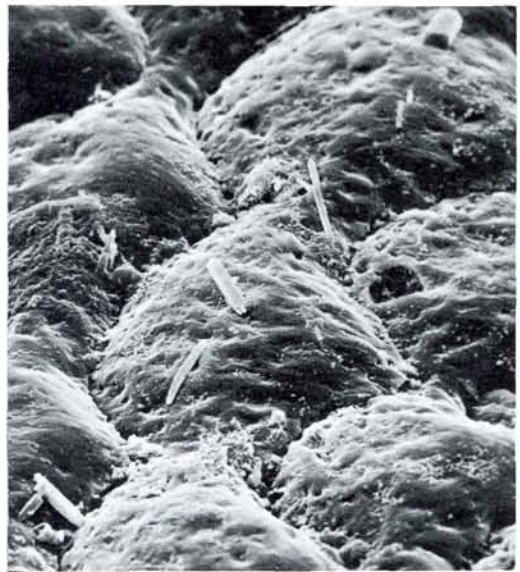


Fig. 8. *M. vertebralis* (Batsch). Less pitted wall of the later portion of the shell. 822 x.



Fig. 9. *M. vertebralis* (Batsch). Detail of fractured specimen showing thickness of the wall of about $6\ \mu\text{m}$. 820 x.



Fig. 11. Detail of fig. 10 showing ribs and furrows with associated pits. 833 x.



Fig. 10. *Peneroplis pertusus* (Forskål). Off Toaal, Kei Islands. Lateral view of fractured specimen showing presence of ribs oriented in the direction of coiling. 59 x.



Fig. 12. Detail of fig. 10 showing apertures, foramina and thickness of lateral wall of final chamber. 490 x.

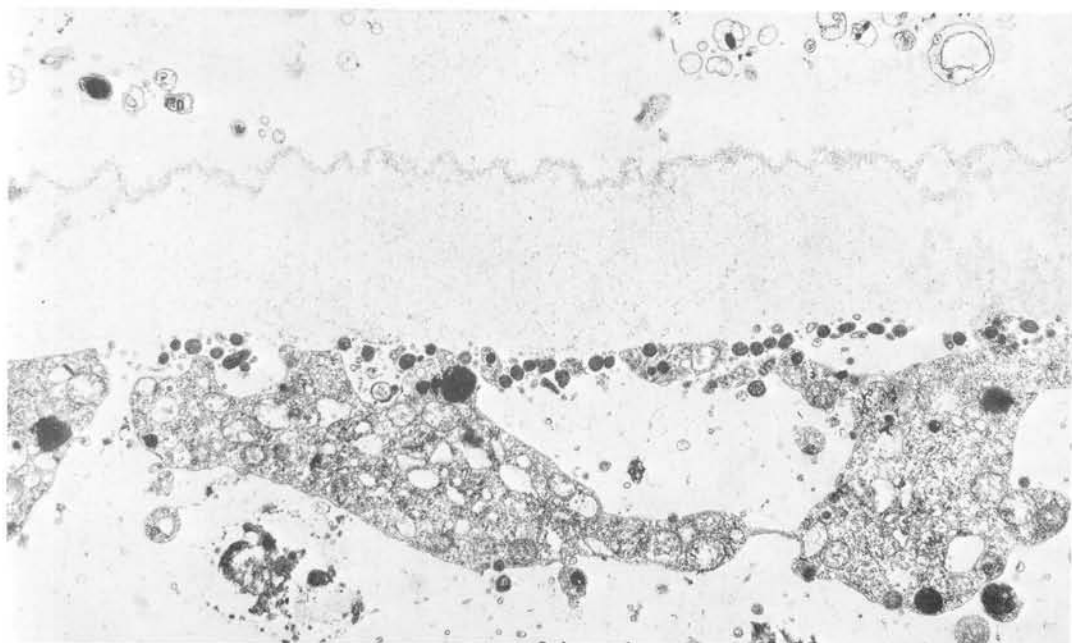


Fig. 13. *P. pertusus* (Forskål). Detail of ultrasection of fixed and decalcified living specimen from the Gulf of Elat. The section is in a pitted furrow between the ribs. 10.700 x.

10 μ m. As in *Spirolina*, the pits in the walls appear to have a smaller diameter at the shell surface than at the bottom of the pits.

The ultrastructure of porcellaneous walls is well described (Hay et al. 1963; Towe and Cifelli 1967; Haake 1971; Leutenegger 1977; Ross and Ross 1978). The walls are constructed of generally randomly oriented calcite laths. The laths are embedded in an organic matrix which, although representing a minor portion of the wall material, constitutes a three-dimensional network evidently allowing CO₂ to diffuse along or through it. The organic wall constituent of the porcellaneous forms here illustrated is considerable when compared to the organic portion seen in decalcified perforate hyaline foraminifera (compare Leutenegger 1977).

We suggest that the morphological features which minimize lateral wall thickness in symbiont-bearing porcellaneous forms are adaptations to enhance and facilitate the passage of CO₂ through the shells for the use of the symbiotic algae in their photosynthesis. In view of the recent find by Leutenegger and Hansen (in press) of passage of CO₂ through the pores of *Amphis-*

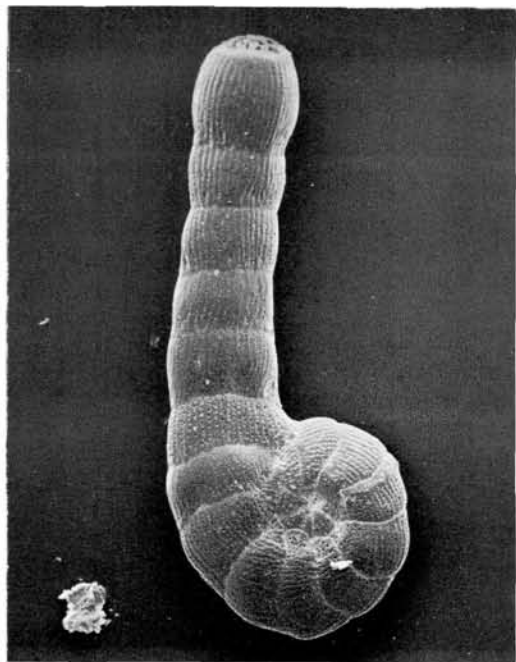


Fig. 14. *Spirolina arietina* (Batsch). Nabek, Gulf of Elat. Lateral view showing ribs oriented parallel with the direction of coiling. 60x.

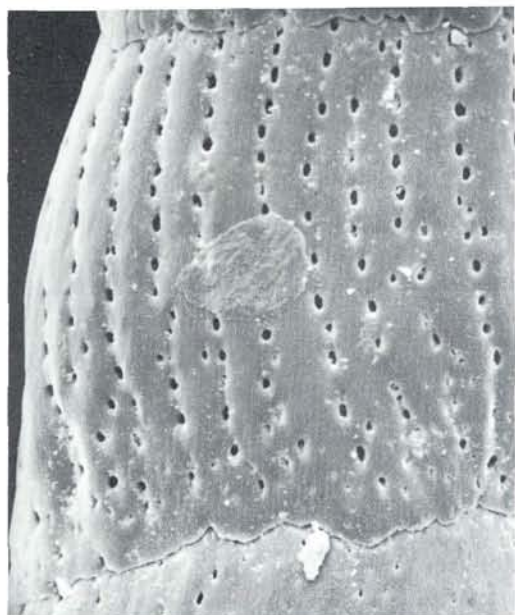


Fig. 15. Detail of fig. 14 showing pits between low ribs. 442 x.

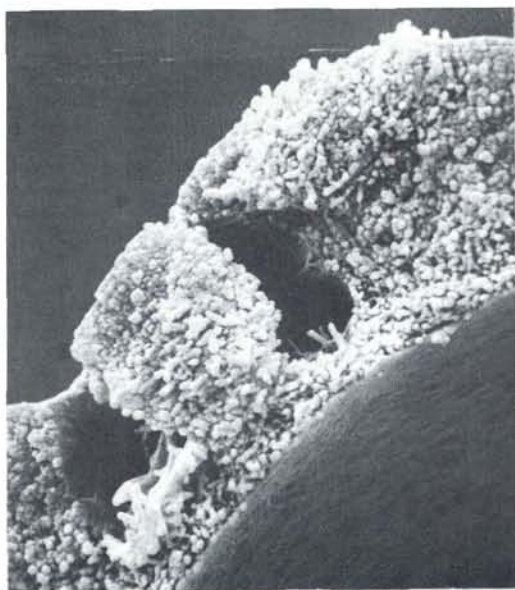


Fig. 17. Detail of fig. 16 showing pits and the 2 μ m wall from the bottom of the pits to the inner surface of the wall. 3400 x.

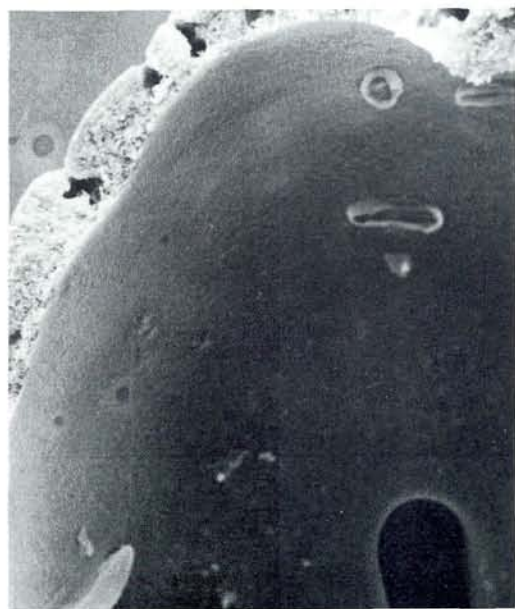


Fig. 16. *S. arietina* (Batsch). Detail of fractured chamber showing the pits and the smooth inner chamber wall. 930 x.

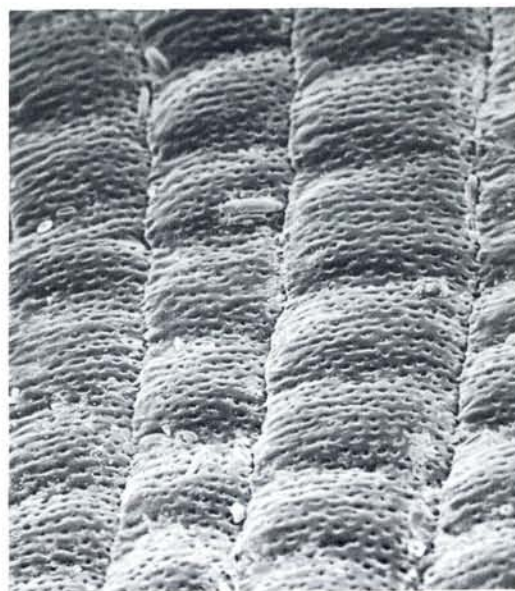


Fig. 18. *Cyclorbiculina compressa* (d'Orbigny). Off Thatch Islands. Detail of lateral wall showing pitted surface. 420 x.

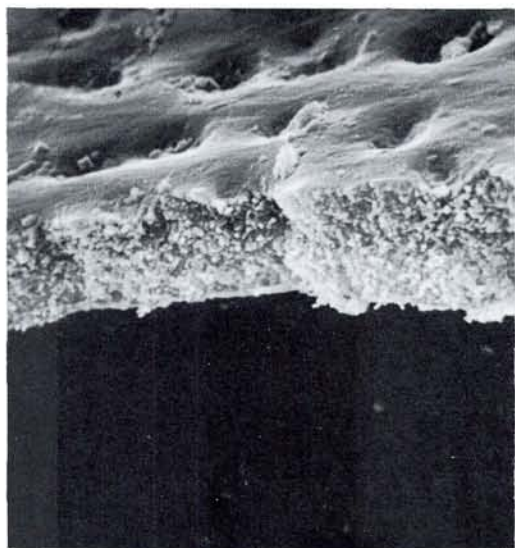


Fig. 19. *C. compressa* (d'Orbigny). Detail of fractured specimen with a wall thickness of about $3\ \mu\text{m}$. 4025 x.

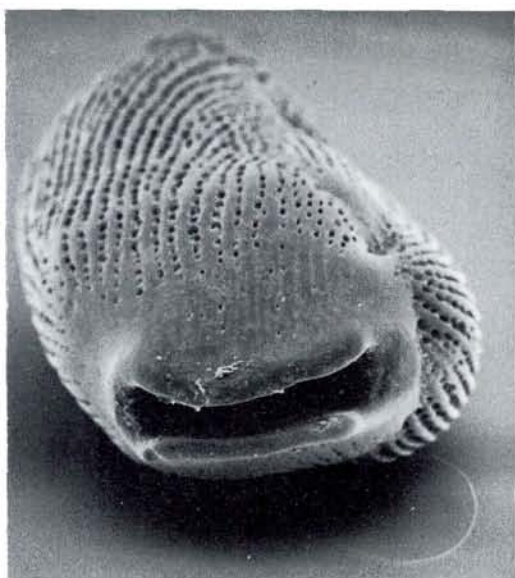


Fig. 21. Apertural view of specimen in fig. 18 showing smooth apertural surroundings 88 x.

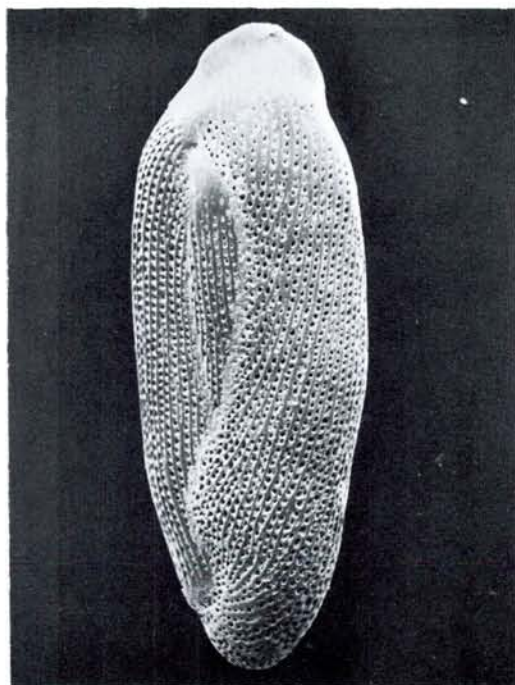


Fig. 20. *Triloculina rupertiana* (Brady). Nabek, Gulf of Elat. Lateral view showing ribs and intermediate pits. 43 x.



Fig. 22. *T. rupertiana* (Brady). Detail of fractured specimen showing thickness of wall of about $10\ \mu\text{m}$ from the bottom of the pits to the inner shell surface. Note the inner organic lining detached from the wall. 910 x.

tegina across the inner organic lining, it is not surprising that a parallel phenomenon takes place through the very thin walls of certain porcellaneous foraminifera.

The causal relationship between morphology and function as here suggested offers an additional paleoecological interpretative tool for estimating depth of deposition of fossil non-transported assemblages from within the shallower part of the euphotic zone where all the forms mentioned are found to live.

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Dansk sammendrag

Ved eksperimenter med ^{14}C kan det vises at CO_2 diffunderer direkte gennem den imperforate, porcelænsagtige, tynde væg hos foraminiferen *Amphisorus hemprichii* og optages af de

symbiotiske alger som ligger plastret op til skallernes inderside. Identiske skalstrukturer findes hos *Sorites* og *Marginopora*. Det er velkendt at begge former ligeledes har symbiotiske alger. Det antages derfor at de tynde vægge hos disse former tillader diffusion af CO_2 .

Peneroplis, *Spirolina*, *Triloculina rupertiana* og *Cyclorbiculina*, som alle er imperforate, porcelænsagtige med symbiotiske alger, viser speciel adaptation i retning af tynde skaller gennem tilstedeværelsen af tæt placerede gruber, som nedbringer skaltrykkelsen til 0.01 mm eller mindre.

De her nævnte former omfatter næsten alle de dominerende, imperforate, symbiontbærende lavtvandsformer fra troperne og subtropenerne.

Det påpeges at de beskrevne skaludformninger vil kunne benyttes ved dybdetolkninger af fossile foraminiferselskaber.

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