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A STUDY OF SOME PRIMITIVE NEOSCHWAGERINA BY A NEW SERIAL SECTION TECHNIQUE

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Abstract. A new serial section method which is a combination of etching by inorganic salt solution and the Bioten replica technique has been invented for use to study the microstructure of fossils. The first trial of the technique has been made on some primitive neoschwagerinids such as *Minoella eonipponica* HONJO, *M. nipponica* (OZAWA) and *Neoschwagerina simplex* OZAWA. Some interesting results obtained from that study are illustrated for reference in respect to the classification of neoschwagerinids.

Previous Techniques

There are several essential problems in the serial section technique of calcified fossils. The first is how to grind fossils successively maintaining the parallelism, second, how to manage the ground fossil surface, and the last is how make a permanent records of the surface.

According to CROFT (1950), a dozen different instruments for parallel grinding have been described within the last half century. The general principles of construction of parallel grinding apparatus were first used by crystallographers (WULFÜNG 1890, etc.). Those principles were first introduced into paleontology by SOLLAS (1903) and have been followed until recently by many paleontologists.

Recently, CROFT (1950) invented a very precise instrument. It is a combination of the large micrometer of ZDANSKI (1938) and a tripod with sliding specimen holder. According to him, fossils can be ground at exact intervals down to ten microns. A concise review of previous instruments and details of his method are described in CROFT (1950). Readers are referred to that work.

The peel method has been used to record the etched surface permanently. Peel solution is viscous and generally composed of a mixture of acetone and amyl acetate with celluloid melted in. It was first employed by paleobotanists, and later, paleontologists have used the technique with a combination of serial grinding techniques.

The disadvantages of the ordinary peel method were that the solution takes a long time to dry after it is dripped on the surface, and that dried peels keep many small bubbles. BUTLER (1935), a paleobotanist, invented the celluloid film method to avoid those disadvantages. The principle of his method is that on a piece of thin celluloid which is covered with acetone, the specimen is firmly pressed against the celluloid, etched surface down. This technique is more excellent than the ordinary peel method. STERNBERG and BELDING (1942) applied this method in micropaleontology, especially to their study of bryozoans; they gave this method a new name "dry peel technique." Recent MORIKAWA's SUMP method (1955) is an application of this method. He has studied Japanese schwagerinid fusulinids by this technique with considerable success.

Although the parallel grinding method and recording technique have been gradually improved what is described above, the etching technique seems to have been kept at the beginning. Dilute hydrochloric acid or organic acid, like acetic acid, has been simply used for the last half century. However, the etching of a ground or polished surface seems to be the most important key to reveal the inside micro-texture of fossils.

Many workers have employed serial section techniques combining those three kinds of techniques in various ways. They are convenient for the study of rather coarse structure of fossil. However, extremely fine textures in fossil are almost impossible to be removed into replica by them. It may be caused by the instability and excess ability of etching solution, and want of plasticity of celluloid which are used in them.

A new serial section method which is a combination of etching by inorganic salt solution and Borden replica technique has been developed by the author. The hydrogen ion, which is produced by the hydrolysis of inorganic salt of strong acid and weak base makes very fine relief of microstructure of organism without destroying them. The salt solution keeps constant pH during reaction. Specially prepared plastics membrane, Borden RFA* makes precise replica of the etched surface, and it takes only a few seconds to dry.

When a piece of treated Borden replica is highly magnified under microscope, figures which are presented in the replica have enough shade contrast and resolving power. Photomicrographs of them are clear as well as those from thin sectioned preparation.

* A new "two step positive replica method" for electron microscopic observation using plastic material of high swelling character, was developed by FUKAMI, A., (1955). "Borden RFA" is the trade name of the special acetylcellulose film which is used for the first step replica in this method.

Results which are described in this paper have been obtained by this technique. Details of the new technique will be explained in a separated paper.

The intervals of sectioning are required to be less than ten microns in this study. CROFT's apparatus with a more improved micrometer is applicable for such purpose. In the present study, the abraded amount was estimated by a specially prepared microscopic attachment which is applied simple electronics following ordinary abrasion.

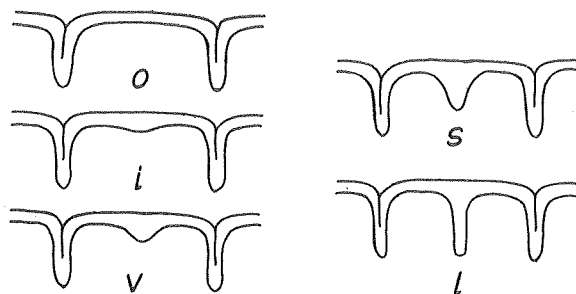
Some new knowledge on the texture of primitive neoschwagerinids, which has been obtained from recent study, are discussed below.

Axial Septula

It is not the aim of this report to mention the difference in biocharacters between different species of primitive neoschwagerinids. The structure of them are discussed.

MINATO (1959) discovered that the axial septula may be a quite useful biocharacter to aid in the classification of neoschwagerinids. He classified axial septula into five types from the viewpoint of the ontogeny of neoschwagerinids (text-fig. 1). Details of the study and the application of the result to the phylogeny of neoschwagerinids are available in MINATO and HONJO (1959).

There was some question as to the stability or continuity of axial



Text-Fig. 1. Five types of axial septula. MINATO distinguished axial septula of neoschwagerinid into these five types from the viewpoint of the ontogeny. The most advanced type which is found in a specimen is one of the best biocharacters to classify neoschwagerinids. For example, the axial septula of *Cancellina primigena* HAYDEN is expressed as *i*, *Gifuella gifuensis* HONJO, *l+s*, and *Yabeina katoi* OZAWA, *6l+s*. Also MINATO expressed the ontogeny of each species in concise logarithmic formula. The phylogeny of neoschwagerinids has been systematized on the basis of those formula.

septula in axial direction. This technique will much contribute to such study. One of the examples is illustrated in text-fig. 2.

In the No. 16 section of text-fig. 2, a second axial septula in embryonic state is found. Very slight swelling of keriotheca appears near transverse septula, and the primary axial septula develops at the side of it. This embryonic axial septula does not occur at all in the middle part of the vault of the chamberlet.

Transverse Septula

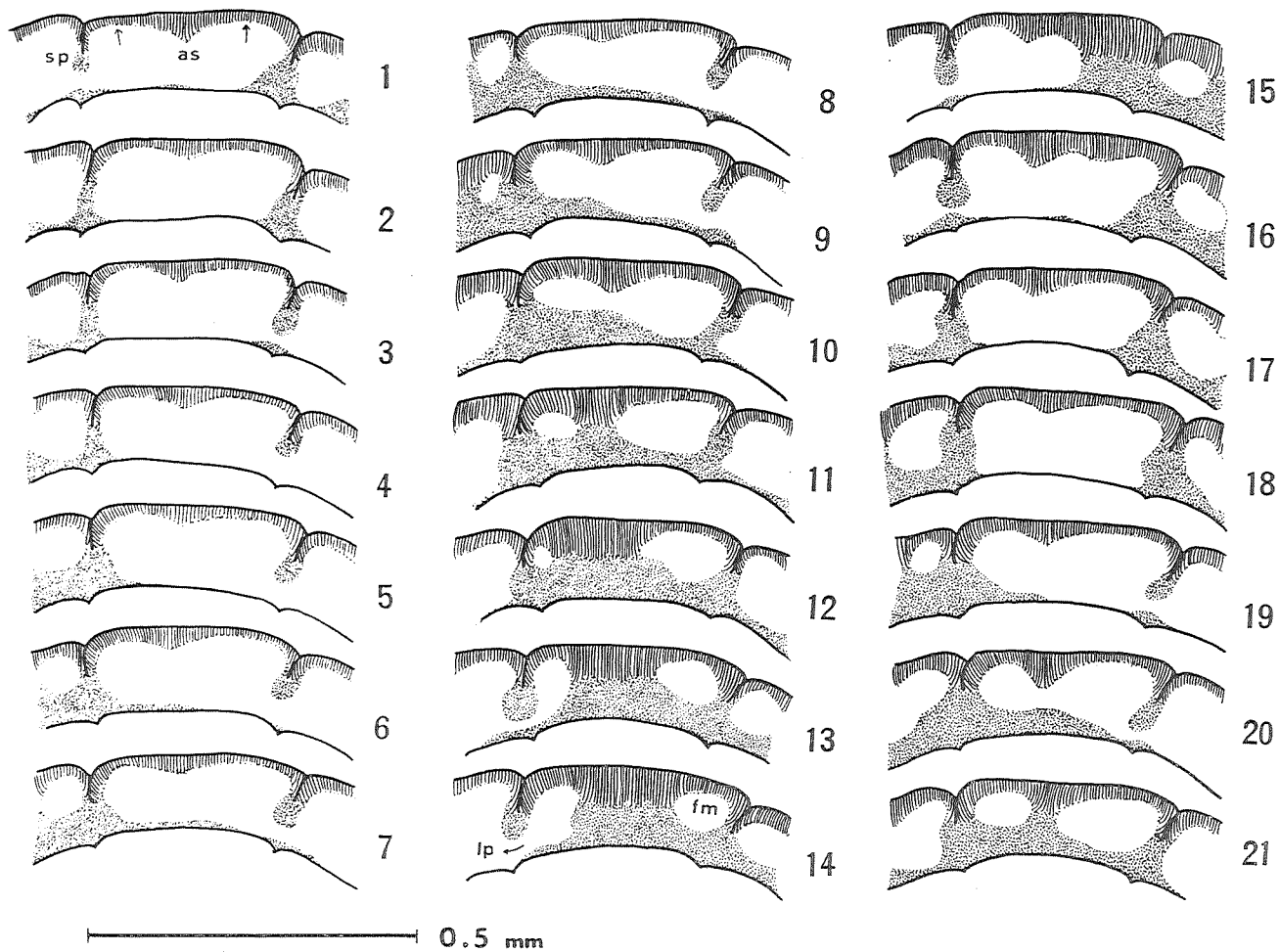
The difference in the shape of transverse septula for each species of neoschwagerinids is very interesting from the viewpoint of the classification and phylogeny. The present author has classified neoschwagerinids from the Akasaka limestone, central Japan, chiefly on the basis of differences of the ontogeny of transverse septula and discussed the evolutionary importance of them (HONJO 1959, MINATO and HONJO, 1959).

As is clear from plate 2 and 3, the shape of the transverse septula differs in every section, therefore when the shape of those parts of a species is used for comparison with other species, one should define the standard section of chamberlet.

In plate 2 and 3, a part of serial axial sections of a chamberlet of *Minoella nipponica* (OZAWA) are displayed. Three volutions with three spirotheca are seen in every microphotograph of different parts of sections. Three sets of transverse septula and corresponding parachomata are found in each volution. The change of the shape of transverse septula in the 10th volution (upper most volution in each section) is described below.

Sec. 1. In the initial stage of serial sectioning of this chamberlet,

Text-Fig. 2. Part of a continuous series of sagittal section through a chamber of *Minoella eonipponica* HONJO, at intervals of 8 microns. The illustrated chamber was adopted without any particular intent from the ninth volution of a well oriented specimen. ax; an axial septulum, sp; septa, lp; lateral passage, fm; foramina. Drawings by projector. *Minoella eonipponica* is one of the most primitive neoschwagerinids having V type of axial septula in the most developed chamber. In the continuous series of sections above illustrated, an axial septulum which bisects the spirotheca keeps V shape in Section 1 to 9 and 17 to 19. When the section was cut through or near transverse septula, the transverse septula vanished. Table 1 presents measurements of the thinnest portions of keriotheca at a point indicated by two small arrows in Section 1 of this text-figure. The thickness of the keriotheca of *M. eonipponica* is the least among neoschwagerinids, the same as that of *Cancellina primigena* HAYDEN or *Gifuella amicola* HONJO.



the transverse septula are connection with the corresponding parachomata. Parachomata are very large in this section; each parachomatum touches the next one by foot.

- Sec. 2. The lower ends of transverse septula are still connected with parachomata, but have become much narrower.
- Sec. 3. The axial section of transverse septula seems to take the form of small equilateral triangles. The tops of the transverse septula and parachomata touch at a point. Parachomata become small.
- Sec. 4. The size of transverse septula is suddenly reduced to merely slight swellings of keriotheca. The transverse septula and parachomata are separated by a certain distance. The height and width of the parachomata are at their minimum in this section.
- Sec. 5 & 6. Transverse septula and parachomata are still separated. Both transverse septula and parachomata are very small. Especially in Section 5, the lower surface of keriotheca is almost flat.
- Sec. 7. The transverse septula and parachomata come close again. Parachomata become large, especially, the width of the top of the parachomata has become wider than that of the root. And at last, two adjacent parachomata touch each other by their sides. A slight aperture between them becomes a foramina.
- Sec. 8. The keriotheca extend into the top of connected parachomata. Each alveolum stands parallel each of the others. This is the lateral section of septa.
- Sec. 9. The lower surface of keriotheca is not straight but waved. The diameter of foramina is the minimum in this section. This section runs almost through the center of the septum.
- Sec. 10. Foramina are increased in size. The black line is the tectum of neighbouring chamberlet. The structure which is seen in this section is schematically illustrated in text-fig. 3.
- Sec. 11 & 12. The section has already passed the septa. The shapes of transverse septula and parachomata become like those of Section 1.

Comparing the shape of transverse septula with those of other species, they should not be ignorant of such solid idea of the inside structure. The state of Section 4 or 5 should be regarded as the standard in classification. In those sections the transverse septula are at the minimum in size. The Section 3, in which the transverse septula touch with parachomata by a point, is good for a reference beside the standard.

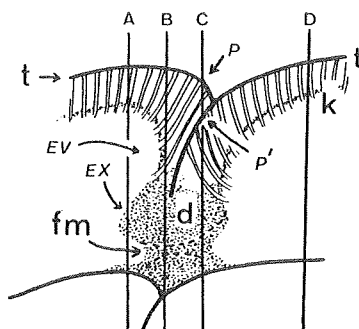
A secondary transverse septulum is first found in the right part of

of the 9th volution of Section 3, plate 2. The continuous series of that septulum is presented in Section 6 to 12. When the section is cut near the center of the vault of the chamberlets, it is not seen. In more advanced neoschwagerinids, secondary transverse septula are found where the spirotheca is minimum in thickness.

On the so called "trabeculus" of neoschwagerinids. DOUTKEVICH and KABAKOV reported "trabeculus" in *Neoschwagerina craticulifera* var. *haydeni* from Pamir. According to the serial section observation by the present author, trabeculus seem to be formed near the septa of any neoschwagerinids.

Both sides of septa, especially the front side are engraved by two holes which are between two adjacent septula. The upper hole is formed by the concaved keriotheca. The lower hole is bored into the basal deposit which is attached to the septa. The constricted part of this hole is called the foraminum.

Therefore, if the section is cut very near the septa, those two holes



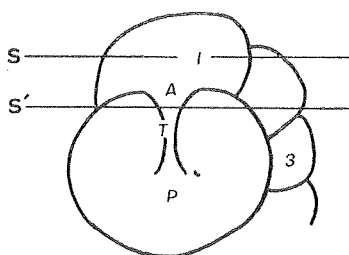
Text-Fig. 3. Diagram to illustrate septa. Septa are formed when tectum (t), as well as keriotheca (k), bend down inwardly. The next chamber begins at the front of the septa. The lower margin of septa is covered by the thick concentration of basal deposit (d), and connected with the tectum of the inner volution. The lower part of this deposit is perforated by foramina (fm). Foramina never cut the tectum. When a transverse section cuts a chamber through A plane, a figure is presented as Section 7, when B, as Section 8 or Section 9, when C, as Section 10, and when D, it is presented as Section 4, Section 5 or Section 6 of plate 2 and 3. (The 10th volution), respectively. In Section 10 of plate 3, waving dark line runs parallel with the tectum. The upper line is the section of the tectum (p), and the lower is bent tectum of the former chamber (p'). Ex is the swelling of the basal deposit developing around the entrance of foramina. The upper half of the front side of septa seems to be sunk (Ev).

are found in the same section separately, as it is seen in the 9th volution of Section 2 of plate 2.

When the section is cut through at a little distance from the septa, those two holes are connection into one, and the upper part of the parachomata seems to be extended laterally, as is seen in the 9th volution of Section 3, plate 2. The trabeculus is such an extended part of parachomata (text-fig. 3).

Proloculus

The megaspheric neoschwagerinids proloculus is a minute, commonly subspherical chamber with a single round aperture in one side to which is attached a long proloculus tube developing to the center of the proloculus. The aperture opens in the equatorial direction. In fig. a and fig. b of plate 1, the lateral sections of the proloculus tube are found. The real size of the proloculus tube of neoschwagerinids has been for the first time estimated by this serial section method (text-fig. 4).



Text-Fig. 4. Diagrammatic equatorial section through proloculus and part of first volution of a megaspheric individual. P; proloculus, A; proloculus aperture, T; proloculus tube. Commonly the first chamber of a primitive neoschwagerinid and of *Gifuella* is comparatively large. It may be noted that the axial section of the first chamber (Section s) has a round contour. Therefore, sometimes it is easily confused with the section of the proloculus in thin axial section. In the axial section s', the lateral contour of the proloculus tube is figured (fig. a, plate 1).

During the study of fusulinids, the exact measurement of the proloculus is required. However, it should be remarked that this property in this sectioned preparation is easily influenced by accidental errors (HONJO, 1959). By using the serial section method, the worker can easily learn the real diameter of a proloculus with least error (fig. c, plate 1).

In some axial thin section of primitive neoschwagerinids, the section of the first chamber is presented as a circle quite confusedly with the real section of a proloculus. Workers should be careful in respect to such incorrect figure of the proloculus.

Spirotheca

Thickness of spirotheca. The thickness of the spirotheca is not constant in the same chamber. Observing the change of the thickness of the spirotheca in text-fig. 2, one sees that the spirotheca is thinnest at Section 3. On the contrary, when the section seems to cut the center of transverse septula which are developing parallel to the section, the keriotheca reaches almost half of the height of the chamber and the lower surface is touched by a lower deposit.

The following table presents the thickness of the spirotheca as measured in each serial replica illustrated in text-fig. 2.

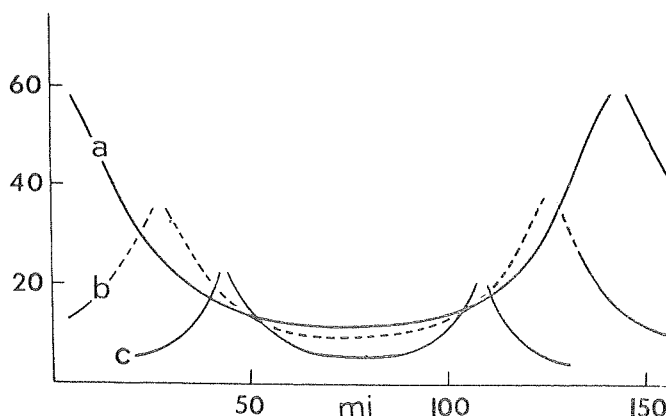
TABLE I.

Section	1 Thickness of Spirotheca	2 Percentage	1' Thickness of Spirotheca	2' Percentage
1.	11	17	14	22
2.	13	21	18	28
3.	8	12	13	21
4.	15	23	11	17
5.	13	19	13	21
6.	18	27	10	15
7.	13	19	13	19
8.	14	22	16	24
9.	13	19	12	18
10.	24	37	20	31
11.	38	58	17	26
12.	65	100	14	23
13.	19	30	17	26
14.	28	42	17	26
15.	13	19	17	103
16.	17	26	32 29	49 45
17.	10	15	15	23
18.	10	15	16	24
19.	13	19	10	15
20.	18	27	17	26
21.	14	22	19	30
	microns		microns	

The numbers in columns 1 and 2 are the thickness of the thinnest portions (indicated by small arrow marks in Section 1, text-fig. 2) in the left and the right area of the axial septula in microns, respectively. Columns 1' and 2' represent the percentage of the thickness of spirotheca in each section in proportions that of Section 12.

As is clear in this table, the thickness of a spirotheca changes by a large margin even within the same chamber. Further, it similarly changes in transverse section as a result of the influence of axial septula and septa. Workers should be very careful of the real thickness of the thinnest portion and the apparent thickness.

The thickness of the keriotheca in relation to lateral change measured in three different volutions are plotted in text-fig. 5.

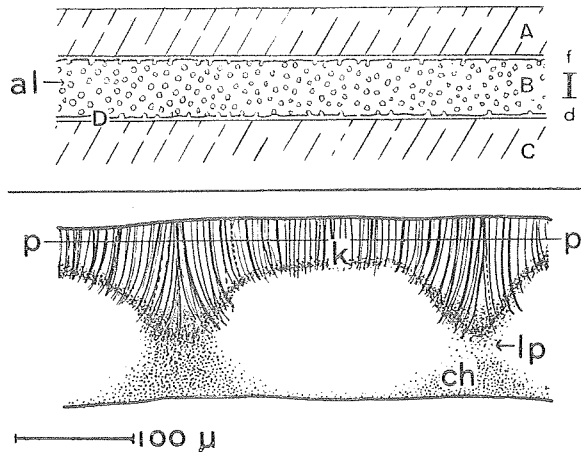


Text-Fig. 5. Graph showing the transition of thickness of the thinnest portion of a keriotheca of *Minoella conipponica*, measured in sagittal section, in microns. Two peaks of each curve represent transverse septula. If a secondary transverse septulum is developed in a chamber, three peaks would be seen. The values corresponding to mi are true "thickness of the thinnest portion of keriotheca". a: measurement in a chamber of the tenth volutions; b: eighth volution; c: third volution.

Alveolar structure of the neoschwagerinids. Using the ordinary method of etching by a comparatively active acid, it is very difficult or impossible to secure for observation the real figures of extremely fine textures like the alveolar texture of neoschwagerinids or verbeekinids.

A thin section of the spirotheca, which cut a specimen in an axial direction, is also unsuitable for such study. As illustrated in text-fig. 6, a microscopic figure of alveoli in transverse or axial thin section in the contrast of the total of the opaque and transparent parts of the spirotheca. This can be understood when the focal depth of an ordinary microscope (Table II) and the thickness of an alveolus which is generally 1.5 to 3.5 microns (μ), are compared.

In other words, a microscopic figure does not represent the outline of one alveolus, but of several. By preparing an extremely thin section, or



Text-Fig. 6. Diagram illustrating the influence of thickness of thin section upon microscopic figure of fine texture in spirotheca. The upper diagram is the longitudinal section of a keriotheca through p-p. B is thin sectioned sample covered with cover-glass A, and deck-glass C. The microscopic figure is the total of all shadows which are inside of focal depth of microscopic lens. For example, f-d is the focal depth when 10× objective lens and 7× ocular are combined. Under a microscope which has such combination of lens, individual alveoli (al), lower tectorium (lt in text-fig. 7), etc. are mixed up in the keriotheca (K). Also lateral passages (lp) etc., are not distinct.

using an objective lens which has short focus, one can avoid this influence of thickness, however, the shade contrast is much reduced and almost non-observable by ordinary microscope. (If the thin section which has been cut normal to alveoli is available, the thickness of each alveolus will be measurable.)

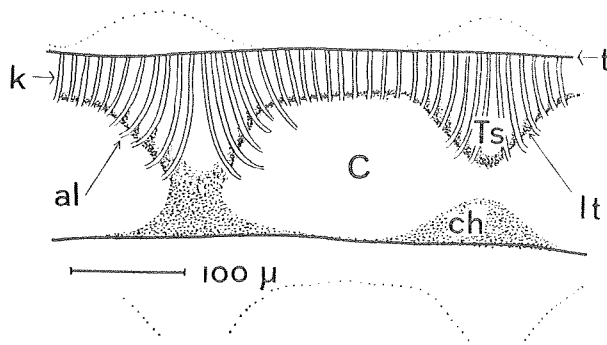
One of the advantages of this new peel technique lies in the fact that the etching solution etches the polished surface very mildly to make slight relief, and the Bieden peel covers every nock and corner of the relief exactly.

According to the new peel and thin section observation by special method, the alveolar texture of those primitive *Neoschwagerina* does not

TABLE II.

	Ocular	4×	7×	10×	15×
Objective Lens	10× (N. A. 0.25)	36 μ	21 μ	15 μ	10 μ
	40× (N. A. 0.70)	3.3 μ	1.9 μ	1.3 μ	0.9 μ
	100× (N. A. 1.25)	0.83 μ	0.42 μ	0.29 μ	0.19 μ

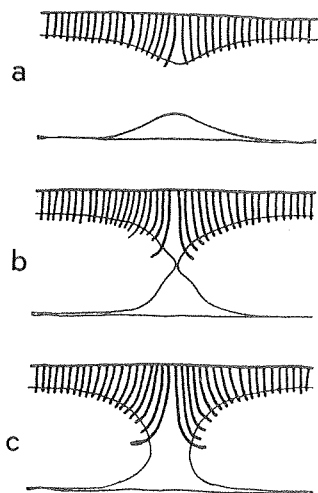
N. A.: numerical aperture.



Text-Fig. 7. Diagram of a part of an axial section of a volution in Bident replica, *Neoschwagerina simplex*. Each alveolus (al, slightly larger than natural) stands separately from others, some lower part of the alveoli sticks out of the lower tectorium (lt). Lower tectorium does not seen inside of an alveolus. C: chamberlet cavity; t: tectum. As is seen in text-fig. 2, alveoli bend in both axial and sagittal planes. Alveolar divergency only in an axial plane is expressed in this diagram.

consist of closely piled tubes, while each alveolus stands separately from the others. Some lower parts of the alveoli stick out of the lower tectorium which covers the lower surface of the spirotheca. The lower end of each alveolus opens to a chamberlet (text-fig. 7.).

Alveolar divergency. In transverse septula and axial septula, each alveolus bends independently to the left and right sides of axial septula, transverse septula or secondary transverse septula without additional



Text-Fig. 8. Diagram illustrating alveolar divergencies in different sections. a: A transverse section which is cut through the center of a lateral passage. Alveoli bend slightly. As the section approaches to septa which develop parallel to the sections, a transverse septulum becomes large, long, and is connected with parachomata. In such sections, alveolar divergency is large (b), Especially, in section c, alveoli bend more and more to become parallel with the tectum.

alveoli. This phenomenon has been described by various authors (THOMPSON 1942, etc.),

Alveoli always stand normal to both tectum and the tangent of the vault of chamberlets at the inside of keriotheca. It is very interesting that each alveolus sticks out of lower tectorium; sometimes the projected portions of some alveoli bend more and more, and they become parallel to the surface of the spirotheca.

The angle of bent alveoli to the normal of the tectum which is measured within lower tectorium is called alveolar divergency. Employing this technique, the present author traced the change of alveolar divergency. As is schematically illustrated in text-fig. 8, according as the transverse or axial septula become longer, alveolar divergency increases.

Acknowledgment

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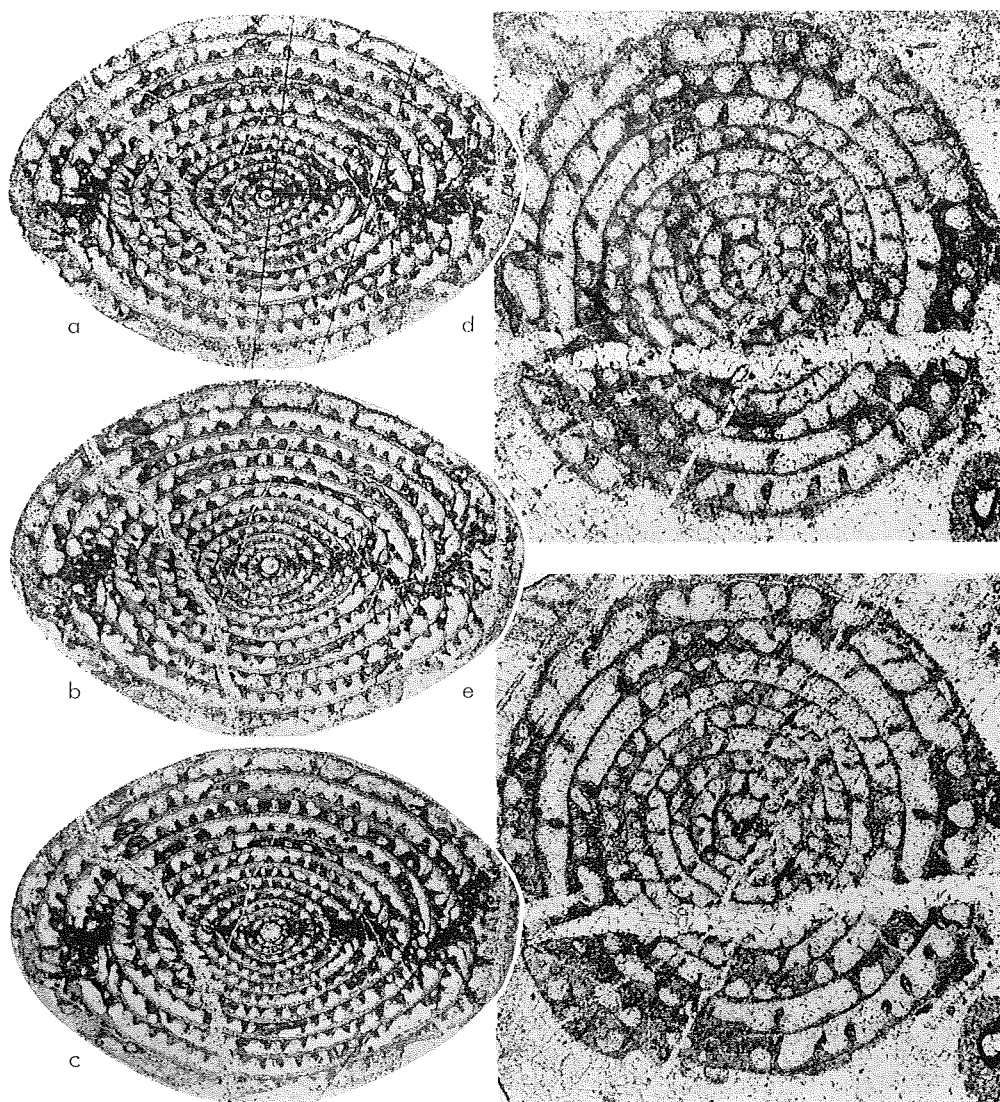
Explanation of
Plate I

Explanation of Plate I

All figures are unretouched photographs.

Figs. a, b and c. Part of a continuous series of sections through *Minoella nipponica* (OZAWA) in Bieden replica. Axial sections. Ground at intervals of 5/1000 mm. Notice at the change of the contour of the proloculus in each section. $\times 15$

Figs. d and e. Sagittal sections of Neoschwagerina *Minoella eonipponica* Honjo in Bieden replica. Ground interval between Fig. d and Fig. e is approximately 20/1000 mm. $\times 37$



Explanation of
Plate II and III

Explanation of Plate II and III

All figures are unretouched photographs.

Part of a continuous series of sections through the ninth volution of *Minoella nipponica* (OZAWA) in Bioten replica. Axial section. Ground at intervals of 10/1000 mm. See "transverse septula" in the text. $\times$ ap. 90

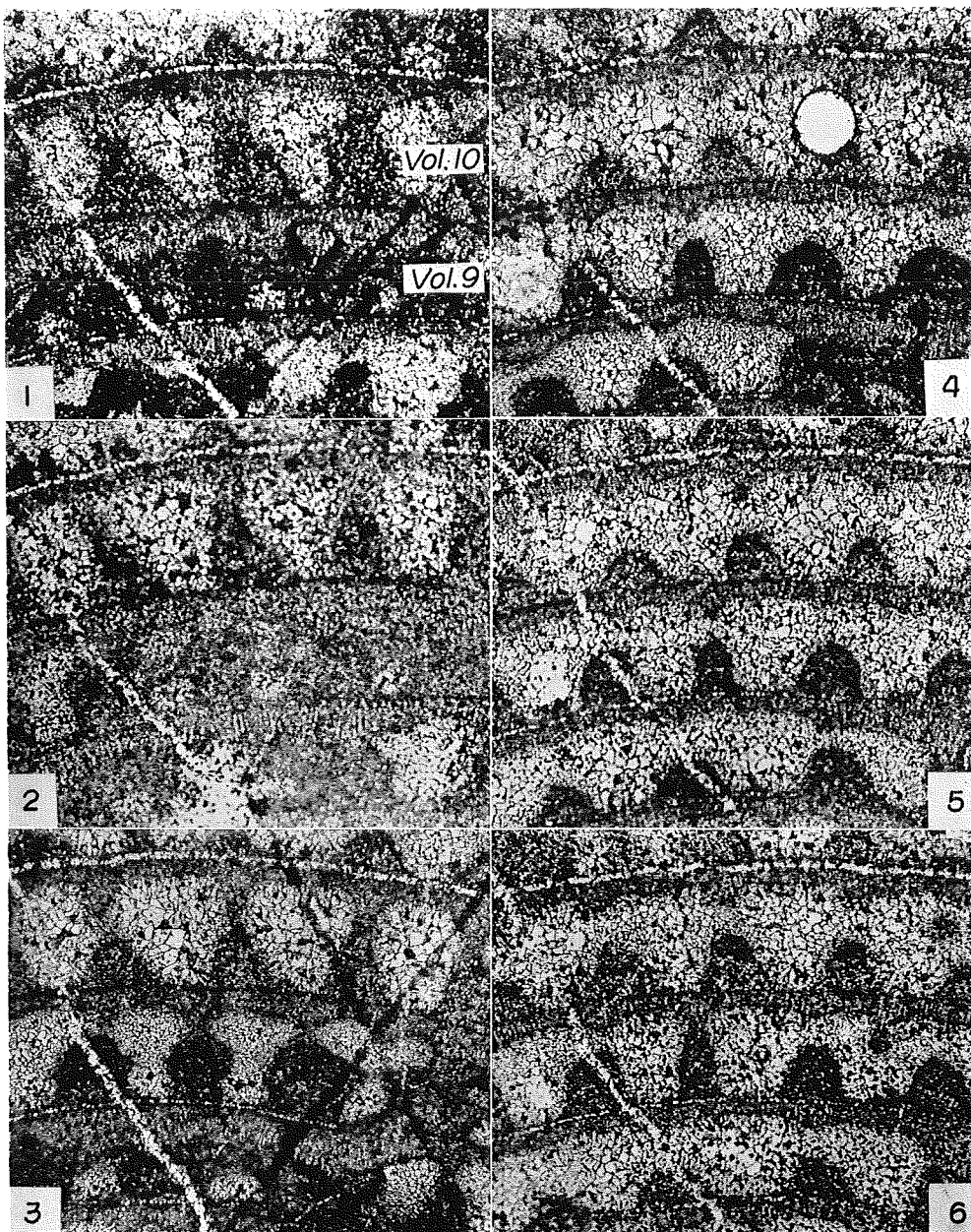


Photo. S. HONJO

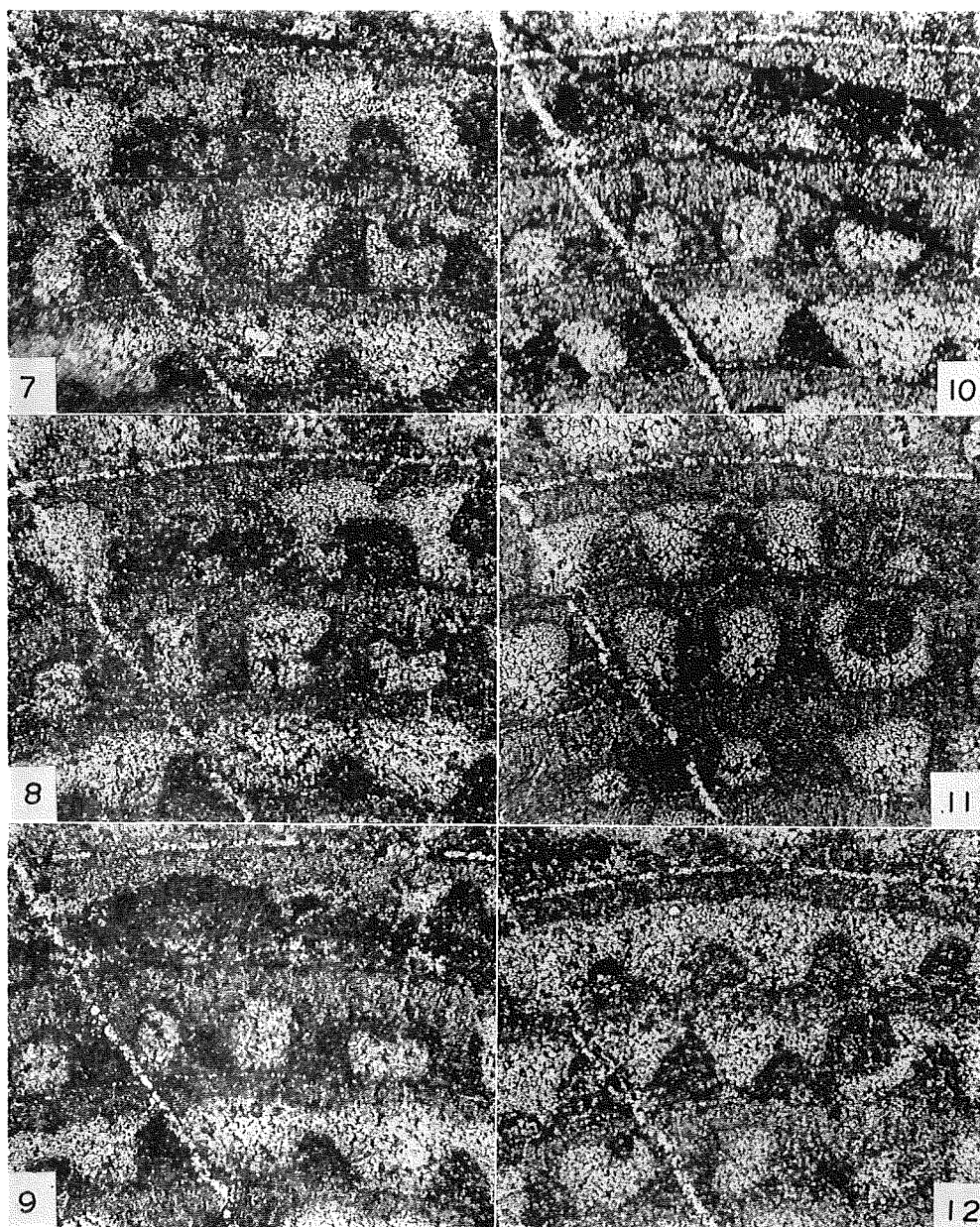


Photo. S. HONJO