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ULTRASTRUCTURE AND MORPHOLOGICAL STUDIES OF EARLY MESOPROTEROZOIC *CHUARIA CIRCULARIS*: A CASE STUDY FROM THE VINDHYAN SUPERGROUP

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ABSTRACT

Specimens of *Chuaria circularis* occurring in the Suket Shale of the Vindhyan Supergroup are subjected to Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Transmitted/Reflected Light Microscopy (LM). Contrary to the previous SEM and TEM studies of *C. circularis* from Rampura that reported no radial pores, our observations conform to the presence of distinct radial canals penetrating the wall and single layered cell wall structure. The thickness of the wall is almost the same in ultra thin sections of the well preserved *C. circularis* from the Rampura area. Post preservation, the lower and upper layers are continuous, parallel and very close to each other. Both the parallel running layers have characteristic structural properties of organic matter i.e. density, robustness and texture. The details on surface ornamentation and the cell wall structure of *C. circularis* are provided. Presence of the radial canals and the trabecular ultrastructure should be treated as an additional taxonomical attributes for *C. circularis*.

Keywords: *Chuaria circularis*, Suket Shale, Semri Group, Vindhyan Supergroup, India, Mesoproterozoic

INTRODUCTION

Observations on the carbonaceous macrofossils recorded from the Precambrian strata are mostly based on the Light Microscopic (LM) studies. And the ultrastructure studies of such carbonaceous remains are limited. Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) studies have an advantage over the LM. SEM provides the information regarding the details of surface morphologies, microstructure and the wall surface microelements. TEM provides insight into the cell wall ultrastructure. Widely distributed Precambrian discoidal form *Chuaria circularis* owes its description to Walcott (1899) that was subsequently emended several times. Ford and Breed (1973) provided complimentary morphological information on the genus and its type species. Detailed synonymies of the genus and species include those of Hofmann (1976, 1992), Duan (1982), Maithy and Shukla (1984), Vidal and Ford (1985), Jankauskas (1989), Steiner (1994), Talyzina (2000). In these papers authors debated the 'organic nature', 'acid resistance, and the size limit of the microfossils and in general the morphology and the cell wall ultrastructure of the forms', 'lower size limit at 1 mm' etc. about *C. circularis*. We confined our studies to *C. circularis* and considered only those carbonaceous remains as *C. circularis* which are acid resistant and extractable by palynological processes; the size range criterion proposed by Jankauskas (1989) appears to be arbitrary and therefore not adopted in the present study.

SEM and TEM studies have been conducted to gain additional information on the morphological features of these carbonaceous macrofossils. The study suggests that all the discoidal forms described in literature as *Chuaria* may not in fact be *Chuaria*. Among the circular discs, specimens with radial canals indicate that the populations of discs are constituted of at least two distinct biological entities.

PREVIOUS STUDIES

The first record of carbonaceous discs from the Vindhyan rocks derives from the Suket Shale of the Semri Group in Rampura locality of Madhya Pradesh (Jones 1909). Since beginning, these discs were interpreted variously, and subsequently established as *Chuaria circularis*-an acritarch taxon. Various carbonaceous remains are known from the Vindhyan Supergroup of central India, including carbonaceous macrofossils viz. *Tawuia*, helically coiled macrofossils described as *Katnia singhii* and *Grypania spiralis* are well known from the Semri Group (see Kumar, 1995).

C. circularis is one of the few carbonaceous remains on which ultrastructure studies have been attempted. The earliest ultrastructure studies by Jux (1971) have been performed on some Palaeozoic baltisphaerids and related that it has a thin wall that was characterized by a thin marginal zone of condensed material and densely spaced system of radially arranged canals. Later, Jux (1977) conducted SEM and TEM studies on the topotype material of *C. circularis* from the Chuar Group, Arizona, USA and compared them with that of the Middle Devonian acritarchs *Tasmanites* from Brazil and the Upper Devonian *Tapajonites* from the Ohio state, USA. Those SEM studies demonstrated no lamellar structure; however, trabecular ultrastructure and rare canal-like structure were recorded by TEM at high magnification (X 38,000) in the Chuar Group material.

SEM studies of *Chuaria* sp. from the Pendjari Formation, Benin and Burkino-Faso, West Africa by Amard (1992) showed three new features viz. considerable wall thickness, a lamellar ultrastructure of the wall and a canal like structure in the cell wall. At the same time TEM studies did not reveal the presence of trabecular ultrastructure as reported by Jux (1977). TEM studies on *C. circularis* recorded from the Visingsö Group demonstrated that the vesicle wall is single-layered, electron dense and homogenous (Talyzina, 2000, p. 129). But the holes present in the fossil wall, however, were considered to be caused by degradation (Talyzina, 2000, p. 128). She mentioned that these features are insufficient for a firm biological classification. Similar studies were carried on spherical vesicles known as *Tasmanites* recorded from the Lower Cambrian Lükati Formation, Estonia (Talyzina and Moczydłowska, 2000). These

†Deceased

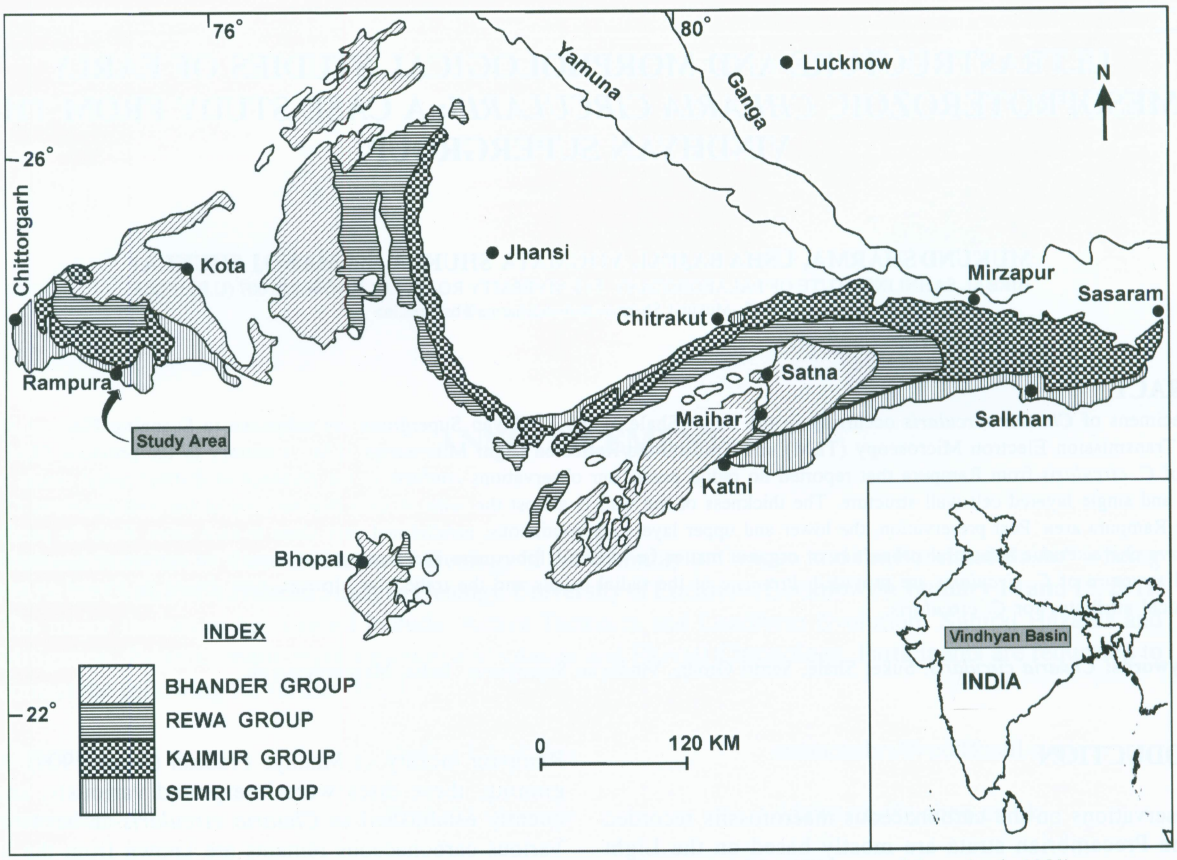

Fig.1. Generalized geological map showing distribution of the Vindhyan Supergroup (after Krishnan and Swaminath, 1958).

studies demonstrated clearly the presence of abundant small pores distributed evenly but without any determined pattern. TEM observations on the same specimen suggest that the vesicle wall is single-layered, electron dense and homogeneous.

TEM studies of leiosphaerids from the Roper Group by Javaux *et al.* (2004) showed that they comprise single and multi-layered forms thereby representing distinct clades, implying a level of taxonomic diversity. Arouri *et al.*, (2000, p. 80, 82) suggested that *C. circularis* is single-layered and recorded absence of multilayered wall under TEM study. Similar observations on wall structure were noted by Peat (1981), Talyzina and Moczydłowska (2000). Dutt *et al.* (2006) conducted SEM and TEM on *C. circularis* of the Suket Shale of Rampura of Semri Group, Vindhyan Supergroup. SEM studies revealed no

Table 1 Recent radiometric dates of different horizons of Lower Vindhyan

Formation	Geographical Position	Method	Age	Reference
Rohtas Limestone	Tikaria, Katni, M.P.	Pb-Pb, isochron	1599 ± 48 Ma	Sarangi *et al.* 2004
Rohtas Limestone	Different localities in Son Valley, M.P. & Rajasthan	Pb-Pb, isochron	1601 ± 130 Ma	Ray *et al.* 2003
Rampur Shale	Sidhi district, M.P.	SHRIMP, U-Pb, Zircon	1599 ± 8 Ma	Rasmussen *et al.* 2002
Rampur Shale (Tuff Bands)	Sidhi district, M.P.	SHRIMP, U-Pb, Zircon	1602 ± 10 Ma 1628 ± 12 Ma	Rasmussen *et al.* 2002
Deonar Formation (Two rhyolitic volcanic horizons)	Sidhi district, M.P.	U-Pb, Zircon, $^{86}Sr/^{86}Sr$ Isotope	1631 ± 1 Ma 1631 ± 5 Ma	Ray et al. 2002
Deonar Formation (Porcellanite Formation)	Sidhi district, M.P.	SHRIMP, U-Pb, Zircon	1628 ± 8 Ma	Rasmussen *et al.* 2002
Base of Semri Group	Chitrakoot area, U.P.	Rb-Sr Model ages	1409 ± 14 Ma to 1531 ± 15 Ma	Kumar *et al.* 2001
Basement rocks	Bundelkhand Granite	Pb-Pb Zircon (SIMS)	2492 ± 10 Ma	Mondal *et al.* 2002

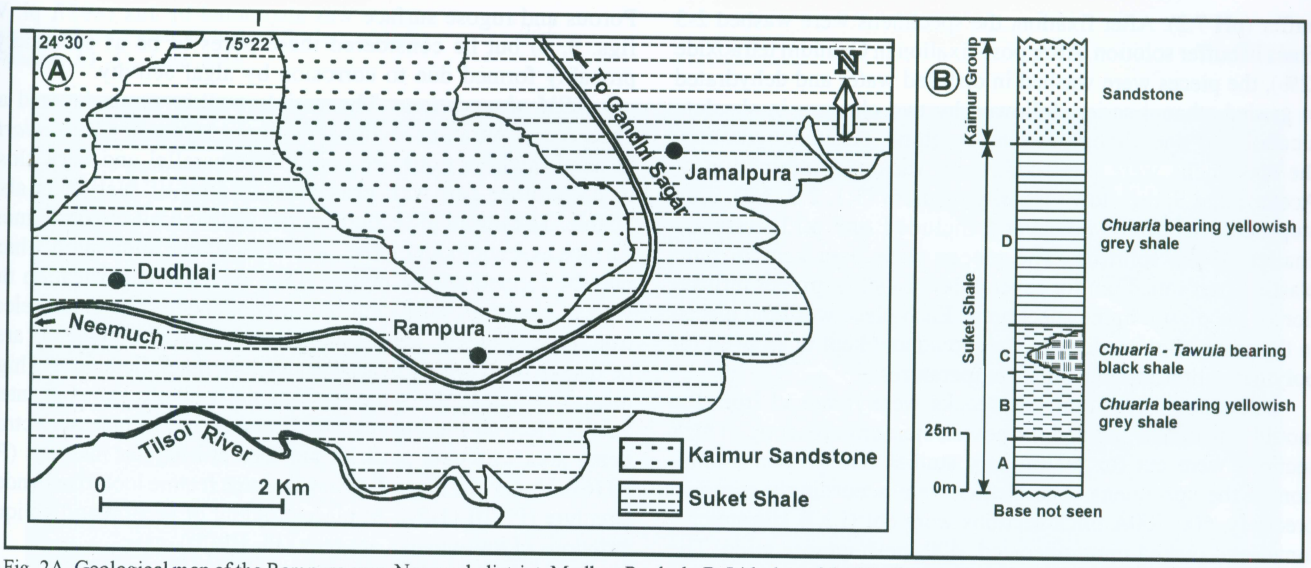

Fig. 2A. Geological map of the Rampura area, Neemuch district, Madhya Pradesh; B. Litholog of the Vindhyan succession exposed at Rampura (A, B after Kumar, 2001).

spiral ornamentation on the surface of *C. circularis* except of a few pits which were interpreted as impression of sediments grains or bio-degradational marks. According to their SEM studies, the internal wall structure of *C. circularis* seems to be single layered, whereas the TEM studies suggest the presence of lamellar structure. However, the authors are not sure of these features and mentioned that these lamellar structures might have formed during the sectioning process. Fine network of trabecular ultrastructure of wall is also absent.

A review of different studies mentioned above shows no consistency on the understanding of the ultrastructures of the carbonaceous remains. Considering the importance of the early fossils, the ultrastructure studies have been undertaken to show more details on the subject.

GENERAL GEOLOGY AND AGE

The Vindhyan Supergroup is well exposed in central India (Fig. 1). Sedimentary rocks constituting the supergroup are unmetamorphosed and tectonically mildly deformed. This supergroup unconformably overlies the Bundelkhand massif and slightly metamorphosed Bijawar Group (~2500 Ma, Crawford and Compston, 1970; Mondal *et al.*, 2002). The Vindhyan sediments comprise of a thick pile of sandstone, porcellanite, shales and limestone. The entire succession is divided into four groups, namely the Semri, the Kaimur, the Rewa and the Bhander in ascending order. The Semri Group is traditionally known as Lower Vindhyan whereas other three groups are collectively referred to as Upper Vindhyan. Each group is further divided into formations and members. The Semri Group comprises of the Deoland Formation, the Arangi Formation, the Kajrahat Limestone, the Deonar Formation, the Koldaha Shale, the Salkhan Limestone, the Rampur Formation, the Rohtasgarh (Rohtas) Limestone and the Bhagwar Shales in ascending order. The Rohtas Limestone is exposed along the southern flank of the Vindhyan basin in the Son Valley area. The Suket Shale is generally correlated with the Rampur Shale in the Son Valley (Bose *et al.*, 2001).

In Rampura locality, the Suket Shale occupy the low lying areas. The main lithologies of the Suket Shale are green shales, grayish yellow shales, black shales, siltstone and light brownish gray sandstones. *C. circularis* specimens are recovered from black as well as yellow shales (Figs. 2 A, B). Kumar (2001) suggested that the Suket Shale-Kaimur sandstone succession represent a subtidal lagoonal deposit overlain by coastal sand complex.

The U/Pb SHRIMP age dating of the zircon crystals recovered from the ash beds at the top of the Rampur Shale has yielded an age ~1.6 Ga (Rasmusssen *et al.*, 2002), while the *Grypania*-bearing Rampur Shale of the Katni area provide ~1.6 Ga (Pb/Pb technique, Sarangi *et al.*, 2004). No dates are available for the Suket Shale of the Rampura area. However on the basis of the correlation, the Suket Shale can be estimated at roughly ~1.6 Ga old (See Table - 1 for summary of the radiometric dates of the Semri Group).

MATERIAL AND METHODS

C. circularis bearing samples were collected from the Suket Shale of the Semri Group extended over the vast tract of eastern to central Son Valley (Fig. 2A). Samples were processed to take out the specimens. Well preserved carbonized specimens were encircled by 3-4 mm high carnauba wax rim, hydrofluoric acid (40 %) was poured into the encircled rimmed area and left for 5 minutes. Due to controlled maceration, carbonized *C. circularis* was liberated from the bedding surface and floated on the acid. All completely detached specimens were collected with the help of single hair brush, washed with distilled water and quickly dried by compressed air. Some of the individual specimens were mounted on glass slides for light microscopic studies and others were mounted on aluminium stubs and coated with 22 nm layer of gold palladium coating for SEM studies. Specimens were studied using Phillips 505 microscope. Similarly individual specimens were separately treated for TEM studies as mentioned below.

For preparing ultra thin sections, individual carbonaceous discs of *C. circularis* were kept in 40 percent HF to remove last vestiges of silica particles (Maheshwari and Bajpai, 1996). No further acid or alkali treatment followed to avoid any possible alteration in the structural pattern on the surface. After thorough washing in distilled water the specimens were fixed overnight in Gluteraldehyde (4%) prepared in 0.1M Cacodylate

buffer (pH 7.2). After fixation, the specimens were washed 2-3 times in buffer solution. After post-fixation in Osmium tetraoxide (2%), the pieces were washed in distilled water and dehydrated in graded ethanol series, followed by two changes in absolute alcohol and one change in pure Acetone. After dehydration, the specimens were passed through various combination of Acetone and Spurr's low viscosity medium (3:1, 2:2, 1:3), each step being of 10-12 hours and included one mid -duration change of the solution. The pieces were then kept in pure plastic Spurr's medium for several days to allow proper infiltration of the plastic in the specimens. Each piece was then placed in the embedding medium in the mould and kept in an oven for polymerization at controlled temperatures.

After polymerization, the blocks were removed from the moulds, trimmed under low power stereomicroscope. Thick sections were cut for preliminary studies and proper orientation of the specimens. The blocks were accordingly trimmed precisely. 600-700Å thick sections were cut (LKB Ultramicrotome) and picked upon uncoated, 400 mesh copper grids. The sections were stained in aqueous uranyl acetate for 15 minutes and in lead citrate for 5 minutes. Sections were investigated under TEM Phillips 410 LS.

SYSTEMATIC DESCRIPTIONS

Group **ACRITARCHA**

Genus **Chuaria** Walcott 1899 emend. Vidal and Ford 1985.

Type Species ***Chuaria circularis*** Walcott 1899.

Locality: Rampura, Neemuch District, Madhya Pradesh, India.

Stratigraphic Position: The Suket Shale (= Rampur Shales), Rohtas Formation, the Semri Group, the Vindhyan Supergroup.

Chuaria circularis

(Pl. I, figs. 1-7; Pl.; II, figs. 1-6; Pl. III, figs. 1-6)

DESCRIPTION

Light Microscope observations: Circular to elliptical carbonaceous compressions and impressions on the bedding surface of yellow and black shales. Discs are smooth to concentrically wrinkled. A few specimens show irregular folds and 'V' shaped median rupture (opening). Rarely three dimensional or mineralized (pyritized) specimens are also noted. Diameter of discs ranges between 0.25 mm to 10 mm. Size distribution is unimodal with maximum at 3.5 mm for both shorter and longer diameters.

SEM observations: No characteristic ornamentation has been noted on the surface of vesicle except minor granulations, corrugation and undulations (Pl. I [1-3]). The cracked surfaces show lamellar features. Most of these are affected by degradational processes. At high magnification a rugose and porous surface is observed that seems to be communicating with inner body of the vesicle and between lamellae (Pl. I [4-6] and Pl. II [2, 5, 6]. Unlike several layers of lamellae reported by Amard (1992), we could notice two to three layers of lamellae. Porous and rugose surface was also noted by Jux (1977, pl. V, figs. 3, 4) but he considered these pores to be as secondary, probably formed due to corrosion by algal activity.

TEM observations: The vesicle wall is single layered as observed in ultrathin sections. Both the layers of *C. circularis* in semi-thick sections are continuous parallel and very close to each other and well-preserved folding of section is also visible. The ultrathin sections of the wall exhibit the outer most surface as smooth and intact, at places shows undulation which may be formed during preservation. At high magnification the wall is electron translucent and homogeneous. At places electron dense area with some dotted area is seen, it may be the area of darker band which is seen under low magnification. At high magnification, in the same sections the wall structures at intervals is electron translucent, homogenous and at places electron dense area with dark particles are seen at irregular intervals (Pl. III (6)). Under low magnification the same feature look-like banded structure (Pl. III (1-5)). At places folding of section and oblique striations of knife marks are seen (Pl. III (5)).

Jux (1977) made very critical observations on TEM studies of *C. circularis* at high magnification (X 38,000). He recorded fine network, trabecular ultrastructure of the wall which was considered as fundamental feature. Such features, though not observed in Pendjari Formation specimens (Amard, 1992), are noticed in the Suket Shale *Chuaria* (Pl. III (6)). Though rarely, Jux (1977, pl. 5, fig. 3) in TEM studies observed, canal like structures of 0.1-0.2 μm in diameter. There is no agreement among other workers on these 'canal like features' to be original and genuine feature and not the artifacts. We have also noted such features in the Suket *Chuaria* at very high magnification (X 38,000), but do not consider them as primary.

CONCLUSIONS

LM, SEM and TEM observations on *C. circularis* extend our knowledge of morphology and ultrastructure of these forms. Our studies on ultrastructure of *C. circularis* reinforce another parameter on wall structure that should be treated as further emendation on the taxonomy of the genus and species. Despite a large inventory on the morphology and taxonomy of Precambrian micro and macrofossils, ultrastructure studies are only few and the present study provides much needed supplementary information. Ultrastructure studies are also required to trace the early history of eukaryotic diversification, through the recording of complexity at the level of wall structure and surface ornamentation.

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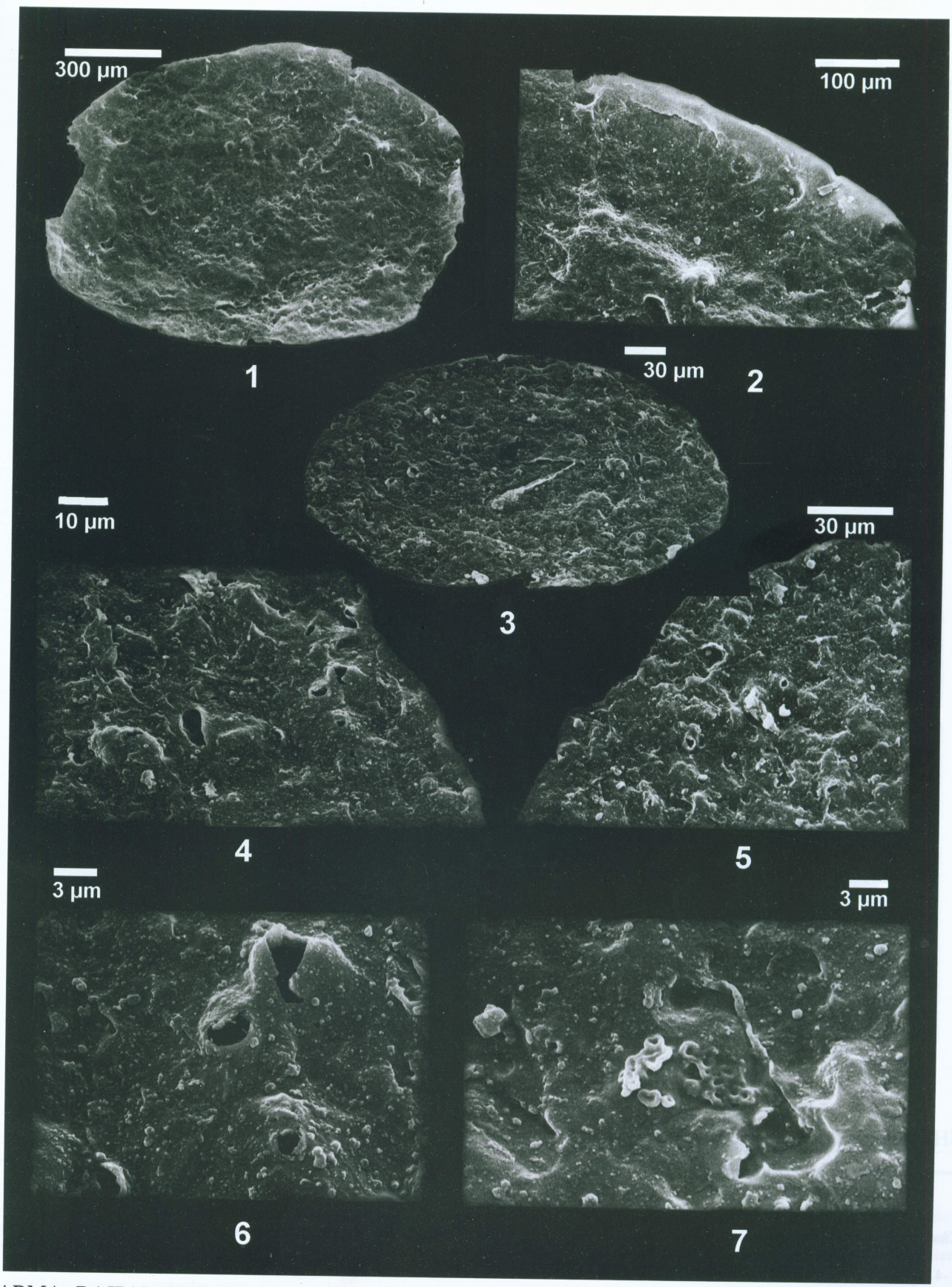
EXPLANATION OF PLATE I

Chuaria circularis from the Suket Shale, Rampura, Neemuch district, Madhya Pradesh.

1-3. SEM showing granulated, corrugated and rugose surface with light and dark areas with few folds.

4-7. Gradually enlarged images of surface area shown in 1-3. these figures show the pores and folds. Pores are nearly circular, randomly present on the surface.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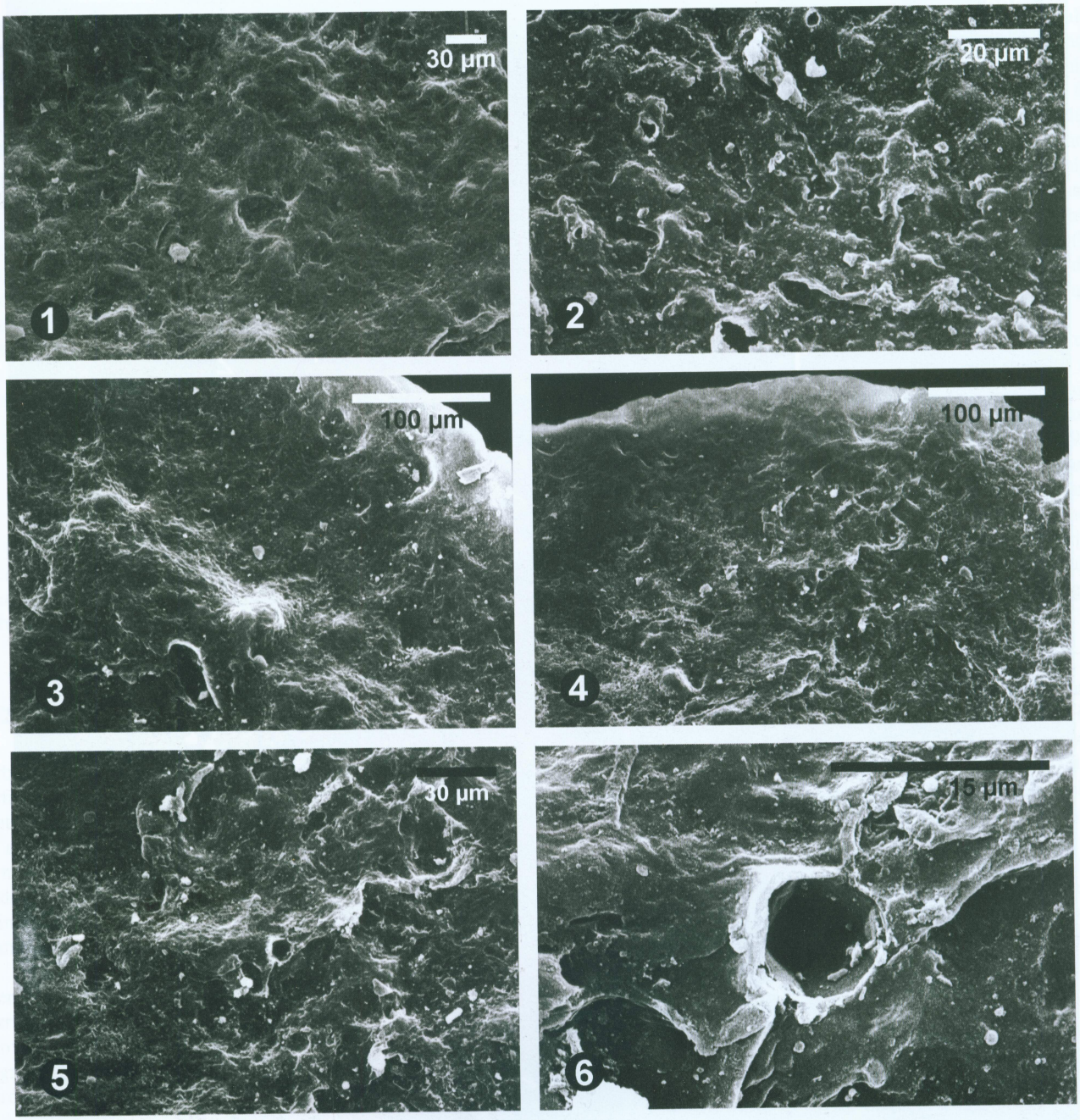
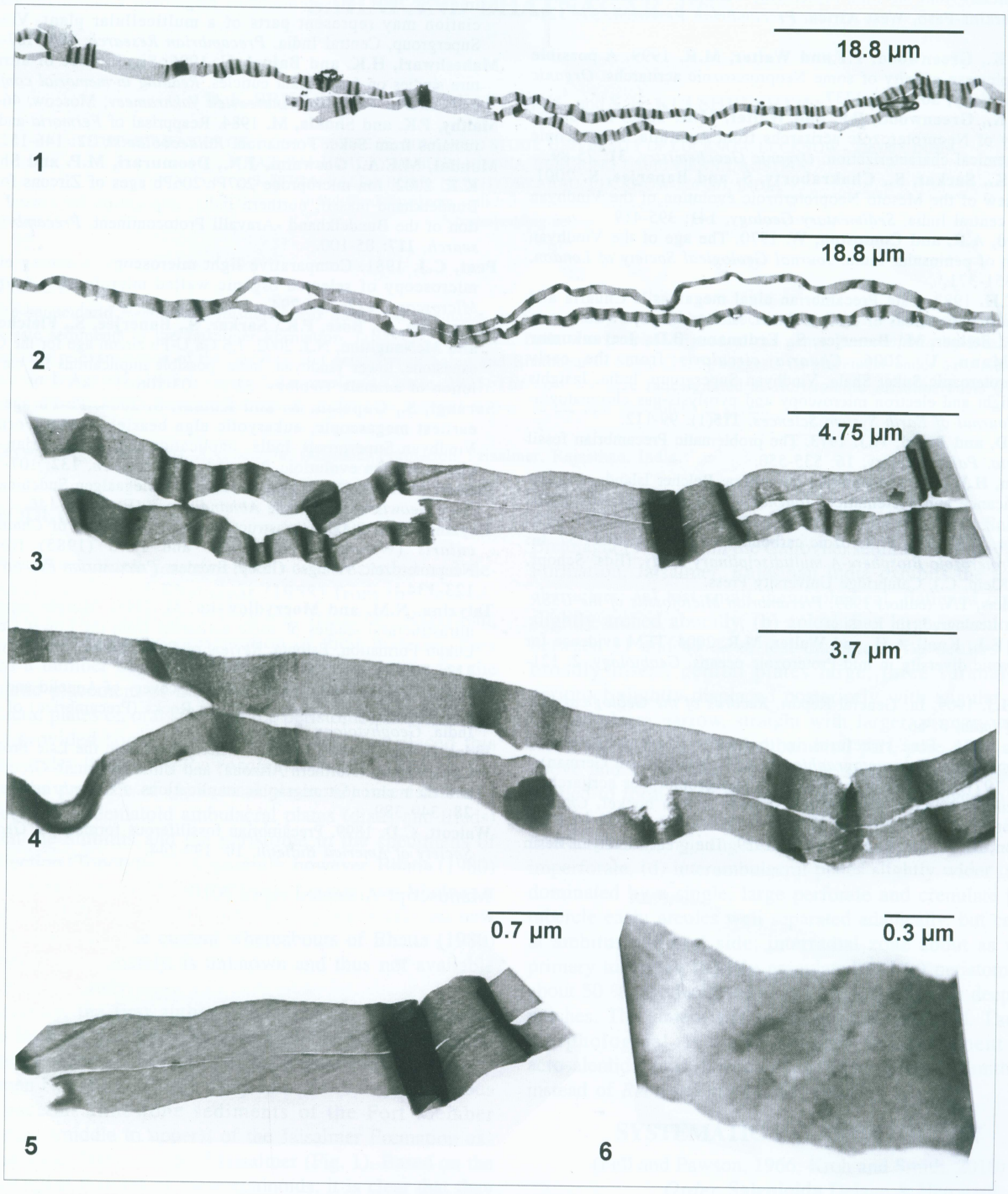


EXPLANATION OF PLATE II

Chuaria circularis from the Suket Shale, Rampura, Neemuch district, Madhya Pradesh.

1-4. SEM showing granulated, corrugated and rugose with multilayered surface.

5-6. SEM showing gradual enlargements of the pore structure showing nearly circular outline.



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EXPLANATION OF PLATE III

TEM images of ***Chuaria circularis*** from the Suket Shales, Rampura, Neemuch district, Madhya Pradesh. Boxes indicate the area, with number, of the figure magnified in subsequent figures.

1-2. TEM images of ***C. circularis*** showing single layered vesicle wall with folds present at irregular interval.

3-5. TEM images show the intact, smooth outer surface at places show undulations that were formed during preservation.

6. TEM at high magnification shows the wall is electron translucent and homogeneous.

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