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Confocal Laser Scanning Microscopy (CLSM) of newly recovered microfossil assemblage from the Kurnool Group, South India: New insights on microfossil morphology

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Morphological studies were conducted on the newly recovered organic-walled microfossils being reported for the first time from the Neoproterozoic Owk Shale of the Kurnool Group, South India. New observations are made using CLSM studies on the *Valeria lophostriata, Plicatidium latum* and *Octoedryxium truncatum* and previously reported *Obruchevella.* New information is added to the original taxonomical attributes of these entities. Presence of intricate morphological ornamentation is linked to the evolutionary adaptations for complex mode of life. The presence of *Octoedryxium truncatum* and abundance of *Obruchevella* is also discussed to strengthen the arguments that the fossil-bearing horizon is Neoproterozoic in age. The study also demonstrates the advantage of Confocal Laser Scanning Microscopy (CLSM) over the conventional Optical Microscopy observations in the microfossils studies.

Keywords: Organic-Walled Microfossils, Confocal Laser Scanning Microscopy, Owk Shale, Kurnool, Neoproterozoic, India

ARTICLE HISTORY

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INTRODUCTION

In the past two decades, advances in analytical techniques including several high spatial resolutions and holistic imaging techniques, such as Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Focused Ion Beam preparation; and in situ nanoscopic studies, Nano Sims, Scanning Tunneling Microscopy, and Atomic Force Microscopy allowed the researchers to determine the morphology more accurately in comparison to transmitted light Optical Microscope (OM) (Kempe *et al*., 2002; Schopf *et al*., 2008; Javaux *et al*., 2010; Kremer *et al*., 2012; Sharma *et al.,* 2009; Singh and Sharma, 2014; Schopf *et al*., 2016; Delarue *et al*., 2018; Singh *et al*., 2019a). A few researchers attempted the use of such techniques to study both Proterozoic and Archaean material, revealing details of the complex architecture of the Precambrian biosphere. These tools provide new insights on the morphology and ultrastructure of different microfossils (Vidal, 1976, 1979; Butterfield *et al*., 1994; Arouri *et al*., 1999; Javaux *et al*., 2003; Kempe *et al*., 2005; Moczydłowska and Willman, 2009; Sharma *et al*., 2009; Peng *et al*., 2009; Javaux *et al*., 2013; Tang *et al*., 2013; Agić *et al*., 2015; Tang *et al*., 2015; Singh and Sharma, 2014; Agic *et al*., 2017; Loron *et al*., 2019; Li *et al*., 2019). In the last two decades the non-destructive and non-intrusive Confocal Laser Scanning Microscope (CLSM) technique, allowing *in situ* analysis of the microfossils, has been applied to document the high-resolution three-dimensional morphology of the poorly preserved taxa (Schopf *et al*., 2011). As compared to conventional optical microscopy, CLSM has a depth-discriminating property and three-dimensional reconstruction function. This allows it to perform three-dimensional imaging for the accurate analysis of the spatial structures of the fossils (Teng *et al*., 2020). There are several CLSM studies of Precambrian fossils carried out on thin sections (Schopf *et al*. 2006; Chi *et al*. 2006; Schopf *et al*., 2008, 2011, 2016; Hackley and Kus, 2015; Czaja *et al*., 2016; Schopf and Kudryavtsev, 2009; Herisse *et al*., 2017, Guo *et al*., 2018; Singh *et al*., 2019a). However, there are only a few reports of studies carried out on the macerated material (Kempe *et al*., 2005; Singh *et al*., 2019a). We conducted Optical Microscopy and CLSM studies on the newly recovered microfossils taxa from the Owk Shale of the Kurnool Group South India, *i.e.*, *Valeria lophostriata, Plicatidium latum, Octoedryxium truncatum, Obruchevella delicata,* and *Obruchevella parva*, which provides insights into the evolutionary advancements (morphological), their cellular complexity and the visualization of the different structural features in three dimensions (3D).

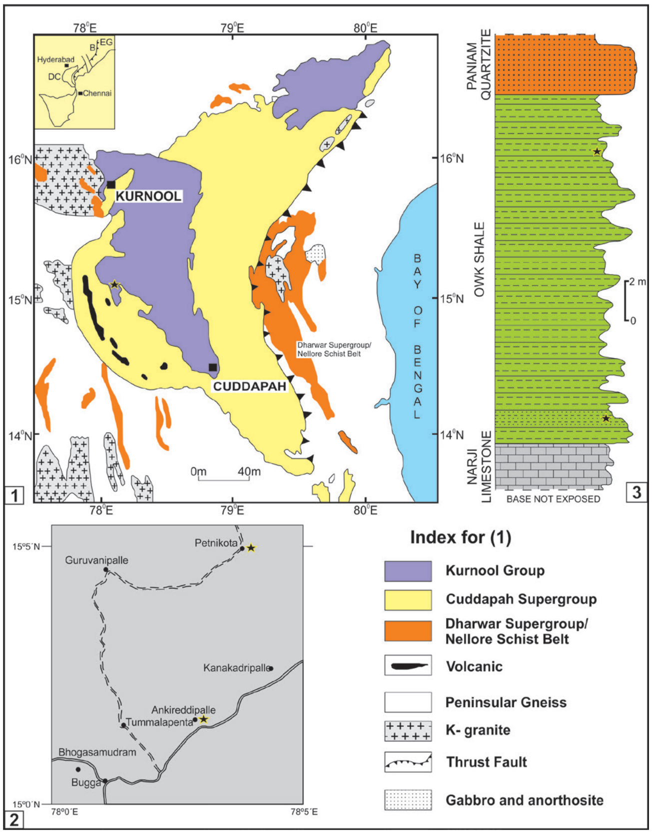


Fig. 1. 1.1 Generalized geological map of the Kurnool basin (after Mishra, 2011), inset: Bhandara Craton and DC – Dharwar Craton separated by Godavari Graben and EG – Eastern Ghat; 1.2 Geographical localities of the fossil yielding areas in Ankireddipalle village, Kurnool district, Andhra Pradesh; 1.3 Lithostratigraphic succession exposed at Ankireddipalle village showing the Narji Limestone, Owk Shale and Paniam Quartzite. Stars indicate the carbonaceous fossils yielding levels.

GENERAL GEOLOGY AND AGE

Stratigraphic framework

Cuddapah Basin is a distinct N-S trending crescent-shaped basin in the southern part of India (Fig. 1). The southern and western boundary of the Cuddapah Basin is marked by the Eparchaean unconformity where it rests on the granite-greenstone terrain of the Eastern Dharwar Craton (EDC) and in the eastern part, it is concave and is marked by the prominent boundary thrust parallel to the Nellore Schist Belt (NSB), Eastern Ghats Mobile Belt (EGMB) and East Coast of India (Nagaraja Rao *et al*., 1987). King (1872) provided the earliest comprehensive lithological record of the Cuddapah Supergroup, and a century later it was revised, and additional information was provided (Meijerink *et al*., 1984; Nagaraja Rao *et al*., 1987). The Cuddapah Supergroup has been broadly divided into three groups, namely the Papaghani Group, Chitravati Group, Nallamalai Group (Nagaraja Rao *et al*., 1987). The Kurnool Group is the youngest group of the Cuddapah Basin occupying a large area and deposited with angular unconformity. It has been subdivided into six formations, which in stratigraphic order are: the Banganpalle Quartzite, Narji Limestone, Owk Shale, Paniam Quartzite, Koilkuntla Limestone, and Nandyal Shale; and attain a thickness of about 450 m (Nagaraja Rao *et al*., 1987; Fig. 2 and Table-1). The Banganpalle Quartzite forms the basement of the Kurnool Group which is overlain by massive to flaggy Narji Limestone. It is followed by the buff, purple and khaki-Coloured Owk Shale unit. These three litho-units constitute the first cycle of deposition; the second cycle of sedimentation is marked by the occasional conglomerate at the base of the Paniam Quartzite, overlain by siliceous to flaggy Koilkuntla Limestone which gradually passes into the purple coloured Nandyal Shale.

Table 1. Generalized lithostratigraphy of the Kurnool Group (after Nagaraja Rao *et al.,* 1987).

Geological Unit	Formation	Thickness of the Unit
Kurnool Group	Nandyal Shale	(50–100 m)
	Koilkuntla Limestone	(15–50 m)
	Paniam Quartzite	(10–35 m)
	Owk Shale	(10–15 m)
	Narji Limestone	(100–200 m)
	Banganpalle Formation	(10–50 m)
	------------- ---- Unconformity ------------- ----	
	Cuddapah: Srisailam Quartzite	

Age

The Banganpalle Quartzite of the Kurnool Group is a diamond-bearing unit. The source of these diamonds is considered to be the Vajrakarur Kimberlite, occurring in the nearby area, which has been dated as 1140 Ma (Crawford and Compston, 1973). Therefore, the Kurnool Group sedimentation is believed to be younger than 1140 Ma. However, Dongre *et al*. (2008) found limestone xenoliths included in the adjoining Siddanpalle Kimberlite. It is considered that during the emplacement of this kimberlite, only two sedimentary successions having limestone units were present in the vicinity, i.e. the Bhima Group and the Kurnool Group (~50 Km apart). They suggested that the limestone xenolith may be the remnants of either of the limestone sequences and suggested that the Kurnool Group is Late Mesoproterozoic age (> 1090 Ma). Raman and Murthy (1997) proposed the minimum age of 500 Ma (K/Ar) and maximum age of 980 Ma (Rb/Sr) for the shales of the Kurnool Group. Gururaja *et al*. (2000) based on certain fossils, proposed that the Pc-C boundary lies within the Kurnool Group. At present, the Kurnool Group is considered to be Neoproterozoic in age (Sharma and Shukla, M., 1999; Sharma and Shukla, Y., 2012, 2016; Shukla *et al*., 2020).

MATERIALS AND METHODS

The present study is based on the microfossils recovered from the Owk Shale of the Kurnool Group present in south India. The samples of the Owk Shales were collected from Ankireddipalle (15°07′; 78°03′) village of the Kurnool

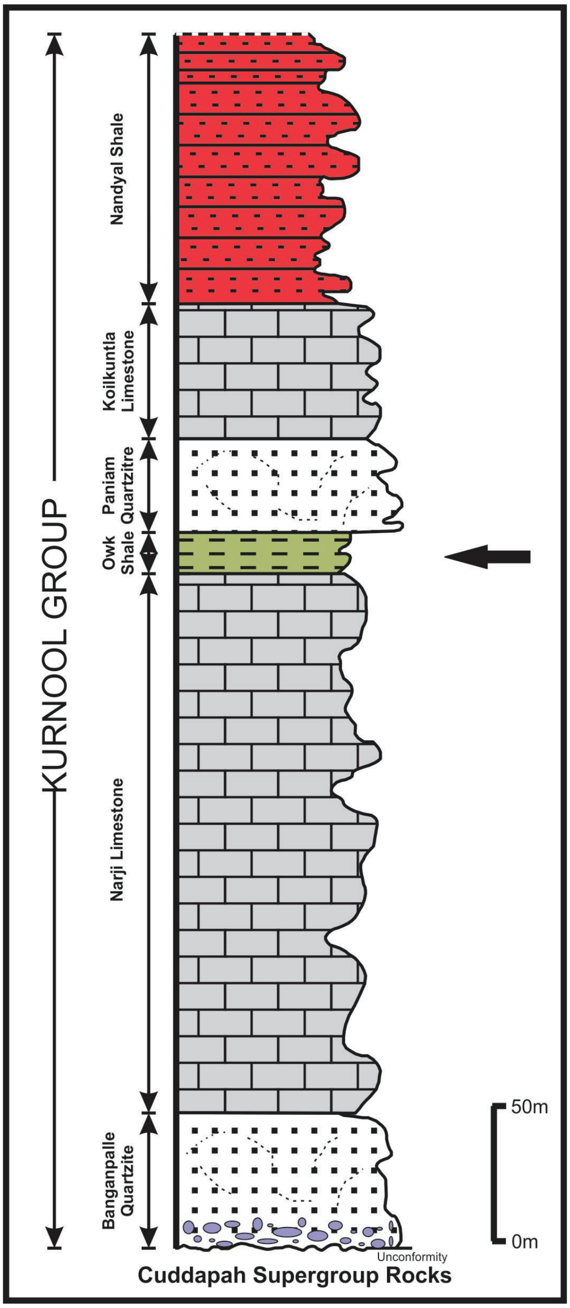


Fig. 2. Generalized lithostratigraphic succession of the Kurnool Group, lying above the Cuddapah Supergroup rocks (after Nagaraja Rao *et al.*, 1987). Arrow shows the studied horizon.

district of Andhra Pradesh in south India. At this spot, the thickness of the section is 15 m, and it is exposed on the eastern part of the hillock Δ1445. The samples of shale were macerated by refined palynological maceration techniques devised by (Grey, 1999). The organic residue so obtained was mounted on the glass slide with the help of PVC and Canada Balsam (R.I. = 1.5).

Optical microscopy (OM)

About 60 palynological slides of the Owk Shale fossils were examined and studied under the Optical Microscope Olympus BH2 at 100X (under oil immersion lens), for documenting the morphological details of the microfossils. All the measurements of the microfossils described in this paper were made under the Cell Cense Standard Software, and photographs were taken with the microscope mounted software supported Olympus DP 26 digital camera. For all the described microfossil specimens in this paper, England Finder coordinates and slide numbers are provided in the parenthesis of each figure caption. All the slides containing demonstrated specimens are deposited in the repository of the Birbal Sahni Institute of Palaeosciences, Lucknow, India under Museum Statement Number 1536.

Confocal Laser Scanning Microscopy (CLSM)

CLSM was used with standard protocols (Schopf *et al*., 2006) to decipher additional morphological information at high spatial resolution and to generate the 3D image of the microfossils. Three-dimensional Confocal fluorescence imaging was acquired on Leica TCS SP8 Confocal Laser scanning biological microscope system equipped with two Melles Griot lasers, a 448 nm 20 mW-output argon ion laser, and a 633 nm 10mW-output helium-neon laser (Melles Griot, Carlsbad CA). The images were obtained using a 100X oil-immersion objective (Numerical Aperture = 1.4). The images acquired by this system were subsequently processed and examined on LAS-X imaging software.

PALAEOBIOLOGY OF THE OWK SHALE

The present study reports the presence of *Octoedryxium truncatum*, *Valeria lophostriata* and *Plicatidium latum.* A few specimens of *Obruchevella, viz. O. delicata,*and *O. parva* are also examined. Morphological features are based on observations gathered through both the microscope, i.e. OM as well as CLSM studies.

SYSTEMATICS

Genus ***Valeria*** **Jankauskas, 1982, emend. Nagovitsin, 2009**

Type species ***Valeria lophostriata*** (Jankauskas, 1979) 1982

Valeria lophostriata (Jankauskas, 1979) Jankauskas, 1982
(Pl. I, Figs. 1-5)

Kildinella lophostriata Jankauskas, 1979, p. 153, fig. 1.13–1.15; *Valeria lophostriata*- Jankauskas, 1982, p. 109, pl. 39, fig. 2; Hofmann and Jackson, 1994, p. 24, pl. 17, figs. 14–15, pl. 19, fig.4; Nagovitsin, 2009, p. 144, Fig. 4E; Nagovitsin *et al*., 2010, p. 1195, Fig. 2.11; Tang *et al*., 2015, p. 315, fig. 11; Pang *et al*., 2015, p. 254–255, figs. 3A–H, 6C–E; Baludikay *et al*., 2016, p.174, fig.7H; Riedman and Porter, 2016, p. 875, fig. 4.1; Javaux and Knoll, 2017, p. 218, fig. 7.1–7.4; Agić *et al*., 2017, p. 119, fig. 12I. Miao *et al.,* 2019, fig: 11A-F

(For additional synonymy see Jankauskas *et al*., 1989, Javux and Knoll, 2017 and Miao *et al*., 2019)

Microscopic observations - Thin-walled vesicles, spheroidal to elliptical in shape with lanceolate folds. Vesicle size ranges from 90–123 µm (width), and 95–160 µm (length). Distinctive ornamentation of concentric ridges is visible on the inner surface of the walls. The ridges are almost evenly spaced (1 µm apart; Pl. I, Figs. 1, 4). The outer surface appears smooth in optical microscopy.

CLSM observations - The CLSM image shows that the outer surface of *Valeria* is uneven. It also confirms the very well developed striations as the small ridges of close to 1 µm in size on the inner wall of the vesicle (Pl. I, Figs. 2-3, 5).

Materials - Six well preserved and a few broken specimens have been observed in the shale samples of the Owk Shale from the Kurnool Group, the Cuddapah Basin, India.

Remarks - *Valeria* is a long-ranging taxa (Late Palaeoproterozoic to Cryogenian) with unique morphological ornamentation. The presence of concentric striations on the inner wall makes it easy to identify this species. The biomechanical analyses were conducted by Pang *et al*. (2015) on the *Valeria lophostriata* from the Ruyang Group. They suggested that the presence of these concentric rings is biologically programmed to perform the excystment mechanism.

Occurrence and stratigraphic range - This taxon occurs in the late Palaeoproterozoic (Javaux *et al*., 2004; Lamb *et al*., 2009; Peng *et al*., 2009) to the Cryogenian (Nagy *et al*., 2009) age geological formations of the world (Hofmann, 1999) and is even reported in some cases up to early Cambrian Gouhou Formation (518 Ma) North China (Tang *et al*., 2015; He *et al*., 2017). Late Palaeoproterozoic: Changcheng Group, North China (Miao *et al*., 2019); Late Palaeoproterozoic or early Mesoproterozoic: Ruyang Group in North China (Yan, 1995; Pang *et al*., 2015; Agić *et al*., 2017); Bylot Supergroup in Canada (Hofmann and Jackson, 1994); Mallapunyah Formation, Roper Group and Alinya Formation in Australia (Riedman and Porter, 2016; Javaux and Knoll, 2017); Mesproterozoic: Kamo Group, Zigazino-Komarovo Formation, Dashkinsky Formation, Tonian Ust'-Kirbin, Chapoma and Karuyarvinskaya Formations in Russian Federation (Jankauskas, 1979, 1982; Pyatiletov, 1980; Volkova, 1981; Samuelsson, 1997; Khabarov *et al*., 2002; Pavlov *et al*., 2002; Nagovitsin, 2009); Mbuji-Mayi Supergroup in Democratic Republic of Congo (Baludikay *et al*., 2016); Qaanaaq Formation in Northwest Greenland (Samuelsson *et al*., 1999); Batsfjord Formation in Norway (Vidal and Siedlecka, 1983); Greyson Formation and Chuar Group in USA (Vidal and Ford, 1985; Nagy *et al*., 2009; Porter and Riedman, 2016; Adam *et al*., 2017).

Genus ***Plicatidium*** **Jankauskas, 1980**
Type species ***Plicatidium latum*** **Jankauskas, 1980**

Plicatidium latum Jankauskas, 1980
(Pl. II, Figs. 1-5; Pl. III, Figs.1-6)

Plicatidium latum- Jankauskas, 1980, p. 109, 110, pl. 12, Fig. 15; Jankauskas, 1989, p. 139, pl. 41, Figs. 3 and 4; Veis *et al.,* 2000, pl. 2, Fig. 10; Sergeev *et al*., 2007, pl. 1, Fig. 19; Pang *et al*., 2015, Fig. 2A and 2B; Vorob'eva *et al*., 2015, p. 216, Fig. 6.6–6.9; Sergeev *et al*., 2016, p. 101, Fig. 6.10. Sergeev *et al*., 2017, p. 296, Fig. 7.1-3, 7.5.

Microscopic observations - Compressed, unbranched tubes with thin elastic walls having numerous cross-ribs parallel to each other. Incomplete tubes 132-184 µm in diameter, up to 126 µm long (incomplete specimen); tube wall translucent, medium-grained, 1 µm thick. Ribs opaque, 0.6–1.8 µm wide; distance between ribs is 0.6–2 µm (Pl. II, Fig. 1).

CLSM observations - The majority of specimens show that ribs present on the surface of the body are parallel but in some specimens they are intermingled too. So, the CLSM study confirms that the ribs were initially parallel and present on both sides of the body, but due to some preservational difficulties they appear intermingled or overlapping sometimes. The three-dimensional reconstruction also reveals that the ribs are present all around the body with 0.5-1.5 µm high relief (shown by the arrow in Pl. II, Fig. 3).

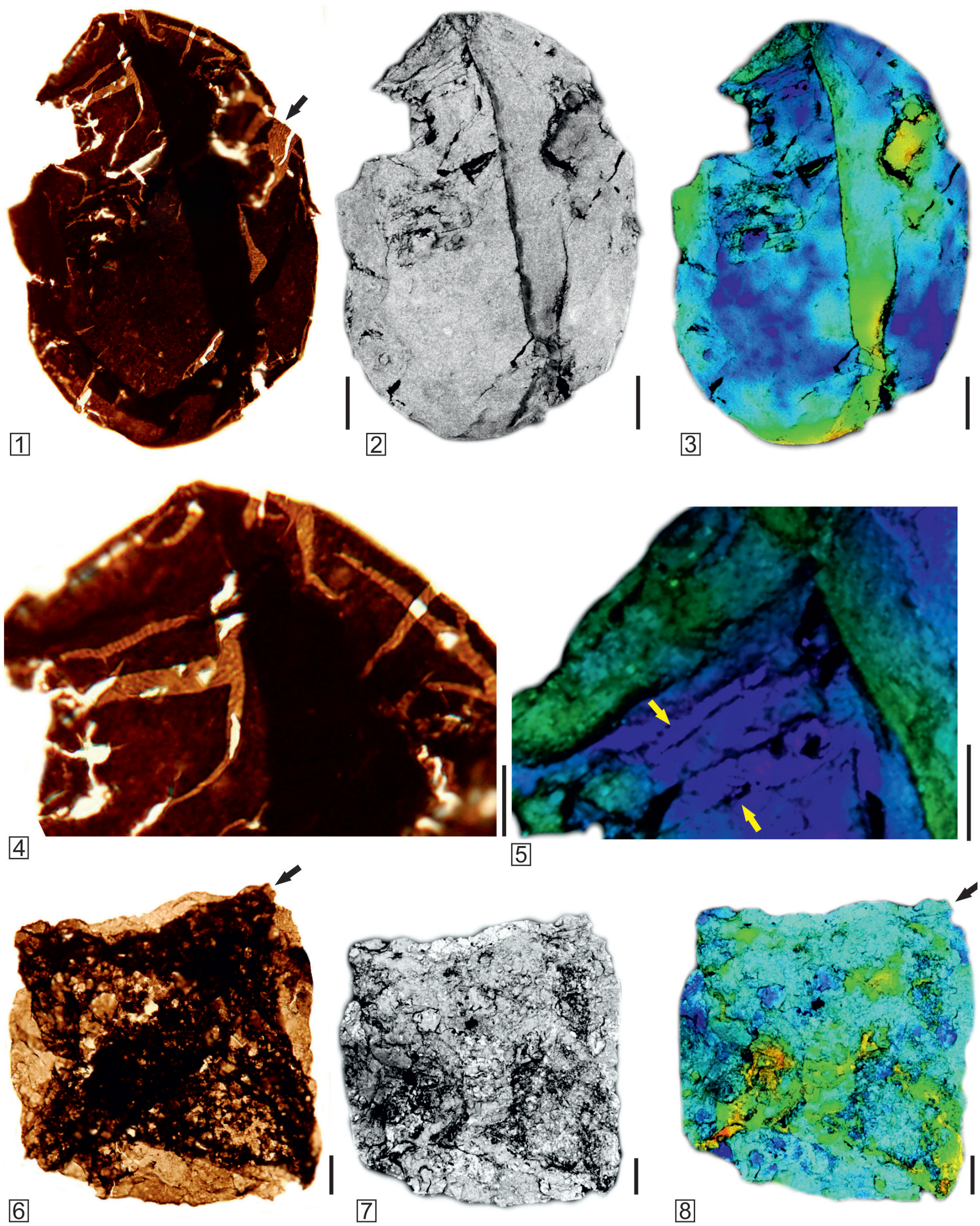
Material examined - 5 specimens, two well-preserved and three incomplete specimens.

Remarks - Pyatiletov (1988) and Butterfield *et al*. (1994) emended the genus by merging *Plicatidium* and *Rugosoopsis,* which was further supported by Moczydłowska (2008). However, Vorob'eva *et al*. (2015) considered them as two separate entities having different mechanical properties which reflect their biological affinity (elastic and rigid tubes, respectively; Vorob'eva *et al*., 2015). We are also inclined to treat *Plicatidium* as a distinct entity. Pang *et al*. (2015) suggested that the *Plicatidium* ribs are secondary in origin, formed as a result of dehydration and compaction of tubes as post-mortem decay. The CLSM study suggests that possibly the ribs present over the smooth sheath are primary (Pl. II, Fig. 3) and parallel (Pl. III, Fig. 4) in origin which otherwise appear intermingled or overlapping under optical microscopy.

Occurrence and stratigraphic range - Widely distributed in Proterozoic microfossils assemblages. This species has been reported from the different horizons of the world; Mesoproterozoic: Ruyang Group, North China (Pang *et al*., 2015); Kotuikan Formation, northern Siberia (Vorob'eva *et al*., 2015; Sergeev *et al*., 2007); Ust'-Il'ya Formation, northern Siberia (Sergeev *et al*., 2017).

Genus ***Octoedryxium*** **Rudavskaja, 1973**
Type species ***Octoedryxium truncatum*** **(Rudavskaja) Vidal, 1976**

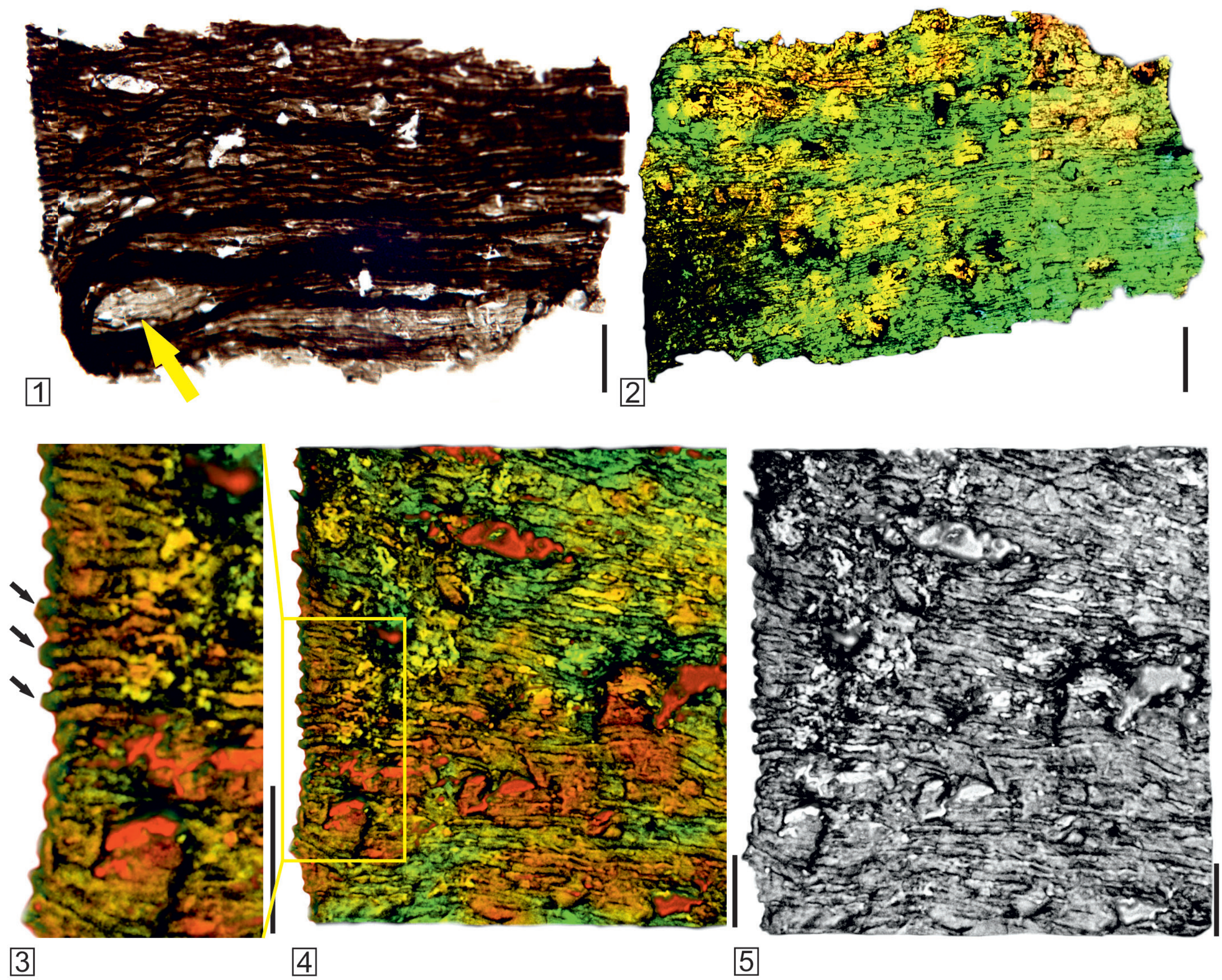
Plate I



EXPLANATION OF PLATE I

Plate–I. Photomicrographs of *Valeria lophostriata* and *Octoeodryxium truncatum* from Owk Shale, Kurnool Group under transmitted light Optical Microscope (OM) and Confocal Laser Scanning Microscope (CLSM).Optical photomicrographs (1, 4, 6), Confocal Laser Scanning micrographs (2, 3, 5, 7, 8). 3, 5, 4 are recorded under false colour imagery showing projected and depressed areas and 2, 7 are in grey colour CLSM image. (1-5) *Valeria lophostriata,* BSIP slide no.-16579, N41/1 (4-5) enlarged view of *Valeria lophostriata*; (6-8) *Octoeodryxium truncatum,* BSIP slide no. 16580, N26/4. Scale bar= 20µm for (1, 2, 3, 6, 7, 8); (4-5) Enlarged view, Scale Bar=10 µm.

Plate II



EXPLANATION OF PLATE II

Plate–II. Photomicrographs of *Plicatidium latum* from Owk Shale, Kurnool Group under transmitted light Optical Microscope (OM) and Confocal Laser Scanning Microscope (CLSM). Optical photomicrographs (1), Confocal Laser Scanning Micrographs (2, 3, 4, 5). 2, 3, 4 are recorded under false colour imagery showing projected and depressed areas and 5 is grey colour CLSM image. (1-5) *Plicatidium latum,* BSIP-16581, W51/1 (3-5) enlarged view of *Plicatidium latum*; (3) Enlarged view showing well developed plicatids. Scale Bar=10 μm (1-2) 20 μm; (3-5) Enlarged view Scale Bar=10 μm. (3) Arrow shows positive relief of Plicatids.

Octoedryxium truncatum (Rudavskaja) Vidal, 1976
(Pl. VI, figs.U-Z)
(Pl. I, Figs. 6-8)

Octoedryxium truncatum Rudavskaja, gen. et sp. nov., Rudavskaja, 1973, p.7, pl. l, fig.1-3; Vidal, 1976, p.22-25, fig. ll: A- P; Timofeev *et al*., 1976, p.49, pi. 19, fig. 2-5; Vidal, 1979, p. 22, pl. 2, figs. f, g; Vidal and Knoll, 1982, fig. 2E-F; Vidal and Siedlecka, 1983, p. 63, fig. 7E-G; Rudavskaja, 1989, p. 65, pl. VII, 2-6, 11, 13, 15, 16; Zang and Walter, 1992, Fig: Plate 6. K-P; Prasad and Asher, 2001, Pl 13; Fig: 5, 6 & 10; Vavrdová, 2006, p. 117, Figure 2.A-B; Moczydłowska, 2008, p. 10, Fig: 5A; Nagovitsin, *et al*., 2008, p. 425, Fig. 3d; Babu *et al*., 2009, Fig. 2B; Serezhnikova *et al*., 2014, p. 72, Fig: 6C; Ragozina *et al*., 2016, p. 1316; plate-1, Fig: 3.

Microscopic observations - Vesicle is octahedral with smooth or psilate surface texture; diagonal ridges observed; wall spongy or porous, occasionally with a slit-like aperture; corners are the square or blunt; the diagonal ridges are dark brown with in colour spongy appearance. The specimen is 171 μm long and 164 μm wide.

CLSM observation - CLSM image confirms the octahedral shape of the vesicle with diagonal ridges and reveals that the body of the *Octoedryxium* is spongy (Pl. I, Fig. 7 and 8)

Material - Single specimen.

Remarks - The species is distinguished due to its peculiar octahedral shape, spongy surface texture, and diagonal ridges. These features are very well seen in the Confocal Laser Scanning Microscopic images (Pl. I, Fig. 6-8). The spongy surface texture is distinct in the present specimen under the CLSM as well. The size of the specimen reported here is larger than the type specimen.

Occurrence and stratigraphic range - Neoproterozoic – Early Cambrian. Vidal (1976) and Vidal and Knoll (1983) reported *Octoedryxium* in abundance from the upper Visingsö Beds in Sweden, which is dated as ca. 700-610 Ma (Vidal, 1976). Later, *Octoedryxium truncatum* has been reported from several other localities: Liulaobei Formation of the Huainan Group, (Zang and Walter, 1992); Bahraich Group, Ganga Basin, India (Prasad and Asher, 2001); Měnín-1 borehole from southern Moravia (Vavardoa, 2006); Włodawa Formation on the Lublin Slope in Poland (Moczydłowska, 2008); from Member 3 of the Kandyk Formation of the Ui Group, Siberia (Nagovitsin *et al*., 2008); Gotan Limestone Formation, Bilara Group, Marwar basin (Babu et al., 2009); Maikhanuul Formation, Zavkhan basin, Western Mongolia (Serezhnikova *et al*., 2014); Tsagaanolom Formation of the Zavkhan Basin in western Mongolia (Ragozina *et al*., 2016).

Type species ***Obruchevella delicata*** **Reitlinger, 1948**
(Pl. IV, Figs. 1-2)

(For detailed synonymy see Sharma and Shukla, 2012)

Microscopic observation - Tubes empty, rarely septate, coiled into a regular cylindrical spiral; do not taper towards ends; walls usually jointed to one another or loosely adpressed; some spirals may twist in the opposite direction to the main spiral direction. Total 10 specimens was recorded. The diameter of the tubes ranges from 2 to 4 µm; the spiral outer diameter is 18-40 µm, the spiral length is up to 140 µm.

CLSM observations - CLSM studies revealed that these are very simple isodiametric tubular sheaths with open ends found intermingled with each other to form a cylindrical helix and occasionally septate (shown by the arrow in Pl. IV, Fig. 2). It also reveals that the tube walls are tightly joined to one another.

Remarks - Tubes of *Obruchevella* are interpreted to be either sheaths or trichomes transformed into empty structures. Due to diagenetic alteration, septa could rarely survive post-mortem degradation and observed in a few cases.

Age and distribution - Ediacaran (Vendian): Tinna Formation, Patom Uplift, Siberia; Nagod Limestone, India; Lower Cambrian: Sinna Formation, Patom Uplift, Siberia; Chulaktau Formation, South Kazakhstan; Yuhucun Formation, China (Song, 1984); Burgess Shales, Canada; Owk shale, Kurnool Group, India (Sharma and Shukla, 2012); Saradih Limestone, Raipur Group, Chhattisgarh Supergroup, India (Singh *et al*., 2019b)

Type species ***Obruchevella parva*** **Reitlinger, 1959**
(Pl. IV, Figs. 3-7)

Obruchevella parva Reitlinger, 1959, emend. Golovenok and Belova, 1989, emend. Burzin, 1995
(For detailed synonymy see Sharma and Shukla, 2012; Schopf *et al*., 2015)

Microscopic observation - Tubes are empty, sometimes with rare septa forming a spiral. The tubes do not taper towards ends. Total 20 specimens recorded. The diameter of the tubes ranges from 4 to 9 µm; spiral outer diameter is up to 70 µm, the spiral length is up to 150 µm, incomplete specimens. In comparison to *Obruchevella delicata, Obruchevella parva* is loosely coiled empty sheaths.

CLSM observation - CLSM studies also revealed that these are empty tubes sometimes with rare septa. The loose coiling of the tubes is also very well documented in the 3D reconstruction under the CLSM (Pl. IV, Figs. 4, 6-7).

Remarks - Sergeev *et al*. (2012) and Sharma and Shukla (2012) described in detail this species, as well as other species of the genus *Obruchevella*. We also agree with the observations made by them with regard to numerous emendations in *Obruchevella* species, especially of *O. parva* and *O. delicata* which have complicated the situation of the fossil cyanobacterium genus. Therefore, we followed the practice adopted by Sergeev *et al*. (2012) and Sharma and Shukla (2012).

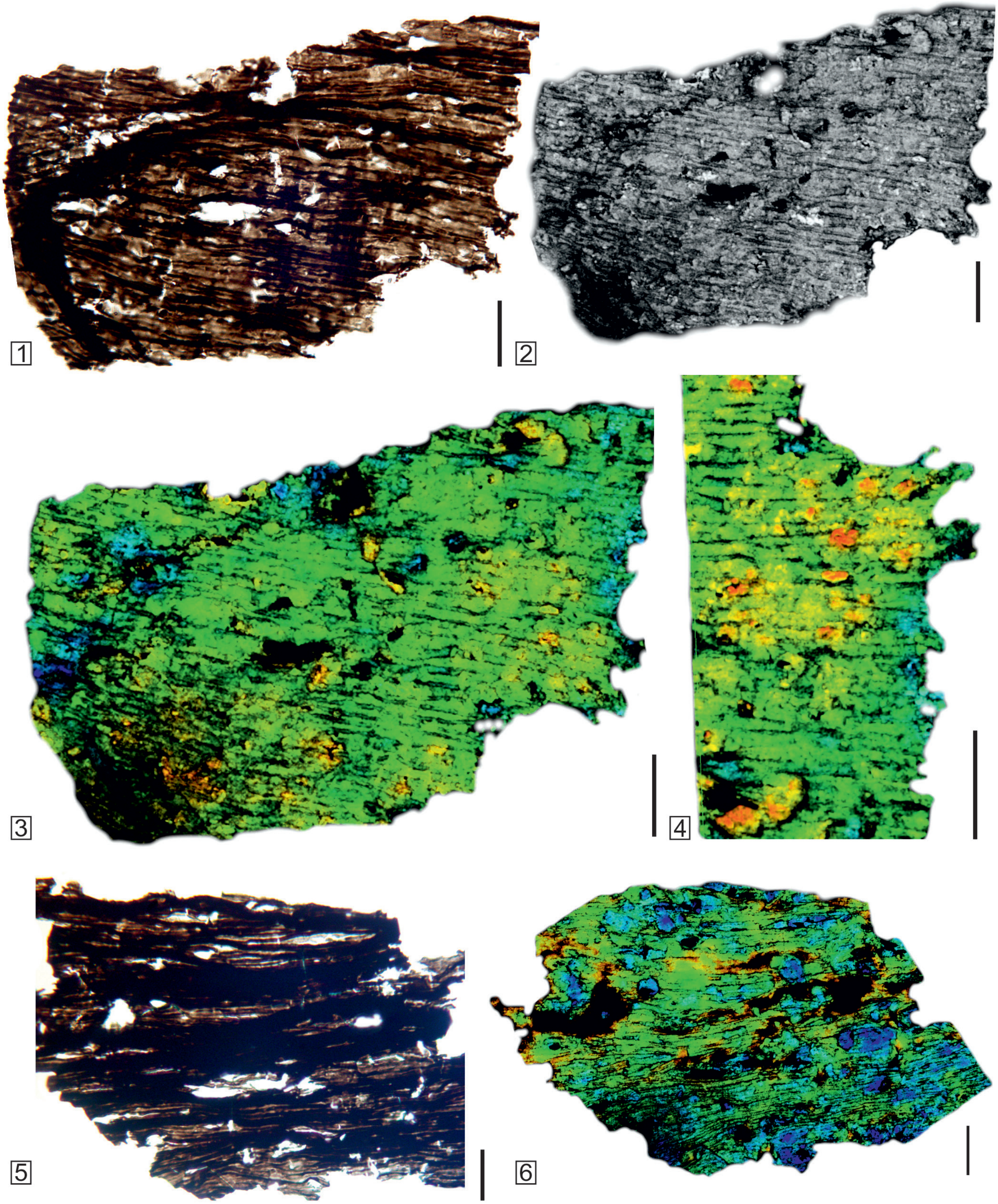
Age and distribution - Widely distributed in Ediacaran (Vendian) to lower Cambrian silicified and organic-walled assemblages. Doushantou Formation, Western Hubei, China (Zhang, 1984); Owk Shale, Kurnool Group, Cuddapah Supergroup, India (Sharma and Shukla, 2012); Saradih Limestone, Raipur Group, Chhattisgarh Supergroup, India (Singh *et al*., 2019b)

DISCUSSION

Ornamentations and their functions

Morphological complexity in organisms started manifesting around the late Palaeoproterozoic (Han and Runnegar, 1992; Sharma and Shukla 2009). Many features are visible under transmitted light OM; whereas recent CLSM studies have also revealed some additional morphological features and confirmed previously reported features in the three-dimensional view; in the organic-walled microfossils, which are discussed in the present paper. *Valeria lophostriata* is having circular striations on the inner surface of its body (Pl. I, Figs. 4, 5) whereas *Plicatidium latum* is having horizontal plicatids or striations on its outer surface (Pl. II, Figs. 3-5). The CLSM image (Pl. I, Fig. 5) provides better

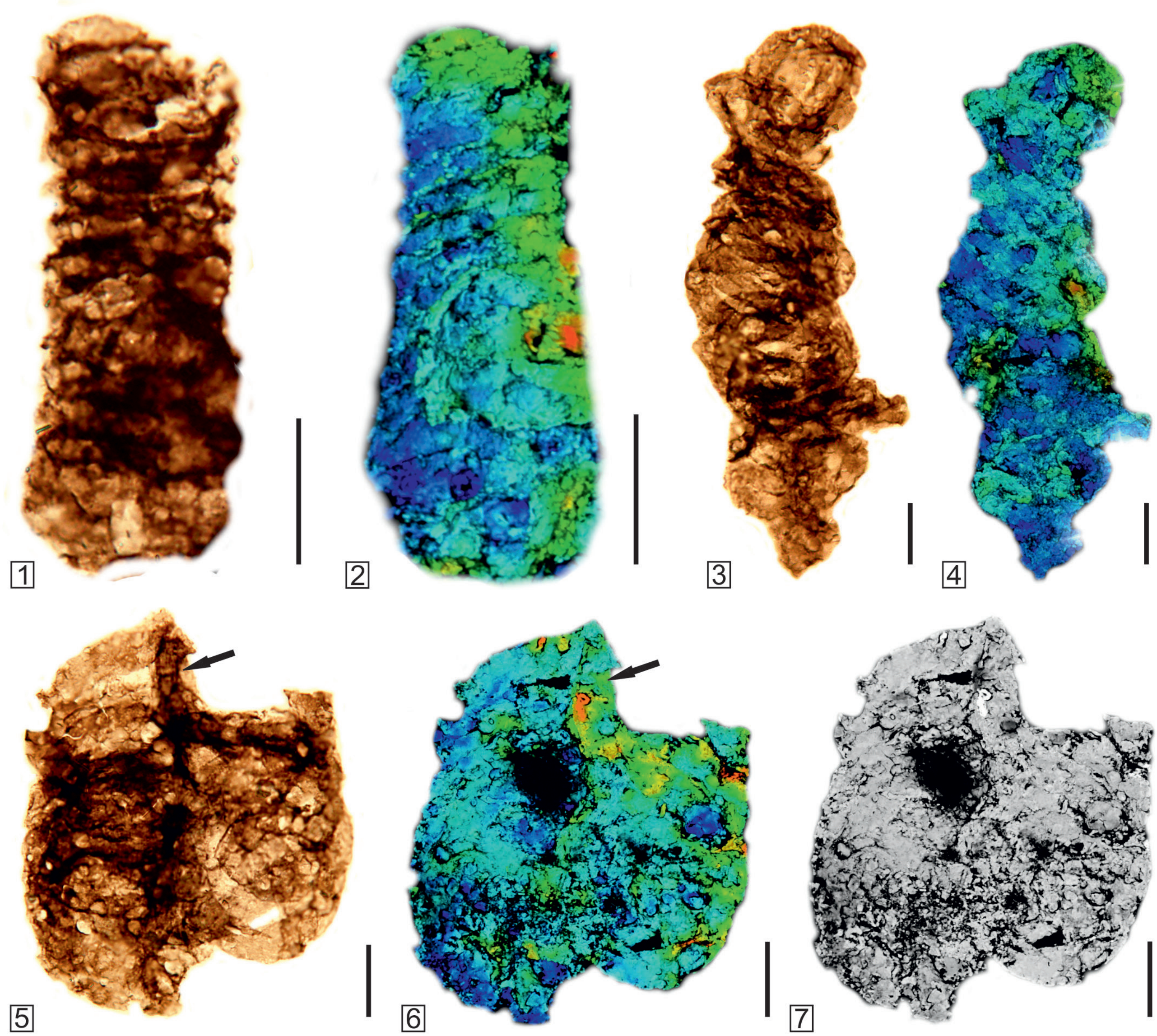
Plate III



EXPLANATION OF PLATE III

Plate–III. Photomicrographs of *Plicatidium latum* from Owk Shale, Kurnool Group under transmitted light Optical Microscope (OM) and Confocal Laser Scanning Microscope (CLSM). Optical photomicrographs (1, 5), Confocal Laser Scanning Micrographs (2, 3, 4, 6); (1 and 5) *Plicatidium latum* under OM, (2, 3, 4 and 6) under CLSM. 3, 4, 6 are recorded under false colour imagery showing projected and depressed areas. (1) BSIP slide no. 16582, U47/4; (2) BSIP slide no. 16581, R35/3 (4) Cross section showing equidistant parallel plicatids. Scale bar= 20 µm for (1-6).

Plate IV



EXPLANATION OF PLATE IV

Plate–IV. Photomicrographs of *Obruchevella delicata* and *Obruchevella parva* from Owk Shale, Kurnool Group under transmitted light Optical Microscope (OM) and Confocal Laser Scanning Microscope (CLSM). Optical photomicrographs (1, 3, 5), Confocal Laser Scanning micrographs (2, 4, 6, 7). 1-*Obruchevella delicata* under OM, 2-same specimen under CLSM, BSIP slide no. -16583, P 28/4; 3-*Obruchevella parva* under OM, 4-same specimen under CLSM, 5- *Obruchevella parva* under OM and 6 & 7 same specimen under CLSM. 2, 4, 6 are recorded under false colour imagery showing projected and depressed areas. (3) BSIP slide no. 16584, R24/4; (5) BSIP-slide no. 16585, E40/4. Scale bar= 20 µm for (1-7); (5-6) Arrow shows presence of septa.

observable details of the striations of *Valeria*. An alternative explanation/advantage of these striations at micron-level depth could likely be, increase in the surface area without enlarging the size of the organism for metabolic functions and uptake of nutrients. CLSM images of the well-preserved areas of the *Plicatidium latum* show that the striations on the outer surface of the organisms are equidistant in origin (Pl. III, Fig. 4); however, post mortem decay and compaction resulted in disturbing this feature, and striations appear placed at uneven distances. These striations, as in the case of *Valeria,* are also likely to increase the surface area of the organism without increasing its size significantly. Increased surface area, through striations, also strengthens the body of the organisms.

The affinity of microfossils and their biostratigraphic significance

The Neoproterozoic Era shows an increased record of the eukaryotic diversification and complex life forms (Huntley *et al*., 2006; Knoll *et al*., 2006; Parfrey *et al*., 2011; Cohen and Macdonald, 2015; Yang *et al*., 2017; Riedman and Sadler, 2018; Xiao and Tang, 2018). The morphologically complex acritarchs having wall ornamentations are considered as eukaryotic fossils (Javaux *et al*., 2001; Knoll *et al*., 2006). The palaeontological records suggest that the eukaryotic forms originated in the late Palaeoproterozoic time (Runnegar, 1994; Lamb *et al*., 2009; Moczydłowska *et al*., 2011; Singh and Sharma, 2014; Butterfield, 2015). Among them, the most convincing examples are fossil taxa *Valeria* and *Plicatidium*. *Valeria* is a unicellular microfossil and widely accepted as a unicellular eukaryote assigned to different affinities, i.e. protistan-grade phytoplanktonic species (Butterfield and Chandler, 1992); prasinophycean microalgae (Moczydłowska *et al*., 2011; Moczydłowska, 2015), or resting or reproductive cysts of an unidentified microorganism belonging to the eukaryotic domain (Javaux and Knoll, 2017).

Plicatidium latum is the filamentous microorganism that is constituted of broad empty tubes, occasionally folded, with closely placed transverse plications (Pl. II, Fig. 1) along the primary transverse axis. The affinity of these fossil remains is obscure, but certainly not the cyanobacteria as they possess complex morphology and large size, which strongly suggests a eukaryotic origin. Biostratigraphically *Plicatidium latum* and *Valeria lophostriata* are not very significant since they are present throughout the Proterozoic Time span but advanced morphological features in these eukaryotes make them a viable source to understand the advent of the complex sculpture in nature, documented in the form of these organic-walled microfossils.

Octoedryxium truncatum is a new entity recovered from the Owk Shale of the Kurnool Group. The spongy surface texture, octahedral form and diagonal ridges are very well documented and observed under the Confocal Laser Scanning Microscopy. This form has been reported from Cryogenian to Ediacaran or even up to Early Cambrian (Vidal, 1976; Vidal and Knoll, 1983, Vavardova, 2006; Nagovitsin *et al*., 2008). Prasad *et al*., (2005) reported the appearance of *Octoedryxium truncatum* in the Rewa Group of the Vindhyan Supergroup and suggested the late Neoproterozoic age for the Rewa Group.

Obruchevella shows morphological similarity with the extant cyanobacterial group *Lyngbya–Phormidium–Plectonema* (LPP) and is also comparable with the modern cyanobacteria *Spirulina*. Genus *Obruchevell*a has been recorded from the different parts of the world which includes Australia, Alaska, Canada, China, Greenland, India, Mongolia, Russia, and Saudi Arabia (See Peel, 1988; Mankiewicz, 1992; Burzin, 1995). The species of *Obruchevella* discussed in this paper is known from the Late Neoproterozoic to the Early Cambrian.

CONCLUSIONS

The present study provides the three-dimensional structural information of the organic-walled microfossils *Valeria lophostriata, Plicatidium latum, Octoedryxium truncatum*, *Obruchevella delicata*, and *Obruchevella parva* from the Owk Shale of the Kurnool Group, India. New Data is taxonomically very significant as surface texture and fine structural wall morphology were otherwise not documented under the OM studies. As the taxonomy of fossil palynomorph is currently based on the flattened compression-preserved specimen, these new observations provide additional taxonomical characters to be included in the traditional observations.

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