

Spore tetrads, possible indicators of intense climatic regimes: case study from an early Permian stratum of Singrauli Coalfield, Son-Mahanadi Basin, India

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Abstract – A large number of naked, fossil spore tetrads assignable to the dispersed microspore genera *Indotriradites*, *Microbaculispora* and *Microfoveolatispora* are reported for the first time from an early Permian stratum (Lower Barakar Formation) of Singrauli Coalfield, Son-Mahanadi Basin, Central India. This is also the first record of tetrads from any Artinskian strata in the world. There is no evidence of any kind of sporangia or related plant parts in the present investigation that could ascertain the affinity of these tetrads; however, the presence of a trilete mark in the spores of the tetrads demonstrates their alliance at least with the pteridophyte group. The present study suggests possible factors affecting the sporogenesis process in the past, considering other available global records pertaining to fossil spore tetrads. The results of significant physiological and biochemical analyses performed on the anthers of modern plants related to reproductive biology, in order to understand the conditions and changes responsible for the formation of tetrads, are also considered. We analysed the globally occurring fossil tetrads and the palaeoclimates prevailing during their deposition. A correlation between extreme climatic conditions, specific pH values inside microsporangium and the formation of tetrad is envisaged. It is deduced that extreme climatic conditions (extreme cold/extreme hot) might have triggered some sort of malfunctioning in the sporogenesis process that altered the specific pH values inside the microsporangium. Any restraint of the activity of the callase enzyme, responsible for dissolution of callose walls laid between the individual spores, may therefore have apprehended the dissociation of tetrads into individual spores.

Keywords: callase, early Permian, extreme climatic conditions, pH value.

1. Introduction

Living embryophytes mostly produce reproductive structures known as spore and pollen in the form of tetrads. The four haploid spores of the tetrad are formed as a result of meiotic division of a diploid spore mother cell. Each generally becomes dispersed in the environment as an individual spore following a dissociation process which is widespread in most of the non-angiospermous plant world. However, very rarely the tetrads do not dissociate to form a single spore (as seen in some of the modern hepatics) and are dispersed as permanent tetrads (Edwards, Wellman & Axe, 1999).

The earliest evidence for the first land plants or the embryophytes comes from the microscopic dispersed spores generally known as cryptospores. They lack haptotypic features such as a trilete mark or a monoete mark (Stemans, 2000); however, they possess sporopollenin in their exine. Cryptospores are preserved in the form of monads, permanent dyads or as permanent tetrads. Permanent tetrads and dyads happen to be an important part of Ordovician – Early Devo-

nian dispersed spore assemblages and were habitually dispersed intact in the environment with all four individual spores attached firmly (Edwards, Wellman & Axe, 1999). The tetrads are either naked or enclosed within a thin cover. Naked tetrads are mostly laevigate; however, some ornamented forms also existed.

The existence of spore tetrads can be traced back to the Cambrian period based on a recent discovery of Cambrian palynomorphs from the Bright Angel Shale, Arizona, USA by Taylor & Strother (2008). However, these palynomorphs (preserved as dyads and tetrads) are considered as protoembryophytes by these authors, rather than embryophytes, as their overall morphology is different from the definite embryophytes. The tetrad taxon *Tetrahedraletes* cf. *Medinensis*, identified in Dapingian deposits (early Middle Ordovician, c. 473–471 Ma) from the Zanjón and Labrado formations in NW Argentina, is the oldest tetrad record; it existed for c. 70 Ma on this land during Llanvirn (earliest Ordovician) – Lochkovian (earliest Devonian) times (Rubinstein *et al.* 2010). The tetrads were abundant and diverse throughout the Ordovician and Silurian periods.

Until late Middle Ordovician time (463–461 Ma), the signatures of the earliest land plants are only

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recorded in the form of cryptospores and not as a complete plant. The earliest plant fragments (sporangia) that also possessed *in situ* preserved naked permanent tetrads and naked dyads were documented for the first time in Upper Ordovician (Caradoc) rocks of Oman. These spores, produced in large numbers inside very small sporangia, confirm their affinity with the land plants. Transmission electron microscopy (TEM) analysis of the spore walls of these tetrads further proves their affinities with the liverworts (Wellman, Osterloff & Mohiuddin, 2003). Oman fossils therefore represent the oldest sporangia and the oldest *in situ* tetrads and dyads on the Earth.

In comparison to a large number of reports on the dispersed spore tetrads, the data on *in situ* preserved tetrads is meagre. Edwards, Duckett & Richardson (1995), Edwards *et al.* (1996), Edwards, Wellman & Axe (1999), Wellman, Osterloff & Mohiuddin (2003) and Edwards *et al.* (2012) recorded various types of *in situ* spore tetrads (*Velatitetras*, *Synorisporites*, *Tetrahedraletes*, *Acontotetras* and *Cheilotetras* from different kinds of mesofossils (sporangia, bifurcating axes or irregular spore mass) collected from a number of early Palaeozoic (Ordovician–Devonian) localities in the Welsh Borderland and in Oman.

In contrast to the abundance of lower Palaeozoic spore tetrads regarded as basal embryophytes the tetrads at the Permian–Triassic transition and from the Upper Triassic rocks are comparatively less represented and belong to the lycopods, whereas spore tetrads are scarce in Permian sequences (the only taxon is *Jayantisporites* from the Indian Permian stratum). The lower Palaeozoic spore tetrads have been assigned to the genera *Synorisporites*, *Tetrahedraletes*, *Velatitetras*, *Acontotetras*, *Rimosotetras*, *Stegambiquadrella* and *Cheilotetras*, whereas the tetrads of the Triassic period belong to the form genera *Densoisporites*, *Lundbladisporea*, *Uvaesporites*, *Verrucosisporites*, *Lapposporites*, *Decisporis*, *Kraeuselisporites* and *Otynisporites* (megaspore tetrad).

Here we report and describe the biggest ever collection of unseparated naked spore tetrads from the lower Permian (Artinskian) Lower Barakar Formation of Bina Colliery, Singrauli Coalfield, Central India (Fig. 1), assignable to the dispersed microspore genera *Indotriradites*, *Microbaculisporea* and *Microfoveolatispora*. Two types of tetrads, ornamented and zonate, are recognized in this study along with other pollen and spores. There are a few records of spore tetrads in the Indian Triassic sequences (Banerji & Maheshwari, 1973; Maheshwari & Banerji, 1975; Tiwari & Meena, 1989; Ram-Awatar, 2011); however, they have never been reported from any Permian strata in India apart from a solitary record of *Jayantisporites* recorded from the lowest Permian (Asselian–Sakmarian) Talchir Formation (Lele & Makada, 1972). There is no evidence of any kind of sporangia or related plant parts that could help to determine the affinity of these tetrads. However, the presence of a trilete mark in some of our tetrads demonstrates their alliance with the pter-

idophytes. The study also includes a comparison of the present tetrad assemblage with the similar assemblages reported globally of Palaeozoic and Triassic age. The complete palynoassemblage of Bina Colliery is depicted in the frequency chart of Figure 2.

We analysed the global records of fossil tetrads and the palaeoclimates prevailing during their deposition, and observed that they mostly occur in sediments which are deposited under extreme climatic conditions (extreme cold or extreme hot). This is supported by the fact that most of the occurrences of spore tetrads reported globally are found in Ordovician–Lower Devonian sediments; in the lower Permian Talchir (Asselian–Sakmarian) and the Lower Barakar (Artinskian) formations; at the Permian–Triassic boundary; and in the Lower Triassic Panchet (Induan–Olenekian) Formation. These occurrences have been correlated with extreme cold due to Late Ordovician (Finnegan *et al.* 2011) and Carboniferous–Permian glaciations, and extreme hot due to Early Triassic Siberian volcanic activity respectively.

Extreme cold conditions during the deposition of Talchir Formation (Asselian–Sakmarian) are inferred by the abundance of monosaccate pollen taxa (*Parasaccites* and *Plicatipollenites*) along with the spore taxa *Callumisporea* and *Jayantisporites* in the Talchir palynoassemblages (Tiwari & Tripathi, 1987). Similarly, a very cold to cool phase must have prevailed at the transition zone of the Karharbari and Lower Barakar formations in Bina Colliery, as is evidenced by the presence of pollen taxa *Scheuringipollenites*, *Parasaccites*, *Faunipollenites* and *Striatopodocarpites* along with the spore genus *Callumisporea* in the present assemblage (Tiwari & Tripathi, 1987; Vijaya *et al.* 2012).

The recent physiological and biochemical studies carried out on the extant plants *Allium sativum* and *Petunia hybrida* (Izhar & Frankel, 1971; Winiarczy, Jaroszuk-Ścisiel & Kupisz, 2012), correlating adequate specific pH values inside microsporangium, the release of callase enzyme, disintegration of the callose walls and the ultimate release of the single spore from the tetrads, are applied on the presently studied material from Bina Colliery. It is deduced that unfavourable conditions such as hot and cold extremes of climate might have induced changes in the plant physiological processes (i.e. non-functional tapetum/altered pH values in the microsporangium or the inactive callase) that prevented the dissociation of tetrads into individual spores. Such results imply that extreme climatic conditions might have affected the formation of spore tetrads in the past.

2. Geological setting

The Singrauli Coalfield (Fig. 1) lies at the northernmost boundary of the Son-Mahanadi Master Basin that stretches from the east coast to the centre of Peninsular India. This coalfield embodies the last deposits of Gondwana sedimentation. No Gondwana deposits

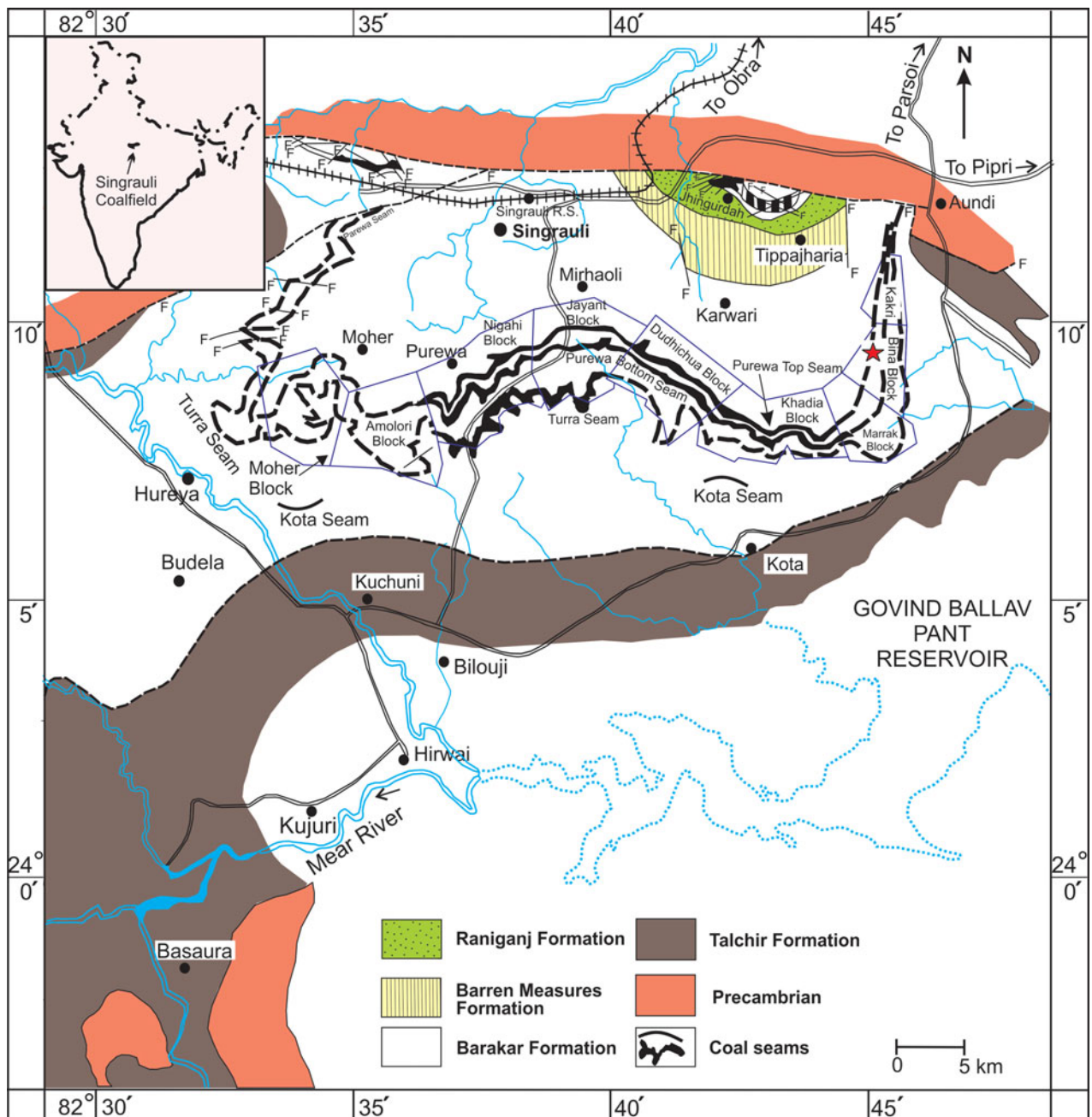


Figure 1. (Colour online) Location Map of the Singrauli Coalfield showing Bina Colliery (after Raja Rao, 1983).

occur beyond this coalfield area in the northern part of Peninsular India. The coalfield lies between latitudes $23^{\circ}47'N$ and $24^{\circ}12'N$ and longitudes $81^{\circ}48'E$ and $82^{\circ}52'E$ and is located in the drainage area of the Son and Rihand rivers. The total geographical area of this coalfield is $c. 2200 \text{ km}^2$, $c. 80 \text{ km}^2$ in the Sonbhadra District of Uttar Pradesh State and the remainder in the Singrauli District of Madhya Pradesh State.

The coalfield is structurally divided into two tectono-sedimentary sub-basins: (1) Moher sub-basin on the north-eastern side; and (2) the Singrauli main sub-basin to the west. There is no clear-cut demarcation between these two sub-basins because all the lower Gondwana formations are exposed uninterruptedly in these sub-basins. The only difference lies in the amount of coal

reserves found in each. The coal reserves in the Moher sub-basin, covering an area of $c. 220 \text{ km}^2$, is $c. 9 \times 10^9$ tonnes, of which 2.724×10^9 tonnes are proven reserves (Raja Rao, 1983). Singrauli main sub-basin covers an area of $c. 1900 \text{ km}^2$. All ten working open-cast mines of Singrauli Coalfield (namely Dudhichua, Jayant, Kakri, Bina, Krishnashilla, Amlohri, Khadia, Block B, Nigahi and Jhingurdah) lie within the Moher sub-basin. The first nine collieries are in Barakar Formation and have three coal seams: the lowermost Turra seam; the middle Purewa bottom; and the uppermost Purewa top. Below Turra, a thin seam referred to as Kota also exists within the Karharbari Formation (Sakmarian–Artinskian). Jhingurdah Colliery is in the Raniganj Formation (Lopingian) and has the

Table 1. General stratigraphic succession of Singrauli Coalfield (after Vijaya *et al.* 2012).

Age	Formation/ group	Thickness (m)	General lithology
Recent			Alluvium
Cretaceous	Basic intrusive		Dolerite dykes and sills
Late Triassic (Norian)	Parsora	>500	Medium to coarse grained ferruginous quartzose sandstone
Early Triassic (Anisian)	Pali	>700	Greenish- to reddish yellow, medium to coarse-grained sandstone with variegated siltstone and clay
Late Permian (Lopingian)	Raniganj	215–400	Fine to medium-grained dirty to buff-coloured subarkosic to feldspathic wacke with alternation of thin lamination of grey and carbonaceous shale along with non-persistent coal seams
Middle Permian (Guadalupian)	Barren Measures	110–300	Dark brown to brownish yellow to greenish grey, medium to coarse-grained flaggy sandstone with thin grey clay bands in between
Early Permian (Kungurian–Artinskian)	Barakar	325–550	Dirty white fine to coarse-grained subarkosic to arkosic sandstone along with siltstone, shale, carbonaceous shale and coal seams
Early Permian (Sakmarian–Asselian)	Talchir	75–230	Dark greenish grey to grey shale, fine-grained sandstone diamictite, siltstone pebbly sandstone and boulder bed
<i>Unconformity</i>			
Precambrian	Mahakoshal		Granite, gneiss, quartzite, phyllite, schist and pegmatite

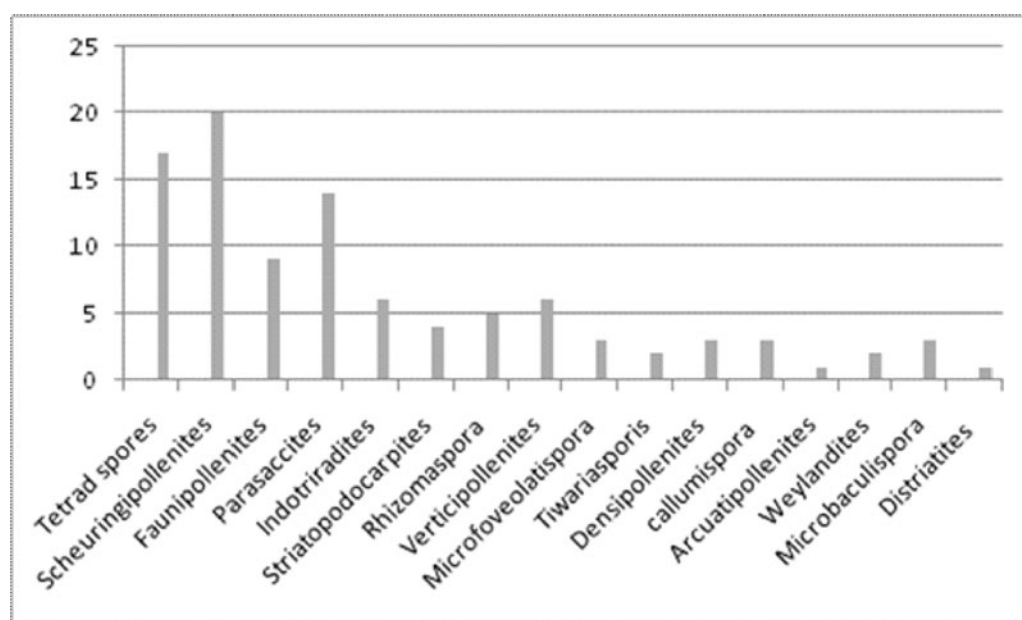


Figure 2. Frequency chart showing all the palynomorphs and spore tetrads recovered from Bina Colliery.

thickest coal seam (134 m) in India. It also has the deepest basinal area of all the collieries of this coalfield. The stratigraphic sequence found within the Singrauli Coalfield is described in Table 1.

3. Materials and methods

Morphological studies have been performed on the microspores recovered from the macerals belonging to a lower Permian Gondwana exposure in central India. Five samples were collected from each of three grey-carbonaceous fine-grained shale horizons designated A, B and C (24°9'36.3"N; 82°44'39.1"E) from the lowermost Turra coal seam exposed in the Bina Colliery located in the eastern part of Singrauli Coalfield, Son-Mahanadi Basin, Madhya Pradesh State (Fig. 1). These exposures belong to the Lower Barakar Forma-

tion. The spore tetrads have only been found in shale horizon A; B and C are completely devoid of them (Fig. 3). Other palynomorphs have been recovered from all three horizons. The shale sediments are fluvial in origin. Microspores including the spore tetrads are extracted by digesting the shale samples following standard palynological processes using HF (40%) and HNO₃ (63.01%) for different time durations. The potential maceral specimens are permanently mounted on slides using polyvinyl and Canada balsam for light microscopic analysis. The morphological characters of the microspores are examined under an Olympus (BX-61) high-power binocular microscope and photography was performed using Olympus DP-20 digital camera.

In all, 34 spore tetrads have been isolated in the present investigations which are assignable to the dispersed microspore genera *Indotriradites*,

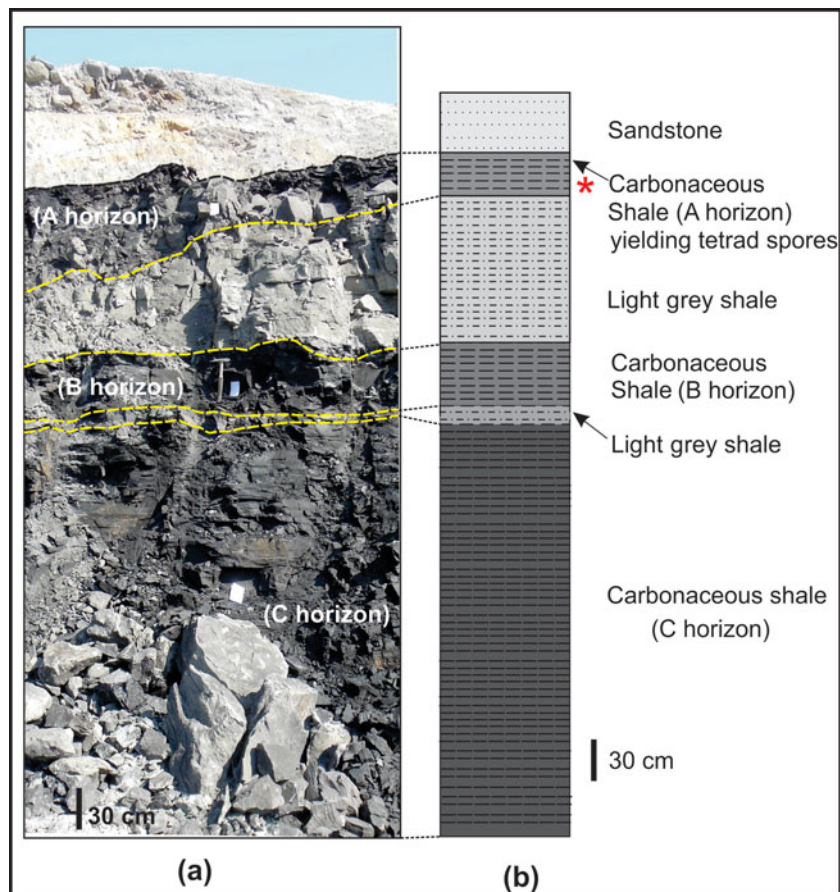


Figure 3. (a) (Colour online) Photograph showing measured section of a part of Turra Seam, Bina Colliery, Singrauli Coalfield. (b) Litho-column depicting different litho-units of a part of Turra Seam. Asterisk indicates the spore-tetrads-yielding horizon.

Microbaculispora and *Microfoveolatispora*. Tetrads form a sizeable chunk, about 17% of the total palynoassemblage, consisting of 15 spore/pollen genera (Fig. 2). Plant megafossils comprising *Noeggerathiopsis hislopi* and ten species of *Glossopteris* (namely *G. gigas*, *G. tenuifolia*, *G. browniana*, *G. cf. G. feistmanteli*, *G. communis*, *G. recurva*, *G. nakkarea*, *G. pantii*, *G. leptoneura* and *G. searsolensis*) have also been recovered from the same shale horizons of the Turra seam of Bina Colliery in which the present palynomorphs were recorded.

All the slides containing spore tetrads described in this paper are deposited in the repository of Birbal Sahni Institute of Palaeobotany, Lucknow, vide statement no. 1366 and Museum Slide no. 15134–15136.

4. Results

4.a. Observations of the present spore tetrads

Complete palynoassemblage recovered from the Bina colliery consists of 15 spore/pollen genera with a dominance of non-striate bisaccate pollen *Scheuringipollenites* (20%) and spore tetrads (17%) (Fig. 2), and a subdominance of girdling monosaccate pollen *Parasaccites* (14%) and striate bisaccate *Faunipollenites* (9%). Other associated palynomorphs are *Indotriradites* (6%), *Verticypollenites* (6%), *Rhizomaspora*

(5%), *Striatopodocarpites* (4%), *Microbaculispora* (3%), *Microfoveolatispora* (3%), *Densipollenites* (3%), *Callumisporea* (3%), *Weylandites* (2%), *Tiwariasporeis* (2%), *Arcuatipollenites* (1%) and *Distriatites* (1%) (Fig. 2).

The occurrence of pollen taxa *Scheuringipollenites*, *Parasaccites* and *Striatopodocarpites* and spore taxon *Callumisporea* in abundance along with other species (namely *Indotriradites*, *Microfoveolatispora*, *Rhizomaspora* and *Densipollenites*) in the present palynoassemblage place these beds at the transition of the Karharbari Formation and Lower Barakar Formation (Artinskian).

All the spore tetrads recovered from the Turra seam are permanent, naked and tetrahedral in shape. The individual microspores of the tetrads appear to be morphologically mature as they possess well-developed ornamentation and are almost of equal size to that of the dispersed microspores of concerned tetrad genera. Some tetrads are compactly arranged (Fig. 4g, h), whereas a few are very open (Fig. 4a, d). They are mostly fused; however, a distinct cleavage can be noticed between the individual spores in some of them (Fig. 4a, d, g). They range from 59 to 66 μm in diameter with an average of 62.50 μm along the *x* axis (horizontal). Individually, the tetrad genus *Indotriradites* has an average diameter of 62.50 μm , *Microbaculispora* 61 μm and *Microfoveolatispora* 62 μm .

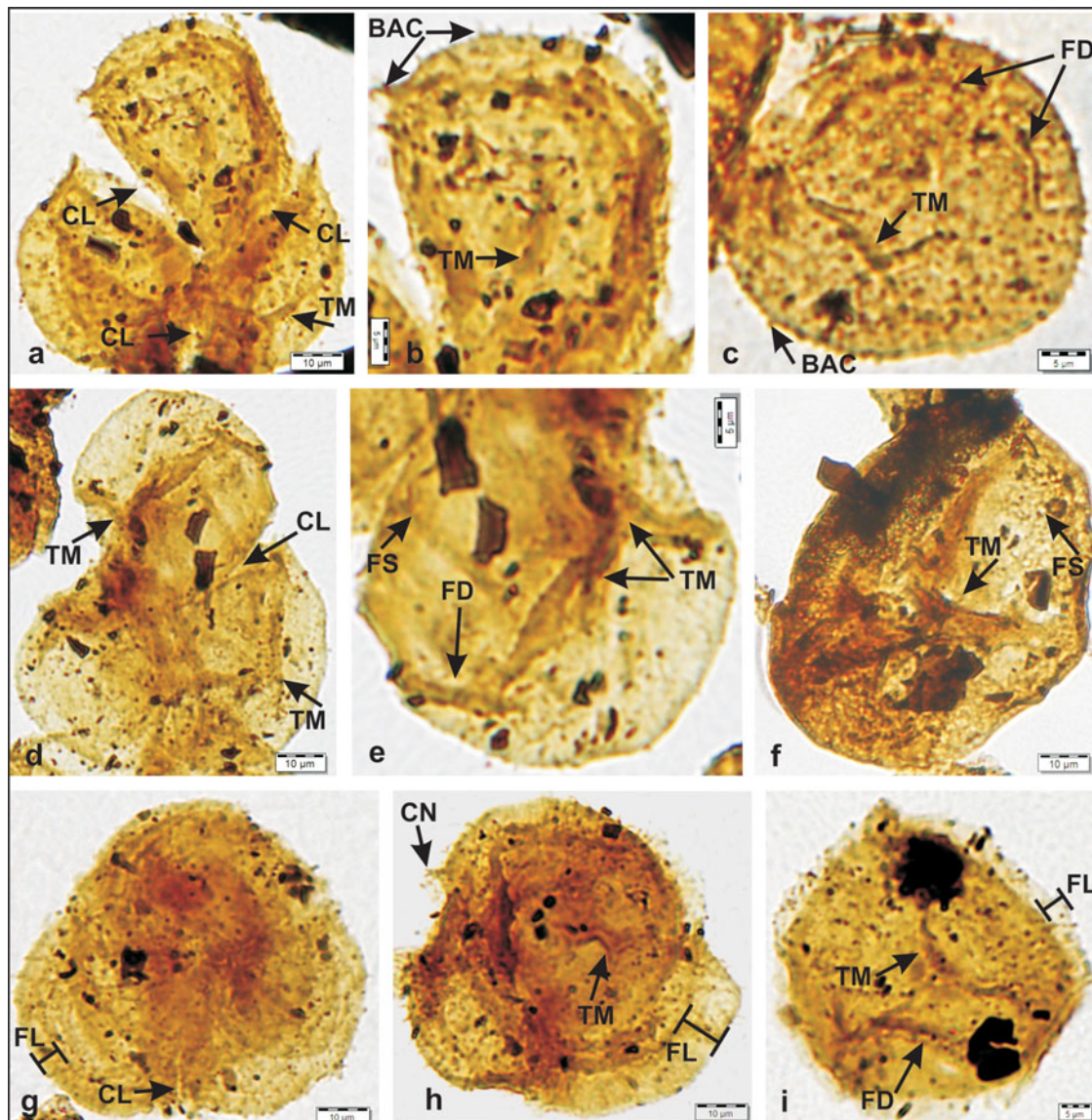


Figure 4. (Colour online) Spore tetrads recovered from the A horizon of Turra Seam, Bina Colliery. (a) *Microbaculispora* tetrad showing individual spores arranged tetrahedrally, prominent cleavages (signs of splitting) seen between spores (CL – cleavage; TM – trilete mark). Scale bar = 10 μm (Q46/2–4). (b) Upper spore of the *Microbaculispora* tetrad in (a), showing faint trilete mark and baculae on the exine (BAC – Baculae). Scale bar = 5 μm (Q46/2–4). (c) Dispersed spore of *Microbaculispora* showing trilete mark, fold and baculae on the exine (FD – Fold). Scale bar = 5 μm (S50/1). (d) *Microfoveolatispora* tetrad showing tightly adhered individual spores having trilete mark, spores forming a prominent tetrahedral. Scale bar = 10 μm (M42/2). (e) Individual spore (upper) of *Microfoveolatispora* tetrad in (d) showing foveolae structure on the exine with distinct trilete mark (FS – foveolae structure). Scale bar = 5 μm (M42/2). (f) Dispersed spore of *Microfoveolatispora* showing trilete mark with foveolate exine. Scale bar = 10 μm (W33/1–3). (g) *Indotriradites* tetrad showing firmly attached individual spores, sign of cleavage seen between two lower spores (CL), well-preserved flange (FL) seen on the periphery of the spores, faintly preserved trilete mark on the exine. Scale bar = 10 μm (N31/3). (h) *Indotriradites* tetrad showing tightly adhered spores with faintly preserved trilete mark, coni and flange on the periphery of individual spores. Scale bar = 10 μm . (i) Dispersed zonate spore of *Indotriradites* showing well-preserved flange (FL) on the periphery and a distinct trilete mark. Scale bar = 5 μm (T35/1).

The spore tetrads found in the present study are divided into two groups of ornamented and zonate forms.

4.a.1. Ornamented forms

Ornamented forms are represented by the genera *Microbaculispora* and *Microfoveolatispora*.

Microbaculispora Bharadwaj 1962: Individual spores in this tetrad (Fig. 4a–c) are subtriangular in shape, have broadly rounded corners and unequal axes;

exine thin, baculate, measures $\pm 1.5 \mu\text{m}$ in thickness; bacula small $\pm 1 \mu\text{m}$ high and 2–3 μm broad at base with flat to sharp tip, densely distributed on the exine; trilete mark distinct, rays extend up to corners, faint secondary folds exist along the rays. Based on these morphological characters, this spore tetrad is assigned to the microspore genus *Microbaculispora* Bharadwaj 1962.

Microfoveolatispora Bharadwaj 1962: Individual spores in *Microfoveolatispora* (Fig. 4d, e) are

triangular to broadly rounded, angled, sides are convex; trilete mark distinct, thick lipped, ending just before the corner; rays are associated with folds, secondary fold prominent; exine thin and foveolate, foveolae uniformly distributed, foveolae measures $\pm 1 \mu\text{m}$. All these morphological characters confirm that this spore tetrad belongs to the microspore genus *Microfoveolatispora* Bharadwaj 1962.

4.a.2. Zonate forms

Zonate forms are represented by the genus *Indotriradites*.

Indotriradites Tiwari 1964: Individual spores in this zonate tetrad, *Indotriradites* (Fig. 4g–i), are roundly triangular to subrounded in shape, show central body and inner body; exine 2–3 μm thick in the central body, uniformly micropunctate and coniate, conical 1–2 μm high, broad at base; trilete mark distinct, rays extend up to margin of the flange, flange broad with a smooth to slightly wavy margin. Based on these characters this tetrad compares well with the microspore genus *Indotriradites* Tiwari 1964.

4.b. Observations of the global distribution of spore tetrads

As compared to the substantial representation of the early Palaeozoic (Ordovician – Early Devonian) spore tetrads (cryptospores, mostly of bryophytic origin) recorded globally (Richardson & Lister, 1969; Strother & Traverse, 1979; Gray, 1985; Johnson, 1985; Richardson, 1988, 1996; Vavrdová, 1988, 1990; Burgess, 1991; Burgess & Richardson, 1991; Strother, 1991; Wellman & Richardson, 1993; Edwards, Duckett & Richardson, 1995; Edwards *et al.* 1996, 2012; Strother, Al-Hajri & Traverse, 1996; Wellman, 1996; Wang, Li & Wang, 1997; Edwards, Wellman & Axe, 1999; Steemans 2000, 2001; Steemans, Higgs & Wellman, 2000; Wellman, Higgs & Steemans, 2000; Wellman, Osterloff & Mohiuddin, 2003; Steemans & Wellman, 2004; Taylor & Strother, 2008; Rubinstein *et al.* 2010), their occurrence has never been recorded in the rocks of similar age in India.

Potonie & Lele (1959) reported *Quadrisporites horridus* from the Talchir Formation (Asselian) near Gorai village in the South Rewa Gondwana Basin as the first spore tetrad from any locality in India. However, it was not a true tetrad as the four bodies in *Q. horridus* (Potonie & Lele 1959, plate 1, figs 26–36) lie close together in the bedding plane, forming a rounded square or rounded rhombus and not a tetrahedron as is found in tetrads of embryophytes and tracheophytes. No germinal aperture (either monolete or other marks) can be seen in these spore tetrads. Pant & Singh (1990) kept this taxon in Bryophyta (*Riccia*), whereas Tiwari & Meena (1989) identified *Quadrisporites* as related to the acritarchs and not the spore tetrads of embryophytes.

The first ever true tetrad to be described from Indian Gondwana was by Lele & Makada (1972) who recor-

ded the tetrads of the microspore genus *Jayantisporites* represented by three species (*J. pseudozonatus*, *J. indicus* and *J. conatus*) from the lowest Permian Talchir Formation (Asselian) in Jayanti Coalfield, Bihar State. They suggested that these tetrads were immature because of the smaller size of the spores they hold. A well-developed trilete mark in the individual spores of the tetrad of *J. pseudozonatus* (Lele & Makada, 1972, plate 1, fig. 13) proves their affinity with the group lycopods. *Jayantisporites* therefore stands as the only spore tetrad taxon represented in Indian Permian strata prior to the present study. The genera *Jayantisporites*, *Indotriradites*, *Microbaculispora* and *Microfoveolatispora* are the only tetrad taxa found in the Permian sequences of India.

The records of the spore tetrads in the Triassic sediments of India are also meagre. The tetrads of the spore genus *Decisporis* are reported from the Panchet Formation of Auranga Coalfield and Maitur Formation of Raniganj Coalfield by Banerji & Maheshwari (1973) and Maheshwari & Banerji (1975), respectively. Tiwari & Meena (1989) recorded *Densoisporites*, *Lundbladispota* and *Verrucosisporites* from the Panchet Formation of Raniganj Coalfield. Spore tetrads of the microspore genera *Densoisporites*, *Lundbladispota*, *Verrucosisporites* and *Lapposporites* have recently been reported from the Upper Pali Formation (Lower–Middle Triassic) in Sohagpur Coalfield by Ram-Awatar (2011).

A large number of unseparated tetrads belonging to definite tracheophytes (lycopods) have been reported from many end-Permian and Triassic localities in the continents: (1) Sverdrup Basin in Arctic Canada (Utting, 1994); Barents Sea, Arctic region (Mangerud, 1994); and East Greenland (Looy *et al.* 2001; Visscher *et al.* 2004); (2) Europe, including Italy (Massari *et al.* 1994; M. Smit, unpub. MSc thesis, Utrecht University, 1996; B. Van de Schootbrugge, unpub. MSc thesis, Utrecht University, 1997; K. De Graaf, unpub. MSc thesis, Utrecht University, 1999); and Hungary (Góczán, Oravecz-Scheffer & Szabó, 1986; Haas *et al.* 1986, 1988); (3) Asia, including Pechora Basin, Urals, Russia (Tuzhiakova, 1985); Moscow Basin, Russia (Afonin, 2000); Jungar Basin, North China (Qu & Wang, 1986; Ouyang & Norris, 1999); Meishan, South China (Ouyang & Utting, 1990); Sri Lanka (Dahanayake *et al.* 1989) and India, including Damodar Basin (Lele & Makada, 1972; Banerji & Maheshwari, 1973; Maheshwari & Banerji, 1975; Tiwari & Meena, 1989) and Son Basin (Ram-Awatar, 2011 and present study); (4) Africa: Mombassa Basin, Kenya (Hankel, 1992); and (5) Madagascar (Wright & Askin, 1987).

These end Permian – Triassic tetrads have been assigned to the form genera *Densoisporites*, *Lundbladispota*, *Uvaesporites*, *Verrucosisporites*, *Lapposporites*, *Decisporis* and *Otynisporites* (megaspore tetrad).

With the exception of a few examples of the spore tetrads – *Tetrahedraletes medinensis* from Upper Ordovician Southern Ohio (average diameter 28.1 μm ;

Taylor, 1995) and *Velatitetras* 17–38 μm and many *in situ* tetrads contained in the sporangium or as spore masses recovered from the upper Silurian rocks at Ludford Lane, Shropshire, UK (30–35 μm) and from the Early Devonian strata located at north Brown Cleve Hill locality, Welsh Borderland (17–20 μm ; Edwards, Wellman & Axe, 1999; Edwards *et al.* 2012) – most of the lower Palaeozoic tetrads (*Cheilotetras* 48–53 μm , *Tetraedraletes* 49–67 μm , *Rimosotetras* 49 μm , *Stegambiquadrella* 52 μm and *Acontotetras* 39–46 μm ; Edwards, Wellman & Axe, 1999; Edwards *et al.* 2012; Steemans *et al.* 2012), Permian tetrads (*Indotriradites* 59–66 μm , *Microbaculispora* 61 μm and *Microfoveolatispora* 62 μm , present study) and Triassic tetrads (*Densoisporites* 57–67 μm , *Lundbladispota* 54–62 μm , *Uvaesporites* 55.29 μm , *Verrucosisporites* 68 μm , *Lapposporites* 50–57 μm , *Decisporis* 55–64 μm and *Otynisporites* megaspore tetrad 800 μm ; Banerji & Maheshwari, 1973; Tiwari & Meena, 1989; Looy *et al.* 2005; Ram-Awatar, 2011) range from 39 μm to 68 μm in diameter.

5. Discussion

Two successive cleavages are responsible for the formation of a tetrad from spore mother cell. Dyad is formed in the first stage when the wall formed during meiosis I divides the spore mother cell. The subsequent division of this dyad during meiosis II gives rise to the tetrad. Callose (β -1, 3-glucan, a polysaccharide) is deposited on the external walls of these developing meiocytes during the prophase stage of meiosis. The callose wall isolates meiocytes from other tissues and keeps them separated by preventing their underlying walls from fusing (Li, Gong & Wang, 2010). The quantity of callose increases during meiosis; however, at the end of microsporogenesis the callosic walls are disintegrated due to the hydrolytic activities of an enzyme called callase (β -1,3-d-glucanase) (Stieglitz, 1977; Chen & Kim, 2009) that hydrolyses the β -D glycosidic linkages present in the callose. Ultimately, the spores of the tetrad are separated from each other and released as mature pollen grains/spores (Xie, Wang & Hong, 2010; Wan *et al.* 2011). Stieglitz & Stern (1973) and Pacini, Franchi & Hesse (1985) suggested that the tapetum layer in the sporangium produces as well as releases the callase enzyme necessary for tetrad dissolution. If the activity of the callase enzyme diminishes or ceases for some reason, the dissolution process of the callose walls is also affected, thereby preventing the release of the spores of the tetrad.

In a significant study carried out on the anthers of sterile *Allium sativum* (garlic), a species producing sterile pollen and propagating only through vegetative methods and fertile *Allium atropurpureum*, Winiarczy, Jaroszuk-Ścisiel & Kupisz (2012) demonstrated the significance of pH values inside the microsporangium in regulating the activities of callase. Callase activity was monitored in both the species during microsporogen-

esis, and *A. atropurpureum* (which possesses functional male gametophytes) was used as a control. The existence of callose in the microsporocyte was detected using fluorescence microscopy and aniline blue staining. In this microscopic observation a callose wall is seen encircling the microsporocyte in prophase I, whereas during prophase II each dyad showed a thin intersporal callose wall and a thicker external callose wall (Winiarczy, Jaroszuk-Ścisiel & Kupisz, 2012, fig. 1). Complete separation of the four microspores involved the development of a callose wall between them. The detailed studies carried out on callase confirmed the existence of three isoforms of this enzyme that are active at specific pH values. Winiarczy, Jaroszuk-Ścisiel & Kupisz (2012) believed that at least one of the callase isoforms might be responsible for the degradation of the callose walls. As the callose wall is not degraded and it stays around the tetrads in *Allium sativum* for more than 2 weeks, gametogenesis could not take place and as a result no functional male gametophyte is formed. The degeneration of the microspore cytoplasm is also observed, which results in the abortion of the microspore (Winiarczy, Jaroszuk-Ścisiel & Kupisz, 2012). They further demonstrated that the callose wall stays around meiotic cells in *A. sativum* when an acidic environment of pH value 5.0 or less prevails in the microsporangium, and only one callase isoform is active with optimum activity at pH 4.8. However, disintegration of the callose wall takes place only when the pH rises up to 6.0 and simultaneously the two other callase isoforms that are active at pH 6.0 and 7.3 appear.

The correlation between pH values and the callase activity is also established in an extant *Petunia hybrida* plant. Due to higher pH values (6.8–7.0) prevailing in the anthers of the sterile *Petunia hybrida* during meiosis, the callase remained inactive and the spore tetrads therefore remained enclosed within the callose wall for a longer time. However, slightly lower pH values (5.9–6) at the tetrad stage in fertile genotype of this plant enabled the microspores to disperse (Izhar & Frankel, 1971). Working on the reproductive biology of the extant plant *Arabidopsis* (family Brassicaceae), Fei & Sawhney (1999) proposed that the existence of callose wall in the tetrads for longer periods may lead to the degeneration of microspore cytoplasm. They also postulated that the tapetum, which produces enzymes to be used in the development of the male gametophyte, remained non-functional and therefore callase could not be synthesized; as a result the callose wall was not degraded and remained intact.

Since the present study is related to fossil tetrads, it is impossible to postulate the pH values in the microsporangium of the fossil plant to which these belonged. However, it is convincing from the above studies on the extant plants *Allium* and *Petunia* that the pH values were not adequate. Either the callase could not be produced by the tapetum or it did not work optimally; ultimately the callose walls could not be dissolved, which is required to facilitate the release of microspores.

The tetrad genera reported from the Triassic sequences are mostly aligned to the orders Selaginellales and Isoetales (Balme, 1995) on morphological details. The ultrastructural analysis carried out on the tetrad genera *Densoisporites*, *Lundbladispota*, *Otyisporites* and *Uvaesporites* collected from the Permian–Triassic transition of Italy and Greenland also confirm Isoetalean (Pleuromeiaceae) affinity to the first three spores and selaginalean to *Uvaesporites* (Looy *et al.* 2005). The tetrads *Verrucosisorites*, *Lapposisorites* and *Decisporis* are broadly affiliated to the pteridophytes as they possess trilete marks. The affinities of Permian tetrads (namely *Jayantisorites*, *Indotriradites*, *Microbaculispora* and *Microfoveolatispora*) could not be determined due to the absence of any sporangia or related plant parts in the assemblage; however, the presence of well-developed trilete marks in them at least demonstrate their alliance with the pteridophytes.

Different hypotheses have been proposed to evaluate the reasons for the release of intact spore tetrads from the sporangia. Most of the workers who reported tetrads from the Permian–Triassic transition and from Triassic strata advocated extreme hot conditions as being responsible for the tetrad formation. While investigating the Lower Panchet (Induan–Olenekian) palynomorphs from the Raniganj Coalfield, Tiwari & Meena (1989) observed a high incidence of tetrads in the palynoassemblage; however, they were missing from the upper Permian (Lopingian) Raniganj palynoflora. They opined that warmer conditions due to high temperature and low rainfall at the beginning of the Triassic period might be responsible for the inertness of the callase enzyme. They further stated that it could also be an adaptive strategy of certain taxa to produce tetrads instead of monads so that they could withstand the changing climatic conditions and protect individual spores from perishing in such disastrous conditions.

Looy *et al.* (2005) also linked the enormous production of tetrads during the Triassic period to environmental changes that affected the regular sporogenesis cycle in some specific taxonomic groups. Foster & Afonin (2005) opined that the formation of such unusual palynomorphs around the Permian–Triassic boundary is the result of deteriorating atmospheric conditions. Visscher *et al.* (2004) referred to the permanent tetrads found in the end-Permian rocks as unusual mutated palynomorphs, where mutations were brought about by the worldwide increase in near-surface ultraviolet-B radiation (UV-B, 280–315 nm). Extensive volcanic eruptions over a large area of Siberia at the beginning of the Triassic period resulted in the release of huge amounts of organo-halogens (CH_3Cl) into the atmosphere. These organo-halogens depleted the stratospheric O_3 globally, allowing the harmful ultraviolet-B radiation into the troposphere, thereby changing the nucleotide sequencing of the genome of the plants in question and causing mutations to occur (Visscher *et al.* 2004; Looy *et al.* 2005). While applying the Cambridge two-dimensional chemistry–transport model to assess the specific causes of O_3 depletion,

Beerling *et al.* (2007) projected that both HCl released during eruption of the Siberian Traps, as well the release of massive amounts of CH_3Cl by the combustion of dispersed organic matter entrapped in the Siberian rocks, were responsible for the O_3 depletion. They also concluded that the combined effect of these chemicals vigorously depleted the O_3 of the stratosphere and the resulting high-intensity UV-B radiation destabilized the plant genomes.

In our opinion, the spore tetrads are simply the unseparated miospores stacked together due to non-dissolution of the callose walls and not the mutated palynomorphs as stated by Visscher *et al.* (2004) and Looy *et al.* (2005). If the mutations had occurred in the plants inhabiting the Early Triassic palaeoforests, they would have transformed genetically and not produced normal spores during Middle–Late Triassic time. This assumption is based on the fact that some of the miospore genera (namely, *Lundbladispota* and *Densoisporites*), whose spore tetrads existed at the Permian–Triassic boundary or in the Lower Triassic rocks, are found to occur again in the Middle and Upper Triassic rocks. The miospore taxa *Lundbladispota raniganjensis*, *Densoisporites contactus* and *D. playfordi* are recorded in the borehole RD-1/4, depth 532.48 m, near Durgapur City, Raniganj Coalfield (Tiwari & Rana, 1981). These authors correlated this section as belonging to the Mahadeva Formation (lower Middle Triassic–Anisian) on the basis of the palynoassemblage. Hermann *et al.* (2012) recently reported *Densoisporites* spp. (as dispersed miospore) from the Landa Member (Anisian) of the Salt Range and Surghar Range sections in Pakistan. Similarly, Ram-Awatar *et al.* (2014) reported miospore genera *Lundbladispota* and *Densoisporites* from the C Member of Lashly Formation (Middle–Upper Triassic) of Allan Hills, central Transantarctic Mountains, South Victoria Land, Antarctica. If some kind of mutagenic activity occurred in the plants that produced the genera *Lundbladispota* and *Densoisporites* during Early Triassic time, they would have changed genetically and would not have produced the normal miospores of these genera during Middle–Late Triassic time; instead, they would have produced the so-called mutated tetrads. Similarly, the theory of mutagenesis does not apply to the tetrads reported from Ordovician–Lower Devonian rocks and early Permian strata as no volcanic activity has been reported during these time scales, despite the presence of a large number of tetrads in the rocks of these periods.

6. Conclusions

1. A good assemblage of naked, permanent tetrad spores has been reported for the first time from a cold-regime depositional stratum, that is, the Lower Barakar Formation of early Permian (Artinskian) age in Singrauli Coalfield, Son-Mahanadi Basin, central India. This is also the first record of tetrads from any early Permian locality in the world. The affinity of these

tetrads could not be ascertained due to the absence of sporangia or related plant parts in the assemblage; however, the presence of well-developed trilete marks in the tetrads at least demonstrates their alliance with the pteridophyte group.

2. The study also supports the hypothesis that the formation of tetrad spores is somewhat linked to extreme climatic conditions as evidenced by the global records of their occurrences in Ordovician – Lower Devonian sediments, in lower Permian (Asselian–Artinskian) rocks, at the Permian–Triassic boundary and thereafter in Lower Triassic sediments, all correlated with extreme cold (due to Ordovician–Silurian and Carboniferous–Permian glaciations) and hot (due to Early Triassic Siberian volcanic activity) climatic conditions.

3. It is proposed that the extreme climatic conditions prevailing during the above mentioned regimes might have triggered some sort of malfunction in the sporogenesis process, which altered the pH values inside the microsporangium and inhibited the release of callase enzyme from the tapetum or diminished its optimal activity. As a consequence, large number of tetrads could not be dissociated into individuals or monads and remained intact.

4. The hypothesis of mutagenesis based on the identification of spore tetrads as the mutated palynomorphs is not justified, as similar spore tetrads also occur in the Ordovician – Lower Devonian sediments and in early Permian rocks where there is no evidence of any kind of volcanic activity that might have induced the mutations as has been established for the tetrads of the Triassic period. We prefer to refer to them as the unseparated miospores, adhered together due to the non-dissolution of the callose walls.

5. The occurrence of spore tetrads in the sediments can be linked to the prevalence of intense climatic conditions during the geological past.

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