RESEARCH ARTICLE

A New Pollen Aperture in *Schleichera oleosa* (Lour.) Oken from Madhya Pradesh, Central India, and its Taxonomic and Evolutionary Significance

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Abstract

Angiosperms demonstrate a surprisingly frequent phenomenon of variability in the gross pollen morphological characteristics. In the present study, we recorded and described a new aperture in the pollen grains of *Schleichera oleosa* (Lour.) Oken, a monotypic genus of the soapberry family Sapindaceae, from Madhya Pradesh, central India, based on the LM and CLSM observations. This condition is termed the tetra-zono-parasyncolporoidate condition. A new pollen aperture has been recorded in this monotypic genus with the usual one (tri-zono-parasyncolporoidate condition), and this phenomenon of the occurrence of a new with increased number of pollen aperture is known as pollen aperture heteromorphism. The study will further help identify this taxon from the sedimentary archives as one of the indicators of the warm and humid (moist) climate and increased monsoonal rainfall.

Keywords: *Schleichera oleosa* (Lour.) Oken, Monotypic genus, Tetra-zono-parasyncolporoidate pollen, Sapindaceae, Central India

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Introduction

Angiosperms display striking variations in the gross pollen morphological attributes, such as grain size, shape, number of apertures, exine thickness, and ornamentation within and among populations of the same species, as well as within an individual plant (Wodehouse, 1959; Erdtman, 1966; Dajoz *et al*., 1991; Pire and Dematteis, 2007; Yasmin *et al*., 2010; Bhowmik and Datta, 2012; Sousa *et al*., 2013). Among the various variations, variation in the number, arrangement, and dispensation of apertures is the most common (Mignot *et al*., 1994; Pire and Dematteis, 2007).

Schleichera sp. Willd. (Sapindaceae) is a deciduous tree found naturally distributed from the Himalayan foothills, western Deccan to Sri Lanka and China. It is also found growing in Central India, Bihar and Southern parts of India (Mabberly, 1987; Kundu and Schmidt, 2011; www.SL.ku.dk; PROSEA). It is also rarely cultivated in many parts of the tropics, especially in India. *Schleichera oleosa* has tri-zono-parasyncolporoidate pollen, in general. In the present study, we recorded a new tetra-zono-parasyncolporoidate pollen, although comparatively in lesser value, besides the usual record of tri-zono-parasyncolporoidate pollen, in the same monotypic genus *Schleichera oleosa* (Lour.) Oken.

It is the first study of its kind from central India using the Herbarium specimen.

Material and Methods

Pollen sample of *Schleichera oleosa* (Lour.) Oken was acquired from the Herbarium specimen of the Botanical Survey of India (BSI), Central Region Circle, Prayagraj, India (Voucher specimen number 34414; Ajaygarh, 24.3.83, Ram Lal 34414). For light microscopic (LM), and confocal laser scanning microscope (CLSM) studies, the pollen samples were prepared after employing the methods of extraction of palynomorphs, devised by Erdtman (1960), and also followed by Quamar *et al.* (2017, 2021a, 2022). The dried floral material after soaking in glacial acetic acid in a vial, crushing (gently) using a glass rod and sieving (with a 150 mesh sieve) was subjected to an acetolysis mixture comprising a 9:1 ratio of acetic anhydride ($C_4H_6O_3$) and concentrated sulphuric acid (H_2SO_4) in a sterilized centrifuge tube in the hot water bath for 5 min (Erdtman, 1943, 1960). At every stage, centrifugation and decantation were accomplished. Finally, the material was treated with 50% glycerine solution. The mounting of the material was done in glycerine jelly on a glass slide and sealed with wax for a permanent record. The prepared reference slides of pollen have been labelled, numbered (reference laboratory number11804) and deposited at the sporothek of the Birbal Sahni Institute of Palaeosciences (BSIP) (Lucknow) and used for the LM study at the Quaternary Palynology Laboratory of the BSIP. For the CLSM study and also for taking micro-photographs, the same slide (prepared for the LM study) was used. The CLSM

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study was carried out at the CLSM and Raman Spectroscopy Laboratory of the BSIP. The pollen identification was assisted by authored reference materials (Chauhan and Bera, 1990; Nayar, 1990; Quamar and Chauhan, 2011, 2012; Quamar and Bera, 2017). Regional reference collections held at the BSIP Herbarium, Lucknow were also consulted to substantiate the identification. The terminology used for describing the morphology of the studied plant pollen taxon follows Erdtman (1952), Faegri and Iversen (1964), Walker and Doyle (1975), and Punt *et al.* (2007) and unless otherwise stated.

Results and observation

Pollen grain of *S. oleosa* (Lour.) Oken: Type I

Tetra-zono-parasyncolporoidate, shape: oblate spheroidal/ sub-spheroidal (8x9 µm; variable), bilateral (symmetry), isopolar (polarity), colpi (brevicolpi; long and broad (Length 3 µm, Width 1 µm), apices acute; margin smooth; os indistinct, four colpi fused together forming a quadrangular structure (apocolpium; 4x3 µm; variable), exine 0.9 µm thick, sexine slightly thicker than nexine, pattern striate (Fig. 1- A, B, F, H, I, J).

It has been encountered in a comparatively lower value of 24% of the Total Pollen Count.

Pollen grain of *Schleichera oleosa* (Lour.) Oken: Type II

Tri-zono-parasyncolporoidate, shape: oblate spheroidal/sub-spheroidal (7.5x8µm; variable), bilateral (symmetry), isopolar (polarity), colpi long and broad (brevicolpi; Length 3 µm, Width 0.5 µm), apices acute; margin smooth; os indistinct, colpi (all the three colpi) fused together and form a triangular structure (apocolpium; 3x4 µm; variable), exine 0.7µm thick, sexine slightly thicker than nexine, pattern striate (Fig. 1- C, D, E, G).

It has been encountered in higher values, 76% of the Total Pollen Count.

Table 1: Representation of both Type I and Type II pollen of *Schleichera oleosa* (Lour.) Oken, its voucher specimen number and reference laboratory number.

Schelichera oleosa (Lour.) Oken	Relative abundance (%)	Voucher specimen number	Reference laboratory number
Type I	24 %	34414; Ajaygarh, 24.3.83, Ram Lal	11804
Type II	76%	34414	

The relative abundance (%) of the recovered Type I and Type II pollen, voucher specimen number and reference laboratory number of the studied monotypic genus i.e. *Schleichera oleosa* (Lour.) Oken has been shown in Table 1.

Discussion and Conclusion

Tetra-zono-parasyncolporoidate pollen of *Schleichera oleosa* (Lour.) Oken was found relatively in a lesser frequency (24%), whereas the usual form as tri-zono-parasyncolporoidate pollen has 76% representationin the prepared palynoslide (Table 1). The occurrence of a new aperture in the studied sample is a new record in this monotypic genus and this phenomenon is known as "pollen aperture heteromorphism" (Erdtman, 1966; Wodehouse, 1965; Pire and Dematteis, 2007). The study has taxonomic significance as *Schleichera oleosa* (Lour.) Oken is one of the common and usual associates of the moist and dry tropical deciduous forests in central India. The correct identification of this moist tropical deciduous forest element of the said forest types could not only help differentiate it from the species of *Syzygium* R.Br. ex Gaertn., and *Eucalyptus* L Her.(of the family Myrtaceae), but also suggest, in association with other moist forest elements, a warm and humid (moist) climate with intensified monsoon around the study area. The present study clearly demonstrated that the use of LM and

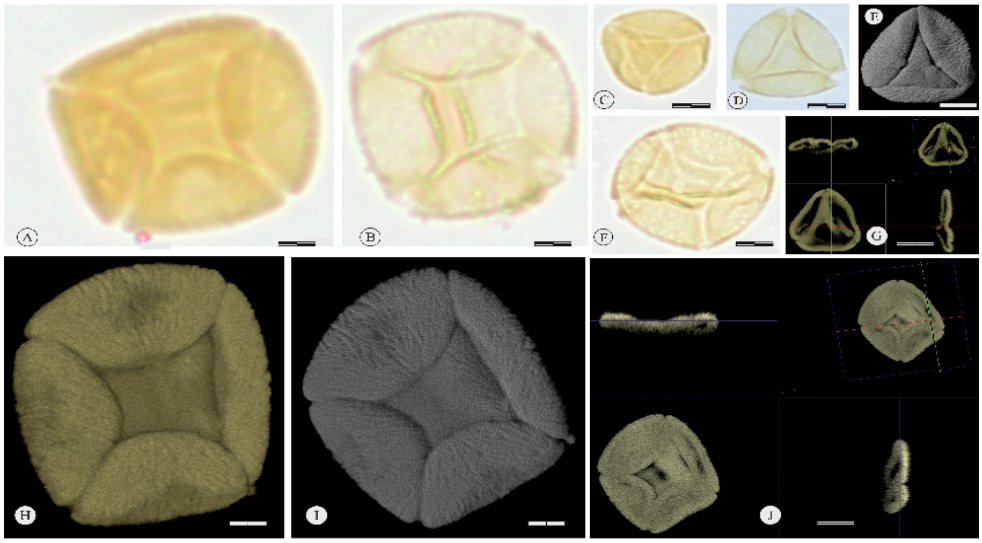

Fig. 1: *Schleichera oleosa* (Lour.) Oken (Type I): figures A, B & F. LM microphotographs; figures H & I. CLSM images, showing the tetra-zono-parasyncolporoidate condition and the striate pattern; figure J. Cross section of the tetra-zono-parasyncolporoidate grain, showing the exine thickness.
Schleichera oleosa (Lour.) Oken (Type II): figures C &D. LM microphotographs; figure E. CLSM image, showing the tri-zono-parasyncolporoidate condition and the striate pattern; figure G. Cross section of the tri-zono-parasyncolporoidate grain, showing the exine thickness.
(Scale bar=10 µm.)

CLSM has provided high-resolution pollen morphological characteristics and surface ornamentation of *Schleichera oleosa* (Lour.) Oken, thus, providing a separate taxonomic identity of this monotypic genus (with a unique phenomenon of pollen aperture heteromorphism). This aspect could help understand the pollen deposition pattern around the study area (Quamar and Kar, 2020a), as well as in interpreting the fossil pollen records for the reconstruction of past vegetation dynamics and associated climate change (Quamar, 2021; Quamar and Bera, 2020, 2021; Quamar and Kar, 2020b; Quamar *et al*., 2021a; Kar and Quamar, 2019, 2020 and references cited therein). The increased number of aperture has also evolutionary implications also, as it was postulated that the higher the number of apertures (colpi and/or pores) along with extremely ornamented exine surface, the advanced (derived) the grain is, and vice-versa (Nadot *et al.,* 2000; Yildiz *et al.,* 2009). Therefore, it can be suggested that *Schleichera oleosa* (Lour.) Oken Type I (Fig. 1- A, B, F, H, I, J) having tetra-zono-parasyncolporoidate condition is advanced (derived), whereas *Schleichera oleosa* (Lour.) Oken Type II having tri-zono-parasyncolporoidate condition (Fig. 1- C, D, E, F) is primitive (ancestral).

The ploidy level of this monotypic genus is 2 (2n= 32 [n=16]) and the C-value‡ of DNA of this monotypic genus also varies (Ohri *et al*., 2004; PROSEA). Changes in ploidy level, the C-value of DNA, and meiosis (Stainier *et al*., 1967; Mignot *et al*., 1994; Dajoz *et al*., 1995; Nadot *et al*., 2000; Ohri *et al*., 2004; Campos *et al*., 2011; Sousa *et al*.,2013; Siddhanta *et al*., 2018; Quamar *et al*., 2021b) could be driving the occurrence of the phenomenon of "pollen aperture heteromorphism". However, the effect of environment and/or pollination ecology (Till- Bottraud *et al*., 1994; Nadot *et al*., 2000; Pire and Dematteis, 2007) cannot be ruled out. The morphological variations obtained in the present study (Fig.1- A, B, C, D, E, F, G, H, I, J) may be positively related to diversity in polyploidy level of *Schleichera oleosa* (Lour.) Oken, (2C DNA amount/ploidy level [picogram; pg**]: 1.17, 4C DNA amount ± s.d. [pg]: 4.66 ± 0.30, 1C DNA amount [mega-base pairs; Mbp]: 1142, **1 pg= 980 Mbp (Cavalier-Smith, 1985; 'C' value: C value is the amount of nuclear DNA in the unreplicated gametic nucleus, irrespective of the ploidy level of the species. In simple words, it is the amount of DNA [in 'pg'] contained within a haploid nucleus (e.g., a Gamete) or one-half the amount in a diploid somatic cell of a eukaryote. Borsch and Barthlott (1994) and Kreunen and Osborn (1999) were of the opinion that pollen aperture heteromorphism could be attributed to late aperture ontogeny and the resulting weak control over the developmental process of the aperture. Till-Bottraud *et al.* (1994) asserted that the pollen aperture heteromorphism is predicted to be an Evolutionary Stable Strategy (ESS) if there is a trade-off between germination speed and life expectancy of the pollen grain. Natural selection should favour many-aperturate quickly germinating pollen grains when pollinators are abundant, compared to longer-lived pollen grains with fewer apertures when pollinators are scarce. Till-Bottraud *et al.* (1994) and Nadot *et al.* (2000) further put forth a hypothesis that aperture number varies with several traits of pollination ecology and pollen physiology. Moreover, detailed molecular, as well as phylogenetic studies are required to confirm these possibilities.

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