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(Short Communication)

**Pollenkitt ropes of *Notopora schomburgkii* Hook. f. (Ericaceae, Vaccinieae)**

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Pollen morphology of *Notopora schomburgkii* Hook. f. was examined using light (LM), scanning (SEM) and transmission electron microscopy (TEM). Pollenkitt ropes were observed and reported for the first time on pollen grains of *N. schomburgkii*, Ericaceae. With TEM these ropes show lipidic (“pollenkitt-like”) electron density but also show some resistance to acetolysis.

**Key words:** *Notopora schomburgkii*, pollen morphology, pollenkitt ropes

**Introduction**

The genus *Notopora* Hook. f. (Ericaceae: Vaccinioideae: Vaccinieae) is a genus composed of five species of Neotropical blueberries<sup>(1-2)</sup> and it is endemic to the Guayana highland of Venezuela and adjacent Guyana<sup>(3-4)</sup>. Maguire, Steyermark and Luteyn<sup>(3)</sup> are the only workers who have previously studied the pollen morphology of four species of this genus including *N. schomburgkii*, under both light (LM) and scanning electron microscopes (SEM). They reported that pollen tetrads of the genus *Notopora* were 42 – 56 $\mu$ m in size under LM, without viscin threads, exine sculpturing rugulate/verrucate becoming psilate along the aperture margins and at distal poles.

The transfer of pollen grains in zoophilous angiosperms depends mainly on the adherence of sticky, clumping pollen grains to the flower visitors. Pollenkitt and viscin threads are two different modes, by which this clumping is occurred. Pollenkitt is to be found in all investigated angiosperm families <sup>(5)</sup>. On the other hand, viscin threads occur only in three not closely related angiosperm families, the Onagraceae, Ericaceae, and Caesalpinioideae (Leguminosae), and members of the family Onagraceae produce both pollenkitt and viscin threads <sup>(5)</sup>. Hesse <sup>(5)</sup> also stated that “pollenkitt is ubiquitous, and most probably also the other two families (means Ericaceae and Leguminosae), produce both pollenkitt and viscin threads”. In Ericaceae, viscin threads are found on pollen grains of members of the tribes Bejarieae, Phyllodoceae and Rhodoreae of subfamily Ericoideae <sup>(2)</sup>, and in a single genus *Gaylussacia* of the tribe Vaccinieae, subfamily Vaccinioideae <sup>(6)</sup>. During a detailed study on pollen morphology of the tribe Vaccinieae, we have found “viscin thread-like ropes”, which are latter identified as to be pollenkitt ropes (the terminology follows Hesse <sup>(5)</sup>), in pollen tetrads of *N. schomburgkii*.

### Material and Methods

Polliniferous material used in this investigation was taken from the dried herbarium specimen of the Herbarium of Botanical Museum, Göteborg, Sweden (GB). Collection information for the specimen examined is given below:

*Notopora schomburgkii* Hook. f., Venezuela: Edo. Bolivar, Gran. Sabana, 1280m alt. 17.11.1978. J.L. Lutyen, M. Labron-Lutyen & J.A. Steyermark 6286 (GB)

Pollen sample was acetolysed following the technique of Erdtman <sup>(7)</sup> modified by Takahashi <sup>(8)</sup>. After the dehydration in an ethanol series, acetolysed pollen was mounted in silicone oil (viscosity 3000 cs) for LM observations. Pollen was examined and measured with a Nikon Eclipse E200 microscope. Tetrad diameter (D), polar length (P) and equatorial length (E) of pollen, and length (2f) and width of colpus were measured based on at least 10 grains. Pollen slides are deposited at the Hokkaido University Museum, Sapporo, Japan.

For SEM, acetolysed pollen grains were dehydrated in an ethanol series, and mounted and air dried on aluminum stubs from 70% ethanol, and sputter coated with Gold-Palladium by a Hitachi E102 ion sputter. Subsequently the prepared pollen was examined and photographed with a Jeol JSM-5310LV scanning electron microscope operated at 15 KV.

To evaluate the resistance to acetolysis, we acetolysed the pollen grains up to 10 minutes or more, and the acetolysed grains were observed by SEM following the procedure stated above.

For TEM, the material from herbarium specimen was rehydrated in 3% Aerosol-OT solution for more than one week, and then fixed overnight in 1% osmium tetroxide solution. Fixed materials were dehydrated through an ethanol series and embedded in Epon 812 epoxy resin. Sections were cut on a Reichert-Jung Ultracut N ultratome, and post-stained with saturated uranyl acetate and lead acetate solution for 23 min (20 min and 3 min, respectively), and observed and photographed on Hitachi H-800 transmission electron microscope operated at 75 KV. Descriptive terminology follows Punt *et al.* <sup>(9)</sup>.

## Results and Discussion

Acetolysed pollen grains are united at tetrahedral tetrad (Fig. 1A), sometimes with “viscin thread-like ropes” (Figs. 1B, 1C), most grains broken, D (49.5–) 53.7 (–60.2)  $\mu\text{m}$ , P (22.3–) 26.7 (–28.9)  $\mu\text{m}$ , E (37.2–) 40.1 (–44.9)  $\mu\text{m}$ , 3-colporate, colpus distinct, (31.4–) 34.0 (–38.0)  $\mu\text{m}$  long, width (3.0–) 3.5 (–5.0)  $\mu\text{m}$ , exine sculpture verrucate or coarse verrucate to rugulate in LM.

We could not study the exine sculpture very clearly by SEM as acetolysed pollen grains were partly covered with pollenkitt debris (Fig. 1E). Electron dense pollenkitt was also recognized on the non-acetolysed pollen grains in TEM observations (Fig. 1D). The apocolpial exine is composed of ektexine; tectum, columellae (rod-like elements distinct), and foot layer, and endexine. Ektexine is ca. 1.5  $\mu\text{m}$  thick and total exine is ca. 1.7  $\mu\text{m}$  thick (Fig. 1F).

After the routine usual acetolysis treatment (about 5 min.), “viscin thread-like ropes” are observed on some pollen tetrads, which are likely to mislead the presence of viscin threads for the

*Notopora* pollen. Many pollen grains of *N. schomburgkii* also did not show any evidence of these ropes, and it might be due to the breakdown of these ropes at or near base during acetolysis. Like viscin threads of Ericaceae, the surface of these “thread-like ropes” is also smooth under both LM and SEM <sup>(5)</sup>. “Viscin thread-like ropes” are attached, commonly one per grain, on surface of pollen grains at near or around the colpus region and the maximum lengths observed attain between 252.2 – 325.0  $\mu\text{m}$ . The length of the ropes is difficult to determine as they are frequently broken, folded, or portions overlain by the grains (Fig. 1B, 1C), and is likely to vary between grains. Viscin threads of Ericaceous pollen are comparatively more fragile than those of the Onagraceae, and are attached commonly on distally polar surface <sup>(10)</sup>. However, the diameter of these “thread-like ropes” is unusually thick and varies from 3.0 – 9.3  $\mu\text{m}$ . From TEM micrographs, both “viscin thread-like ropes” and pollenkitt on exine surface show more or less similar electron density which might indicate the similarity in their composition, but different from that of exine (Figs. 1F, 1G).

Our pollen morphological observations on *N. schomburgkii* largely agree with the earlier description <sup>(3)</sup>, except for the presence of “viscin thread-like ropes” under both LM and SEM, although most of the grains were broken. Pollen grains of this species might be susceptible to acetolysis treatments. Lack of reference to viscin threads or “thread-like ropes” in previous study <sup>(3)</sup> might be due to the fragile nature and/or breakdown of such ropes during routine microtechnical procedures like acetolysis treatment. Moreover, another problem might be the chemical constituent of these “thread-like ropes”, as both the thread-like ropes and the pollenkitt on the exine surface were dissolved after strong acetolysis at the present study (for a period of 10 min or longer). Probably the “viscin thread-like” appearances are artifacts and should be termed as pollenkitt ropes (the term by Hesse <sup>(5)</sup>). The possibility of presence of pollenkitt in Ericaceae and Caesalpinioideae pollen was also previously argued <sup>(5)</sup>. Chemical composition of these pollenkitt ropes might be a complex mixture of lipid and other viscous substances (including carotenoids and other unknown substances) as suggested for pollenkitt <sup>(5)</sup>, but both the pollenkitt and pollenkitt ropes of *Notopora* pollen have somewhat resistance to acetolysis. The chemical composition of these (pollenkitt and the ropes) might be a topic for further research, as the pollenkitt ropes or tryphine

was never previously found in glutaraldehyde-fixed material or herbarium specimens<sup>(11)</sup>. Therefore, we should be careful about this kind of “viscin thread-like ropes” which do not contain sporopollenin, during our routine palynological works. It is assumed that the presence of viscin thread-like structures reflects an adaptation to zoophilous pollination<sup>(5, 10 and 12)</sup>, but the pollination biology of *Notopora* has not been studied yet. Luteyn<sup>(4)</sup> reported that Neotropical species which belong to the tribe Vaccinieae, are adapted to pollination by hummingbirds and rarely by bumble bees, butterflies or other insects.

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**Legends**

**Fig. 1.** Pollen features of *Notopora schomburgkii*. A. whole tetrad (LM), B – C & G. pollenkitt rope (LM, SEM and TEM, respectively), D whole tetrad (TEM), E. apocolpial exine (SEM), and F. exine structure (TEM).



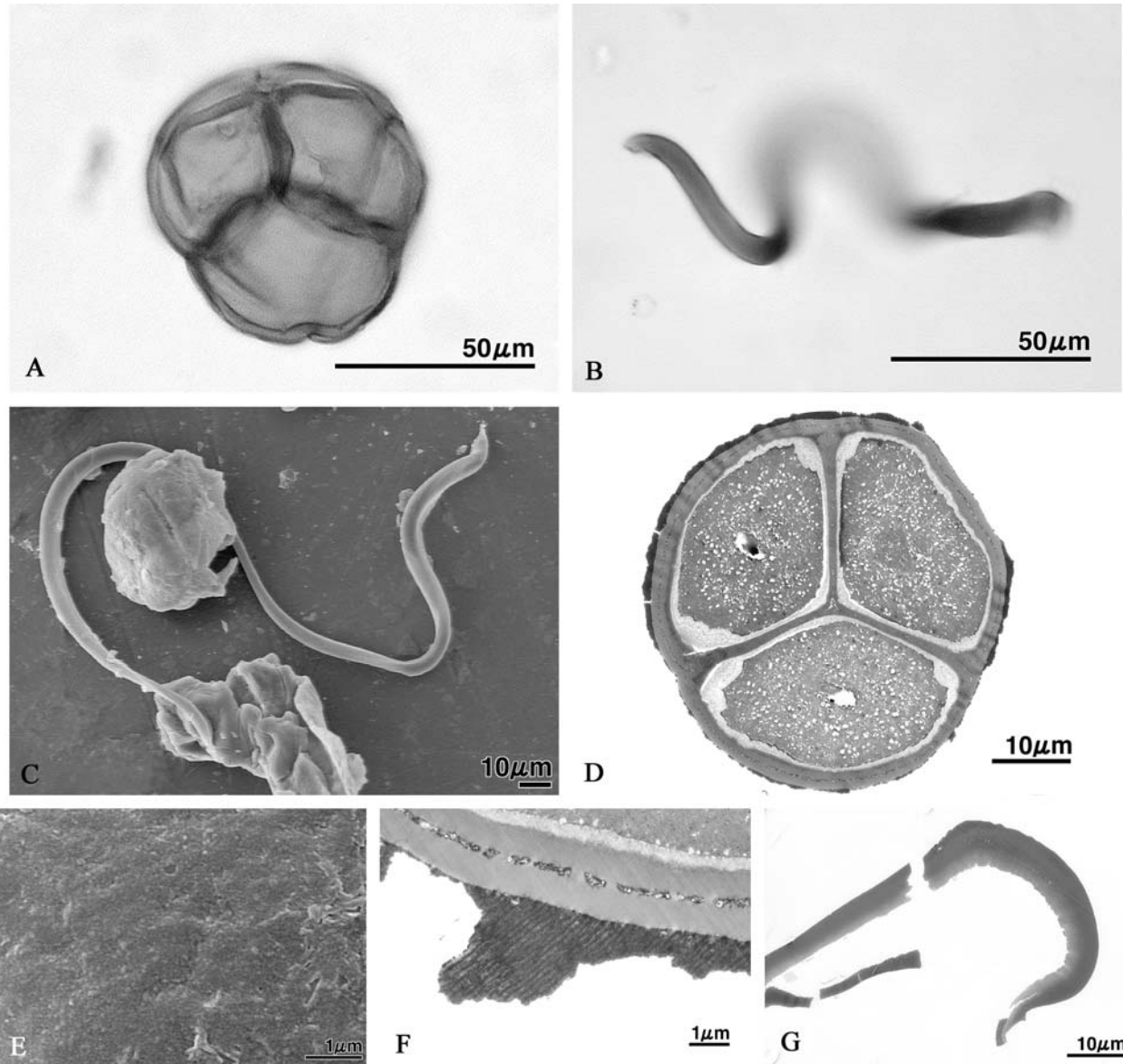


Fig. 1.