

POLLEN MORPHOLOGY OF *RHODODENDRON* L. AND RELATED GENERA AND ITS TAXONOMIC SIGNIFICANCE

A.K.M. GOLAM SARWAR¹ AND HIDEKI TAKAHASHI²

*Laboratory of Systematic Botany, Graduate School of Agriculture,
Hokkaido University, Japan.*

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Abstract

Pollen grains of 40 taxa of *Rhododendron* L. and its closely related genera, *Therorhodon* L. and *Menziesia* Sm., were examined by means of light microscopy and scanning electron microscopy (SEM), or SEM alone. Pollen tetrads of *R. japonicum*, *R. schlippenbachii*, *R. tsusiophyllum* and *M. pentandra* were examined also with transmission electron microscopy. In all the genera studied, 3-colporate, oblate to suboblate pollen grains are arranged in tetrahedral tetrads. The apocolpial pollen wall is composed of the exine - well developed tectum, columellae, foot layer and endexine, and the intine. On the contrary, the septal exine is composed of fragmentary tectum, and the two foot layers of adjacent grains are sometimes connected by columellae and endexine. Among different infrageneric taxa only the subsect. *Ledum* (sect. & subgen. *Rhododendron*) was characterized by small sized pollen tetrads, higher 2f/D value and rugulate exine sculpture. The pollen morphological characteristics overlapped each other in all other taxa. Thus palynological features showed little usefulness in the infrageneric classification of *Rhododendron*, however, they gave additional support to the individual generic status of *Menziesia* and *Tsusiophyllum*, and the sister relationship between *Rhododendron* and *Therorhodon*.

Introduction

The genus *Rhododendron* L., one of the largest and diverse genera of Ericaceae (Ericoideae, Rhodoreae), comprises over 1000 species (Chamberlain *et al.*, 1996). The centre of diversity of the genus is in the Himalaya, but *Rhododendron* sect. *Vireya* (Blume) Copel. f. is also diverse in Malesia (Sleumer, 1966). Since Linnaeus (1753) established *Rhododendron*, this large genus has posed systematic problems in terms of infrageneric circumscription and rank (for details see Kurashige *et al.*, 2001). *Rhododendron* and closely related genera are included in the tribe Rhodoreae (Kron *et al.*, 2002). The taxonomic history of the Rhodoreae is complex (Gillespie and Kron, 2010), but a brief introduction may help to illustrate the variations in generic composition of this group. According to Stevens (1971), tribe Rhodoreae comprises the genera *Rhododendron*, *Therorhodon*, *Ledum*, *Tsusiophyllum* and *Menziesia*. The genus *Therorhodon* was hypothesized as sister to *Menziesia* + *Tsusiophyllum* + *Rhododendron* (including *Ledum*) (Kron and Judd, 1990). In contrast, Chamberlain *et al.* (1996) recognized only 2 genera, namely *Rhododendron* and *Menziesia*. The recent classification of the Ericaceae (Kron *et al.*, 2002) recognized four genera in this tribe, namely *Diplarche*, *Menziesia*, *Rhododendron* (including *Ledum* and *Tsusiophyllum*) and *Therorhodon*. *Therorhodon* is often placed in *Rhododendron* (Chamberlain *et al.*, 1996; Kurashige *et al.*, 2001; Goetsch *et al.*, 2005); the cladistic analyses of molecular data

¹Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. E-mail: drsarwar@bau.edu.bd.

²The Hokkaido University Museum, N10 W8, Sapporo 060-0810, Japan.

also support reduction of the genus *Therorhodium* to a subgenus within the genus *Rhododendron* (Kurashige *et al.*, 2001; Gao *et al.*, 2002a; Goetsch *et al.*, 2005). Recently Craven (2011) has suggested the inclusion of *Diplarche* and *Menziesia* to *Rhododendron*, that will make the tribe Rhodoreae monogeneric.

Pollen morphology has been shown to be useful for taxonomic and phylogenetic analysis of some Ericaceous taxa (Kron *et al.*, 2002; Sarwar, 2007). The pollen of *Rhododendron* has also been studied by many researchers for taxonomic purpose as well as a part of regional flora (Huang, 1972; Vasanthi and Pocock, 1987; Fuhsung *et al.*, 1995; Mao *et al.*, 2000; Terzioğlu *et al.*, 2001; Gao *et al.*, 2002b, c; Wang *et al.*, 2006; Zhang *et al.*, 2009; Miyoshi *et al.*, 2011). All these results showed that pollen tetrads of *Rhododendron* were diverse in size and exine sculpture; sometime sufficient to differentiate the sections (Gao *et al.*, 2002b; Zhang *et al.*, 2009), but insufficient to differentiate the subgenera (Gao *et al.*, 2002c; Wang *et al.*, 2006). Moreover, our knowledge of pollen morphology and ultrastructure is still very limited for such a large genus as *Rhododendron*. Therefore, the objectives of this study were to clarify the differences of the pollen grains among *Rhododendron* and closely related genera of the tribe Rhodoreae, and to study the systematic significance of the micromorphology of pollen grains for the genus *Rhododendron*.

Materials and Methods

Pollen morphology of a total of 40 taxa of *Rhododendron* and its closely related genera of the tribe Rhodoreae, *Rhododendron* (34 spp.), *Therorhodium* (2 spp.) and *Menziesia* (4 spp.), was examined by means of light microscopy (LM) and scanning electron microscopy (SEM), or SEM alone (Table 1). The pollen tetrads of *R. japonicum*, *R. schlippenbachii*, *R. tsusiophyllum* and *M. pentandra* were examined with transmission electron microscopy (TEM) to study the exine stratification of respective genera. Polliniferous materials used in this investigation were taken from the dried specimens from the herbaria C, GB, SAPS, SAPT and TUS. Abbreviation of the herbarium names except for SAPT (the Botanic Garden, Hokkaido University, Sapporo) are according to the Index Herbariorum (Holmgren *et al.*, 1990).

Table 1. List of taxa used in this study along with their voucher specimens.

No.	Taxa	Voucher specimens
1.	<i>Rhododendron albrechtii</i> Maxim.	Japan: Hokkaido, Inaho pass, 22.05.1983, Takahashi 3975 (SAPS)
2.	<i>R. arborescens</i> Torr.	USA: no locality, (Herb. Arbor. Harvard Univ.) Fl. 28.06.1892. Unknown <i>s.n.</i> (SAPS)
3.	<i>R. aureum</i> Gergi.	Japan: Hokkaido, Mt. Daisetsu; Mt. Asahi-dake, 20.06.1982, Takahashi 2512 (SAPS)
4.	<i>R. brachycarpum</i> D. Don	Japan: Honshu, Mt. Zao, 08.07.1983, Takahashi <i>et al.</i> 40 (SAPS)
5.	<i>R. dauricum</i> L.	Japan: Hokkaido, Iburi-shicho, Hobetsu-cho, 11.05.2004, Kanayama <i>et al.</i> 04-9050 (SAPS)
6.	<i>R. davidsonianum</i> Rehd. & Wils.	Scotland: Royal Botanic Garden, Edinburg, noday, 05.1971, C9180 (GB)
7.	<i>R. decorum</i> Franch.	China: Prov. Sze-chuan, Teng-hsiang-ying, 20.05.1922, Smith 2016 (GB)
8.	<i>R. degronianum</i> Carr.	Japan: Nagano Pref., Mt. Kimpu-san, 22.06.1975, Iketani 1763 (TUS 129348)
9.	<i>R. dilatatum</i> Mig.	Japan: Yamanashi Pref., Minamitsuru-gun, 01.05.1983, Togashi <i>s.n.</i> (SAPT)
10.	<i>R. diversipilosum</i> (Nakai) Harma	Japan: Prov. Mutsu, Mt. Hakkoda, 30.06.1978, Takahashi 206 (SAPS)

Table 1 contd.

No.	Taxa	Voucher specimens
11.	<i>R. formosanum</i> Hemsl.	Taiwan: Taichung Co., Gukan-Chinsan, 16.03.1985, Murata 17561 (TUS)
12.	<i>R. groenlandicum</i> (Oeder) Kron & Judd	Greenland: Godthabsfjord, Ilulailik, Igdlorssuit, 17.7.1976, Hansen & Fredskild 1007 (SAPT)
13.	<i>R. hidakanum</i> Hara	Japan: Hokkaido, Hidaka, Syoya, 10.5.1977, Tateishi & Togashi <i>s.n.</i> (TUS 66107)
14.	<i>R. indicum</i> Sw.	Japan: Tokyo, cult., no day.05.1882, Miyabe <i>s.n.</i> (SAPS)
15.	<i>R. japonicum</i> (A. Gray) Suring.	Japan: Nagano Pref., bet. Shirakaba-ko and Mt. Tateshina-yama, 29.05.1983, Takahashi 3998 (SAPS)
16.	<i>R. kaempferi</i> Planch.	Japan: Miyagi Pref., Mts. Abukuma, Wariyama pass, 10.05. 1986, Iketsu <i>et al.</i> 95 (SAPT)
17.	<i>R. keiskei</i> Miq.	Japan: Kagoshima Pref., Yaku Is., Mt. Tachudake, 10.05.1984, Murata <i>et al.</i> 17861 (TUS)
18.	<i>R. lapponicum</i> (L.) Wahlenb.	Canada: Manitoba, Churchill, 26.06.1984, Gillett 1835 (C)
19.	<i>R. macrosepalum</i> Maxim.	Japan: Shokoku Isl., Kagawa Pref., Kida-gun, 05.05.1982, Takahashi 1033 (SAPS)
20.	<i>R. macrostemon</i> Maxim.	Japan: Hondo, Yamamoto in Settsu, cult., 10.05.1953, Togasi 688 (SAPS)
21.	<i>R. maddenii</i> Hook. f.	Bhutan: Thimphu–Nimchling–Tanalum Bridge–Bunakha–Chima Khoti, 01.06.1967, Kanai <i>et al.</i> 346? (TUS 57346)
22.	<i>R. mucronulatum</i> Turcz. var. <i>ciliatum</i> Nakai	Korea: Keisho-Nando, 20.05.1039, Yokoyama 299 (SAPS)
23.	<i>R. nudipes</i> Nakai.	Japan: Shiga Pref., Mts. Hirasan, 06.05.1981, Murata 10910 (SAPT)
24.	<i>R. parvifolium</i> Adams.	Japan: Prov. Nemuro, Ochii-shi, 16.06.1934, Tatewaki 20940 (SAPS)
25.	<i>R. quinquefolium</i> Bisset <i>et</i> Moore	Japan: Rikuzen, Mt. Funagata, 06.05.1972, Ogura 1637 (TUS 68874)
26.	<i>R. schlippenbachii</i> Maxim.	Japan: Hokkaido, Sapporo-shi, Hokkaido University campus, cult., 18.05.2004, Sarwar & Takahashi <i>s.n.</i> (SAPS)
27.	<i>R. semibarbatum</i> Maxim.	Japan: Kyushu, Mts. Sobo-kutamuki, 07.07.1979, Murata 7987 (TUS 57400)
28.	<i>R. subarcticum</i> Harmaja	Japan: Hokkaido, Mt. Taira-yama, 30.06.1982, Takahashi <i>et al.</i> 2643 (SAPS)
29.	<i>R. trinerve</i> Fr.	Japan: Niigata, Iwafune-gun, Takanosu-yama, 10.07.1974, Togashi <i>s.n.</i> (TUS 67214)
30.	<i>R. tschonoskii</i> Maxim.	Japan: Honshu, Mt. Zao, 08.07.1983, Takahashi <i>et al.</i> 33 (SAPS)
31.	<i>R. tsusiophyllum</i> Sugim.	Japan: Hakone, Mt. Koma, 31.07.1926, Sawada <i>s.n.</i> (C) Japan: Sagami, Komagatake in Mt. Hakone, 10.08.1927, Asahina & Hisauchi <i>s.n.</i> (TUS 4578)
32.	<i>R. viscistylum</i> Nakai var. <i>amakusaense</i> Tak. <i>ex</i> Yam.	Japan: Kumamoto Pref., Mt. Nokogiridake, 30.04.1978, Minamidani 29613 (TUS 100748)
33.	<i>R. wadanum</i> Makino	Japan: Prov. Rikuzen, Sendai-shi, Aoba-yama, 29.04.1977, Takahashi 550 (SAPS)
34.	<i>R. weyrichii</i> Maxim	Japan: Shikoku, Kagawa Pref., Goshikidai, 28.04.1973, Shimamura <i>et al.</i> <i>s.n.</i> (SAPT)
35.	<i>Therhodion camtschaticum</i> (Pall.) Small	Japan: Hokkaido, Mt. Chiroro, 07.08.1985, Takahashi <i>et al.</i> 5836 (SAPS)
36.	<i>T. redowskianum</i> (Maxim.) Hutch.	Russia: South Sakhalin, Poronaysk, 15.7.1937, Yoshimura & Hara <i>s.n.</i> (SAPS)
37.	<i>Menziesia cicalyx</i> (Miq.) Maxim.	Japan: Shiga Pref., Mt. Anzouyama, 03.05.1988, Tateishi & Hoshi 13689 (TUS)
38.	<i>M. goyozanensis</i> Kikuchi	Japan: Iwate Pref., Mt. Goyozan, Tatamiishi-top, 07.07.1984, Mieno 445 (TUS)
39.	<i>M. multiflora</i> Maxim.	Japan: Prov. Rikuzen, Miyagi-gun, Izumigatake, 14.06.1978, Takahashi 767 (SAPS)
40.	<i>M. pentandra</i> Maxim.	Japan: Hokkaido, Sapporo-shi, Mt. Muine, 06.07.1982, Takahashi 2687 (SAPS)

Pollen grains were acetolysed following Sarwar and Takahashi (2012a). For LM, the dehydrated (in an ethanol series) pollen was mounted in silicone oil (viscosity 3000 cs), and examined and measured with a Nikon Eclipse E200 microscope. The dimensions “D”, “P”, “d(E)” and “2f”, corresponding to the tetrad diameter, polar length, equatorial length and colpus length of pollen grain were measured, and the D/d, P/E and 2f/D ratio was calculated (Oldfield, 1959). The arithmetic mean, standard deviation and the maximum and minimum values were calculated using the XLSTAT 2009.3 program. Pollen slides of all collections are deposited at the Hokkaido University Museum, Sapporo, Japan. Pollen size and shape classes were used following Erdtman (1986) and descriptive terminology follows Sarwar *et al.* (2006) and Punt *et al.* (2007).

For SEM, the acetolysed pollen samples were dehydrated in an ethanol series, mounted and air dried on aluminum stubs from 70% ethanol, and sputter coated with Platinum-Palladium using a Hitachi E102 ion sputter. Subsequently, these were examined and photographed with a Jeol JSM-5310 LV scanning electron microscope operated at 15 KV. The SEM micrographs of apocolpial exine sculpture from similar positions were used for the purpose of description and comparison.

For TEM, material from herbarium specimens 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 using 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 using a Hitachi H-800 transmission electron microscope operated at 75 KV.

Results

Pollen morphology of Rhododendron:

Pollen grains are in tetrahedral tetrads, rarely compact or lobed, grains somewhat shrunk in some species (severly in *R. groenlandicum*), rarely with other configurations, sometimes in giant dyads in *R. tsusiophyllum*; viscin threads present; D 30.9-67.1 μm , P 16.3-35.8 μm , E 21.8-47.5 μm , D/d 1.31-1.51, P/E 0.66-0.81, oblate or suboblate; 3-colporate, rarely 4-colporate in *R. kaempferi*, finely demarcated, 2f 14.5-30.4 μm , W 0.7-2.2 μm , 2f/W 6.59-35.43, 2f/D 0.31-0.54, costae present, distinct or indistinct, colpus margin distinct; endocracks present; endoaperture lalongate, 0.6-2.6 μm long, 6.8-15.2 μm wide; apocolpial exine 1.7-3.6 μm thick, septum 0.6-3.6 μm thick; tectate, apocolpial exine sculpture from verrucate to rugulate (Table 2). In SEM, the pollen surface varies from uneven and rugged to flat, primary apocolpial exine sculpture indistinct, secondary sculpture finely (0.1-0.25 μm) to moderate (0.25-0.4 μm) gemmate-pilate (Type GP; Figs. 1F, G, I-O, 2A-D, G-O, 3A-E); or surface rugged to flat, apocolpial exine sculpture coarsely rugulate, grooves distinct (Type R; Figs 2E, F, 3F); or intermediate (Fig. 1H); colpus membrane granulate to granuloid or rarely smooth.

Three species of *Rhododendron*, viz. *R. japonicum*, *R. schlippenbachii* and *R. tsusiophyllum* were studied with TEM. The apocolpial exine is composed of ektexine and endexine (Figs 4A - I). Sexine is c. 1.1-1.3 μm thick, tectum canalized in *R. japonicum* (Fig. 4E), and a total exine is c. 1.8-2.1 μm thick. The septum is c. 0.9-1.9 μm thick. The intine is almost evenly thick around the pollen tetrad, showing lower electron density than the endexine beneath both the apocolpial and septal exine.

Pollen morphology of Therorhodion:

Pollen of *T. redowskianum* was studied only with SEM. Pollen grains are in lobed tetrahedral tetrads; viscin threads present; D 50.0 μm , P 26.5 μm , E 35.0 μm , D/d 1.43, P/E 0.76, suboblate;

3-colporate, colpi short and narrow in *T. redowskianum*, 2f 14.8 μm , W 2.9 μm , 2f/W 5.1, 2f/D 0.3, costae present and distinct, colpus margin distinct; endocracks present; endoaperture lalongate, 1.7 μm long, 9.7 μm wide; apocolpial exine 2.2 μm thick, septum 1.4 μm thick; tectate, apocolpial exine sculpture from verrucate to rugulate (Table 2).

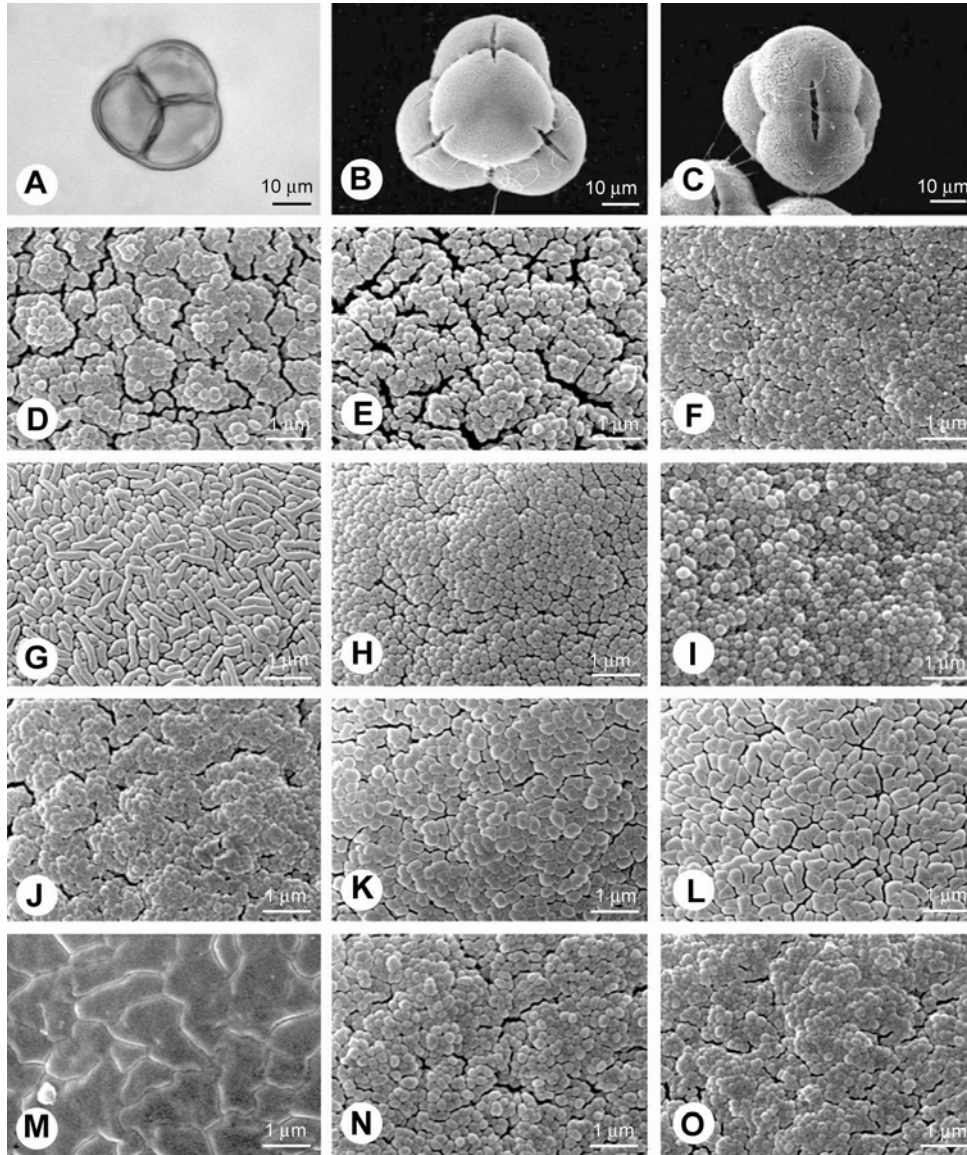


Fig. 1. LM and SEM micrographs of *Rhododendron* pollen. A) *R. formosanum* (Murata 17561); B) *R. maddenii* (Kanai *et al.* 346?). C) *R. tsusiophyllum* (Sawada *s.n.*); D) *R. aureum* (Takahashi 2512); E) *R. kaempferi* (Iketsu *et al.* 95); F) *R. aureum* (Takahashi 2512); G) *R. brachycarpum* (Takahashi *et al.* 40); H) *R. decorum* (Smith 2016); I) *R. degonianum* (Iketsu 1763); J) *R. formosanum* (Murata 17561); K) *R. macrostemon* (Togasi 688); L) *R. viscistylum* var. *amakusaense* (Minamidani 29613); M) *R. semibarbatum* (Murata 7987); N) *R. arborescens* (Unknown *s.n.*); O) *R. albrechtii* (Takahashi 3975). Pollen tetrads at polar view (A-D); tetrads with viscin threads (A, B, D); pollen tetrad at equatorial view with viscin threads (E); micrographs with apocolpial exine sculpture details (F-O).

In SEM, pollen surface is uneven and rugged, primary apocolpial exine sculpture indistinct, secondary sculpture finely (0.1-0.25 μm) to moderate (0.25-0.4 μm) gemmate-pilate (Type GP; Figs 5D-E); colpus membrane granuloid or smooth.

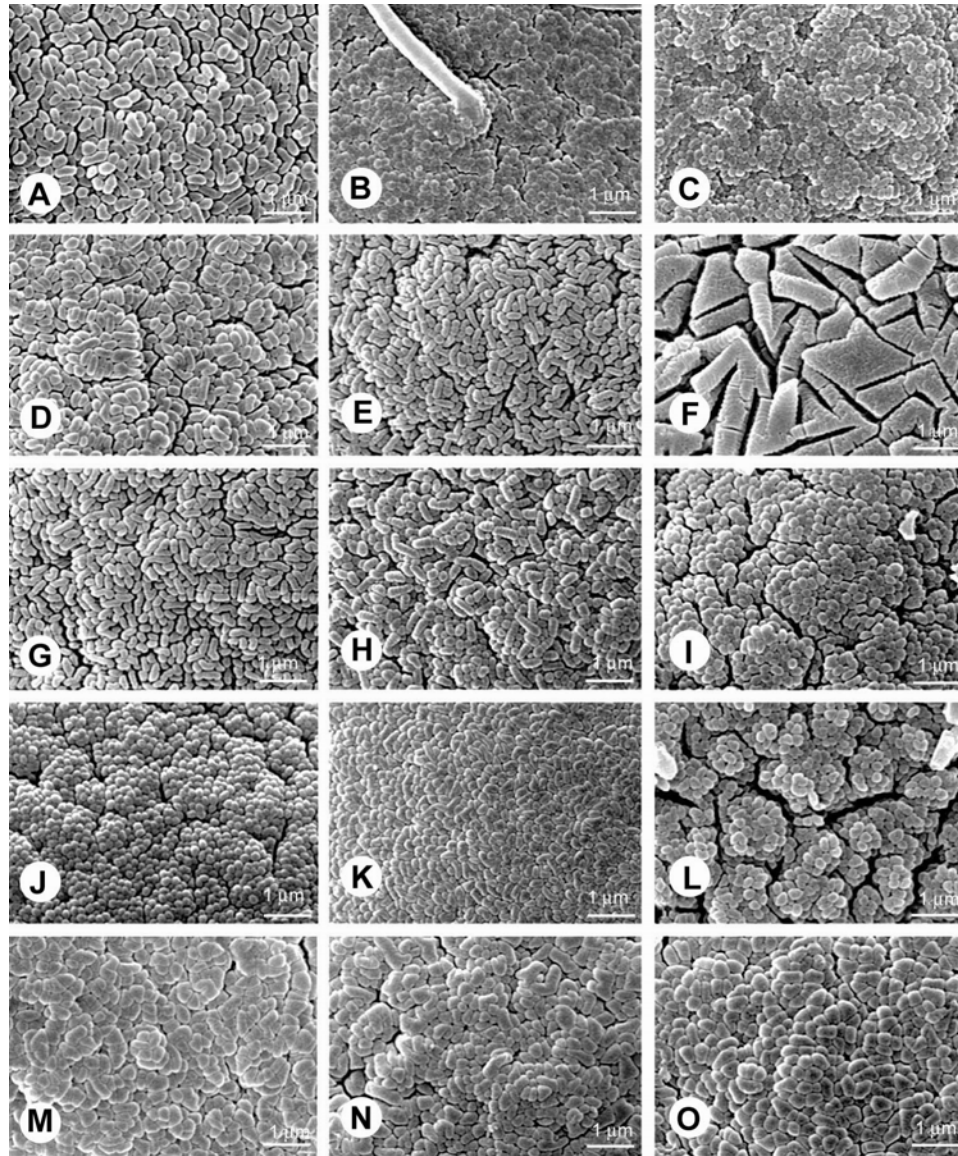


Fig. 2. SEM micrographs of *Rhododendron* pollen. A) *R. quinquefolium* (Ogura 1637); B) *R. schlippenbachii* (Sarwar & Takahashi *s.n.*); C) *R. lapponicum* (Gillett 1835); D) *R. parvifolium* (Tatewaki 20940); E) *R. diversipilosum* (Takahashi 206); F) *R. subarcticum* (Takahashi 2643); G) *R. dauricum* (Kanayama *et al.* 04-9050); H) *R. mucronulatum* var. *ciliatum* (Yokoyama 299); I) *R. davidsonium* (C. 9180); J) *R. keiskei* (Murata *et al.* 17861); K) *R. dilatatum* (Togashi *s.n.*); L) *R. hidakanum* (Tateishi & Togashi *s.n.*); M) *R. wadanum* (Takahashi 550); N) *R. weyrichii* (Shimamura *et al. s.n.*); O) *R. indicum* (Miyabe *s.n.*). Micrographs with apocolpial exine sculpture details (A-O); base of viscin threads attached with apocolpial exine (tectum) (A).

Pollen morphology of Menziesia:

Pollen of *M. cilicalyx* and *M. goyozanensis* was studied only with SEM. Pollen grains are in tetrahedral tetrads, lobed or compact; viscin threads commonly absent except in *M. pentandra*; D 34.3-36.7 μm , P 17.4-18.7 μm , E 24.0-27.5 μm , D/d 1.33-1.43, P/E 0.68-0.73, oblate; 3-colporate, 2f 15.4-17.6 μm , W 1.1-1.7 μm , 2f/W 9.06-16.0, 2f/D 0.42-0.51, costae present and distinct, colpus margin distinct; endocracks present; endoaperture lalongate, 1.0-1.8 μm long, 6.2-8.7 μm wide; apocolpial exine 1.7 μm thick, septum 1.0-1.2 μm thick, with faint perforations in *M. pentandra*; tectate, apocolpial exine sculpture finely verrucate (Table 2).

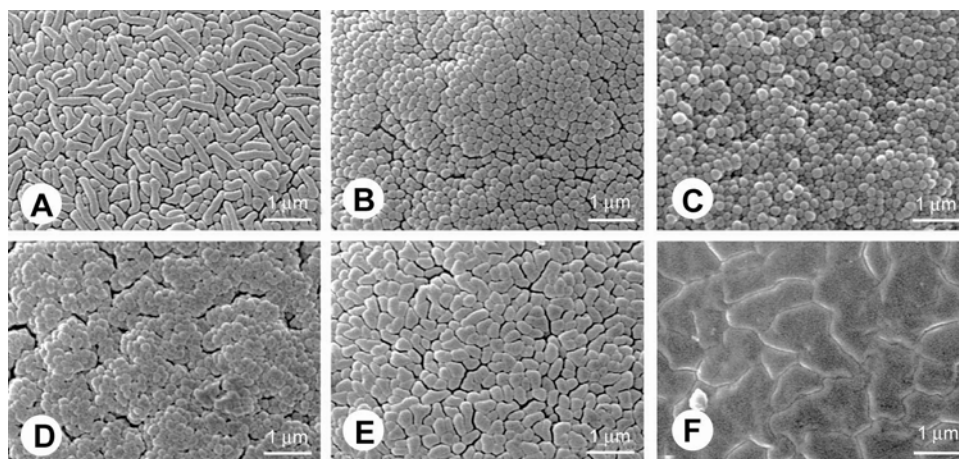


Fig. 3. SEM micrographs of *Rhododendron* pollen. A) *R. japonicum* (Takahashi 3998); B) *R. kaempferi* (Iketsu *et al.* 95); C) *R. macrosepalum* (Takahashi 1033); D) *R. nudipes* (Murata 10910); E) *R. trinerve* (Togashi *s.n.*); F) *R. tsusiophyllum* (Sawada *s.n.*). Micrographs with apocolpial exine sculpture details (A-F).

In SEM, pollen surface is flat, primary apocolpial exine sculpture indistinct, secondary sculpture unit narrowly straight-edged striate (Type NS; Figs 5I, K); or coarsely rugulate, lirae striate (Type R; Fig. 5L); colpus membrane granuloid to smooth.

In TEM of *M. pentandra*, the apocolpial exine is composed of ectexine and endexine (Figs. 5M-O). Sexine is c. 1.0 μm thick, and the total exine is c. 1.9 μm thick (Fig. 5N). The septum is c. 1.3-1.9 μm thick. The intine is almost evenly thick around the pollen tetrad, showing lower electron density than the endexine beneath both the apocolpial and septal exine.

Discussion

The genus *Rhododendron* is stenopolynous, having 3-colporate and medium pollen tetrads with viscin threads. A continuous and serial variation was revealed in all quantitative palynological characters within the genus (Tables 2 & 3). The size of *Rhododendron* pollen tetrads varies widely between 30.9 μm and 67.1 μm (Table 2).

No distinct difference in tetrad size was observed among the subfamilies, however, the subgenera *Hymenanthes* and *Pentanthera* produced relatively larger (48 μm) pollen tetrads (Table 3). Variations in ploidy level might be one of the probable causes of this wide variation in pollen size among *Rhododendron* species. In *Rhododendron*, enormous species diversification has

Table 2. Variation in pollen characters of *Rhododendron* and related genera showing mean value in μm and standard deviation. Minimum-maximum value in μm in parenthesis, D= tetrad diameter, P= polar length, d(E)= equatorial diameter, 2f= aperture length, W= aperture width, Apo.= apocolpium, n.d.= not discerned

Name of taxa	Config-uration ¹	D	P	d(E)	D/d	P/E	2f	W	2f/D	Apo. exine thickness	Septum thickness	Ornamentation ²	Fig. No.	Re- marks ³
Rhododendron														
Subgenus Hymenanthes														
Section Ponticum														
<i>R. aureum</i>	LT(+V)	57.0±2.5 (51.2-59.4)	29.1±1.4 (26.7-31.0)	37.7±1.8 (35.5-40.9)	1.51	0.77	20.9±2.4 (18.2-24.8)	1.0±0.4 (0.5-1.5)	0.37	2.4±0.4 (1.8-3.0)	1.4±0.3 (0.7-1.8)	GP	1F	
<i>R. brachycarpum</i>	T(+V)	48.5±2.9 (44.2-52.8)	25.5±2.1 (22.3-28.1)	35.6±1.7 (34.3-38.0)	1.36	0.72	20.2±2.0 (17.3-23.1)	0.7±0.2 (0.5-1.2)	0.42	3.0±0.5 (1.8-3.3)	1.1±0.4 (0.5-1.7)	GP	1G	
<i>R. decorum</i>	LT(+V)	65.0±2.3 (62.0-69.3)	34.9±1.9 (31.0-38.0)	44.6±0.9 (42.9-45.9)	1.46	0.78	24.8±3.5 (18.2-31.4)	0.7±0.3 (0.5-1.2)	0.38	2.6±0.4 (2.0-3.0)	2.4±1.0 (1.3-4.1)	NS/RS	1H	1
<i>R. degronianum</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	1I	
<i>R. formosanum</i>	LT(+V)	65.2±1.9 (62.9-68.4)	35.8±1.5 (33.1-38.9)	44.3±1.1 (42.7-46.6)	1.47	0.81	27.3±3.1 (22.8-31.9)	2.2±0.3 (1.9-2.9)	-	2.7±0.3 (2.4-3.1)	3.6±0.6 (2.4-4.3)	GP	1J	
<i>R. macrostemon</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	1K	
<i>R. visciatylum</i> var. <i>amatsuaense</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	1L	
Subgenus Mumeazalea														
<i>R. semibarbatum</i>	CT(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	1M	
Subgenus Pentanthera														
<i>R. arborecens</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	1N	
Section Sciadorhiodion														
<i>R. albrechtii</i>	T(+V)	67.1±3.3 (61.1-72.6)	35.3±3.1 (30.7-41.3)	47.5±4.3 (41.3-54.0)	1.41	0.74	30.4±4.8 (24.8-38.8)	2.2±0.6 (1.3-3.0)	0.45	2.7±0.5 (1.7-3.1)	1.6±0.4 (1.2-2.5)	GP	1O	
<i>R. quinquefolium</i>	T(+V)	52.4±2.8 (48.0-57.6)	28.7±1.9 (25.2-30.7)	38.8±1.9 (35.0-41.3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	GP	2A	
<i>R. schlippenbachii</i>	T(+V)	52.4±2.8 (48.0-57.6)	28.7±1.9 (25.2-30.7)	38.8±1.9 (35.0-41.3)	1.35	0.74	18.7±1.9 (16.3-21.1)	2.1±0.4 (1.4-2.6)	0.36	2.6±0.2 (2.4-2.9)	1.9±0.3 (1.4-2.4)	GP	2B	
Subgenus Rhododendron														
<i>R. lapponicum</i>	T(+V)	45.7±0.7 (44.9-46.2)	24.9±0.4 (24.4-25.2)	32.3±0.9 (31.4-33.0)	1.41	0.77	17.7±0.6 (17.3-18.2)	1.5 n.d.	0.39	2.1±0.3 (1.8-2.5)	0.6±0.3 (0.3-0.8)	GP	2C	2, 3
<i>R. parvifolium</i>	CT(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	2D	
Subsection Ledum														
<i>R. diversipilosum</i>	LT(+V)	30.9±1.5 (29.4-33.5)	16.3±0.9 (14.9-18.2)	21.8±0.9 (20.6-23.1)	1.42	0.75	16.7±1.7 (13.2-19.0)	1.4±0.6 (0.3-2.5)	0.54	1.9±0.2 (1.7-2.1)	1.2±0.3 (0.7-1.8)	R	2E	
<i>R. subarcticum</i>	LT(+V)	31.8±1.1 (30.0-33.2)	17.3±0.9 (15.8-18.5)	23.2±0.5 (22.3-23.9)	1.37	0.75	16.6±1.2 (14.0-19.0)	0.8±0.3 (0.5-1.2)	0.52	1.7±0.2 (1.3-2.0)	0.6±0.3 (0.3-1.2)	R	2F	2, 3
Subsection Maaddenia														
<i>R. maddenii</i>	LT(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	-	
Subsection Rhodorastra														
<i>R. dauricum</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	2G	
<i>R. mucronulatum</i> var. <i>ciliatum</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	2H	

Table 2 contd.

Name of taxa	Config-uration ¹	D	P	d(E)	D/d	P/E	2f	W	2f/D	Apo. exine thickness	Septum thickness	Orname-ntation ²	Fig. No.	Re-marks ³
Subsection Triflora														
<i>R. davidsonianum</i>	T(+V)	51.2±2.6 (46.7-54.5)	26.5±1.2 (24.8-28.1)	37.4±1.2 (35.5-39.6)	1.37	0.71	18.6±2.8 (14.9-23.1)	1.0±0.4 (0.5-1.5)	0.36	3.1±0.3 (2.5-3.5)	1.2±0.1 (1.0-1.3)	GP	2I	3
<i>R. kieskei</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	2J	
Subgenus Tsutsusi Section Brachycalyx														
<i>R. dilatatum</i>	T(+V)	56.4±1.8 (54.1-59.4)	29.7±2.1 (26.4-32.7)	39.6±1.8 (38.0-42.9)	1.42	0.75	17.8±2.4 (14.9-23.1)	0.7±0.3 (0.5-1.3)	0.32	3.4±0.4 (3.0-4.3)	1.7±0.3 (1.3-2.1)	GP	2K	
<i>R. hidakamum</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	2L	
<i>R. wadamum</i>	T(+V)	54.1±2.5 (50.8-59.4)	28.8±1.6 (26.4-31.4)	37.2±1.5 (34.7-39.6)	1.45	0.77	18.6±2.0 (14.9-20.6)	0.8±0.3 (0.5-1.2)	0.34	3.6±0.3 (3.1-4.3)	1.5±0.3 (0.8-2.0)	GP	2M	
<i>R. weyrichii</i>	T(+V)	55.7±1.8 (52.8-57.8)	29.3±2.1 (27.2-33.7)	39.4±2.6 (36.3-43.2)	1.41	0.74	18.6±3.1 (13.2-23.1)	1.1±0.4 (0.7-1.7)	0.33	3.3±0.1 (3.1-3.5)	2.2±0.7 (1.5-3.3)	GP	2N	
Section Tsutsusi														
<i>R. indicum</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	2O	
<i>R. japonicum</i>	CT(+V)	63.2±2.2 (59.7-66.3)	31.8±1.1 (30.5-33.3)	48.3±1.6 (45.4-49.5)	1.31	0.66	19.6±1.4 (18.2-21.5)	2.1±0.7 (1.2-3.0)	0.31	3.0±0.2 (2.8-3.3)	1.5±0.2 (1.3-1.7)	GP/R	3A	
<i>R. kaempferi</i>	CT(+V)	53.0±3.4 (48.7-58.6)	27.9±2.8 (23.1-33.3)	37.4±3.0 (33.0-44.6)	1.42	0.75	18.4±2.5 (14.9-22.3)	1.2±0.5 (0.5-2.1)	0.35	2.6±0.4 (2.2-3.5)	1.6±0.3 (1.2-2.0)	GP	3B	4
<i>R. macrosepalum</i>	T(+V)	56.7±2.7 (54.8-60.3)	29.9±2.6 (26.4-32.2)	40.9±1.6 (39.6-42.9)	1.39	0.73	18.6±4.1 (15.7-21.4)	0.9±0.6 (0.5-1.3)	0.33	3.2±0.2 (3.0-3.3)	1.7±0.5 (1.3-2.0)	GP	3C	4
<i>R. nudipes</i>	T(+V)	54.6±2.5 (51.2-59.6)	28.1±1.7 (25.6-30.9)	36.7±1.7 (34.7-39.6)	1.49	0.77	22.6±3.2 (19.8-28.1)	1.7±1.0 (0.8-3.3)	0.41	3.4±0.3 (3.0-4.0)	3.2±1.0 (2.2-5.0)	GP	3D	3
<i>R. trinerve</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	3E	
<i>R. tschonoskii</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	-	
<i>R. isisophyllum</i>	T(+V)	35.0±1.3 (33.0-37.0)	18.1±1.3 (15.2-20.1)	26.0±1.6 (23.1-28.1)	1.35	0.70	14.5±2.1 (12.4-19.0)	2.2±0.8 (1.3-3.6)	0.41	2.1±0.2 (2.0-2.5)	1.8±0.6 (1.3-3.0)	P/R	3F	3
<i>Therorhodion camischaticum</i>	LT(+V)	50.0±4.2 (43.9-57.8)	26.5±1.9 (23.3-29.5)	35.0±3.6 (29.4-41.3)	1.43	0.76	14.8±2.0 (12.4-17.3)	2.9±0.6 (2.1-4.0)	0.30	2.2±0.4 (1.8-3.0)	1.4±0.1 (1.2-1.5)	GP	5D	2, 3
<i>T. redowskianum</i>	T(+V)	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	GP	5E-F	
<i>Menziesia ciliicadylex</i>	T	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	NS	5I-J	
<i>M. goyozanensis</i>	T	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	-	n.d.	n.d.	NS	-	
<i>M. multiflora</i>	T	36.7±2.5 (32.8-39.8)	18.7±1.6 (17.2-21.6)	27.5±1.3 (25.6-29.7)	1.33	0.68	15.4±1.5 (13.2-17.3)	1.7±0.4 (1.3-2.5)	0.42	1.7±0.2 (1.3-2.0)	1.0±0.4 (0.3-1.3)	NS	5K	P
<i>M. pentandra</i>	CT(+V)	34.3±1.5 (31.7-36.6)	17.4±0.7 (16.7-18.6)	24.0±1.2 (21.6-25.1)	1.43	0.73	17.6±1.0 (16.5-19.8)	1.1±0.4 (0.5-1.5)	0.51	1.7±0.1 (1.5-2.0)	1.2±0.1 (1.0-1.5)	NS/R	5L	3, P

¹ T: Tetrahedral tetrad, CT: Compact tetrahedral tetrad, LT: Lobed tetrahedral tetrad, (+V): Viscin threads present.

² Exine ornamentation type by SEM. GP: Gemmate-pilate, P: Psilate, R: Rugulate, NS: Narrow straight-edged striate.

³ 1: Number of endoaperture more than one, 2: Costae indistinct, 3: Endocracks absent/indistinct, 4: Rarely 4-aperturate, P: Perforated septum.

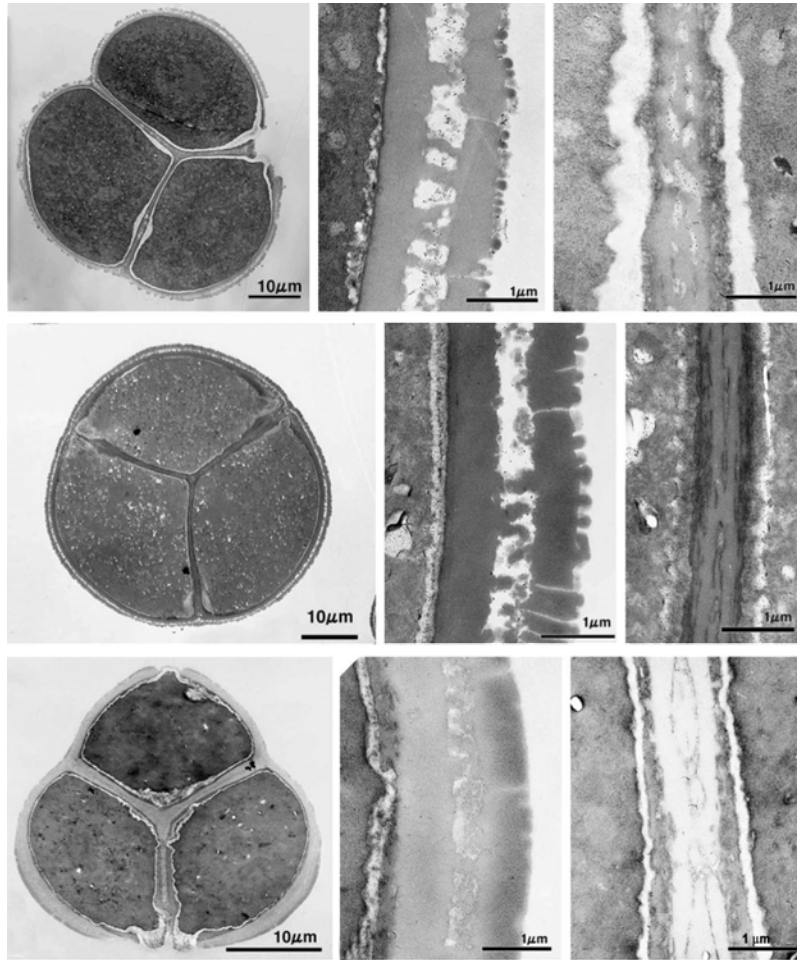


Fig. 4. TEM micrographs of *Rhododendron* pollen. A-C) *Rhododendron schlippenbachii* (Sarwar & Takahashi *s.n.*); D-F) *R. japonicum* (Takahashi 3998); G-I) *R. tsusiophyllum* (Sawada *s.n.*). Whole tetrad (A, D, G); apocolpial exine showing thick canalized tectum with supracteal fine gemmae-pila, thick columellae, thick foot layer and thin endexine (B); in septum, tectum fragmentary, two foot layer of adjacent grains sometimes connected by columellae, endexine thick (C); apocolpial exine showing canalized thick tectum with supracteal fine gemmae-pila, thick columellae, thick foot layer and thin endexine (E); in septum, tectum lacking, two foot layer of adjacent grains connected by thin or rudimentary columellae, endexine thick (F, I); apocolpial exine showing thick tectum, columellae, thick foot layer and endexine with (endo) cracks (H).

clearly occurred at the diploid level ($2n=26$), and polyploidy occurs among one third of cytologically examined lepidote species, the degree of polyploidy ranging from triploids ($2n=3x=39$) to dodecaploids ($2n=12x=156$) (Janaki Ammal, 1950). Aneuploidy ($2n=30$) has been reported in one case (Jones and Brighton, 1972) and two species namely, *R. wallichii* Hook. f. (as *R. campanulatum* var. *wallichii* Hook. f.) and *R. grande* Wight were reported to have $n=12$ (i.e. $2n=24$) (Mehra, 1976), although the number of *Rhododendron* species having diploid level ($2n=24$) should be increased after the inclusion of *Therorhodion* (Kron and Judd, 1990). Cockerham and Galletta (1976) reported that the mean pollen diameter was 11% larger in the tetraploids compared to that in the diploids in certain *Vaccinium* species.

Viscin threads occur among the pollen tetrads in *Rhododendron* and *Therorhodion*, and presumably play a role in pollen removal from the anthers and its adhesion to pollinators. Any pollen material with viscin threads points to the highly specialized (entomophilous) pollination mode. It has been suggested that viscin threads increase the efficiency of pollination, and their presence implies highly specific pollinators for accurate delivery of pollen to stigma (Hesse *et al.*, 2000). The viscin threads would also play a role in pollen presentation. According to Skvarla *et al.* (1978) there is significant association between the structure of viscin threads in Onagraceae and the pollen vector: beaded viscin threads associated with birds and moth pollinated taxa whereas smooth ones occur in bee pollinated taxa. No viscin threads were found in *Menziesia* (despite reports to the contrary in Copeland, 1943; Wood, 1961), except in *M. pentandra* (Table 2). Viscin threads are not to be expected in species with urceolate or tubular corollas, since there they might obstruct cross-pollination (Stevens, 1971). Thus they are found in *M. pentandra*, which has broadly urceolate (to campanulate) corollas, but not in other species of the genus with urceolate and/or tubular corollas.

Table 3. Variation in pollen characters of different subgenera of *Rhododendron* showing minimum-maximum (mean) value in μm . D: tetrad diameter, 2f: aperture length, W: aperture width.

Name of subgenera	D	2f	W	Exine sculpture	Reference
<i>Azaleastrum</i>	39.18 - 56.93	8.89 - 23.92	0.86 - 2.36	GP	Gao <i>et al.</i> (2002b)
<i>Candistrum</i>	37.97 - 39.95	13.80	1.29 - 1.38	GP	Gao <i>et al.</i> (2002b)
<i>Hymenanthes</i>	48.50 - 65.20	16.53 - 20.20	0.70 - 2.90	GP, R	This paper; Gao <i>et al.</i> (2002c)
<i>Mumeazalea</i>	35.85 - 39.95	16.10	1.85 - 1.98	GP	Gao <i>et al.</i> (2002b)
<i>Pentanthera</i>	49.04 - 67.10	18.70 - 30.40	1.56 - 3.44	GP	This paper; Gao <i>et al.</i> (2002c); Zhang <i>et al.</i> (2009)
<i>Rhododendron</i>	30.29 - 54.84	9.63 - 20.23	0.80 - 1.50	GP, R	This paper; Gao <i>et al.</i> (2002c)
<i>Tsutsusi</i>	35.00 - 63.20	13.97 - 22.60	0.70 - 2.20	GP, R	This paper; Gao <i>et al.</i> (2002c); Zhang <i>et al.</i> (2009)

Usually, apocolpial exine is thicker than the septal exine, but thinner apocolpial exine has been observed in *R. formosanum* (Table 2). Similar relatively thinner apocolpial exine also has been observed in some taxa of the subfamily Vaccinioideae (Sarwar and Takahashi, 2006; Sarwar *et al.*, 2006) and it may have some taxonomic value in the infrageneric classification of the respected genera. In the lobed tetrads of *R. formosanum*, single pollen grains might be loosely attached together and the septum has not been reduced. A similar cause for comparatively thicker septum has been discussed for tetrads of the family Annonaceae (Le Thomas *et al.*, 1986). However, no significant correlation was found between compactness of tetrad and septum thickness in the present study or published literature (Kim *et al.*, 1988).

The apocolpial exine sculpture can be divided into two distinct groups - pollen surface is uneven and rugged to somewhat flat, apocolpial exine sculpture of Type GP (Figs 1F, G, I-O, 2A-D, G-O, 3A-E); and pollen surface flat or rugged, apocolpial exine sculpture of Type R (Figs 2E, F, 3F). The latter type of exine sculpture characterized *Rhododendron* subsect. *Ledum*, and all other species have almost similar exine sculpture except *R. tsusiophyllum* (Fig. 3F). The subsect. *Ledum* was also characterized by smaller pollen tetrads (30.9-31.8 μm) and a higher value of 2f/D ratio (0.52-0.54) (Table 2). Neither tetrad size nor exine sculpture was able to be used to differentiate among the subgenera and/or sections of *Rhododendron* (Tables 2, 3). Thus, palynological characters showed little usefulness in the infrageneric classification of

Rhododendron (Goetsch *et al.*, 2005), but could be used for identification of individual *Rhododendron* species (Table 2; Gao *et al.*, 2002b, c).

Generic delimitation of the tribe Rhodoreae is a subject of dispute until now (Gillespie and Kron, 2010). The phylogenetic analyses of *Rhododendron* based on molecular data did not support the individual generic status of *Menziesia* and *Therorhodion*, or even *Diplarche* (Craven, 2011), but suggested their inclusion within the genus *Rhododendron* (Kurashige *et al.*, 2001; Goetsch *et al.*, 2005). The results of this palynological study added some new points of disagreement within the present generic alignment of this tribe (Gillespie and Kron, 2010). As expected, the quantitative palynological features vary to a large extent in a large genus like *Rhododendron*, and give a little support for the individual generic status of *Menziesia*, *Rhododendron* and *Therorhodion* (Table 2). However, the specialized exine sculpture of Type NS and perforated septum of *Menziesia*, clearly distinguish the genus from other two genera of this tribe, *Rhododendron* and *Therorhodion* (Table 2; Figs 1-3, 5; Gao *et al.*, 2002c; Miyoshi *et al.*, 2011). Both the exine sculpture and septum with perforations have already been identified as taxonomically important characters in different Ericaceous genera (Sarwar, 2007). Along with other morphological and molecular characters (Kron *et al.*, 2002; Gillespie and Kron, 2010), the exceptional exine sculpture may also give additional support to the individual generic status of *Menziesia* (Sarwar and Takahashi, 2012b; Takahashi and Sarwar, 2013). Palynological features of the other two genera, *Rhododendron* and *Therorhodion* are very similar (Table 2; Figs 1-3, 5), and they might support the sister relationship between these two genera as identified by Gillespie and Kron (2010).

The pollen morphological features e.g., tetrad size, exine sculpture, etc. of *R. tsusiophyllum* of sect. *Tsutsusi* are different from those of other members of the same section as well as subgen. *Tsutsusi* (Table 2; Type GP; Figs 2K-O, 3A-E vs. Type R; Fig. 3F). Taking pollen morphology into account *R. tsusiophyllum* might be transferred from the subgen. *Tsutsusi* (Chamberlain *et al.*, 1996) to subsect. *Ledum* of the subgen. *Rhododendron* (Table 2; Type R; Figs 2E, F). Similar transfer of *R. huadingense* from sect. *Brachycalyx* of the subgen. *Tsutsusi* to subgen. *Pentanthera* has also been proposed based on palynological features (Zhang *et al.*, 2009). In TEM, the pollen wall structure of *R. tsusiophyllum* especially the thickness of the columellae and the sexine-nexine ratio also showed a distinct difference (data not shown) compared to the two other taxa of *Rhododendron* (Fig. 4). When considering the differences in the breakdown of the separating wall of the pollen sac, opening of the anther during maturity and the three-locular ovary as well as differences in other morphological characters between *R. tsusiophyllum* and other *Rhododendron* species (Stevens, 1969; Yamazaki, 1991); *R. tsusiophyllum* might be recognized as a separate monotypic genus *Tsusiophyllum*; *T. tanakae* Maxim., which is sister to whole of *Rhododendron* (including *Ledum*) (Kron and Judd, 1990). The recent molecular phylogenetic study of subfamily Ericoideae (Gillespie and Kron, 2010) may also support this supposition. *Rhododendron tsusiophyllum* forms a clade with *Menziesia pilosa*, which is well-supported in Bayesian and Maximum Likelihood analyses, instead of other *Rhododendron* species (Figs 1, 3 in Gillespie and Kron, 2010).

Based solely on molecular data, the classification and evolutionary relationship between plants is not always completely reliable (Stace, 2005), especially in genera like *Rhododendron* where polyploid species are a common phenomenon (Janaki Ammal, 1950). Hörandl (2006) also suggested that clades retrieved by phylogenetic analyses should not be used solely as a basis for classification, but should be regarded primarily as information for a better understanding of relationships. So, detailed phylogenetic analyses, using morphological, palynological and molecular data with larger number of specimens, are necessary to clarify generic circumscription of *Rhododendron* and its relationship with other closely related genera.

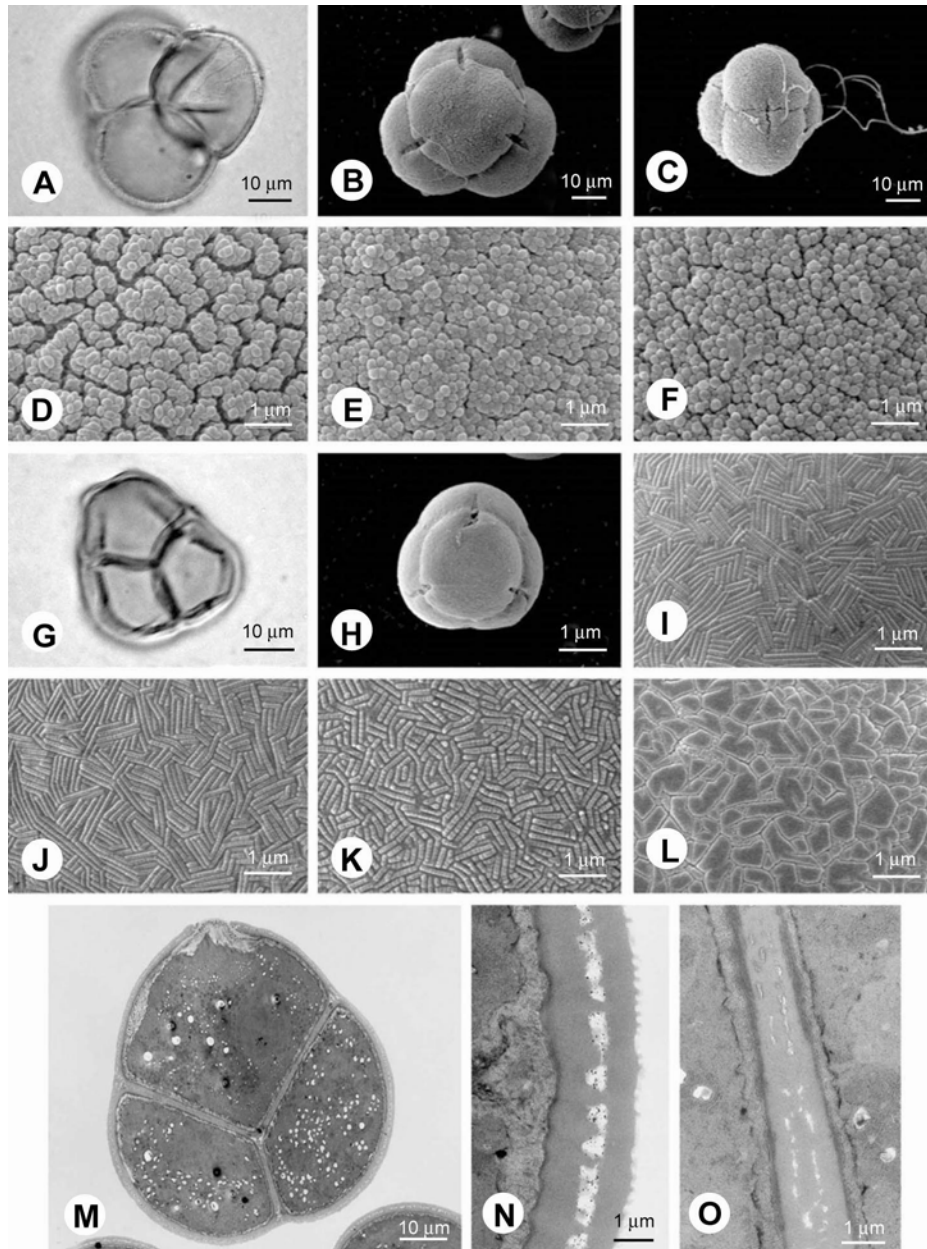


Fig. 5. LM, SEM and TEM pollen micrographs of *Therorhodion* (A-F) and *Menziesia* (G-O). A, B) *Therorhodion camtschaticum* (Takahashi *et al.* 5836); C) *T. redowskianum* (Yoshimura & Hara *s.n.*); D) *T. camtschaticum* (Takahashi *et al.* 5836); E, F) *T. redowskianum* (Yoshimura & Hara *s.n.*). G) *Menziesia pentandra* (Takahashi 2687); H) *M. multiflora* (Takahashi 767); I, J) *M. cilicalyx* (Tateishi & Hoshi 13689); K) *M. multiflora* (Takahashi 767); L) *M. pentandra* (Takahashi 2687); M-O) *M. pentandra* (Takahashi 2687). Pollen tetrads at polar view (A, B, G, H); pollen tetrads at equatorial view showing aperture (C); micrographs with apocolpial exine sculpture details (D, E, I, K, L); micrograph with mesocolpial exine sculpture details (F, J); whole tetrad (M); apocolpial exine showing tectum with narrow straight-edged striae, columellae, foot layer and thin undulated endexine (N); septum with tectum, and well defined columellae and foot layer of two adjacent grains (O).

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