

## POLLEN MORPHOLOGY OF *KALMIA* L. (PHYLLODOCEAE, ERICACEAE) AND ITS TAXONOMIC SIGNIFICANCE

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### Abstract

Pollen morphology of seven taxa of *Kalmia* was examined using light and scanning electron microscopy (LM and SEM, respectively), or SEM alone, in search of new characters that might contribute to infrageneric classification of the genus. The *Kalmia* species are stenopalynous and characterized by 3-colpor(oid)ate, medium, oblate pollen united in tetrahedral tetrads with rugulate exine sculpture. However, a continuous and serial variation in all the quantitative characters and exine sculpture was revealed within the genus. *Kalmia buxifolia* is characterized by having the smallest pollen tetrads, largest aperture, largest 2f/D ratio and septum thicker than apocolpial exine. On the other hand, *K. latifolia* produces the largest pollen tetrads with smallest aperture and smallest 2f/D ratio. An evolutionary trend from rugulate to psilate has also been observed in apocolpial exine sculpture of the genus *Kalmia*. On the basis of acetolysed pollen characteristics, a dichotomous key for *Kalmia* was prepared.

### Introduction

*Kalmia* L. (Family Phyllodoceae, subfamily Ericaceae) comprises eleven quite distinct species of evergreen or deciduous shrubs (rarely tree) and is restricted to North America and Cuba, the only circumboreal species is *K. procumbens* (Kron *et al.*, 2002; Stevens *et al.*, 2004). The number of species (including *Leiophyllum* Pers. and *Loiseleuria* Desv.) varies from seven to eleven (Ebinger, 1974; Southall and Hardin, 1974; Judd, 1995; Stevens *et al.*, 2004). Linnaeus described the genus *Kalmia* (Linnaeus, 1753, 1754) as well as its first two species, *K. latifolia* and *K. angustifolia*. Drude (1889) placed *Kalmia* in the tribe Phyllodoceae, and its position has not been changed by most of the subsequent authors (see Ebinger, 1974). There have been few nomenclatural complications in the genus, and only two other generic names have been proposed. Kuntze (1891) used Catesby's pre-Linnean name of *Chamaedaphne* and made numerous transfers to it, while Small (1914) divided *Kalmia* into two genera. He proposed the genus *Kalmiella*, segregating *Kalmia hirsuta* and *K. ericoides* in the latter genus on the basis of their inflorescence structure (i.e., solitary flowers) and deciduous calyx (Small, 1914). All subsequent authors (Copeland, 1943; Wood, 1961), except Alain (1957), have treated *Kalmiella* as a synonym of *Kalmia*.

*Leiophyllum* Pers. and *Loiseleuria* Desv. are two monotypic genera of the tribe Phyllodoceae, Ericaceae (Drude, 1889; Stevens, 1971). *Leiophyllum buxifolia* (Berg.) Elliott is a shrub endemic to the southeastern United States (New Jersey). Due to its disjunct distribution and morphological variability, *Leiophyllum* has long been a subject of debate and speculation about its taxonomy and evolutionary history (Strand and Wyatt, 1991). *Loiseleuria procumbens* (L.) Desv. has Holarctic circumpolar-boreal range with a large gap in North-West Asia. *Loiseleuria* and *Leiophyllum* despite differences in the leaf veneration and fusion of corolla, show many similarities in anatomy

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and anther structure, and described as a closely related pair of genera (Stevens, 1971). Recent molecular phylogenetic studies included these two monotypic genera within *Kalmia* (Kron and King, 1996; Kron *et al.*, 2002). Here, we adopt a broad circumscription of *Kalmia* which includes *Leiophyllum* and *Loiseleuria* [see Kron *et al.* (2002) for the formal transfers].

Pollen of *Kalmia* is shed in tetrahedral tetrads which vary in size from 45  $\mu\text{m}$  in *K. latifolia* to 23  $\mu\text{m}$  in *K. angustifolia* var. *carolina*; the tetrads are associated with viscin strands in *K. latifolia*, *K. hirsuta*, *K. ericoides* and *K. angustifolia*, while such threads have not been seen in the remaining species (Ebinger, 1974; Southall and Hardin, 1974; Judd, 1995). Exine sculpturing is generally coarsely reticulate or rugulate in *K. microphylla* (Hook.) Heller, while finely so in *K. latifolia* and *K. ericoides*. The pollen morphology of some taxa has been described in previous literature, but little is known about the use of palynological features in taxonomy/systematics.

Hitherto, no formal infrageneric classification of *Kalmia* has been proposed, but some distinctly different groups, based on different features, have been recognized within this genus (Stevens, 1971; Ebinger, 1974; Southall and Hardin, 1974; Judd, 1995; Kron and King, 1996). Previously, the pollen morphological features were found to be important in the infrageneric as well as subfamilial classification of some Ericaceae taxa (Sarwar and Takahashi, 2006a, b, 2009; Sarwar *et al.*, 2006; Sarwar, 2011). Therefore, we present here a general pollen survey on *Kalmia* based on LM and SEM to search new character that could add information pertinent to infrageneric classification of this genus.

### Materials and Methods

Pollen morphology of seven taxa (and 11 specimens) out of 11 species of *Kalmia* was examined by means of LM and SEM, or SEM alone (Table 1). The remaining four species were not examined due to unavailability of herbarium specimens (and/or pollen samples) in the herbaria consulted here as well as others (Sarwar, 2011; for the complete list of herbaria consulted). Polliniferous materials used in this investigation were taken from the dried specimens from the herbaria S, SAPS and SAPT. Abbreviation of the herbarium names except for SAPT (the Botanic Garden, Hokkaido University, Sapporo) are according to the Index Herbariorum (Holmgren *et al.*, 1990).

Pollen grains were acetolysed following the technique of Erdtman (1960) modified by Takahashi (1987). Palynological features of three *Kalmia* species were studied both acetolysed and non-acetolysed condition to find out the effect of acetolysis on its pollen. For LM, the dehydrated (in an ethanol series) pollen (both acetolysed and non-acetolysed) 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. The measurements given in Table 2 are based on at least 10 grains from each specimen. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were also conducted using the same program to visualise the relationships among the studied species based on pollen data. A dendrogram was built by AHC. Pollen slides of all collections are deposited at the Hokkaido University Museum, Sapporo, Japan. Pollen size and shape classes were made following Erdtman (1986) and descriptive terminology follows Punt *et al.* (2007) and Sarwar *et al.* (2006).

For SEM, the pollen samples, both acetolysed and non-acetolysed, were dehydrated in an ethanol series, and mounted and air dried on aluminum stubs from 70% ethanol, and sputter coated

with Platinum-Palladium by 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 of similar position were used for the purpose of description and comparison.

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

| No. | Taxa   | Voucher specimens  |
|-----|--|--|
| 1.  | <i>Kalmia angustifolia</i> L.  | USA: Connecticut, Litchfield Co., Norfolk, Great Mt. Forest, Tobey bog, 23.06.1983. S. DeSimon 415 (SAPT) North America: Grand Harbor, 03.08.1888, K. Miyabe s.n. (SAPS)   |
| 2.  | <i>K. buxifolia</i> (Berg.) Gift, Kron & Stevens                               | USA: North Carolina, Brunswi. Co., 12 miles S of Wilmington, 06.04.1939. R.K. Godfrey & R.N. White 7110 (S)  |
| 3.  | <i>K. ericoides</i> Wright ex Grisebatch var. <i>aggregata</i> (Small) Ebinger | Cuba: Prov. Pinar del Rio, La Grifa, 19.11.1923. E.L. Ekman 18165 (S)  |
| 4.  | <i>K. latifolia</i> L.   | USA: Connecticut, Litchfield Co., Mt. Riga State Park, near Riga lake, moist woods, 16.06.1981. G. Feldman 63 (SAPT) Georgia, Lumpkin Co., Chattahoochee National Park, 11.05.1982, F.H. Utech <i>et al.</i> 82-056 (SAPS) |
| 5.  | <i>K. microphylla</i> (Hook.) Heller var. <i>microphylla</i>                   | USA: Washington, Mt. Rainier, (Flora of Cascade Mountains), 13.08.1894. O.D. Allen s.n. (SAPS)<br>Canada: British Columbia, Garibaldi Park, 23.07.1975, V.J. Krajina s.n. (SAPT)   |
| 6.  | <i>K. polifolia</i> Wangenh.   | USA: Washington, swamp, upper valley of the Nesqually, (Flora of Cascade Mountains), 27.05.1893. O.D. Allen s.n. (SAPS)  |
| 7.  | <i>K. procumbens</i> (L.) Gift, Kron & Stevens                                 | Japan: Hokkaido, Prov. Kitami, Monbestu-gun, Shirataki-mura, Mt. Taira-yama, 30.06.1980. H. Takahashi <i>et al.</i> 2644 (SAPS) Sweden: Torne Lappmark, Abisco, Mt Njulla, 20.07.1989. H. Takahashi 9907 (SAPS)            |

## Results

### *General pollen morphology*

In LM, the medium pollen grains are united in tetrahedral tetrad (Fig. 1A-C); viscin threads present in some species; D 24.4-36.6  $\mu\text{m}$ , P 12.8 -18.8  $\mu\text{m}$ , E 17.3-26.3  $\mu\text{m}$ , D/d 1.31-1.52, P/E 0.70-0.80, oblate or suboblate; 3-colpor(oid)ate, 2f 11.6-19.6  $\mu\text{m}$ , W 0.4-1.2  $\mu\text{m}$ , 2f/D 0.32-0.67, costae present and distinct, colpus margin distinct; endocracks present; endoaperture distinct and lalongate; apocolpial exine 1.6-2.1  $\mu\text{m}$  thick, septum 0.7-1.9  $\mu\text{m}$  thick (Table 2); tectate, apocolpial exine sculpture from fine verrucate to rugulate.

In SEM, the pollen surface is somewhat flat (Fig. 1D-E), apocolpial exine sculpture moderate to coarsely rugulate, with distinct grooves (Type R; Fig. 1F-G, J-K); or primary exine sculpture coarsely rugulate-psilate, the rugulae with moderately (diam. > 0.2  $\mu\text{m}$ ) granulate (Type RGS; Fig. 1H); or psilate (Type P; Fig. 1L); colpus membrane variable, from granulate to smooth, sometime

indistinct. Exine sculpture along the colpi is similar to that appearing at the distal pole (apocolpial region). The mesocolpial exine has a tendency to decrease in lateral extension of rugulae with more distinct unit (Fig. 1I).

### ***Specific pollen description***

#### ***K. angustifolia***

In LM, pollen grains are in lobed tetrahedral tetrad; viscin threads present; oblate, non-acetolysed grains suboblate; exine sculpture rugulate (Table 2). In SEM, exine sculpture moderate to coarsely rugulate, with distinct grooves (Type R; Fig. 1F); colpus membrane smooth or granuloid.

#### ***K. buxifolia***

In LM, pollen grains are in compact tetrahedral tetrad, small; viscin threads absent; oblate; colpus margin faintly demarcated; endocracks absent/indistinct; endoaperture indistinct; exine sculpture rugulate (Table 2). In SEM, exine sculpture moderate to coarsely rugulate, with distinct grooves (Type R; Fig. 1G); colpus membrane granulate.

#### ***K. ericoides* var. *aggregata***

In LM, pollen grains are in lobed tetrahedral tetrad; viscin threads present; suboblate; exine sculpture from coarsely rugulate to psilate (Table 2). In SEM, primary exine sculpture coarsely rugulate-psilate, the rugulae with moderately (diam. > 0.2  $\mu\text{m}$ ) granulate (Type RGS; Fig. 1H); colpus membrane indistinct.

#### ***K. latifolia***

In LM, pollen grains are in tetrahedral tetrad with exceptions; viscin threads present; oblate, suboblate in one non-acetolysed specimen (Utech *et al.* 82-056); rarely 4-colporate; exine sculpture rugulate (Table 2). In SEM, exine sculpture moderate to coarsely rugulate, with distinct grooves (Type R; Fig. 1J); colpus membrane granulate.

#### ***K. microphylla* var. *microphylla***

In LM, pollen grains are in compact tetrahedral tetrad; viscin threads absent; only a few pollen tetrads could be recovered from anther. In SEM, apocolpial exine sculpture rugulate (Table 2).

#### ***K. polifolia***

In LM, pollen grains are in compact tetrahedral tetrad; viscin threads absent; oblate; endocracks absent/ indistinct; exine sculpture rugulate (Table 2). In SEM, exine sculpture moderate to coarsely rugulate, with distinct grooves (Type R; Fig. 1K); colpus membrane smooth.

#### ***K. procumbens***

In LM, pollen grains are in tetrahedral tetrad; viscin threads absent; oblate, suboblate in one specimen (Takahashi *et al.* 2644); endocracks absent/indistinct; endoaperture indistinct in one specimen (Takahashi *et al.* 2644); exine sculpture psilate (Table 2). In SEM, exine sculpture psilate (Type P; Fig. 1L); colpus membrane granulate or sometime indistinct.

In principal component analysis (PCA) using the LM characters, the first and second principal components explain 69.32% of the variance of the sample, 42.18% for the first component - Factor 1; and 27.14% for the second - Factor 2. Among the species, *K. buxifolia* and *K. polifolia* showed the highest values in the first and second components (4.23 and 2.40, respectively), and *K. procumbens* (Takahashi 9907) and *K. ericoides* var. *aggregata* (-2.14 and -2.08, respectively), showed the lowest values in the first and second components. *Kalmia buxifolia* is situated at the

Table 2. Variation in palynological characters of subfamily Ericoideae showing mean value (in  $\mu\text{m}$ ) and standard deviation ( $n = 10$ ). Range (in  $\mu\text{m}$ ) in parenthesis. n.a.: non-acetolysed, D: tetrad diameter, P: polar length, d(E): equatorial diameter, 2f (L): aperture length, W: aperture width, Apo.: apocolpium, n.d.: not discerned.

| Name of Species                               | Config-uration <sup>1</sup> | D                       | P                       | d(E)                    | D/d  | P/E  | 2f                      | W                    | 2f/D | Apo. exine thickness | Septum thickness     | Ornamentation <sup>2</sup> | Remarks <sup>3</sup> |
|---|-----------------------------|-------------------------|-------------------------|-------------------------|------|------|-------------------------|----------------------|------|----------------------|----------------------|----------------------------|----------------------|
| <i>Kalmia angustifolia</i>                    | LT(+V)                      | 32.2±1.4<br>(29.9-34.3) | 16.2±0.8<br>(14.9-17.2) | 23.0±0.9<br>(21.9-24.8) | 1.40 | 0.70 | 14.7±2.0<br>(9.9-16.7)  | 0.8±0.3<br>(0.5-1.3) | 0.43 | 1.9±0.1<br>(1.8-2.1) | 0.9±0.3<br>(0.5-1.3) | R                          | 1, 2                 |
| DeSimon 415                                   | LT(+V)                      | 27.0±1.0                | 15.2±1.3                | 19.4±1.2                | 1.39 | 0.78 | n.d.                    | n.d.                 | n.d. | n.d.                 | n.d.                 | R                          |                      |
| Miyabe s.n. (n.a.)                            | LT(+V)                      | 29.1±1.1                | 15.2±0.7                | 19.1±0.9                | 1.52 | 0.80 | n.d.                    | n.d.                 | n.d. | n.d.                 | n.d.                 | R                          |                      |
| <i>K. buxifolia</i>                           | CT                          | 24.4±0.8<br>(23.1-25.9) | 12.8±0.9<br>(11.4-14.9) | 17.3±0.5<br>(16.5-18.2) | 1.41 | 0.74 | 16.4±1.4<br>(13.2-18.2) | 0.4±0.1<br>(0.3-0.7) | 0.67 | 1.6±0.3<br>(1.3-2.0) | 1.9±0.1<br>(1.7-2.0) | R                          | 1, 2                 |
| <i>K. ericoides</i> var.                      | LT(+V)                      | 32.6±1.3                | 16.7±1.0                | 21.9±0.9                | 1.49 | 0.76 | 12.1±1.7<br>(9.9-14.9)  | 0.6±0.1<br>(0.5-0.7) | 0.37 | 2.1±0.3<br>(1.8-3.0) | 0.8±0.4<br>(0.3-1.5) | RGS                        |                      |
| <i>K. latifolia</i> Feldman 63                | (T)(+V)                     | 36.6±1.6<br>(34.7-39.1) | 18.8±1.1<br>(16.6-20.1) | 26.3±1.1<br>(24.8-28.1) | 1.39 | 0.71 | 11.6±2.0<br>(8.3-14.9)  | 0.6±0.3<br>(0.3-1.3) | 0.32 | 1.8±0.1<br>(1.7-2.0) | 0.7±0.3<br>(0.5-1.3) | R                          | 3                    |
| Feldman 63 (n.a.)                             | (T)(+V)                     | 31.8±1.1<br>(30.6-33.4) | 16.7±1.8<br>(14.6-19.3) | 23.7±0.9<br>(22.8-24.9) | 1.34 | 0.71 | n.d.                    | n.d.                 | n.d. | n.d.                 | n.d.                 | R                          |                      |
| Utech <i>et al.</i> 82-056 (n.a.)             | (T)(+V)                     | 34.3±1.7<br>(32.1-38.6) | 18.6±1.2<br>(16.8-20.5) | 23.5±1.1<br>(22.0-25.0) | 1.46 | 0.79 | n.d.                    | n.d.                 | n.d. | n.d.                 | n.d.                 | R                          |                      |
| <i>K. microphylla</i> var. <i>microphylla</i> | LT                          | n.d.                    | n.d.                    | n.d.                    | n.d. | n.d. | n.d.                    | n.d.                 | n.d. | n.d.                 | n.d.                 | R                          | A few grains         |
| <i>K. polifolia</i>                           | CT                          | 30.2±2.2<br>(27.6-33.6) | 16.8±2.4<br>(12.5-19.7) | 23.0±1.2<br>(21.8-25.2) | 1.31 | 0.73 | 16.3±2.9<br>(13.9-21.1) | 1.2±0.2<br>(1.0-1.9) | 0.54 | 2.1±0.4<br>(1.4-2.6) | 1.6±0.3<br>(1.2-1.9) | R                          | 2                    |
| <i>K. procumbens</i>                          | T                           | 31.5±1.1                | 16.4±1.2                | 22.4±1.4                | 1.41 | 0.73 | 15.7±0.8<br>(14.9-16.5) | 0.8±0.3<br>(0.5-1.2) | 0.50 | 1.8±0.1<br>(1.5-2.0) | 0.7±0.3<br>(0.3-1.3) | P                          | 2                    |
| Takahashi 9907                                | T                           | 30.7±2.3                | 16.6±1.1                | 21.9±1.8                | 1.40 | 0.76 | 19.6±1.7<br>(17.3-23.1) | 0.5±0.1<br>(0.3-0.7) | 0.64 | 1.8±0.2<br>(1.7-2.0) | 0.9±0.4<br>(0.3-1.3) | P                          | 1, 2                 |
| 2644  | T                           | 28.4±34.0               | (14.0-18.0)             | (19.8-25.1)             |      |      |                         |                      |      |                      |                      |                            |                      |
| Takahashi <i>et al.</i>                       |                             | 28.9±2.6                | 15.1±0.9                | 20.1±1.4                | 1.44 | 0.75 | n.d.                    | n.d.                 | n.d. | n.d.                 | n.d.                 | P                          |                      |
| 2644 (n.a.)                                   |                             | (22.6-32.0)             | (13.6-16.1)             | (17.7-21.5)             |      |      |                         |                      |      |                      |                      |                            |                      |

<sup>1</sup> T: Tetrahedral tetrad, (T): Tetrahedral tetrad with other configurations, CT: Compact tetrahedral tetrad, LT: Lobed tetrahedral tetrad, (+V): Viscin threads present.

<sup>2</sup> Exine ornamentation type by SEM. R: Rugulate, RGS: Coarsely rugulate-psilate with moderately granulate, P: Psilate. For details please consult with the text.

<sup>3</sup> 1: Endoaperture indistinct, 2: Endocracks absent/indistinct, 3: Rarely 4-aperturate.

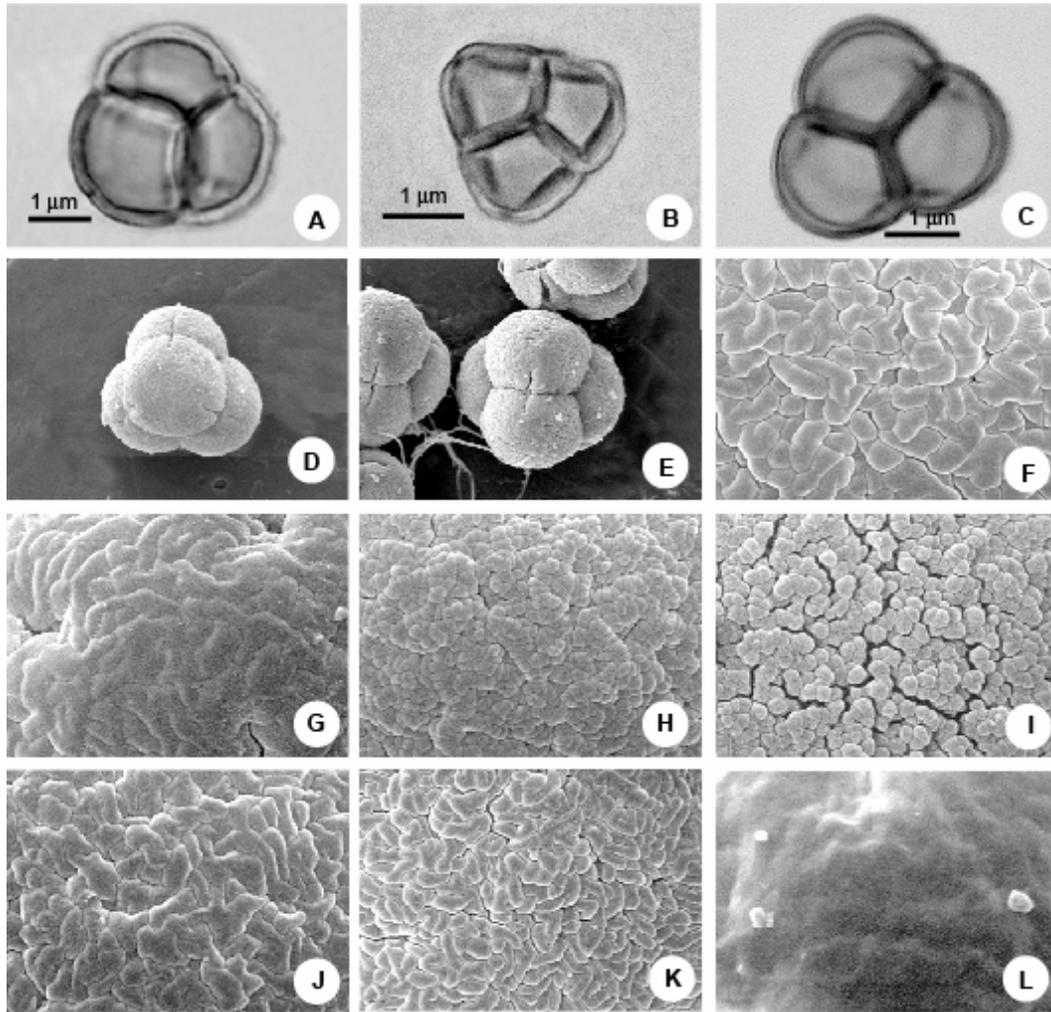


Fig. 1. LM and SEM micrographs of acetolysed *Kalmia* pollen. A. *K. angustifolia* (DeSimon 415); B. *K. buxifolia* (Godfrey & White 7110); C-E. *K. ericoides* var. *aggregata* (Ekman 18165); F. *K. angustifolia* (DeSimon 415); G. *K. buxifolia* (Godfrey & White 7110); H-I. *K. ericoides* var. *aggregata* (Ekman 18165); J. *K. latifolia* (Feldman 63); K. *K. polifolia* (Allen s.n.); L. *K. procumbens* (Takahashi *et al.* 2644). Pollen tetrads at polar view (A-D); pollen tetrad with viscin threads at equatorial view (E); micrographs with apocolpial exine sculpture details (F-H, J-L); micrographs with mesocolpial exine sculpture details (I).

upper right edge of total variation of *Kalmia* (Fig. 2). In agglomerative hierarchical clustering (AHC) using quantitative characters, the taxa studied were distributed in three major clusters (Fig. 3). Apart from the PCA, the *K. buxifolia* pollen also possesses a distinct position in the AHC analysis. The Cluster 1 composed of *K. angustifolia*, *K. polifolia* and *K. procumbens*, and the Cluster 3 of *K. ericoides* var. *aggregata* and *K. latifolia*. *Kalmia buxifolia* is the only member of Cluster 2 (Fig. 3).

## Discussion

### *Variation in palynological characters*

All the *Kalmia* species examined are characterized by 3-colpor(oid)ate, medium, oblate pollen united in tetrahedral tetrads with rugulate exine sculpture indicating that the genus *Kalmia* is a closely related entity. This is in agreement with previous reports (Ebinger, 1974; Southall and Hardin, 1974; Judd, 1995). However, there are significant differences in the value of quantitative palynological characters that may to some extent be related to differences in the mounting media (Meltsov *et al.*, 2008 and references therein). Pollen grains mounted in silicon oil were smaller than grains mounted in glycerine jelly (Faegri and Iversen, 1989; Meltsov *et al.*, 2008).

Although, the majority of palynologists use the acetolysis method of Erdtman (1960) or the slightly modified method of Reitsma (1969) for the preparation of pollen grains for LM and SEM observations, these methods themselves also affect the size of pollen grains. The increase of pollen size after acetolysis varies among genera, sometimes even among species. In *Kalmia* species, we have observed 6-16% difference in tetrad diameter (D) between acetolysed and non-acetolysed pollen of the same taxon (Table 2). However, in some taxa, the size increment of pollen grains ranges between 6% and 30% due to acetolysis has been reported by Schols *et al.* (2004).

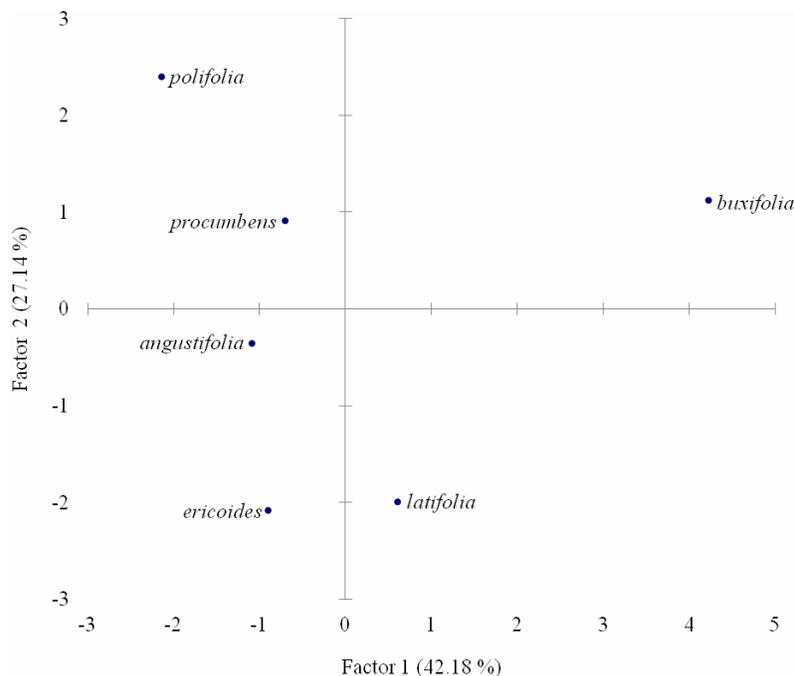


Fig. 2. Two dimensional graph representing species in the Principal Component Analysis.

No correlation between ploidy level and palynological features was found in *Kalmia* (Table 2). Although in Vaccinioideae some tetraploids (*Vaccinium* spp.) produce larger pollen tetrads compared to those of the diploids (Cockerham and Galletta, 1976). *Kalmia polifolia* is tetraploid and all other *Kalmia* species are diploids (Jaynes, 1969 cf. Kron and King, 1996), the pollen tetrads of *K. polifolia* are relatively smaller or similar in size than other diploid species (Table 2).

Interestingly when compared to *K. latifolia*, the chromosomes of *K. polifolia* are about 50% smaller, but contain the same amount of chromatin (Kron and King, 1996).

Previously, the presence of viscin threads on pollen tetrads was reported only for three species of *Kalmia*, *K. latifolia*, *K. hirsuta* and *K. ericoides* (Ebinger 1974; Judd, 1995). However, viscin threads were also observed on pollen tetrads of all three specimen of *K. angustifolia* (Table 2). Southall and Hardin (1974) reported the presence of viscin threads in only *K. latifolia* referring older references and used this character in their systematic treatment of *Kalmia*. However, the scanning electron micrographs of *K. hirsuta* and *K. ericoides* (including *K. simulata*; see Ebinger, 1974) showed viscin threads on their pollen tetrads (Figs 5G, I, J in Southall and Hardin, 1974). Viscin threads on pollen tetrads were also reported in *K. buxifolia* (as *Leiophyllum buxifolia*) and *K. procumbens* (as *Loiseleuria procumbens*) by Stevens (1971), but no viscin threads were observed in this study. This feature needs confirmation with larger number of specimens from both of these two taxa. Hesse *et al.* (2000) reviewed the origin, nature, systematic distribution, and the respective function of highly variable and diverse thread-forming structures including viscin threads in angiosperm anther. Any pollen material with viscin threads points to the highly specialized 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 pollination mechanism of *Kalmia* is very unique; entomophily, anemophily, and possibly autogamy, are all possible (Southall and Hardin, 1974). *Bombus bimaculatus*, *B. ternarius*, *Andrena kalmiae*, *A. vicina* and *A. claytoniae* have been observed pollinating the flowers of various *Kalmia* species of eastern North America (Southall and Hardin, 1974; Jaynes, 1988).

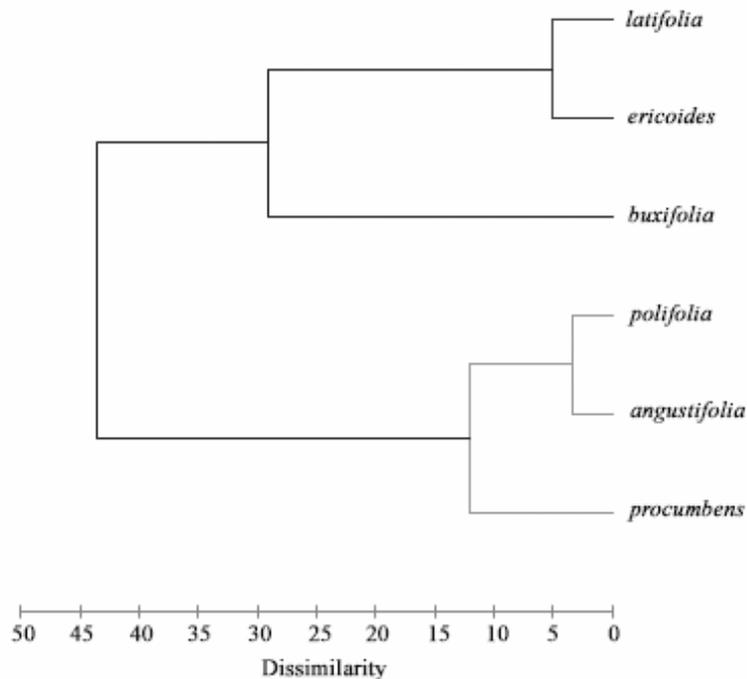


Fig. 3. Dendrogram showing relationships in *Kalmia* species based on Agglomerative Hierarchical Analysis.

### *Taxonomic significance of palynological features*

The cladistic relationships of the species of *Kalmia* were investigated by Southall and Hardin (1974) using “visual ground plan correlation method” of Wagner (1961). Their study indicates that *K. latifolia* is the early-branching taxon and ancestor of all other *Kalmia* species, although *K. latifolia* of today may be slightly different from the original ancestral type (Southall and Hardin, 1974). *Kalmia microphylla* and *K. polifolia* were very closely related and could have been derived from *K. angustifolia* (including *K. carolina*; see Ebinger, 1974), and *K. ericoides* (including *K. aggregata* and *K. simulata*; see Ebinger, 1974) is most closely related to *K. hirsuta* and *K. cuneata* (Southall and Hardin, 1974). In the AHC analysis, *K. angustifolia*, *K. polifolia* and *K. procumbens* made a cluster (Cluster 1 in Fig. 3) which might indicate similarities in palynological features of these three taxa. On the contrary, although *K. latifolia* was indicated as the early-branching and *K. ericoides* as late-branching taxon (Southall and Hardin, 1974), palynological characteristics of these two taxa were very similar, and also positioned in the same cluster (Cluster 3 in Fig. 3).

Inclusion of the two monotypic genera, *Leiophyllum* and *Loiseleuria* in *Kalmia* and, in consequence, the resurrection of *K. buxifolia* and *K. procumbens*, respectively has recently been proposed (Kron *et al.*, 2002). Pollen morphological features, in general, showed similarities and may support the inclusion of these two taxa in *Kalmia*, but also showed some distinct variations viz. thicker septum compared to apocolpial exine, exine sculpture tending to psilate, etc. (Table 2). Pollen tetrads of *K. buxifolia* showed the characteristic rugulate apocolpial exine sculpture of *Kalmia* (Fig. 1G), however, it possesses some exceptional pollen characters within *Kalmia*, viz. smallest tetrads, largest aperture, largest 2f/D ratio and septum thicker than apocolpial exine (Table 2; Fig. 2). In the AHC analysis, *K. buxifolia* is the only member of Cluster 2, which was sister to Cluster 3 (Fig. 3).

The sister relationship of *K. buxifolia* to *K. procumbens* has been recovered from all, both individual and/or combined, analyses of morphological and molecular data (Kron and King, 1996; Kron *et al.*, 2002), although, their palynological characters are distinctly different (Table 2; Figs 1G, L). However, the cladistic analysis of morphological data showed that these two taxa, *K. buxifolia* and *K. procumbens*, are more closely related to the genera *Ledothamnus* and *Bryanthus* than other *Kalmia* species (Fig. 3b in Kron *et al.*, 2002). On the contrary, the *rbcL* molecular data showed that *K. latifolia* is sister to *K. buxifolia* and *K. procumbens*, but the position of *K. angustifolia* and *K. polifolia* within *Kalmia* is not clear (Fig. 5a in Kron *et al.*, 2002). With the inclusion of *Leiophyllum* and *Loiseleuria*, the combined analysis of morphological, anatomical, palynological and molecular data from a larger number of both specimens and species may be useful to identify relationships among the species as well as in infrageneric classification of *Kalmia*, as both genera also possess distinct morphological and anatomical characters (Stevens, 1971; Kron and King, 1996). The apocolpial exine thickness and sculpture have emerged as taxonomically important palynological features for *Kalmia*. An evolutionary trend from rugulate to psilate, or vice-versa, was postulated in apocolpial exine sculpture of the genus *Kalmia* (Figs 1F-L; see also Kron and King, 1996).

Dichotomous key to *Kalmia* taxa examined based on pollen characters:

- |  |   |
|--|---|
| 1. Viscin threads present on pollen tetrads  | 2   |
| 2. Apocolpial exine thickness < 2.0 µm and sculpture rugulate, oblate pollen   | 3   |
| 3. Pollen grains united in lobed tetrahedral tetrads, D 32.2 µm, aperture relatively larger, 2f/D 0.43                           | <i>K. angustifolia</i>                    |
| 3. Pollen grains united in tetrahedral tetrad along with other configurations, D 36.6 µm, aperture relatively smaller, 2f/D 0.32 | <i>K. latifolia</i>                       |
| 2. Apocolpial exine thickness > 2.0 µm and sculpture coarsely rugulate-psilate with moderately granulate, suboblate pollen       | <i>K. ericoides</i> var. <i>aggregata</i> |

- |  |                      |
|--|----------------------|
| 1. Viscin threads absent on pollen tetrads   | 4                    |
| 4. Apocolpial exine sculpture rugulate, tetrahedral tetrads compact                                    | 5                    |
| 5. Septum thicker than apocolpial exine, D 24.4 $\mu\text{m}$ , aperture relatively larger, 2f/D 0.67  | <i>K. buxifolia</i>  |
| 5. Apocolpial exine thicker than septum, D 30.2 $\mu\text{m}$ , aperture relatively smaller, 2f/D 0.54 | <i>K. polifolia</i>  |
| 4. Apocolpial exine sculpture psilate, tetrahedral tetrads normal                                      | <i>K. procumbens</i> |

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