Research Article

# Palynological Studies of *Aconogonon* (Polygonaceae) from Pakistan

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**Abstract** | To better understand systematic characters of Aconogonon (Polygonaceae), we studied pollen morphology in three (A. alpinum, A. rumicifolium and A. tortuosum) of five species distributed in Pakistan using light microscopy and scanning electron microscopy. Results showed that Aconogonon is characterized by tricolpate pollen with microspinulose type of exine ornamentation. One distinctive pollen type was recognized on the basis of aperture number and sculpturing of exine under SEM. Based on analyses in the present study, diagnostic keys to the three species were provided.

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## Introduction

Polygonaceae Juss., the Buckwheat, Smartweed or Knotweed family, are widely distributed from the arctic to the tropics although most species are found in the northern temperate region (Heywood, 1978). The family is represented by 48 genera containing 1,200 species (Freeman and Reveal, 2005; Sanchez and Kron, 2008) while in Pakistan it is represented by 103 species in 19 genera (Qaiser, 2001). Among 19 genera of the family, *Aconogonon* (Meisn.) Reichenb was treated as separate genus due to its characteristic pollen type (Haraldson, 1978, Hong and Hedgeberg, 1990). It is represented by 18-20 species, scattered from north to south Asia, Europe, Japan and North America, and only five species are recorded from Pakistan (Qaiser, 2001). The genus is characterized by having trifid venation, eight stamens 8 arranged in two whorls, clearly visible nectarines, free, short styles and trigonous, ribbed fruits (De Craene and Akeroyd, 1988; De Craene et al., 2000).

The systematic implication of pollen morphology in Polygonaceae has been discussed by a number of workers. Wodehouse (1931) was the first who studied comprehensively palynological characters of the family Polygonaceae, identified different types of pollen grains which were related to each other and developmental tendencies in pollen. On the basis of qualitative and quantitative characters, diagnostic keys were prepared and proved with illustrations to show how tricolporate pollen with thick walls and broad furrow might have given rise to many pored pollen grains with thin walls and narrow or reduced furrow.

Thereafter, pollen morphology of the family was examined as systematic characters (Hedgeberg, 1946; Wang and Feng, 1994; Zhang and Zhou, 1998; Zhou et al., 1999; Zhou et al., 2002). However, pollen morphology of the species of *Aconogonon* from Pakistan has never been investigated. The purpose of the present study is to provide detailed palynological information on *Aconogonon* of the family Polygonaceae using LM and SEM and to find out taxonomic significance of pollen morphology in the delimitation of the species under study.

**Table 1:** *List of voucher specimens investigated for palynologicalstudies with locality, district, collector name and accession numbers arranged in alphabetical order of genera*

| Sr. No. | Species | Locality | District | Collector name | Acc. No. |
|---|---|---|---|---|---|
| 1 | Aconongononalpinum(All.) Schur | Dir<br>BuraiBandi | Dir<br>Hazara | Allahuddin<br>Shoukat and Nisar | 41998<br>34117 |
| 2 | A. rumicifolium(Royle ex Bab.) Hara | Mount Makra (Northern side) | Hazara | Muqarrab Shah and Dilawar Khan | 940044 |
| 3 | A. tortuosum (D. Don) Hara | Naltar<br>Jajoti | Gilgit<br>Gilgit | ShahzadIqbalet al.<br>Mir Ajab Khan et al. | 116866<br>122639 |


**Figure 1:** *A. tortuosum (D. Don) Hara. Flowering plant*


**Figure 2:** *A. rumicifolium (Royle ex Bab.) Hara Flowering plant*

## Materials and Methods

Pollen samples of *Aconogonon* were obtained from herbarium specimens of three (*A.alpinum* [All.] Schur, *A. rumicifolium* [Royle ex Bab.] Hara and *A. Tortuosum* [D.Don] Hara) of the five species distributed in Pakistan, which are housed at Quaid-i-Azam University, Islamabad (Figure 1, 2, 3, Table 1). They were acetolysed following the modified procedure of Erdtman (1960). For light microscopy, pollen grains were mounted in glycerin jelly on a glass slide and stained with 1% safranin. The glass slide was placed on hot plate, where by glycerin jelly was melted–to remove bubble. A cover slip was placed on the pollen-glycerin jelly mixture. When cooled, the glass slide was labelled and edges of the cover slip were sealed with transparent nail vanish. The pollen grains on glass slides were examined under the light microscope. Pollen type, its shape and diameter in polar and equatorial view, P/E ratio, exine thickness and its sculpturing, length of colpi and lumina size were examined. Details of pollen morphology were based on the measurements of 10-15 grains. The data were statistically analysed I-e., range; mean and standard error (±) were calculated using MS excel (Table 2). Their photographs were taken with the Nikon FX-35 microscope equipped with camera. For SEM studies, pollen grains suspended in a drop of 40% acetic acid were transferred to clean metallic stubs and coated with gold using a JEOL JFC 1100 E ion sputtering device. SEM observations were carried out on a JEOL mi-


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croscope JSM5910. The work was carried out in the Centralized Resource Laboratory, University of Peshawar (Pakistan).

Terminology of pollen morphology followed by Faegri and Iversen (1964), Kremp (1965) and Punt et al., (1994, 2007).

## Results

A summary of pollen morphological characters of the three species of *Aconogonon* is given in table 2. Quantitative observations are recorded under light microscope (Figure 4-7). Scanning electron micrographs of selected species of the genera are presented in figure 8-11.

**Figure 3:** *A. alpinum (All.) Schur. Flowering plant*

### Pollen class
Tricolpate

### Size
The size of pollen grains (polar axis × equatorial diameter) varies from 22×21 µm in *A. rumicifolium* to 24×22.5 µm in *A. alpinum* and 24×21.8 µm in *A. tortuosum* (Table 2).

### Symmetry and Shape
Pollen grains are radially symmetrical and isopolar. The outline is mostly prolate-spheroidal, occasionally prolate and sub-prolate in equatorial view while circular, sometimes semiangular in polar view (Figure 4-7). The P/E (polar diameter/equatorial diameter) ratios vary from 1.01 to 1.10. It is maximum in *A. tortuosum* (1.10) and minimum in *A. rumicifolium* (1.01), which shows that there is slight variation in the pollen size (Table 2). Columella is well developed and evenly distributed.

### Aperture
Pollen grains are non-lacunate. Ora is elliptic or slightly elongated. Colpi are very long, depressed and their length varies from 15.04 µm (*A. rumicifolium*) to 16.4 µm (*A. alpinum*).

### Exine thickness
Exine thickness among the taxa of *Aconogonon* ranges from 2.2 to 3.5 µm. In *A. alpinum* (2.2 µm) it appears to be smaller than that of *A. rumicifolium* (3µm) and *A. tortuosum* (3.5 µm).

### Exine Sculpturing
Under light microscope, exine appears granular (Figure 4-7) while scanning electron micrographs shows microspinulose type of ornamentation in all species of the genus (Figure 8-11).

## Discussion

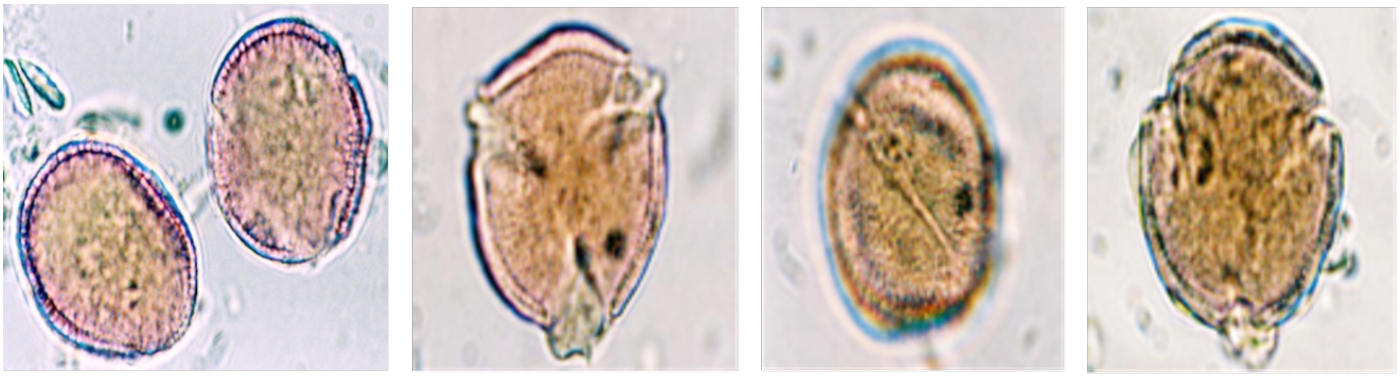
Not only general morphology of plant but also pollen morphology is of great taxonomic significance (Stix, 1960). Pollen morphological characters have been used for the identification of taxa (Erdtmann, 1966) and also for number of phylogenetic studies (Walker and Doyle, 1975).

*Aconogonon* is characterized by Aconogonon type pollen (Wang and Feng, 1994; Zhang and Zhou, 1998; Zhou et al, 2002). The pollen grains of the examined taxa of the genus were tricolpate, circular and circular-semiangular in polar view while equatorial outline was mostly prolate-spheroidal, rarely subprolate as in *A. alpinum* with slight variation in size (Figure 4-7, Table 2). The maximum average diameter in polar view was 24 µm in *A. alpinum* and *A. tortuosum* while 22 µm recorded to be the minimum in *A. rumicifolium*. Similarly, in equatorial view maximum diameter was noted in *A. alpinum* (22.5 µm) and minimum in *A. rumicifolium* (21 µm). However, presently recorded dimensions for *A. alpinum* do not fall within the range specified by Wang and Feng (1994) I-e., 38.4-46.1×30.7-35.8 µm. Zhang and Zhou (1998) gave 28.9-34.0×20.4-28.9 µm size for *A. alpinum* and 30.6-40.8 µm equatorial diameter for *A. tortuosum*. Hong and Hedgeberg (1990) described pollen grains of *A. rumicifolium* as tricolpate grains, spheroidal to subpro

**Table 2:** *Summary of Pollen measurements, shape and sculpturing features in Aconogonon (Meisn.) Reichenb. species. (All measurements are in µm)*

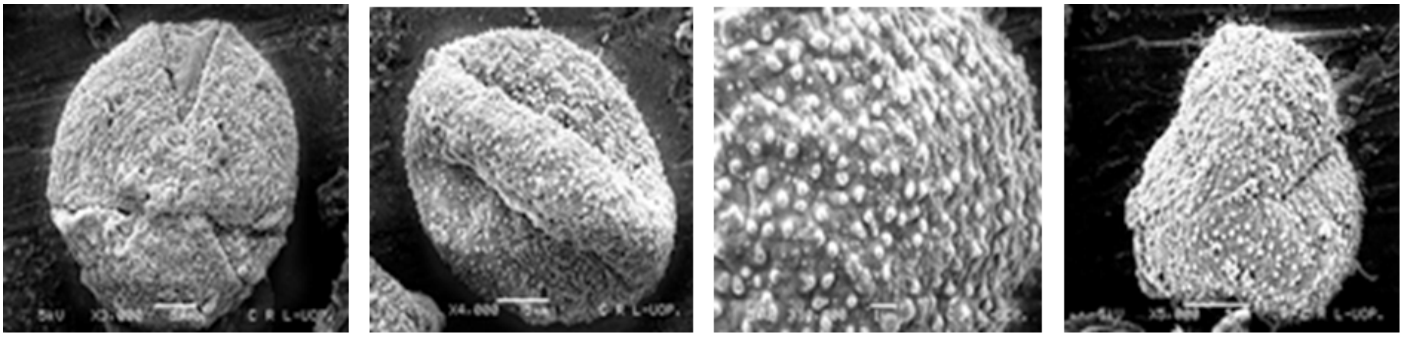
| Sr. No. | Species | Pollen class | Aperture Type | Shape in Equatorial view | Shape in Polar view | Equatorial diameter µm | Polar diameter µm | P/E ratio | Length of colpi µm | Exine thickness µm | Sculpturing | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Under LM | Under SEM |
| 01 | A. rumicifolium | Tricolpate | Non- lacunate | Prolate-spheroidal | Circular, semiangular | *21±0.61 (20-22.5) | 22±1.22 (20-25) | 1.01 | 15.04±0.1 (14.5-16) | 3 | Granulate | Microspinulose |
| 02 | A. alpinum | Tricolpate | Non- lacunate | Prolate-spheroidal to subprolate | Circular, semiangular | 22.5±1.18 (20.5-27) | 24±0.85 (20-28.5) | 1.06 | 16.4±0.8 (15-18.5) | 2.2 | Granulate | Microspinulose |
| 03 | A. tortuosum | Tricolpate | Non- lacunate | Prolate-spheroidal | Circular | 21.8±1.10 (20-24.5) | 24±0.61 (22.5-25) | 1.10 | 16±0.44 (15-17) | 3.5 | Granulate | Microspinulose |

** Mean values followed by min-max in parentheses. P=Polar, E=Equatorial, ± Standard error*



4 5 6 7

**Figure 4-7:** *LM micrographs of the pollen grains of genus Aconogonon at 1000X.*
***A. rumicifolium*: 4**. *Polar and equatorial view.* ***A. alpinum*: 5**. *Polar view,* **6.** *Equatorial view.* ***A. tortuosum*: 7**. *Polar view*



8 9 10 11

**Figure 8-11:** *Scanning electron micrographs of the pollen grain of genus Aconogonon.*
*A.**rumicifolium*: **8.** *Polar view.* ***A. alpinum*: 9**. *Polar view,* **10**. *Equatorial view,* **11**: ***A. tortuosum.*** *Exine ornamentation pattern*

late or oblate to spheroidal-prolate in shape.

Colpi were found to be deep, long and narrow with small variation in length (Table 2). Exine thickness varied between 2.2 µm to 3.5 µm and it proved to be helpful at specific level. When sculpturing of exine was examined with light microscope (LM) it appeared granular. With scanning electron microscope (SEM), microspinulose type of ornamentation was recorded in *A. rumicifolium*, *A. tortuosum* and *A. alpinum* (Figure 8-11). Wang and Feng (1994) recorded 3-4 µm thick exine in *A. alpinum* which appeared negatively reticulate when examined with LM and granulate-perforate under SEM. Zhang and Zhou (1998) and Zhou et al., (2002) measured 3.4µm thick exine in *A. alpinum* and 3.0 µm in *A. tortuosum*. Their observations on exine ornamentation for both species were similar to that of Wang and Feng (1994) observations. Aconogonon type pollen grains are widely accepted to be the basic and most primitive type (Zhou et al., 1999).

Based on the aforementioned analyses, diagnostic keys to the three species of *Aconogonon* are provided as follows:

Key to the species of Aconogonon

| | |
|---|---|
| 1a: P/E ratio 1.01. | 1. *A. rumicifolium* |
| 1b: P/E ratio more than 1.01. | 2 |
| 2a: Exine 2.2 µm thick. | 2. *A. alpinum* |
| 2b: Thickness of exine 3.5 µm. | 3. *A. tortuosum* |

## Conclusion

Based on the present study, it is shown that both qualitative and quantitative micro-morphological characters of pollen are solely sufficient for the characterization and delimitation of the genus *Aconogonon* at species level. There is much scope for further research on pollen morphology of the family Polygonaceae from Pakistan and these characters can be used in combination with other evidences derived from morphology, cytology and anatomy.

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