



**Full Length Article**

## Diversity of Foliar Trichomes and their Systematic Implications in the Genus *Artemisia* (Asteraceae)

MUHAMMAD QASIM HAYAT<sup>1</sup>, MUHAMMAD ASHRAF<sup>†</sup>, MIR AJAB KHAN, GHAZALAH YASMIN, NIGHAT SHAHEEN, AND SHAZIA JABEEN<sup>‡</sup>

Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

<sup>†</sup>NUST Center for Virology and Immunology, National University of Science and Technology, Rawalpindi, Pakistan

<sup>‡</sup>National Center of Excellence in Geology, University of Peshawar, Peshawar, Pakistan

<sup>1</sup>Corresponding author's e-mail: mqasimhayat@hotmail.com

### ABSTRACT

The taxonomic relationships within the genus *Artemisia* L. has been very controversial. In order to understand the systematic implication of foliar trichomes within the genus, the micromorphological characteristics of foliar trichomes from 15 taxa were examined using light microscopy (LM) and scanning electron microscopy (SEM). The observed glandular and non-glandular trichomes were classified into eight main types. Among glandular trichomes, capitate glands had wild distribution on the both surfaces of leaves with notable variations. In case of non-glandular trichomes, T-shaped hairs were most abundant. It was also noted that foliar trichomes of genus *Artemisia* are good taxonomic markers and can be utilized for the discrimination of different taxa within the genus.

**Key Words:** *Artemisia*; *Seriphidium*; Anthemideae; Asteraceae; Anatomy; Trichomes

### INTRODUCTION

*Artemisia* L. is the largest genus of the tribe Anthemideae and even one of the largest genera of the family Asteraceae. It is well known wind pollinated cosmopolitan genus, mainly distributed in temperate areas of mid to high latitudes of the northern hemisphere, colonizing in arid and semiarid environments landscape and has only few representatives in southern hemisphere (McArthur & Plummer, 1978; Valles & McArthur, 2001). Many species of the genus have a high economic value as medicines, food, forage, ornamentals or soil stabilizers in disturb habitats; some taxa are toxic or allergenic and some others are invasive weeds, which can adversely affect harvests (Pareto, 1985; Tan *et al.*, 1998). Most species in the genus are perennial; only approximately 10 species are annuals or biannual (Valles *et al.*, 2003). *Artemisia* is also considered as indicator of steppe climate (Erdtman, 1952) and moderate precipitation (El-Moslimany, 1990).

After various taxonomic rearrangements (Hooker, 1881; McArthur *et al.*, 1981; Ling, 1982, 1991a & b, 1995a & b; Bremer, 1994; Kornkven *et al.*, 1998; Torrell *et al.*, 1999) the genus was divided into five large groups, which have been considered at sectional or subgeneric level; *Absinthium* (Tournefort) de Cand., *Artemisia* Tournefort (= *Abrotanum* Besser), *Dracunculus* Besser, *Seriphidium* Besser and *Tridantatae* (Rydb.) McArthur. *Tridantatae* is only restricted to North America. Ling (1991a & 1995b) separated *Seriphidium* from *Artemisia* as a new genus.

Bremer (1994) accepted this separation but Torrell *et al.* (1999) and Watson *et al.* (2002) again united *Seriphidium* with *Artemisia*. However the classification of *Artemisia* and relationships among its different sections still has been very controversial.

Since the micromorphological characteristics of foliar trichomes have played an important role in plant taxonomy, especially of particular groups at generic and specific levels, more and more studies in this field have attracted the attention of plant morphologists and systematists to resolve the taxonomic conflicts (Hardin, 1979; Fang & Fan, 1993). Although many studies conducted on the histochemistry of the secretory products of the glandular trichomes of *Artemisia* (Smith & Kreitner, 1982; Slone & Kelsey, 1985; Ascensao & Pais, 1987; Duke & Paul, 1993; Duke *et al.*, 1994), but little is known about the systematic significance of trichomes of *Artemisia*. Therefore, in the present paper, the micromorphological characteristics of foliar trichomes in *Artemisia* were studied by means of light microscopy (LM) and scanning electron microscopy (SEM). The specific objectives of this paper were to: (a) compare the micromorphological characteristics of foliar trichomes in different species of this genus and (b) reconstruct the systematic relationships among different taxa of *Artemisia* based on characteristics of foliar trichomes.

### MATERIALS AND METHODS

Leaf material used in this study was obtained from

herbarium specimens as well as freshly collected plants during the expeditions to various parts of Pakistan. Origin and details of studied taxa are given in Table I. Foliar trichomes were initially observed by means of an OLYMPUS/BX-51 light microscope. For this purpose, a modified methodology of Shaheen *et al.* (2009) and Yasmin *et al.* (2009) was followed. Pieces of leaves were soaked in 30% HNO<sub>3</sub> and boiled along with 1.5 g of KCl in a test tube for 2-3 min. Then these leaf pieces were washed with distilled water. Epidermis was peeled and kept in 60% KOH solution for 2 h. Finally, these leaf pieces were suspended in lactic acid and transferred on glass slides for examination. For SEM study, the dried leaves were mounted by double adhesive tape on aluminum stubs, sputter coated with gold by SPI-Module Sputter Coater and examined with a Jeol-JSM 5910 scanning electron microscope.

Basic terminology used in trichomes classification and description was that suggested by Ramayya (1972), Bento *et al.* (2008), Popa and Sipos (2009) and Shaheen *et al.* (2009). However, simple self explanatory terms are included to identify the specific type of trichome.

## RESULTS

Based on the observations obtained by using LM (Fig. 1 & 2) and SEM (Fig. 3 & 4) the foliar trichomes in 15 taxa from *Artemisia* were classified into the following eight main types:

(a). **Capitate trichomes.** These are glandular trichomes. They are ellipsoidal in shape and are characteristic of *A. absinthium* (Fig. 1-A), *A. tournefortiana* (Fig. 2-A), *A. dubia* (Fig. 3-C & 3-F), *A. sieversiana* (Fig. 3-D), *A. amygdalina* (Fig. 3-E), *A. moorcroftiana* (Fig. 3-G), *S. kurramense* (Fig. 3-H), *A. vulgaris* (Fig. 3-I), *A. persica* (Fig. 3-J), *A. biennis* and *A. tangutica*.

(b). **Pluricellular trichomes.** These are also glandular trichomes. They are 2 to 5 cell long, broader at base and tapering toward apex. They are characteristic feature of *A. dubia* (Fig. 1-B) and *A. roxburghiana* (Fig. 1-E & 1-F).

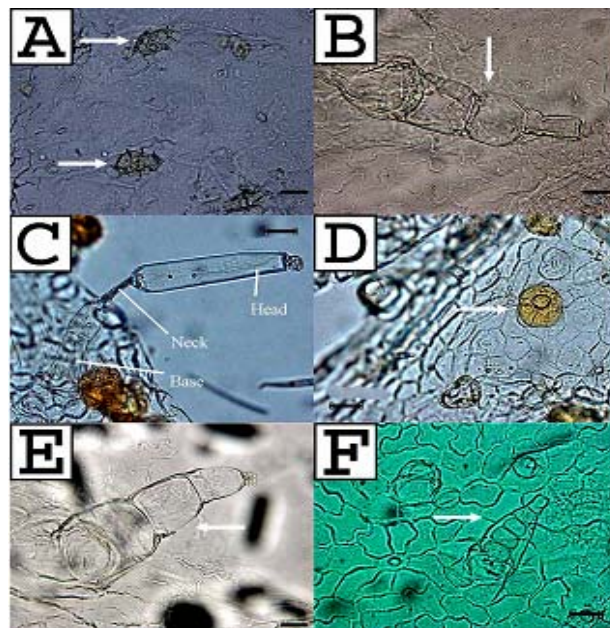
(c). **Peltate trichomes.** They are ball shaped multicellular structures. These glandular trichomes are found in *A. persica* (Fig. 1-D), *A. biennis* (Fig. 3-A & 3-B) and *S. leucotrichum*.

(d). **Thin neck trichomes.** These glandular trichome are the unique feature of *A. persica* (1-C).

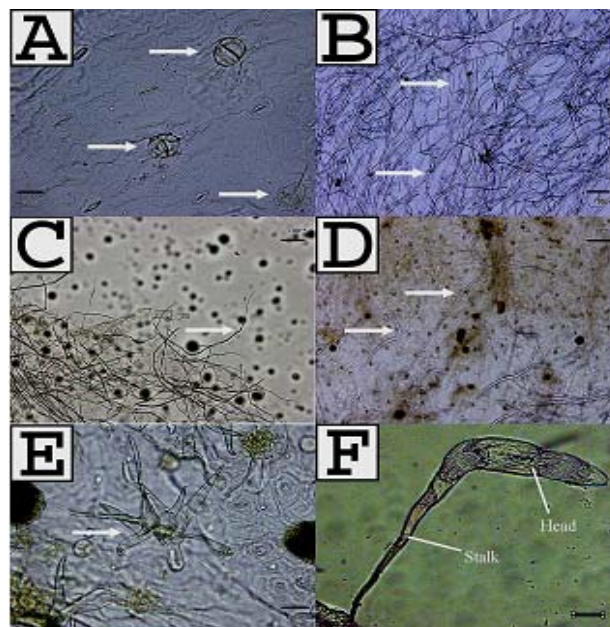
(e). **T-shape trichomes.** These are non-glandular trichomes. They are characteristic feature of *A. roxburghiana* (Fig. 2-B), *A. dubia* (Fig. 2-C & 4-H), *S. leucotrichum* (Fig. 2-D), *A. vulgaris* (Fig. 4-A), *A. moorcroftiana* (Fig. 4-B), *A. tangutica* (Fig. 4-C), *A. biennis* (Fig. 4-D & 4-F), *A. macrocephala* (Fig. 4-E), *A. japonica* (4-G), *A. amygdalina*, *A. absinthium*, *A. sieversiana* and *S. kurramense*.

(f). **Macroform trichomes.** They are multicellular and multi-rayed. These non-glandular trichomes are the unique feature of *A. persica* (4-J).

**Fig. 1.** Types of glandular foliar trichomes in *Artemisia* by means of LM. A, *A. absinthium*; B, *A. dubia*; C-D, *A. persica*; E-F, *A. roxburghiana* (Scale bar = 30µm)



**Fig. 2.** Types of glandular and nonglandular foliar trichomes in *Artemisia* by means of LM. A, *A. tournefortiana* (Scale bar = 30µm); B, *A. roxburghiana* (Scale bar = 100µm); C, *A. dubia* (Scale bar = 100µm); D, *S. leucotrichum* (Scale bar = 100µm); E, *A. persica* (Scale bar = 30µm); F, *A. roxburghiana* (Scale bar = 30µm)

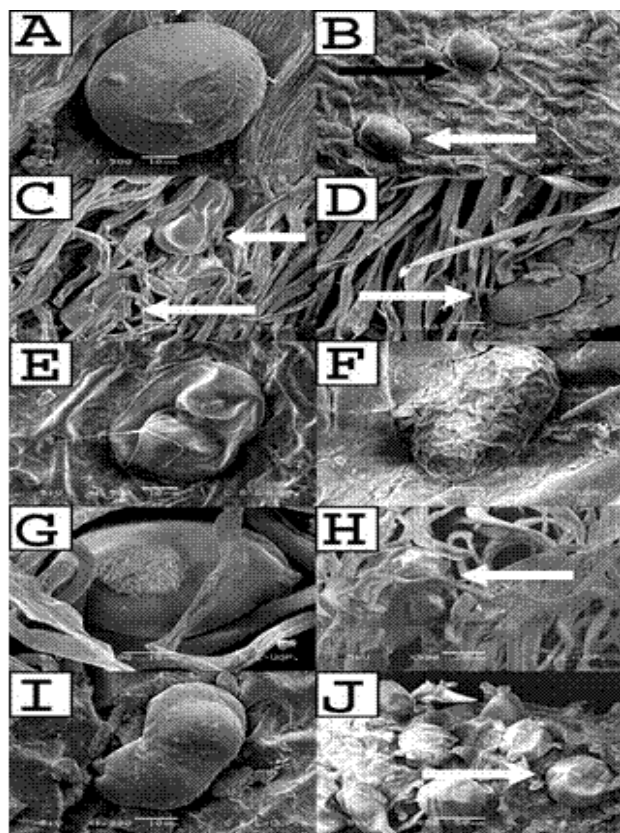


(g). **Unicellular tector trichomes.** These non-glandular thread like long trichome clusters are present in *A. dubia* (Fig. 4-I).

**Table I. List of taxa studied for foliar anatomy and their herbarium vouchers. ISL: Herbarium, Quaid-i-Azam University, Islamabad. PUP, Herbarium, University of Peshawar, Peshawar**

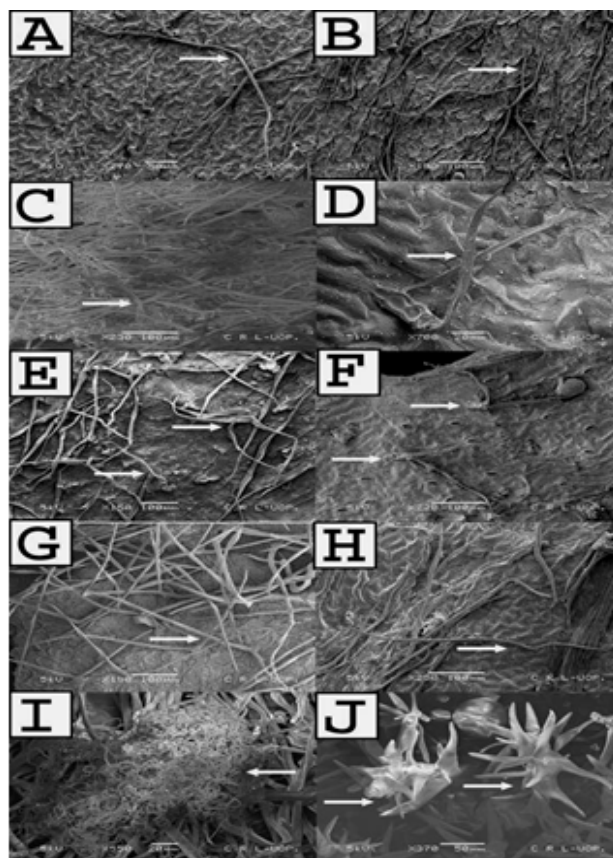
Taxon	Collection data	Herbarium Voucher
<b>Section <i>Artemisia</i> Tournefort</b>		
<i>A. amygdalina</i> Decne.	Mansehra: Naran to Lake Saif-ul-Malook track. <i>T. Malik</i> , 1972.	ISL, 32315
<i>A. biennis</i> Willd.	Rawalpindi: Murree Hills, PLT, Ayubia National Park. <i>M. Q. Hayat</i> , 2007.	PUP, PH005 (ART005)
<i>A. dubia</i> Wall. ex Besser	Rawalpindi: Murree Hills, PLT, Ayubia National Park. <i>M. Q. Hayat</i> , 2007.	PUP, PH002 (ART002)
<i>A. moorcroftiana</i> Wall. ex DC.	Azad Jammu & Kashmir: Muzafabad. <i>T. Malik</i> , 1972.	ISL, 26550
<i>A. roxburghiana</i> Wall. ex Besser	Rawalpindi: Murree Hills, PLT, Ayubia National Park. <i>M. Q. Hayat</i> , 2007.	PUP, PH001 (ART001)
<i>A. tournefortiana</i> Reichenbach	Rawalpindi: Murree Hills, PLT, Ayubia National Park. <i>M. Q. Hayat</i> , 2007.	ISL, 21921
<i>A. vulgaris</i> L.	Azad Jammu & Kashmir: Pearl valley, Mutyal Mara. <i>M. Q. Hayat</i> , 2008.	PUP, PH006 (ART006)
<b>Section <i>Absinthium</i> (Mill.) DC</b>		
<i>A. absinthium</i> L.	Gilgit: Nattar valley. <i>M. Q. Hayat</i> , 2007.	PUP, PH004 (ART004)
<i>A. macrocephala</i> Jacq. ex Basser	Gilgit: Nattar valley. <i>Lal Badshah</i> , 1997.	PUP, 121(556)
<i>A. persica</i> Boiss.	Swat: <i>Nighat Akhter</i> , 1990	PUP, 27
<i>A. siversiana</i> Ehrh.	Gilgit: Nattar valley. <i>A. Rashid</i> , 1986.	PUP, 222 (1057)
<i>A. tangutica</i> Pampanini	Gilgit: Hunza vally. <i>M. Q. Hayat</i> , 2007.	ISL, 32144
<b>Section <i>Seriphidium</i> (Besser) Besser</b>		
<i>S. leucotrichum</i> (Krasch. ex Lady.) K. Bremer & Hum. ex YR Ling	Gilgit: Nattar valley. <i>M. Q. Hayat</i> , 2007.	ISL, 92453
<i>S. kurramense</i> (Qaz.) YR Ling	Kurram Agency: Burki. <i>N. A. Qazilbash</i> , 1937.	PUP, 22419
<b>Section <i>Dracunculus</i> Besser</b>		
<i>A. japonica</i> Thunb.	Rawalpindi: Murree Hills, PLT, Ayubia National Park. <i>M. Q. Hayat</i> , 2007.	PUP, PH008 (ART008)

**Fig. 3. Types of glandular foliar trichomes in *Artemisia* by means of SEM. A-B, *A. biennis*; C, *A. dubia*; D, *A. siversiana*; E, *A. amygdalina*; F, *A. dubia*; G, *A. moorcroftiana*; H, *S. kurramense*; I, *A. vulgaris*; J, *A. persica***



**(h). Clavate trichomes.** They are multicellular with stalk and broad apex. These non-glandular trichomes are the characteristic feature of *A. roxburghiana* (2-F).

**Fig. 4. Types of nonglandular foliar trichomes in *Artemisia* by means of SEM. A, *A. vulgaris*; B, *A. moorcroftiana*; C, *A. tangutica*; D, *A. biennis*; E, *A. macrocephala*; F, *A. biennis*; G, *A. japonica*; H-I, *A. dubia*; J, *A. persica***



The quantitative dimensions of all the trichomes types studied are given in Table II and III.

**Table II. Quantitative characteristics of glandular foliar trichomes of *Artemisia***

Taxa	Capitate	Pluricellular	Peltate	Thin neck
	Height x Width $\mu\text{m}$	Height x Width $\mu\text{m}$	Diameter $\mu\text{m}$	Height x Width $\mu\text{m}$
<i>A. amygdalina</i>	45.67-47.02 x 30.11-32.65	Absent	Absent	Absent
<i>A. biennis</i>	36.04-41.97 x 28.72-30.00	Absent	35.56-40.24	Absent
<i>A. dubia</i>	60.72-62.09 x 30.21-38.34	297.50-525.20 x 42.28-83.47	Absent	Absent
<i>A. moorcroftiana</i>	38.50-40.75 x 28.22-29.96	Absent	Absent	Absent
<i>A. roxburghiana</i>	Absent	60 x 13-30	Absent	Absent
<i>A. tournefortiana</i>	42.69-45-47 x 28.29-30.00	Absent	Absent	Absent
<i>A. vulgaris</i>	40.77-42.70 x 20.94-21.50	Absent	Absent	Absent
<i>A. absinthium</i>	48.08-48.80 x 27.39-33.19	Absent	Absent	Absent
<i>A. macrocephala</i>	Absent	Absent	Absent	Absent
<i>A. persica</i>	61.52-85.25 x 56.00-77.99	Absent	34.66-38.52	286.21-300.00 x 25.08-32.43
<i>A. siversiana</i>	58.34-60.00 x 30.00-31.06	Absent	Absent	Absent
<i>A. tangutica</i>	17.53-18.50 x 15.37-17.00	Absent	Absent	Absent
<i>S. leucotrichum</i>	Absent	Absent	19.02-20.45	Absent
<i>S. kurramense</i>	47.10-72.11 x 31.49-40.38	Absent	Absent	Absent
<i>A. japonica</i>	Absent	Absent	Absent	Absent

**Table III. Quantitative characteristics of non-glandular foliar trichomes of *Artemisia***

Taxa	T-shape	Macroform		Unicellular tector	Clavate
	No. of base cells	Arm length ( $\mu\text{m}$ )	Arm width ( $\mu\text{m}$ )	Height x width ( $\mu\text{m}^2$ )	Height x width ( $\mu\text{m}^2$ )
<i>A. amygdalina</i>	Sessile	340.87-350.23	07.14-09.51	Absent	Absent
<i>A. biennis</i>	2-3	200.50-210.28	20.30-22.73	Absent	Absent
<i>A. dubia</i>	Sessile	228.00-267.76	12.13-15.02	Absent	80.0-83.0 x 2.0-4.0
<i>A. moorcroftiana</i>	Sessile	200.62-190.66	06.08-07.62	Absent	Absent
<i>A. roxburghiana</i>	2	227.50-230.00	27.94-33.00	Absent	382.40 x 41.54
<i>A. tournefortiana</i>	Absent			Absent	Absent
<i>A. vulgaris</i>	2	120.50-130.47	29.72-32.41	Absent	Absent
<i>A. absinthium</i>	4	058.72-060.32	13.70-14.45	Absent	Absent
<i>A. macrocephala</i>	Sessile	295.53-300.43	05.43-15.23	Absent	Absent
<i>A. persica</i>	Absent			143.15 x 30.68	Absent
<i>A. siversiana</i>	Sessile	200.00-210.00	15.13-20.22	Absent	Absent
<i>A. tangutica</i>	3	180.50-200.52	09.95-11.45	Absent	Absent
<i>S. leucotrichum</i>	Sessile	527.70-530.62	07.83-10.11	Absent	Absent
<i>S. kurramense</i>	Sessile	250.63-270.72	10.56-12.16	Absent	Absent
<i>A. japonica</i>	Sessile	298.67-400.54	19.34-21.03	Absent	Absent

## DISCUSSION

Features of trichomes are broadly regarded as useful for establishing the taxonomic relations within the genus *Artemisia* (Hall & Clements, 1923). There various types are reported to occur in different species of *Artemisia* (Ferreira & Janick, 1995).

Capitate glands are widely distributed in the genus *Artemisia*. Remarkable variations were observed among the capitate trichomes of different species studied. Capitate trichomes of *A. absinthium* (Fig. 1-A) and *A. tournefortiana* (Fig 2-A) were embedded in both abaxial and adaxial leaf surfaces. They were ellipsoidal in shape and divided into two halves. The capitate trichomes of *A. dubia* (Fig. 3-C & Fig. 3-F), *A. sieversiana* (Fig. 3-D), *A. amygdalina* (Fig. 3-E), *A. moorcroftiana* (Fig. 3-G) and *S. kurramense* (Fig. 3-H) showed same morphology but did not show the two halves division. In case of *A. vulgaris* (Fig. 3-I) the shape of trichome was same but division into two halves was intermediate. Capitate trichomes, reported by Ferreira and Janick (1995) in *A. annua*, Slone and Kelsey (1985) in *A. tridentata* Nutt., Smith and Kreitner (1982) in *A.*

*ludoviciana* Nutt., Kelsey (1984) in *A. nova* Nelson, Ascensao and Pais (1987) in *A. compestris* L. and Lodari *et al.* (1989) in *A. princeps* Pamp., all were different from those examined in this study.

T-shape trichomes also have wide distribution in genus *Artemisia*. Variations were observed in their base cell numbers, arms height and width (Table III). Several studies revealed their different morphological aspects. Lodari *et al.* (1989) studied T-shape trichomes in *A. princeps*, *A. absinthium*, *A. capillaris* and *A. japonica*. Ferreira and Janick (1995) observed T-shape trichomes in *A. annua*. Ascensao and Pais (1987) reported T-shape trichomes in association with glandular trichomes in *A. compestris*.

In addition to capitate and T-shape trichomes, we also observed some other types of trichomes of taxonomic importance. Of these, pluricellular trichomes of *A. dubia* (Fig. 1-B) and *A. roxburghiana* (Fig. 1-E & 1-F); peltate trichomes of *A. persica* (Fig. 1-D), *A. biennis* (Fig. 3-A & 3-B) and *S. leucotrichum*; thin neck trichomes of *A. persica* (1-C); macroform trichomes of *A. persica* (4-J); unicellular tector trichomes of *A. dubia* (Fig. 4-I) and clavate trichomes of *A. roxburghiana* (2-F), are important one. *A. persica* can

be separated from other species on the bases of thin neck and macroform trichomes. Similarly, unicellular tector trichomes are the unique feature of *A. dubia*. These trichomes occurred in clusters and form yellow spots on the adaxial surface of the foliar leaves.

In conclusion, diversity in the capitate glands, variation in T-shaped hairs and presence of other types of foliar trichomes in *Artemisia* suggests that various glandular and non-glandular foliar trichomes in the genus may serve as a valuable taxonomic tool. There is a need to develop better terminology and detail comparative study of these micromorphological features to resolve taxonomic conflicts in the genus.

**Acknowledgement.** We are thank Dr. Riaz, Department of Physics and Dr. M. Tahir Shah, NCE in Geology, University of Peshawar, Pakistan for their facilitation. We also acknowledge HEC Pakistan for their financial assistance.

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(Received 29 April 2009; Accepted 04 May 2009)