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Phylogenetic Relationships in *Artemisia* spp. (Asteraceae) Based on Distribution of Foliar Trichomes

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ABSTRACT

The phylogenetic relationships within the genus *Artemisia* have been very controversial and need throughout investigations. In continuation to the previous paper, here we report the phylogenetic relationships in the genus *Artemisia* based on the foliar trichomes using light microscopy and scanning electron microscopy. The data from 24 taxa was analyzed for its phylogeny. In addition, eight new types of foliar trichomes in the genus were described. This study also revealed that foliar trichomes of the genus *Artemisia* are good taxonomic markers and can be utilized to resolve the taxonomic conflicts within the genus.

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

INTRODUCTION

Artemisia L. is one of the largest genera of the family Asteraceae. Its members are wind pollinated and mainly distributed in temperate areas of mid to high latitudes of the northern hemisphere, colonize in arid and semiarid environments, and only few representatives are found in southern hemisphere (McArthur & Plummer, 1978; Valles & McArthur, 2001). Many species of the genus have 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; Hayat *et al*., 2009a & b). Except few annuals or biannual, most *Artemisia* species are perennial (Valles *et al*., 2003). The genus *Artemisia* is also considered as indicator of steppe climate (Erdtman, 1952) and moderate precipitation (El-Moslimany, 1990).

Classically the genus has been divided into four groups, which has been treated as sections or subgenera; *Absinthium* (Tournefort) de Cand., *Artemisia* Tournefort (=*Abrotanum* Besser), *Dracunculus* Besser, *Seriphidium* Besser (Hooker, 1881; Martin *et al*., 2001 & 2003). McArthur *et al*. (1981) proposed a new group *Tridantatae* (Rydb.) McArthur, which is endemic to North America. Ling (1982, 1991a & b, 1995a & b) separated *Seriphidium* (Besser ex Hooker) as independent genus. Bremer and Humphries (1993) and Bremer (1994) accepted this separation. But Kornkven *et al*. (1998), Torrell *et al*. (1999) and Watson *et al*. (2002) in their molecular studies again merged *Seriphidium* with *Artemisia*. Nonetheless classification of *Artemisia* and relationships among its different taxa are still controversial.

Scotland *et al*. (2003) opined that rigorous and critical anatomical studied of fewer morphological characters in the context of molecular phylogenies is fruitful to integrating the strengths of morphological data with those of sequence data. Since the micromorphological characteristics of foliar trichomes play an important role in plant taxonomy, especially of particular groups at generic and specific levels, attention of plant morphologists and systematists has been mainly attracted 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, 1983; Slone & Kelsey, 1985; Ascensao & Pais, 1987; Duke & Paul, 1993; Duke *et al*., 1994), little is known about the systematic significance of trichomes in *Artemisia*. In the present paper, we report the micromorphological characteristics of foliar trichomes in *Artemisia* using light microscopy (LM) and scanning electron microscopy (SEM). The specific objectives were to: (a) identify and compare the micromorphological characteristics of foliar trichomes in different species of this genus and (b) reconstruct the phylogenetic relationships among different taxa of *Artemisia* based on characteristics of foliar trichomes.

MATERIALS AND METHODS

All details about the origin and collection of leaf material, processing of the tissues, basic terminology and studies of the foliar trichomes were essentially the same as

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Fig. 1. Types of glandular and nonglandular foliar trichomes in *Artemisia* by means of LM: A, *A. persica*; B, *A. roxburghiana*; C-D, *A. vulgaris* (Scale bar = 50 µm)

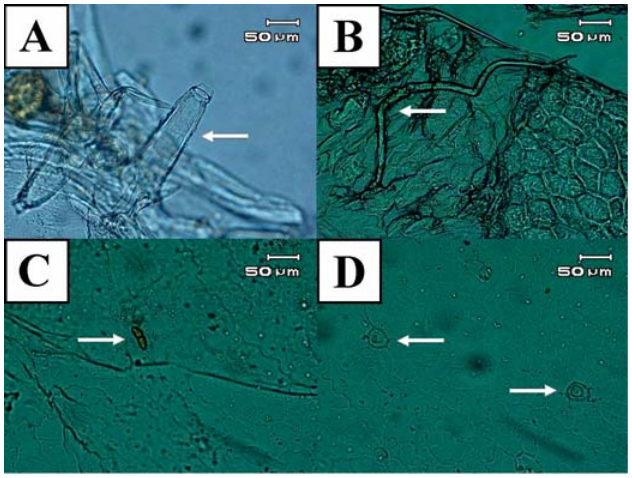
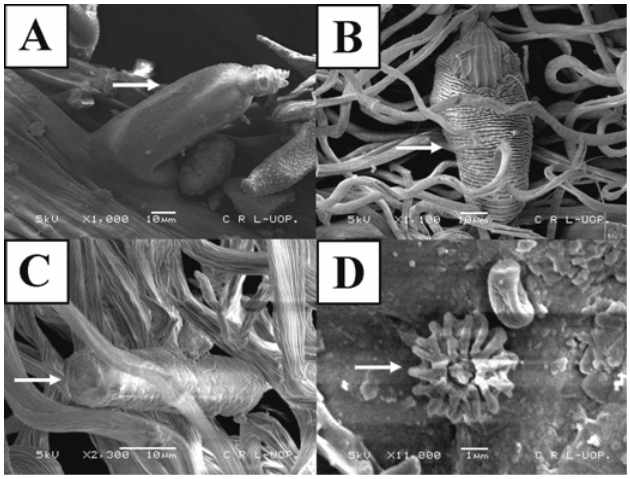


Fig. 2. Types of glandular and nonglandular foliar trichomes in *Artemisia* by means of SEM: A, *A. persica* (Scale bar = 10 µm); B, *A. moorcroftiana* (Scale bar = 10 µm); C, *S. kurramense* (Scale bar = 10 µm); D, *A. stricta* (Scale bar = 1 µm)



we reported in the previous paper (Hayat *et al*., 2009a).

Source and collection information of remaining taxa is given in Table I. Using modified methodology of Shaheen *et al*. (2009) and Yasmin *et al*. (2009) foliar trichomes were at first examined by OLYMPUS/BX-51 light microscope. Pieces of leaves were dipped in 30% Nitric acid and boiled along with 1.5 g of Potassium chloride in a test tube for 2-3 min. Then these leaf pieces were cleaned with distal water. Epidermis was removed and kept in 60% Potassium hydroxide solution for 2 h. Finally, these leaf sections were suspended in Lactic acid and transferred on glass slides for LM observations. For SEM investigations, the dried leaves were mounted on aluminum stubs with the help double adhesive tape, sputter coated with gold by SPI-Module Sputter Coate and studied with a Jeol-JSM 5910 scanning electron microscope. Basic terminology used for trichomes categorization and elaboration was that recommended by Ramayya (1972), Payne (1978), Bento *et al*. (2008), Popa and Sipos (2009) and Shaheen *et al*. (2009). However, straightforward self illustrative terms are incorporated to recognize the particular type of trichome.

On the bases of presence or absence, all 16 foliar trichomes types, noted here, were selected as a character states for phylogenetic analysis of *Artemisia* (Table II). The plesiomorphic or apomorphic state of each trichome was determined using the criteria established by Boudreaux (1979) and further explained by Crisci and Stuessy (1980). In this study an imaginary outgroup was used for comparison that contained all the ancestral characters. An original binary data matrix was produced (Table III) for phylogenetic analysis using the outgroup comparison method (Watrous & Wheeler, 1981). The phylogenetic analysis was done by PHYLIP computer program version 3.67 (Felsenstein, 2007). Most parsimonious trees (MPTs) based on the binary matrix were constructed with MIX program of PHYLIP using Wagner parsimony method (Farris, 1970). A strict consensus phylogenetic tree of the MPTs was generated using the CONSENSE program of PHYLIP (Sokal & Rohlf, 1981). Finally, using DRAWTREE and DRAWGRAM programs of PHYLIP strict consensus phylogenetic tree of *Artemisia* were generated.

RESULTS

Types of foliar trichomes. Based on observations of LM (Fig. 1) and SEM (Fig. 2), the foliar trichomes in 24 taxa from *Artemisia* were classified into 16 main types. Of these a-h were described in Hayat *et al*. (2009a), i-p are illustrated below:

(i). Vase-shape trichomes. These are glandular trichomes. They are cylindrical in shape with constricted apex and are characteristic of *A. persica* (Fig. 1A & Fig. 2A).

(j). Aduncate curly trichomes. These are also non-glandular trichomes. They are long cylindrical in shape. They are characteristic feature of *A. roxburghiana* (Fig. 1B).

(k). Dolabrate trichomes. They have shape like the head of pick, with two divaricated or opposed terminal branches. These non-glandular trichomes are found in *A. vulgaris* (Fig. 1C).

(l). Unicellular peltate trichomes. These glandular trichome are embedded in epidermis and are the characteristic feature of *A. vulgaris* (Fig. 1D), *A. roxburghiana* and *A. japonica*.

(m). Rope rolled trichomes. These are nonglandular trichomes. They are exclusive characteristic feature of *A. moorcroftiana* (Fig. 2B).

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
Section *Artemisia* Tournefort		
A. rutifolia Spreng.	Gilgit: Nattar valley. *A. Rashid*, 1988.	PUP, 244 (1105)
A. santolinifolia Turcz. ex Krasch.	Gilgit: Nattar valley. *A. Rashid*, 1986.	PUP, 239 (1108)
A. vestita Wall. ex DC.	Jahlum: Soon vally, Sakasar. *M. Farooq*, 2005.	ISL, 20093
Section *Seriphidium* (Besser) Besser		
A. maritima L.	Skardu: *M. Q. Hayat*, 2007.	PUP, PH003 (ART003)
S. brevifolium (Wall. ex DC.) Ling & YR Ling	Mansehra: Ujtar, Naran to Lalusar lake track. *M. Q. Hayat*, 2007.	PUP, PH007 (ART007)
S. stenocephalum (Krasch. ex Poljakov) Poljakov	Gilgit: Chalas. *M. Q. Hayat*, 2008.	PUP, PH010 (ART010)
S. turanicum (Krasch.) Poljakov	Gilgit: Nattar vally. *M. Q. Hayat*, 2008.	PUP, PH009 (ART009)
Section *Dracunculus* Besser		
A. scoparia Waldst. et Kit.	Islamabad: Quaid-i-Azam university campus. *M. Q. Hayat*, 2008.	ISL, 32313
A. stricta Edgew.	Kashmir: Muzafrabad. *T. Malik*, 1972.	ISL, 25650

*Partly adapted from Table I, Hayat *et al*. (2009a)

(n). Cylindrical trichomes. They are cylindrical in shape. These non-glandular trichomes are the unique feature of *S. kurramense* (2C).

(o). Floriform peltate trichomes. These glandular trichomes have flower like appearance and are present in *A. stricta* (Fig. 2D).

(p). Cup-shape peltate trichomes. They are unicellular cup like glandular structures with broad apical opening. They are found in *A. tangutica* (Fig 3).

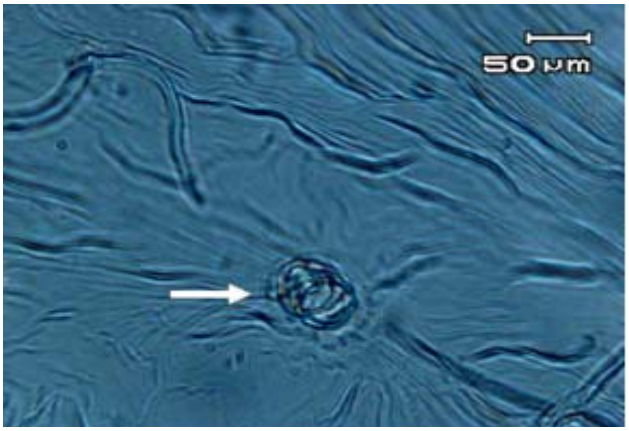
The quantitative dimensions of all the trichomes types studied are given in Table IV and V.

Phylogenetic analysis. Hundred MPTs were generated using Wagner parsimony method (Farris, 1970) and then a strict consensus phylogenetic tree of these MPTs was obtained (Fig. 4 & Fig. 5). In the resulting phylogenetic tree, *Artemisia* and *Seriphidium* is shown to be a monophyletic group.

Table II. Character and character states of foliar trichomes for the phylogenetic analysis of *Artemisia* (the number in brackets represents the codes of character states; plesiomorphic, 0; apomorphic, 1)

Charecter	Character states
a. Capitate	Present (0), Absent (1)
b. Pluricellular	Absent (0), Present (1)
c. Multicellular peltate	Absent (0), Present (1)
d. Thin neck	Absent (0), Present (1)
e. T-shape	Present (0), Absent (1)
f. Macroform	Absent (0), Present (1)
g. Unicellular tector	Absent (0), Present (1)
h. Clavate	Absent (0), Present (1)
i. Vase-shape	Absent (0), Present (1)
j. Aduncate curly	Absent (0), Present (1)
k. Dolabrate	Absent (0), Present (1)
l. Unicellular peltate	Absent (0), Present (1)
m. Rope rolled	Absent (0), Present (1)
n. Cylindrical	Absent (0), Present (1)
o Floriform peltate	Absent (0), Present (1)
p. Cup-shape peltate	Absent (0), Present (1)

Fig. 3. Types of glandular foliar trichomes in *Artemisia* by means of LM: *A. tangutica* (Scale bar = 50µm)



DISCUSSION

Features of trichomes are generally considered as valuable for ascertaining the taxonomic relations within the genus *Artemisia* (Hall & Clements, 1923). Different forms are reported to occur in different taxa of *Artemisia* (Ferreira & Janick, 1995). Different authors reported only two types of trichomes in the genus *Artemisia*: a) capitate glandular trichomes (Smith & Kreitner, 1983 in *A. ludoviciana* Nutt.; Kelsey, 1984 in *A. nova* Nelson; Slone & Kelsey, 1985 in *A. tridentata* Nutt.; Ascensao & Pais, 1987 in *A. compestris* L.; Lodari *et al*., 1989 in *A. princeps* Pamp.; Ferreira & Janick, 1995 in *A. annua*) and b) T-shape nonglandular trichomes (Ascensao & Pais, 1987 in *A. compestris* ; Lodari *et al*., 1989 in *A. princeps*, *A. absinthium*, *A. capillaris* & *A. japonica*; Ferreira & Janick, 1995 in *A. annua*). In this and our previous paper (Hayat *et al*., 2009a), we reported not only 16 new types of glandular and non-glandular foliar trichomes in *Artemisia* but also un-veiled the under lying variations in capitate and T-shape trichomes of the genus.

In addition to capitate and T-shape trichomes and those described in Hayat *et al*. (2009a), here we also observed eight new types of trichomes of taxonomic importance. Of these, unicellular peltate trichomes of *A. vulgaris* (Fig. 1D), *A. roxburghiana* and *A. japonica*, vase-shape trichomes (Fig. 1A & 2A) of *A. persica*; aduncate curly trichomes (Fig. 1B) of *A. roxburghiana*; dolabrate trichomes (1C) of *A. vulgaris*; rope rolled trichomes (Fig.

Table III. Data matrix used in phylogenetic analysis of *Artemisia* (Character & character states are described in Table II)

Taxon	Abbreviation	Trichomes*															
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
Outgroup	Out	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. amygdalina	Amy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. biennis	Bie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. dubia	Dub	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
A. moorcroftiana	Moo	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
A. roxburghiana	Rox	1	1	0	0	0	0	0	1	0	1	0	1	0	0	0	0
A. rutifolia	Rut	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. santolinifolia	San	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. tournefortiana	Tou	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
A. vestita	Ves	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. vulgaris	Vul	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
A. absinthium	Abs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. macrocephala	Mac	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. persica	Per	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0
A. siversiana	Siv	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. tangutica	Tan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
A. maritima	Mar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. leucotrichum	Leu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. brevifolium	Bre	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. kurramense	Kur	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
S. stenocephalum	Ste	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
S. turanicum	Tur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. japonica	Jap	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
A. scoparia	Sco	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
A. stricta	Str	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0

*a, capitate; b, Pluricellular; c, multicellular peltate; d, thin neck; e, T-shape; f, macroform; g, unicellular tector; h, clavate; i, vase-shape; j, aduncate curly; k, dolabrate; l, unicellular peltate; m, rope rolled; n, cylindrical; o, floriform peltate; p, cup-shape paltate

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

Taxa	Vase-shape	Unicellular peltate	Floriform peltate	Cup-shape peltate
	Height x width (µm)	Diameter (µm)	Diameter (µm)	Diameter (µm)
A. roxburghiana	Absent	10-14	Absent	Absent
A. vulgaris	Absent	10-15	Absent	Absent
A. persica	97-101x45-50	Absent	Absent	Absent
A. tangutica	Absent	Absent	Absent	20-25
A. japonica	Absent	9-13	Absent	Absent
A. stricta	Absent	Absent	4-5	Absent

Table V. Quantitative characteristics of nonglandular foliar trichomes of *Artemisia*

Taxa	Aduncate	Dolabrate	Rope rolled	Cylindrical
	Height × width (µm)	Height × width (µm)	Height × width (µm)	Height × width (µm)
A. moorcroftiana	Absent	Absent	200-250x45-50	Absent
A. roxburghiana	700-750x15-20	Absent	Absent	Absent
A. vulgaris	Absent	25-30x10-15	Absent	Absent
S. kurramense	Absent	Absent	Absent	35-40x8-10

2B) of *A. moorcroftiana*; cylindrical trichomes (Fig. 2C) of *S. kurramense*; floriform trichomes (Fig. 2D) of *A. stricta* and cup-shape peltate trichomes (Fig. 3) of *A. tangutica*, are important one.

He *et al*. (2000) resolved the taxonomic conflicts in the genus *Actinidia* Lindl. and related taxa by following the same methodology. In this paper, the results obtained from the phylogenetic tree (Fig. 4 & Fig. 5) based on the micromorphological characteristics of foliar trichomes support the view presented based on molecular studies (Kornkven *et al*., 1998; Torrell *et al*., 1999; Watson *et al*., 2002) that *Artemisia* and *Seriphidium* are monophyletic groups.

From cladogram (Fig. 4) and phylogenetic tree (Fig. 5) it is clear that foliar trichome evolution in *Artemisia* starts form outgroup (assumed as ancestor of *Artemisia*). The first evolved group (1) consist of those taxa, which have both T-shape and capitate trichomes. The second evolve group (2) has only capitate trichome and third group (3) is without trichomes. A possible tendency for foliar trichome evolution is marked in Fig. 4 and Fig. 5. Also we support the idea about general degenerate evolutionary tedency of Asteraceae (Gailing & Bachmann, 2003). we do not believe on old classification of the genus, because it was based only on floral characters and has many objections; for example section *Artemisia* only differ from section *Absinthium* by a

Fig. 4. The strict consensus cladogram of *Artemisia* based on the micromorphological characters of foliar trichomes, Letters represent the abbreviation of taxa (see Table III)

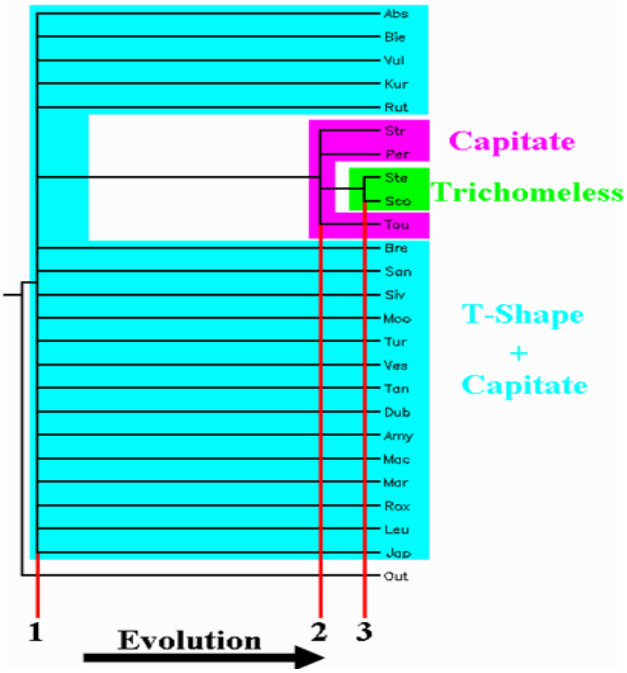
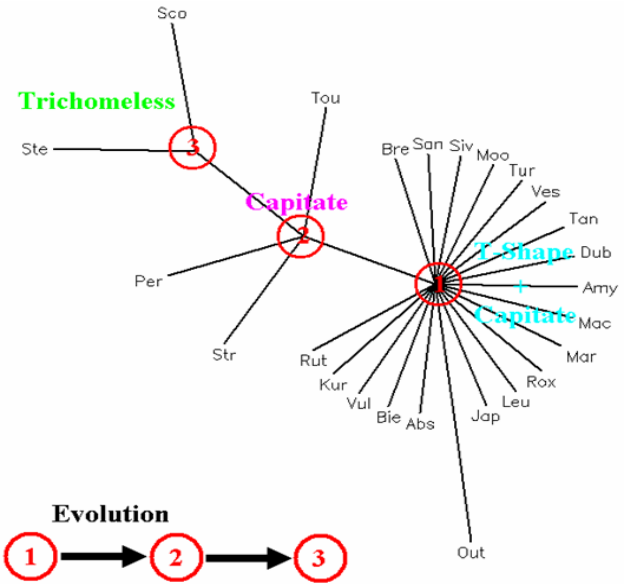


Fig. 5. The strict consensus phylogenetic tree of *Artemisia* based on the micromorphological characters of foliar trichomes, Letters represent the abbreviation of taxa (see Table III)



single character i.e., receptacle naked (*Artemisia*) or receptacle cover with long hairs (*Absinthium*) (Kaul & Bakshi, 1984). However, we believe that more taxa of the genus *Artemisia*, which includes sufficient taxa of each classical section, need to be investigated in more detail and especially focusing on the micromorphological characters of foliar trichomes integrated with molecular approaches.

In conclusion, diversity in glandular and non-glandular foliar trichomes is a valuable taxonomic tool. Non-etheless, there is a need to develop better terminology and detail comparative study of these micromorphological features to resolve taxonomic conflicts in the genus. Their careful anatomical and ontological studies along with the integration of molecular data one can portray the complete picture about the infrageneric classification of *Artemisia*.

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