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Cladistics of Western Himalayan *Artemisia* L. (Anthemideae, Asteraceae) reconstructed on morphological traits

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Abstract

The large size and confounding morphological characters of genus *Artemisia* has created problems of generic delimitations and infrageneric classification for taxonomists. This work suggests the use of additional morphological traits in identification and classification of *Artemisia*. It is observed that same species show polymorphic characters under certain environmental conditions that make them more difficult to classify. To resolve this problem, 52 morphological characters of 42 taxa were selected for phylogenetic analysis of the genus. These include study of life cycle, lifeform, stem, leaf, capitulum, receptacle and achene traits. The results showed that the major variation exist in stem, leaf and capitular characters. These characters were found helpful in discrimination of taxa at specific and subgeneric levels. The phylogeny results revalidated *Artemisia* as a monophyletic lineage. Moreover, *Seriphidium* is recognised as a subgenus within the confines of *Artemisia*. Therefore, regardless of the utilization of modern molecular data, perpetual utilization of morphological information in the field is still required.

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Introduction

Artemisia L. is an economically important, large, cosmopolitan genus (which is present on every continent except Antarctica), belonging to Asteraceae (subfamily Asteroideae, tribe Anthemideae, subtribe Artemisiinae), comprising of approximately 500 species (Bremer, 1994; Valles and McArthur, 2001; Watson *et al.*, 2002; Hayat *et al.*, 2009). The majority of members of *Artemisia* usually have strong aromatic aroma, are subshrubs, shrubs, rarely perennial herbs and occasionally annual or biennial herbs but not trees (Valles and McArthur, 2001). The plant body is often covered with dense hairs. The Leaves are pinnately divided with great variable dimensions. The flowers form disc like structures normally creating paniculate-racemose arrangement. Corolla color varies, ranges from yellow or green to seldom brown. Herbaceous involucral bracts are also present beneath the inflorescences. Receptacle is either naked or hid by hairs, convex or else flat. Ray florets lack stamens so are pistillate. Disk florets are bisexual. Achenes are mostly brown, egg-shaped to oblong (Ghafoor, 2002).

Phylogenetic treatments for *Artemisia* varied over the period, from keeping a completely enormous genus of more than 500 taxa (Cronquist, 1955, 1988; Kornkven *et al.*, 1998, 1999; Torrell *et al.*, 1999; Watson *et al.*, 2002; Oberprieler *et al.*, 2009) to dividing it further into five to eight subgenera (Poljakov, 1961; Bremer and Humphries, 1993; Ling, 1994). Tournefort (1700) distributed *Artemisia* into three genera *(Artemisia, Absinthium and Abrotanum*). Conversely, in 1735, Linnaeus revivified the conception of an all-encompassing genus, henceforth, mentioned as *Artemisia* L. Later, these genera were referred to as sections of *Artemisia*. Rouy in 1903 and Rydberg (1916) raised the sections to the subgenus level. Subgeneric classification faced the same trouble (Torrell *et al.*, 1999). Early classical workers classified the genus into subgenera or sections based on the capitular morphology, florets fertility and receptacle traits (Tournefort, 1700; Linnaeus, 1735; Cassini, 1817; Besser, 1829; DeCandolle, 1837; Hall and Clements, 1923; Ling, 1982, 1991a-b, 1995a-b; Bremer and Humphries, 1993; Bremer, 1994). Five major are usually establishednow(subg. *Artemisia*, subg. *Absinthium* (Miller) Less., subg. *Dracunculus* (Besser) Rydb., subg. *Seriphidium* Besser ex Less. and subg. *Tridentatae* (Rydb.), within the boundaries of *Artemisia* at subgeneric or sectional rank (Torrell *et al.*, 1999; Tkach *et al.*, 2008a,b; Sanz *et al.*, 2008). Comprehensive molecular studies have been conducted on *Artemisia*, some focused on specific subgenera and some concerning the whole genus (and related genera) (Torrell *et al.*,1999; Watson *et al.*, 2002; Vallès *et al.*, 2003; Watson, 2005; Tkach *et al.*, 2008a, b; Sanz *et al.*, 2008, 2011; Shultz, 2009; Garcia *et al.*, 2011; Vallès *et al.*, 2011 and references therein; Hayat, 2011; Riggins and Seigler, 2012; Malik *et al.*, 2017) maintained *Artemisia* as a genus and all others as subgenera within its boundaries. On the other hand, Dobignard and Chatelain, 2011 and Haghighi *et al.*, 2014) continue considering *Seriphidium* an independent genus.

Complete all-inclusive infrageneric classification of genus *Artemisia* has also not yet been established, calling for more researches (Vallès *et al.*, 2011 and references within). Present study highlights the significance of morphological characters in identification and classification of *Artemisia*, collected from Pakistan. Furthermore, it identifies and suggests additional morphological characters in reforming Flora identification keys for the genus for better identification.

Material and methods

Plant specimens used for the study were collected during the excursions to a number of areas of Pakistan (Fig.1). The remaining taxa were also requested from herbaria. Detail about the studied species is listed in Table 1.

The various morphological parts of the specimens listed in Table 2 were measured with hard ruler under a dissecting microscope. Observations were noted under 5X, 10X and 20X magnifiers. Aerial parts of the plant such as leaves, flowers and cypselas were soaked in warm water before dissection. Observations and measurements were made 15-20 times in order to ensure the readings. The resultant data is shown in the results section. The morphological characters were further confirmed and authenticated with the help of previous studies conducted in the area by Ghafoor (2002), Kaul and Bakshi (1984), Mumtaz *et al.*, (2001) and Abid and Qaiser (2008) for phylogenetic analysis.

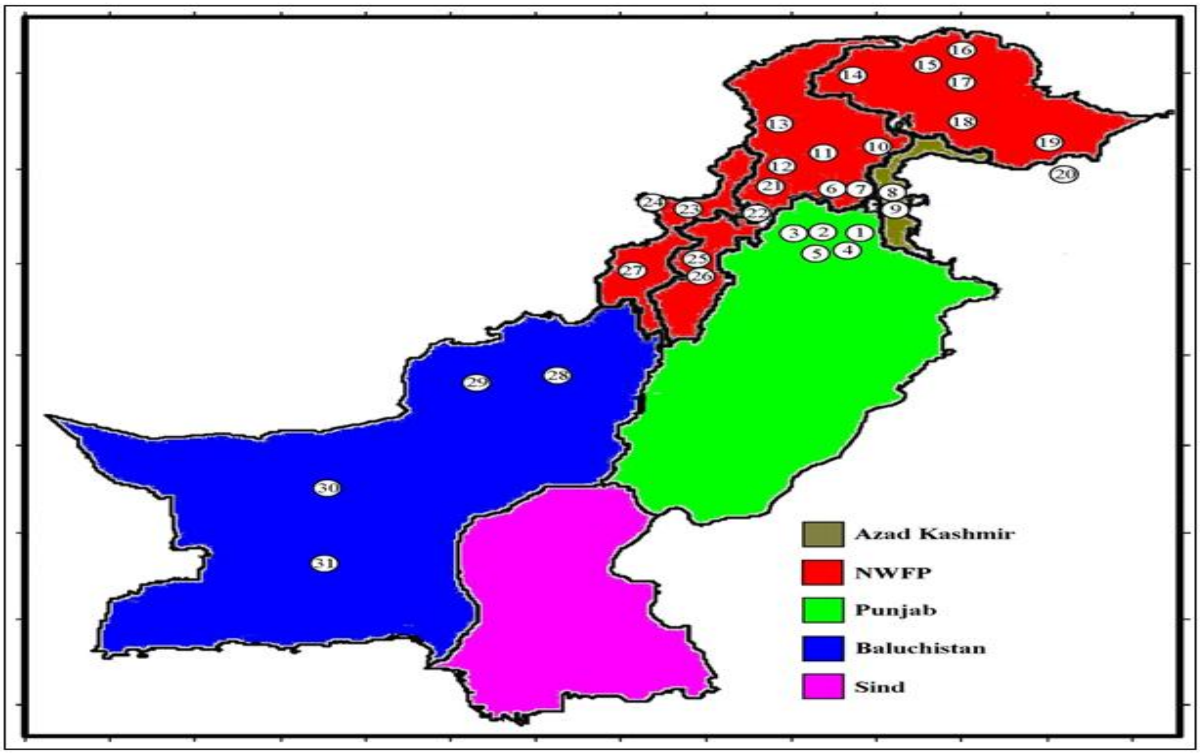

Fig. 1. Regions investigated (white circles) for *Artemisia* all over Pakistan. 1: Islamabad (Quaid-i-Azam University campus); 2: Attock (Fatahjang); 3: Chakwal (Talagang); 4: Rawalpindi (Murree foot hills); 5: Jhelum (Rohtas fort); 6: Abbotabad, (Galiaat); 7: Manshera (Shogran); 8: Azad Jammu and Kashmir (Pearl valley, Mutyal Mara); 9: Bagh (Suddhen Gali); 10: Hazara (Khaghan, Naran); 11: Swat (Kalam); 12: Malakand; 13: Dir (Lowarai pass); 14: Chitral (Ayun); 15: Gilgit (Naltar valley); 16: Hunza valley; 17: Chilas (Nagar); 18: Astor (Astor village); 19: Skardu; 20: Deosai Plains; 21: Peshawar (Sardaryab); 22: Kohat (Khadi Zai); 23: Karak (Mitha khel); 24: Kurram agency (Parachinar); 25, 26: E. Waziristan; 27: W. Waziristan (Burki); 28: Ziarat (Sandman Tangi); 29: Quetta (Hanna Lake); 30: Kalat (Bolan pass); 31: Khuzdar (Khuzdar town).

Based on these morphological observations, for further phylogenetic analysis of *Artemisia*, 52 morphological characters as character states were selected (Table 2). To reveal the intra-specific diversity of the characters, the continuous trait states were taken in to account. The standards established by Cronquist (1955) were used to determine each character state (plesiomorphic (ancestral) state or advanced apomorphic). In this study, *Anthemis arvensis* L. (OUT) was used as outgroup for comparison. The primary data matrix (Table 3) was created following the outgroup comparison method (Watrous and Wheeler, 1981). The using FACTOR program of PHYLIP computer software version 3.67 (Felsenstein, 2007) was used to convert data matrix into binary data matrix for further phylogenetic analysis. The MIX program (Wagner parsimony method) (Farris, 1970) was utilized to construct most parsimonious trees.

The consensus tree was then generated out of the most parsimonious trees using the CONSENSE program (Sokal and Rohlf, 1981) of PHYLIP. The PHYLIP software packages: DRAWGRAM and DRAWTREE programs were used for further assessment of strict consensus phylogenetic tree.

Results

Genus *Artemisia* is a morphologically diverse genus. Its Pakistani representatives also show great diversity. Most of the species are shrubby in nature and few are also herbaceous. Strong aromatic species include *A. herba-alba*, *A. absinthium* and *A. brevifolia*. All other species are either non aromatic or moderately scented. The percentage distribution of life cycles is as follows: perennials 86%, biannuals 7% and annuals 7%. While, 24 of 43 species that is 56% in Pakistani *Artemisia* were shrubby and 19 (44%) were found herbaceous. Fig. 2 illustrates the morphological features in selected *Artemisia* species belonging to Pakistan. Variation in plant height was also found among different Pakistani *Artemisia* species.

Table 1. List of taxa studied and their herbarium codes. (ISL: Herbarium, Quaid-i-Azam University Islamabad; PUP: Herbarium, University of Peshawar, Peshawar).

	Taxon	Herbarium Voucher
1.	*Artemisia amygdalina* Decne.	ISL, 32315
2.	*A. annua* L.	ISL, 16253
3.	*A. biennis* Willd.	PUP, PH005 (ART005)
4.	*A. dubia* Wall. ex Besser	PUP, PH002 (ART002)
5.	*A. elegantissima* Pamp.	ISL, 25652
6.	*A. gmelinii* Weber ex Stechm.	ISL, 23441
7.	*A. incisa* Pamp.	ISL, 56323
8.	*A. laciniata* Willd.	ISL, 56325
9.	*A. moorcroftiana* Wall.	ISL, 56321
10.	*A. roxburghiana* Wall. ex Besser	PUP, PH001 (ART001)
11.	*A. rutifolia* Stephan ex Spreng.	PUP, 244 (1105)
12.	*A. santolinifolia* Turcz. ex Krasch.	PUP, 239 (1108)
13.	*A. tournefortiana* Rchb.	ISL, 21921
14.	*A. vestita* Wall.	ISL, 20093
15.	*A. vulgaris* L.	PUP, PH006 (ART006)
16.	*A. absinthium* L.	PUP, PH004 (ART004)
17.	*A. macrocephala* Jacquem. ex Besser	PUP, 121(556)
18.	*A. minor* Jacquem. ex Besser	ISL, 32145
19.	*A. persica* Boiss.	PUP, 27
20.	*A. sieversiana* Ehrh.	PUP, 222 (1057)
21.	*A. tangutica* Pamp.	ISL, 32144
22.	*A. herba-alba* Asso	ISL, 28615
23.	*A. maritima* L.	ISL, 92830
24.	*A. brevifolia* Wall.	PUP, PH007 (ART007)
25.	*A. chitralensis* Podlech	PUP, 221 (1060)
26.	*A. freitagii* Podlech	ISL, 28620
27.	*A. glanduligera* Krasch. ex Poljakov	PUP, 22400
28.	*A. kurramensis* Qazilb.	PUP, 22419
29.	*A. leucotricha* Krasch. ex Ladygina	ISL, 92453
30.	*A. oliveriana* J.Gay ex Besser	ISL, 20126
31.	*A. quettensis*Podlech	ISL, 27631
32.	*A. sieberi* Besser	ISL, 28625
33.	*A. stenocephala* Krasch. ex Poljakov	PUP, PH010 (ART010)
34.	*A. tecti-mundi* Podlech	PUP, 225 (1065)
35.	*A. turanica* Krasch.	PUP, PH009 (ART009)
36.	*A. vachanica* Krasch. ex Poljakov	PUP, PH110 (ART022)
37.	*A. capillaris* Thunb.	ISL, 26234
38.	*A. desertorum* Spreng.	ISL, 25115
39.	*A. dracunculus* L.	ISL, 19222
40.	*A. japonica* Thunb.	PUP, PH008 (ART008)
41.	*A. salsoloides* Willd.	PUP, 212 (2452)
42.	*A. scoparia* Waldst. & Kitam.	ISL, 32313
43.	*A. stricta* Edgew.	ISL, 25650

The maximum recorded plant height is 250 cm in *A. biennis* while minimum plant height i.e. 10 cm was observed in *A. minor*. Leaf petiole length variations were also observed. For instance, *A. absinthium* was examined with maximum petiole length i.e. 10 cm while few species leaves were observed as sessile.

The ray and disc florets have positive correlation with capitular diameter. All the *Seriphidium* species lack the ray florets. Cypsela diversity was also observed. Cypsela colour varies between light brown and dark brown shades. Most of the cypsela has terminal scar only few were recorded with lateral scar. Few species have exclusive morphology and can distinguish easily form other *Artemisia* relatives. For instance, *A. scoparia* have unique plant morphology. Similarly, *A. amygdalina* has simple leaves while rest of *Artemisia* species has dissected leaves.

Table 2. Morphological characters and character states of *Artemisia* used in the current study. The assigned values of character states are represented by numbers in brackets. The value of plesiomorphic character state is always represented as 0.

Sr.	Character	Character states
1	Life cycle	Perennial (0), Biannual (1), Annual (2)
2	Life form	Herb (0), Shrubby (1)
3	Rootstock	Horizontal (0), Vertical (1)
4	Stem hairs	Hairy (0), Glabrous (1)
5	Stem branching	Absent (0), Present (1)
6	Stem glands	Present (0), Absent (1)
7	Stem groves	Slender (0), Sulcate (1), Striate (2), Costate (3)
8	Stem height	10~40cm (0), 20~80cm (1), 25~100cm (2), 30~200cm (3)
9	Stem colour	Greenish (0), Whitish (1), Violet (2), Yellowish (3), Brownish (4), Reddish (5), Purplish (6)
10	Woody stem base	Absent (0), Present (1)
11	Basal leaf petiole length	3~10cm (0), 1~2cm (1), <1cm (2), Sessile (3)
12	Basal leaf lamina shape	Ovate (0), Lanceolate (1)
13	Basal leaf upper surface	Hairy (0), Glabrous (1)
14	Basal leaf lower surface	Tomentose (0), Sparsely hairs (1), Glabrous (2)
15	Basal leaf upper side colour	Green (0), Dark green (1)
16	Basal leaf lower side colour	Light green (0), Grayish white (1)
17	Basal leaf dissections	Undivided (0), Pinnatifid (1), Pinnatisect (2)
18	Basal leaf lobes shape	Elliptic or ovate (0) Oblong or lanceolate (1)
19	Middle leaf petiole	Present (0), Absent (1)
20	Middle leaf shape	Ovate (0), Lanceolate (1)
21	Upper leaf shape	Lanceolate (0), Linear or filiform (1)
22	Upper leaf margin	Pinnatifid (0), Entire (1)
23	Capitulum shape	Hemispherical (0), Ovoid (1), Glubose (2), Oblong (3)
24	Capitulum length	>5mm (0), 3~4mm (1), 1~2mm (2)
25	Capitulum width	>4mm (0), 3~4mm (1), 2~3mm (2), 1~2mm (3)
26	Capitulum pedunculate	Present (0), Absent (1)
27	No. of involucral seriate	≥5 (0), 4 (1), 4~3 (2), <3 (3)
28	Outer phyllaries texture	Canescent (0), Slightly hairy (1), Glabrous (2)
29	Outer phyllaries margins	Ciliate (0), Membranous (1), Scarious (2)
30	Outer phyllaries shape	Linear or oblong (0), Ovate (1)
31	Inner phyllaries texture	Canescent (0), Glabrous (1)
32	Inner phyllaries margins	Membranous (0), Scarious (1)
33	Inner phyllaries shape	Ovate (0), Oblong or elliptic (1)
34	Receptacle shape	Flattened (0), Convex (1), Hemispherical (2), Conical (3)
35	Receptacle texture	Hairy (0), Glabrous (1)
36	Receptacle diameter	≥2mm (0) 1~2mm (1), <1mm (2)
37	No. of ray florets	>15 (0), 11~15 (1), 6~10 (2), ≤5 (3) Absent (4)
38	Ray florets corolla length	≥2mm (0)1~<2mm (1), <1mm (2) Absent (3)

39	Ray florets corolla shape	Tubular (0), Filiform (1), Urceolate (2), Compressed (3), Absent (4)
40	Ray florets corolla colour	Yellow (0), Brown (1), Greenish (2), Purplish (3), Absent (4)
41	Ray florets	Female (0), Absent (1)
42	No. of disc florets	>40 (0), 31~40 (1), 21~30 (2), 11~20 (3),<10 (4)
43	Disc florets corolla length	>2mm (0), >1~<2mm (1), ≤1mm (2)
44	Disc florets corolla shape	Tubular (0), Clavate (1), Conical (2), Companulate (3)
45	Disc florets corolla colour	Pale (0), Red tinged (1)
46	Disc florets fertility	Bisexual (0), Staminate (1)
47	Cypsela shape	Oblanceolate (0), Oblong (1), Terete (2)
48	Cypsela attachment	Terminal (0), Lateral (1), Oblique (2)
49	Cypsela texture	Striate (0), Glabrous (1)
50	Cypsela Colour	Light brown (0), Dark brown (1)
51	Cypsela length	<1mm (0), ≥1mm (1)
52	Cypsela width	<0.5mm (0), ≥0.5mm (1)

The plant height in different *Artemisia* species ranged between 20 and 80 cm. The minimum observed plant height is 10 cm in *A. minor* whereas *A. roxburghiana* and only few species (*A. biennis*, *A. dubia*, *A. tournifortiana* and *A. vulgaris*) approach to the height of 200 cm and above. Stem of *Artemisia* has notable ridges. Leaves in *Artemisia* are highly variable. The same plant has three forms of leaves, basal, middle and upper. Basal and middle leaves are pinnatifid to pinnatisect. The upper leaves are mostly long and linear, trifid in case of *A. vulgaris*. The analogous leaf dissection in different *Artemisia* species makes leaves a poor taxonomic marker. Leaf petiole length was varies between sessile to 6 cm. The maximum leaf petiole length was 10 cm, observed in *A. absinthium*.

Table 3. The final data matrix used for phylogenetic analysis of *Artemisia* (based on character states designated in Table 2) (The taxa names are same as in Table 1 and are abbreviated here only for convenience).

Taxa	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
A. amyg	0 0 1 1 1 1 3 3 0 0 3 1 0 0 1 1 0 1 1 1 0 1 2 1 1 0 2 1 0 1 1 0 1 1 1 1 2 1 2 1 0 3 1 2 0 0 2 0 1 1 1 1
A. annu	2 0 1 0 0 1 2 2 0 0 0 0 0 0 0 0 2 1 1 1 1 1 0 2 3 0 3 2 2 0 1 1 1 3 1 2 2 1 1 2 0 3 1 0 0 0 0 0 0 1 1 1
A. bien	1 0 1 1 1 1 1 3 3 0 0 0 1 2 0 0 2 1 1 0 0 0 0 1 2 1 3 2 2 0 1 1 1 3 1 1 0 1 0 1 0 1 1 0 0 0 0 2 0 1 0 0
A. dubi	0 1 1 1 1 1 1 3 0 1 2 0 0 1 1 0 1 1 1 0 1 1 3 1 3 1 1 1 2 1 0 1 0 0 1 1 2 1 0 3 0 3 1 0 1 0 1 0 0 0 0 0
A. eleg	0 1 1 0 1 1 1 2 0 1 0 1 0 0 1 1 1 1 0 1 0 1 3 1 2 1 2 2 2 1 1 1 1 3 1 1 2 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0
A. gme	0 0 1 1 1 1 1 2 4 1 2 0 0 0 0 1 1 1 1 1 1 1 2 1 2 0 0 0 1 1 1 1 1 3 1 1 1 2 0 0 0 3 1 2 0 0 1 0 0 1 0 0
A. inci	0 1 1 0 0 1 3 2 0 1 2 0 0 0 0 1 1 1 1 1 0 1 1 1 2 0 2 2 1 1 1 0 1 2 1 1 2 1 0 0 0 3 1 0 0 0 1 0 0 0 0 0
A. laci	0 1 1 0 1 1 3 2 5 1 0 0 1 0 0 1 1 1 0 0 1 1 2 0 1 0 2 2 2 0 1 1 1 2 1 1 1 1 0 0 0 0 1 0 0 0 0 2 0 0 1 1
A. moor	0 1 1 0 1 1 1 1 3 1 0 0 0 0 0 1 2 1 0 0 0 0 0 0 0 1 3 0 2 1 0 1 0 1 1 0 3 1 0 3 0 3 1 1 0 0 1 0 0 0 0 1
A. roxb	0 0 1 0 1 1 1 1 1 1 2 0 0 0 1 1 1 1 1 1 0 1 2 1 2 1 1 1 1 1 1 1 0 2 1 1 2 1 1 1 0 2 1 0 0 0 1 0 0 1 0 0
A. ruti	0 1 1 0 1 1 2 1 1 1 2 0 0 0 0 1 1 1 1 0 1 1 2 1 1 0 2 1 2 0 1 1 1 0 1 2 3 2 0 0 0 2 2 0 1 0 0 0 0 1 0 1
A. sant	0 1 1 0 1 1 2 1 4 1 0 0 0 0 0 1 1 1 0 1 1 1 2 1 1 0 3 1 1 0 0 1 0 1 1 1 1 1 0 0 0 0 1 0 0 0 1 0 0 1 0 0
A. tourn	2 0 1 0 0 1 1 3 6 0 0 0 1 0 0 0 1 1 0 1 1 0 1 2 3 0 2 2 2 0 1 1 1 2 1 1 1 1 0 0 0 3 1 3 0 0 1 0 0 1 1 1
A. vest	0 1 1 1 1 0 2 2 2 1 1 0 0 0 1 1 1 0 0 0 0 1 2 1 1 0 2 0 1 0 0 1 1 1 1 1 2 1 0 0 0 3 1 0 0 0 1 1 0 1 1 1
A. vulg	0 1 0 0 1 1 2 3 5 1 1 0 1 0 1 1 2 0 1 1 0 0 3 1 3 1 1 0 2 1 0 1 1 2 1 1 2 1 0 0 0 3 1 3 1 0 1 0 0 1 0 0
A. absi	0 0 1 0 1 0 3 3 1 1 0 0 0 0 0 0 1 1 1 1 0 0 2 0 0 0 2 0 2 0 0 1 1 0 0 0 0 1 3 0 0 0 1 3 0 0 1 2 0 1 1 1
A. macr	2 0 1 0 1 1 3 0 1 0 1 0 0 0 0 1 1 1 1 1 1 1 0 0 0 0 1 0 2 0 1 1 1 2 0 0 0 1 2 2 0 0 1 0 0 0 0 2 0 0 0 1
A. mino	0 0 0 0 1 1 0 0 2 1 3 1 0 0 0 1 1 1 1 1 1 1 0 0 0 1 2 1 2 1 0 1 1 0 0 0 1 1 0 2 0 0 1 0 1 0 0 1 1 0 1 1

A. persi	0 1 1 0 1 1 3 1 2 1 1 0 0 0 0 1 1 1 1 1 1 1 2 1 2 0 2 0 2 0 0 1 1 1 0 0 2 2 0 0 0 1 1 0 0 0 1 2 1 0 0 1
A. siev	1 0 1 0 0 1 1 2 2 0 0 0 0 0 0 1 1 1 0 1 1 1 0 0 1 0 0 0 2 0 0 1 0 1 0 0 0 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0
A. tang	0 0 1 0 1 0 0 2 1 1 1 0 0 0 0 1 1 0 0 0 0 0 3 1 1 0 3 0 2 0 0 1 1 0 0 2 2 1 3 0 0 3 1 1 0 0 1 2 0 0 1 1
A. herb	0 1 1 0 1 0 1 0 1 1 0 0 0 0 0 0 2 1 0 1 1 1 3 1 3 1 0 0 1 0 1 1 1 2 1 1 4 3 4 4 1 4 1 0 0 0 1 1 1 0 1 1
A. mari	0 1 1 0 1 1 1 0 3 1 0 0 0 0 0 1 2 1 0 1 1 1 3 1 2 1 0 1 1 0 0 1 1 2 1 1 4 3 4 4 1 4 1 1 0 0 1 1 1 0 1 1
A. brev	0 0 1 0 1 1 3 0 2 1 1 1 0 0 0 1 1 1 1 1 1 1 3 1 3 1 0 0 0 1 0 1 0 3 1 1 4 3 4 4 1 4 1 0 0 0 1 1 0 1 0 0
A. chit	0 1 1 0 1 1 0 0 1 1 0 0 0 0 0 1 1 1 0 1 1 1 1 1 2 1 0 1 2 0 1 1 1 3 1 1 4 3 4 4 1 4 1 0 0 0 0 1 1 0 0 0
A. frei	0 1 1 0 1 1 0 0 1 1 1 0 0 0 0 1 1 1 1 1 1 1 1 1 2 1 0 0 0 0 0 0 1 2 1 1 4 3 4 4 1 4 1 0 0 0 1 1 0 1 1 1
A. glan	0 1 1 0 1 1 1 0 3 1 1 0 0 0 0 0 1 1 1 1 1 1 1 1 3 1 0 1 1 1 0 0 1 2 1 1 4 3 4 4 1 4 1 2 0 0 2 1 0 1 1 1
A. kurr	0 1 1 1 1 1 2 1 5 1 2 0 0 0 0 1 1 1 1 1 1 1 3 1 2 1 0 1 1 1 1 1 1 2 1 1 4 3 4 4 1 4 1 0 0 0 0 1 1 1 0 0
A. leuc	0 0 0 0 1 1 3 0 4 1 1 0 0 0 0 1 1 1 0 0 0 1 1 1 2 1 2 0 1 1 0 1 1 2 1 1 4 3 4 4 1 3 1 0 0 0 1 1 1 0 0 0
A. oliv	0 1 1 0 1 1 1 1 3 1 0 0 0 0 0 1 1 1 1 0 1 1 3 1 3 1 0 0 0 1 1 1 1 3 1 1 4 3 4 4 1 4 1 0 0 0 1 1 1 1 0 0
A. quet	0 1 1 0 1 1 1 0 1 1 2 0 0 0 0 1 1 1 0 0 1 1 3 1 2 1 0 1 1 0 0 0 1 3 1 1 4 3 4 4 1 4 1 0 0 0 0 1 0 1 1 1
A. sieb	0 1 1 0 1 1 2 0 4 1 2 1 1 0 0 1 1 1 1 1 0 0 3 1 3 1 0 0 1 1 0 1 1 3 1 1 4 3 4 4 1 4 1 0 0 0 1 1 0 1 0 1
A. sten	0 1 1 0 1 1 0 1 4 1 0 1 1 2 0 0 1 1 0 1 1 1 3 1 3 1 0 2 1 0 1 0 1 3 1 1 4 3 4 4 1 4 1 0 1 0 0 1 0 1 0 0
A. tect	0 1 1 0 1 1 2 1 3 1 2 0 0 0 0 1 1 1 1 1 1 1 1 1 3 1 0 0 0 1 0 1 1 3 1 1 4 3 4 4 1 4 1 0 0 0 0 1 0 1 0 0
A. tura	0 1 1 0 1 1 0 0 2 1 0 0 0 0 0 1 1 1 0 0 1 1 3 1 3 1 0 1 0 0 1 1 1 3 1 1 4 3 4 4 1 4 1 0 0 0 0 1 0 1 1 1
A. vach	0 0 1 0 0 1 1 0 2 1 0 0 0 0 0 1 1 1 1 0 1 1 1 1 3 1 2 0 0 1 1 1 0 3 1 1 4 3 4 4 1 4 1 0 0 0 0 1 0 1 1 1
A. capi	0 0 1 1 0 1 0 2 5 1 2 0 0 0 1 1 1 0 1 1 1 0 1 1 3 0 2 2 1 1 1 0 1 3 1 1 3 2 0 0 0 4 1 0 1 1 1 0 0 0 1 1
A. dese	0 0 0 1 0 1 3 2 4 1 0 1 0 1 1 0 1 1 0 1 1 1 2 1 3 0 2 2 1 1 1 1 0 1 1 0 3 2 0 1 0 3 1 0 0 1 0 0 0 1 0 0
A. drac	0 0 1 0 0 1 1 1 1 0 3 1 0 0 1 0 0 1 1 1 0 1 2 1 2 0 2 2 2 0 1 1 1 2 1 1 1 2 0 0 0 3 0 3 0 1 0 1 0 0 0 0
A. japo	0 1 1 1 1 1 1 2 6 1 3 0 0 1 1 0 2 0 1 1 1 0 1 1 3 0 2 2 2 1 1 1 1 2 1 1 2 2 0 0 0 4 1 0 0 1 0 0 0 1 0 0
A. sals	0 1 0 1 1 1 1 1 3 1 3 1 1 2 0 0 1 1 1 1 1 1 1 1 1 0 1 2 1 1 1 1 0 2 1 1 3 1 0 0 0 3 1 0 0 1 0 0 0 1 0 0
A. scop	1 0 1 0 1 1 0 1 6 1 1 0 0 2 0 0 1 1 1 1 1 1 2 2 3 0 2 2 2 1 1 1 0 3 1 2 2 2 0 2 0 4 1 0 0 1 1 0 0 1 1 1
A. stri	2 0 1 0 1 1 0 0 3 0 0 0 0 1 1 0 1 1 1 1 1 1 1 2 3 0 2 1 2 1 1 1 0 2 1 0 0 1 2 0 0 4 1 1 0 1 1 0 0 1 1 0
OUT	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

A. minor and *A. macrocephala* has maximum capitular diameter. Numbers of ray florets were fewer to about 70 in *A. macrocephala*. The corolla length in ray florets is round about 1 mm. Ray florets in *Seriphidium* were absent. Minimum number of disc florets was the characteristic of *Seriphidium* species while section *Absinthium* has maximum number of disc florets.

The corolla length in disc florets is about 1.5 mm. A positive correlation has been observed in capitular diameter and number of florets. Small cypselas with lateral of terminal scar were observed. The cypselas size was 1x1.5 mm across and they are mostly brown in colour.

Strict consensus tree (Fig. 3) was obtained from 12 MPTs based on morphological traits described and explained in Tables 2 and 3. Fig. 3 also represents the comparison of this morphological work with classical classification of the genus *Artemisia*. The whole genus is divided into four clades. Section *Artemisia* was dispersed between the clades of other sections and appeared as a polyphyletic. All other sections are broadly monophyletic. *Seriphidium* is grouped within the genus in *Artemisia* clade, which confirmed its reunion with the genus *Artemisia*.

Discussion

The morphology of the genus *Artemisia* is complex and many species show close resemblance that leads to identification problems (Hayat *et al*., 2009). This is particularly true in case of section *Seriphidium* species. Single species shows different morphological forms under ecological conditions which cause difficulties for its identification as is the case of *A. vulgaris* complex (Stewart, 1983). In this study, it is observed that during different parts of the year, some of the *Artemisia* species acquire different morphological forms, which cause intricacy for the exact identification of the species. The best example of this phenomenon is *A. scoparia*.

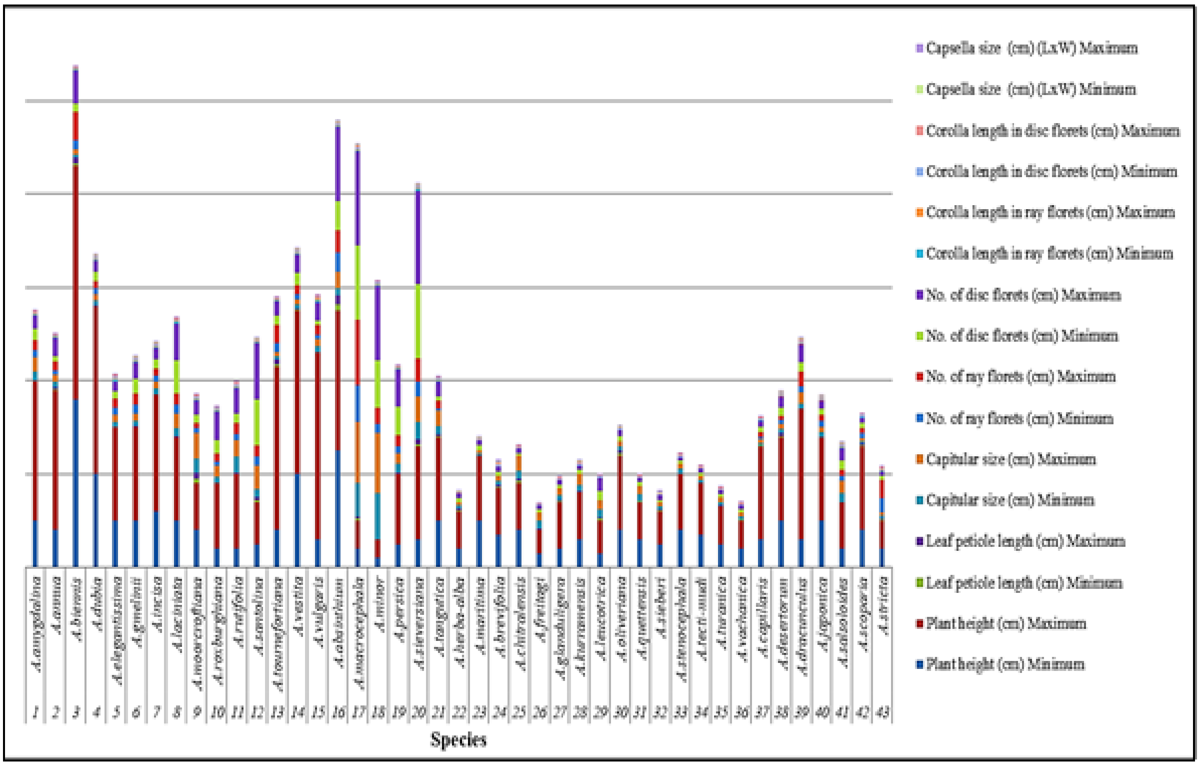

Fig. 2. Diversity in morphological features in selected *Artemisia* species from Pakistan.

This study proves the use of morphological markers in identification of *Artemisia* species supporting Nazar and Mahmood (2010).

Strict consensus phylogenetic tree (Fig. 3) of morphological data is obtained using maximum parsimony phylogenetic analysis method. This shows that subgenus *Artemisia* is polyphyletic and has origin at many points while all other sections are monophyletic of *Artemisia* from this country. This analysis agrees, in a broader sense with the Torrell *et al.*, (1999) sectional division based on molecular studies.

This analysis shows disagreement to some extent with classical sectional classification as some members of section *Artemisia* were classified with other sections. This morphological revision also rejects the separation of *Seriphidium* from *Artemisia* (Bremer and Humphries, 1993; Bremer, 1994; Dobignard and Chatelain, 2011) as an independent genus validating the latest results of Malik *et al.* (2017) and references therein. Here *Seriphidum* appeared to be within *Artemisia* clade and treated as subgenus of genus *Artemisia*. These results also suggests the closeness of subgenus *Artemisia* and *Dracunculus* than *Seriphidium* revalidating the results of Haghighi *et al.* (2014) who also indicated the significance of use of capitulum morphology as marker at subgeneric level.

However, in contrast to Haghighi *et al.* (2014), this study supports the inclusion of *Seriphidium* within *Artemisia*, as mentioned above, following various prominent works (Kadereit and Jeffrey, 2007; Funk *et al.*, 2009; Garcia *et al.*, 2011; Riggins and Seigler, 2012). This uniformity concerning morphological and molecular data promotes the reliability of use of morphological data in solving systematic complexity in the genus, and advocates their mandatory role to revise of taxonomic keys for the genus *Artemisia*.

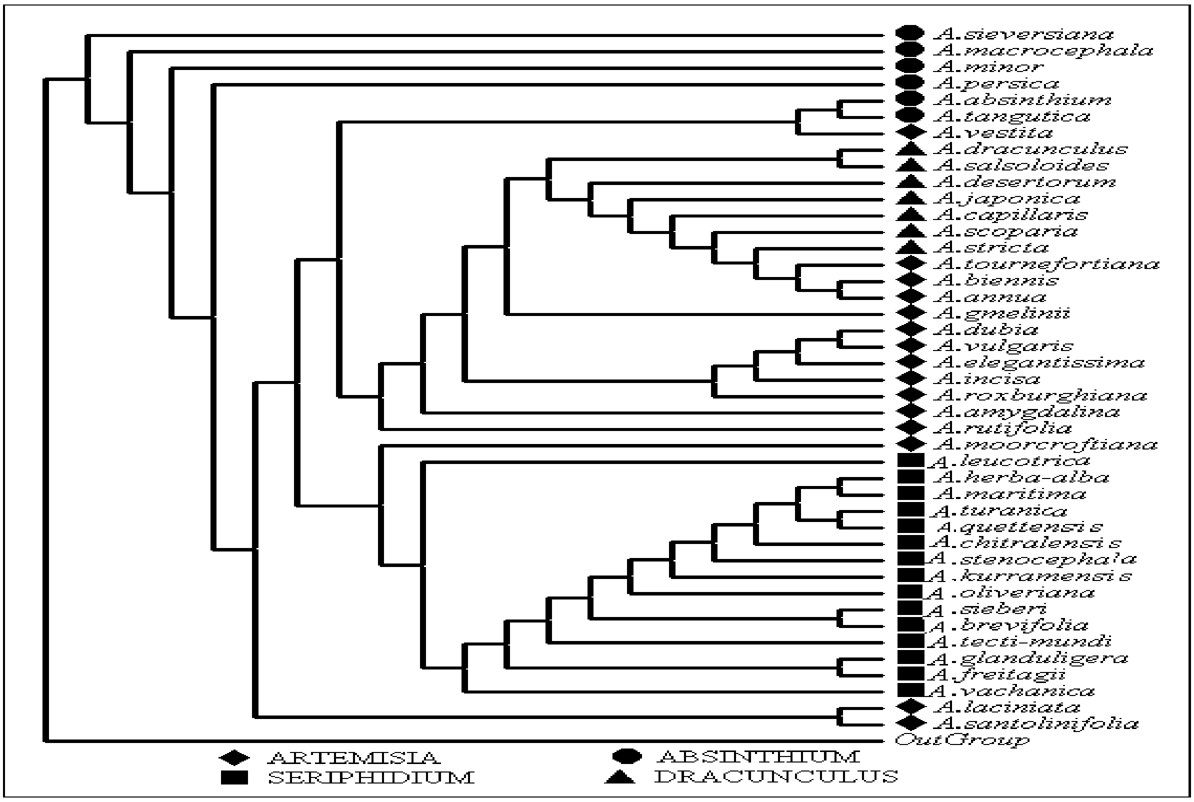

Fig. 3. The final strict consensus tree (of selected *Artemisia* species from Pakistan) obtained by maximum parsimony method. The traditional infrageneric classification of the *Artemisia* is depicted by different geometric shapes.

Present study strongly advocates the use of new characters in morphological data in making taxonomic keys to untie systematic knots still present even after use of molecular data among *Artemisia* species. This study also suggests including *Seriphidium* within *Artemisia* in the Flora of Pakistan, which still recognizes *Seriphidium* as separate genus from *Artemisia*.

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Declaration of Interests

Authors have no conflicts of interest.

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