MORPHOLOGICAL AND PALYNOLOGICAL STUDIES IN SOME ACHILLEA L. SPECIES (ASTERACEAE) OF IRAN

N. Azani, M. Sheidai & F. Attar

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Morphological and palynological studies were performed in 34 populations of 12 *Achillea* species belonging to three sections of *Santolinoidea*, *Millefolium* and *Filipendulinae* growing in Iran. Morphological studies were performed on 34 populations of 12 species. In total 33 morphological characters including 13 quantitative and 20 qualitative were scored. ANOVA test showed significant difference for most of quantitative characters among the species and populations studied. UPGMA clustering of the populations and species studied based on merely quantitative morphological characters and also based on both quantitative and qualitative characters separated populations of each species from the others indicating the use of these characters in species delimitation, however no resolved relationship could be obtained and the species from 3 sections stated in Flora Iranica are intermingled. The size of the pollen grains ranged from 22.4-30.0 µm, with similar P/E ratio, trizonocolporate, radially symmetrical, isopolar, spheroidal. The spines are pointed and are dome shaped broad based. The number of spine rows between colpi varies from 3 to 5 among the species. This feature can be determined easily in the polar view by light microscope. The exine thickness varies from 2.4 µm to 4.5 µm among the species and pollen sculpturing is echinate in all. Clustering and ordination of species based on palynological characters also showed mixing of species from 3 sections mentioned in Flora Iranica.

Nasim Azani and Masoud Sheidai (correspondence, msheidai@yahoo.com*), Shahid Beheshti University, GC, Faculty of Biological Sciences, Tehran, Iran. - Farideh Attar, School of Biological Sciences, University of Tehran, Tehran, Iran.*

Key words. Achillea, morphology, palynology, clustering, ordination.

بررسی مورفولوژیکی و گرده شناسی چند گونه .L *Achillea* در ایران

نسیم اذانی، دانشجوی کارشناسی ارشد دانشکده علوم زیستی، دانشگاه شهید بهشتی.

مسعود شیدایی، استاد دانشکده علوم زیستی، دانشگاه شهید بهشتی.

فریده عطار، دانشیار پردیس علوم، دانشگاه تهران.

بررسی خصوصیات مورفولوژیکی و دانه های گرده در جمعیت های ۱۲ گونه *Achillea* از سه بخش *Santolinoidea* ، *Millefolium* و *Filipendulinae* انجام گرفت. تعداد ۳۳ صفت ریختی شامل ۱۳ صفت کمی و ۲۰ صفت کیفی بررسی شدند. آنالیز واریانس وجود اختلاف معنی دار را در بسیاری از صفات کمی در میان جمعیت ها و گونه های مطالعه شده نشان داد. تجزیه خوشه ای بر اساس صفات کمی و نیز توام با صفات کیفی، جدایی جمعیت های یک گونه را از دیگر گونه ها نشان داد که بیانگر اهمیت این صفات در تاکسونومی و شناسایی گونه های جنس *Achillea* می باشد. روابط گونه ای بدست آمده از آنالیزهای فنتیکی، کلادستیک و روش Bayesian با مرزبندی میان سه بخش ارائه شده در فلورا ایرنیکا همخوانی ندارد و گونه های متعلق به این سه بخش در کنار یکدیگر قرار می گیرند. لذا بنظر می رسد تاکسونومی این گروه نیاز به بازنگری دارد.

Introduction

The genus *Achillea* L. (*Asteraceae*) comprising about 110-140 perennial herb species (Saukel et al. 2004, Guo et al. 2004, 2005, Funk 2007) that are centered in SE Europe and SW Asia with extensions through Eurasia to North America. A large number of species are endemic and restricted to certain regions, in contrast to other species from the genus growing over a

wide geographical range. Plants in this genus are perennial, entomophilous and predominantly out-breeding. The genus exhibits great ecological amplitude ranging from deserts to water-logged habitats and from sea coasts to the high mountains as a result of which, several *Achillea* species show high morphological variability (Ehrendorfer & Guo 2006). The genus *Achillea* is considered to be monophyletic (Guo et al. 2004) within *Asteraceae* and on the basis of a cladistic analysis have been placed into a new sub-tribe, *Achilleinae* (Bremer & Humphries 1993) and (Bremer 1994). The basic diploid karyotype (2x, 2*n* = 18) is quite uniform throughout the genus but polyploid taxa have originated in many clades, often 4x, but sometimes 6x and even 8x taxa.

The pollen grains of *Compositae* are mainly helianthoid, spherical or slightly flattened, tricolporate and echinate with variation in size and colpus number (Wodehouse 1926, 1935). It is a eurypalynous family (Erdtman 1952) and possess zonocolporate pollen (Sachdeva & Malik 1986). Several researchers have studied pollen morphology in *Compositae* species of their regions (Wodehouse 1926, 1935, Feuer & Tomb 1977, Clark et al. 1980, Vincent & Norris 1989, Cilliers 1991, Nakajima & Monteiro 1995, Kaya et al.1996, Pinar & Donmez 2000) and also in *Achillea* species (Yang & Ai 2002, Akyalçın et al. 2007).

Accroding to Flora Iranica (Podlech 1986) nineteen *Achillea* species grow in Iran but there is no biosystematic report on them. Therefore the present study consides morphometric and palynological study of 12 *Achillea* species growing in the country for the first time, trying to reveal the species inter-relationship and the most useful morphological characters for the taxonomy of the genus. Our previous studies on the genus were concerned with cytology of these species (Sheidai et al. 2009).

Materials and methods

Plant material

Morphological studies were performed in 34 populations of 12 *Achillea* species from 3 sections namely: Sect. *Santolinoidea*: 1- *A. wilhelmsii* C. Koch., 2- *A. vermicularis* Trin., 3- *A. conferta* DC., 4- *A. talagonica* Boiss., 5- *A. tenuifolia* Lam., 6- *A. eriophora* DC., Sect. *Millefolium* **:** 7- *A. millefolium* L., 8- *A. setacea* Waldst & Kit., 9- *A. nobilis* L., Sect. *Filipendulinae* **:** 10- *A. filipendulina* Lam., 11- *A. oxyodonta* Boiss., and 12- *A. biebersteinii* Afan. The voucher specimens have been deposited in Tehran and Shahid Beheshti University herbariums (TUH and SBUH respectively, Table 1).

In total 33 morphological characters including 13 quantitative and 20 qualitative characters were studied (Table 2). These were selected based on the species description available in Flora Iranica (Podlech 1986), and related published material (Valant -Vetschera & Kastner 2000), as well as our own observations.

Morphometric studies

In order to determine significant difference for quantitative morphological characters among populations and species, analysis of variance (ANOVA) followed by Least Significant Test (LSD) was performed. In order to determine if quantitative characters can be used in the species delimitation from each other clustering was performed.

In order to study the species relationship, different clustering methods including UPGMA (Unweighted Paired Group using Arithmetic Average), Neighbor Joining (NJ), maximum parsimony and Bayesian clustering as well as ordination plot based on Principal Components Analysis (PCA) were performed by using both quantitative and qualitative characters.

For numerical analyses, the mean of quantitative characters were used while qualitative characters were coded as binary or multistate characters (Table 2). Factor analysis was used to identify the most variable morphological characters among species and populations studied. For clustering, morphological data were standardized (Mean = 0, variance = 1) and used to determine taxonomic and Euclidean distances. The fit of dendrograms were checked by estimating cophenetic correlation and bootstrap values (Podani 2000, Sheidai et al. 2001).

Pollen morphology

Pollen of 11 species of genus *Achillea* was studied by light microscopy (LM) and scanning electron microscopy (SEM). The pollen samples were obtained mostly from fresh collected herbarium specimens, but some dry herbarium materials were also used. Fully matured anthers were removed from the specimens and prepared by the standard acetolysis method (Erdtman 1969), after which they were mounted in glycerin jelly and sealed with paraffin wax for light microscopy (LM). Measurements and morphological observations of the pollen grains were performed by using minimum 20 pollen grains. For scanning electron microscopy (SEM), the pollen grains were attached to the aluminum stubs with double sided cellophane tape, air dried at room temperature and coated with gold. The specimens were examined with PHYLIPS XL 30, LEO 440i at 15 kV and 22 kV and photographed. SPSS Ver. 9 (1998) was used for ANOVA and LSD tets, NTSYS Ver. 2.02 (1998), PAUP ver. 410b10 (2001) and DARwin ver. 5 (2008) was used for clustering and

Table 1. *Achillea* species and their localities used in this study.

Code	Species	Locality
1	*A. wilhelmsii*	Tehran: Damavand road, after Damavand, Jaban, 1800m, 8500130-SBUH
2	*A. wilhelmsii*	Tehran: Lavasan road, Fasham, 2100m, Azani, 8500131-SBUH
3	*A. wilhelmsii*	Tehran: Ghazvin, near Nazar abad, 2000m, Azani, 8500132-SBUH
4	*A. wilhelmsii*	Tehran: Qom road, after Daryache Hoze Soltan, Azani, 8500133-SBUH
5	*A. wilhelmsii*	Tehran: Near Karaj, Chitgar park, Azani,8500134-SBUH
6	*A. wilhelmsii*	Tehran: Haraz road, after Abali, 1800m, Azani,8500135-SBUH
7	*A. vermicularis*	Tehran: Haraz road, Polur, after police station, 2000m,Azani, 8500136-SBUH
8	*A. vermicularis*	Tehran: Karaj, Shahrestanak, 2200m, Azani, 8500137-SBUH
9	*A. conferta*	Khuzestan:10km after Mahshahr to Abadan, Ghahreman & Attar, 28516- TUH
10	*A. talagonica*	Tehran: near Taleghan, 2200m, Azani, 8500138-SBUH
11	*A. tenuifolia*	Ghazvin: Road of Ghazvin to Rasht, 1700m, Azani,8500139-SBUH
12	*A. tenuifolia*	Zanjan: near Sarab, Zehzad,8500140-SBUH
13	*A. eriophora*	Yazd: Marvast, 1900m, Mir Jalili, 20762-TUH
14	*A. eriophora*	Fars: road of Eshkenan to Beiram, 730m, Attar& Khatamsaz, 20432-TUH
15	*A. eriophora*	Khorasan: Birjand, Razg, 1800m, Ali abadi, 22220-TUH
16	*A. eriophora*	Fars: road of Fasa to Jahrom,16km after Fasa,1800m, Ghahreman, Attar & Mehdigholi, 22527-TUH
17	*A. millefolium*	Tehran: Damavand, near Cheshme Aala, 2100m, Azani, 850014-SBUH
18	*A. millefolium*	Tehran: Ammam, 2270m, Attar& Mehdigholi, 14267-TUH
19	*A. millefolium*	Azerbaijan: Khoy, road to Ghotur, 2km after Razi station, 2000m, Ghahreman & Attar, 21997-TUH
20	*A. millefolium*	Tehran: Karaj, Shahrestanak, 2450m, Rezvanian, 23268-TUH
21	*A. millefolium*	Mazandaran: Chalus road, Gachsar, Dizin, 2200-3800m, Ghahreman & Mozaffarian, 6980-TUH
22	*A. setacea*	Tehran: Haraz road, Polur after police station, 2000m, Azani, 8500142- SBUH
23	*A. nobilis*	Azerbaijan: between Khalkhal and Ardebil, Bodalalou, Daryache Neur, 2400m, Ghahreman & Mozaffarian,
24	*A. filipendulina*	Azerbaijan: Arasbaran, Khodaferin: Tatar (protected region) 1350m, Ghahreman, Attar, Dadju, 17316- TUH
25	*A. filipendulina*	Azerbaijan: Marand, Mishodagh, just S. S. W Payam, 1700-2200m, Ghahreman & Mozaffarian, 9688-TUH
26	*A. biebersteinii*	Tehran: Evin,1900m, Azani, 8500143-SBUH
27	*A. biebersteinii*	Tehran: Damavand road, after Ab-e Sard,1750m, Azani, 8500144-SBUH
28	*A. biebersteinii*	Tehran: Haraz road, Polur after police station, 2000m, Azani, 8500145- SBUH
29	*A. biebersteinii*	Tehran: Karaj to Ghazvin road, Hashtgerd, Tregubov, 4512-TUH
30	*A. biebersteinii*	Azerbaijan: Marand to Euoughli, Zangire, 1200m, Ghahreman & Mozaffarian, 9718-TUH
31	*A. biebersteinii*	Azarbaijan: Urumyieh, mountain of Sir, 1770m, Ghahreman, Aghustin & Shyckhole, 9718-TUH
32	*A. oxyodonta*	Tehran: Tochal mountain, 1800m,Azani, 8500146-SBUH
33	*A. oxyodonta*	Tehran: Darbandsar, 2100m, Azani, 8500147-SBUH
34	*A. oxyodonta*	Tehran: Darband, Azani,8500148-SBUH

Table 2. Morphological characters of *Achillea* species and their coding.

	Character	**Character States**
1	Kind of stem	1-striate 2-sulcate
2	Indumentum	1-no 2-slightly 3-densely
3	Type of hair in plant	1-pilose 2-villous 3-tomentose
4	Diameter at base stem	mm
5	Median cauline leaves length	cm
6	Rachis	1-free 2-partly covered 3-covered
7	Rachis diameter	mm
8	Leaf shape structure	1-lax 2-compact 3-imbricate
9	Partition of leaflet	1-entire 2-tripartite-pinnatifid 3-tripartite-pinnatisect 4-pentapartite-pinnatisect 5-multipartite-pinnatisect
10	Form of main lobe (leaflet)	1-oblong 2-cuneate 3-obovate 4-broadly obovate
11	Form of lateral lobe (leaflet)	1-oblong 2-elliptic 3-obovate 4-broadly obovate 5-no form
12	Main lobe (position to rachis)	1-70-90 ^{0} 2-10-07 ^{0} 3-less than10 ^{0}
13	Lateral lobe (position to rachis)	1-70-90 ^{0} 2-10-07 ^{0} 3-less than10 ^{0} 4-no position
14	Leaflet margins	1-edentate 2-dentate 3-spiniform 4-spiniform with elongated teeth
15	Leaflet length	mm
16	Diameter of inflorescence	cm
17	Height of inflorescence	cm
18	Number of capitula	N
19	Form of flower head	1-companulate 2-ovate 3-cupulate 4-obconica 5-cylindrical
20	Length of peduncle	mm
21	Length of median bracts	mm
22	Upper involucral bracts	1-ovate 2-obovate 3-lanceolate 4-spathulate
23	Basal involucral bracts	1-ovate 2-ovate,acute at apex 3-ovate,rounded at base
24	Hair in involucre	1-present 2-absent
25	Density of hair in involucre	1-no 2-slightly 3-densely
26	Margin of involucral bracts	1-whitish 2-brownish
27	Number of ligules	N
28	Length of ligules	mm
29	Form of ligules	1-semicirular 2-obtrateziform 3-broadly obtrateziform
30	Margins of ligules	1-3 crenate 2-3 lobate 3-deeply 3 lobate
31	Colour of ligules	1-yellow 2-white
32	Number of disc floret	N
33	Length of disc floret	mm

PCO analyses. Bayesian clustering was performed by MrBayes ver. 3.1 (2005).

Results

Morphological studies

ANOVA test (Table 3) performed among populations of each species showed significant difference ($p<0.01$) for most of quantitative characters used. UPGMA clustering (Fig. 1) of the populations and species studied based on merely quantitative morphological characters almost separated populations of each species from the others.

Different clustering methods performed using both quantitative and qualitative morphological characters produced similar results. Dendrogram obtained through NJ (Neighbor Joining) method (Fig. 2) showed the best fit of tree obtained to the original morphological data and discussed here.

The populations of *A. wilhelmsii* comprise the first major cluster and stand far from the other species studied. Populations of the other species each form a distinct group and all are placed close to each other. This major cluster shows bootstrap value of 96. The clusters of species and their populations also show high bootstrap value of >0.84. However no resolved relationship can be obtained in this analysis as the species from 3 sections stated in Flora Iranica are intermingled with each other. Similar result was obtained from parsimony analysis of morphological data (Fig. 3). However Bayesian tree showed a better resolution and some species relationship was more evident. For example a close affinity was obtained

between *A. vermicularis* (sect. *Santolinoidea*) and *A. biebersteinii* (sect. *Filipendulinae*), as well as *A. millefolium* and *A. setacea* (both from scet. *Millefolium*), but other species relationship remained unresolved. PCA ordination of the *Achillea* species also supported the clustering results. In general both clustering and ordination analyses separated populations of the species studied from each other indicating their morphological distinctness and the fact that morphological characters used can differentiate the species from each other.

Pollen morphology

Details of pollen morphology in 11 species of *A. willhemsii, A. vermicularis, A. talagonica, A. tenuifolia, A. eriophora, A. millefolium, A. setacea, A. nobilis, A. filipendulina, A. biebersteinii, A. oxyodonta* are given in Table 4, Fig. 4. The size of the pollen grain (Polar axis, equatorial diameter excluding spines) of *Achillea* species ranges from 22.4-30.0 µm. All the species studied had pollen grains with similar P/E ratio, trizonocolporate, radially symmetrical, isopolar and spheroidal. Pollen shape is circular to elliptical in equatorial view and semi-angular in polar view. Colpi are short with broad pores, the spines are pointed and with broad base. The number of spine rows varied from 3 to 5 among the species which could be determined in the polar view by light microscope. The exine thickness varied from 2.4 µm to 4.5 µm among the species while, pollen sculpturing was echinate in all.

Grouping of the species based on UPGMA and NJ clustering as well as ordinaton plots based on PCA (Figs. 5 & 6) produced similar results. In general four major clusters/ groups are formed with *A. wilhelmsii*, *A. oxyodonta*, *A. vermicularis* and *A. eriophora* in the first group and *A. talagonica* and *A. biebersteinii* in the second group. The species of *A. millefolium*, *A. filipendulina* and *A. tenuifolia* comprise the third group while, *A. nobilis* and *A. setacea* show pollen similarity and form the forth group. Therefore the species of 3 sections are mixed together and no clear separation occurs among them.

Discussion

The result of ANOVA test at first produced the idea that these characters may be of no use for species discrimination as they seems to be variable even among populations of a single species, but clustering based on merely quantitative characters separated the species into different groups indicating that quantitative morphological characters may be of use in taxonomy of *Achillea*. However ANOVA test indicates that in order to describe these species based on quantitative morphological characters, large number of populations should be studied and then the mean and range of such characters should be given.

Results of phenetic, parsimony and Bayesian alaysis indicate that the species member of the 3 sections described in Flora Iranica are mixed with each other and no clear separation exists between them. For example in Bayesian tree, two species of *A. millefolium* and *A. setacea* from the sect. *Millefolium* are placed close to each other while, *A. nobilis* from the same section is placed far from them and close to *A. conferta* and *A. talagonica* from the sect. *Santolinoidea*. Similary *A. biebersteinii* from the sect. *Filipendulinae* show close affinity to *A. vermicularis* from the sect. *Santolinoidea* while, two other species of the sect. *Filipendulinae* viz. *A. filipendulina* and *A. oxyodonta* are placed close to each other. This result may show some aspects of taxonomic controversy existing in the geus *Achillea*. For example traditionally, three to six sections were recognized in *Achillea* (Boissier 1875, Heimerl 1884, Hoffmann 1894, Huber- Morath 1975), but Guo et al. (2004) suggested juxtaposition of the two sections *Santolinoidea* and *Filipendulinae* in the form of *Achillea* sect. *Achillea* in a broad sense (s. l.). Ehrendorfer & Guo (2006) on the basis of recent multi-disciplinary (including DNA-analysis) studies transferred the former unispecific genera *Otanthus* and *Leucocyclus* to *Achillea* and a new section was designated as *Achillea* sect. *Otanthus* (Hoffmann & Link), similarly *Otanthus maritimus* (L.) was transferred to *Achillea*, and was placed under a new separate section designated as *Otanthus* and combined as *Achillea maritima* (L.). After the new combination, this section includes only 1 species, *Achillea maritima*. Moreover, traditional sections of *Achillea*, sect. *Arthrolepis*, and *Santolinoideae* DC. were joined together within sect. *Babounya* (DC.) and the genus *Leucocyclus* was arranged under sect. *Babounya* as *Achillea formosa* (Boiss.).

PCA analysis of morphological characters revealed that the first 4 components comprise about 62% of total variance. In the first component with about 26.5% of total variance characters like type of stem, main lobe (position to rachis), leaflet length and number of capitule showed the highest positive correlation (>0.60), while in the second component with about 14.2% of total variance, characters related to leaflet margins, upper involucral bracts shape, density of hair in involucre, margin of involucral bracts and colour of ligules, showed highest positive correlation (>0.60). Therefore, these are the most variable morphological characters of the *Achillea* species and varieties studied which may be used in taxonomy of *Achillea*.

Table 3. Representative ANOVA result for morphological characters among *Achillea millefolium* populations.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
rachis diameter	Between Groups	12.864	4	3.216	13.627	.001
	Within Groups	2.360	10	.236		
	Total	15.224	14			
length of leaflet	Between Groups	52.191	4	13.048	3.220	.061
	Within Groups	40.527	10	4.053		
	Total	92.717	14			
length of median involucral bract	Between Groups	1.369	4	.342	3.950	.035
	Within Groups	.867	10	8.667E-02		
	Total	2.236	14			
length of ligules	Between Groups	5.257	4	1.314	31.294	.001
	Within Groups	.420	10	4.200E-02		
	Total	5.677	14			
length of disc floret	Between Groups	4.091	4	1.023	26.000	.001
	Within Groups	.393	10	3.933E-02		
	Total	4.484	14			
length of median leaves	Between Groups	59.433	4	14.858	10.131	.002
	Within Groups	14.667	10	1.467		
	Total	74.100	14			
number of disc floret	Between Groups	369.733	4	92.433	8.831	.003
	Within Groups	104.667	10	10.467		
	Total	474.400	14			
number of ligules	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
diameter of stem at base	Between Groups	19.389	4	4.847	7.694	.004
	Within Groups	6.300	10	.630		
	Total	25.689	14			
length of peduncle	Between Groups	1.703	4	.426	.324	.855
	Within Groups	13.153	10	1.315		
	Total	14.856	14			
diameter of infelorescence	Between Groups	10.677	4	2.669	1.622	.244
	Within Groups	16.460	10	1.646		
	Total	27.137	14			
high of inflorescence	Between Groups	8.160	4	2.040	13.421	.001
	Within Groups	1.520	10	.152		
	Total	9.680	14			
number of capitule in inflorescence	Between Groups	1704.667	4	426.167	5.632	.012
	Within Groups	756.667	10	75.667		
	Total	2461.333	14			

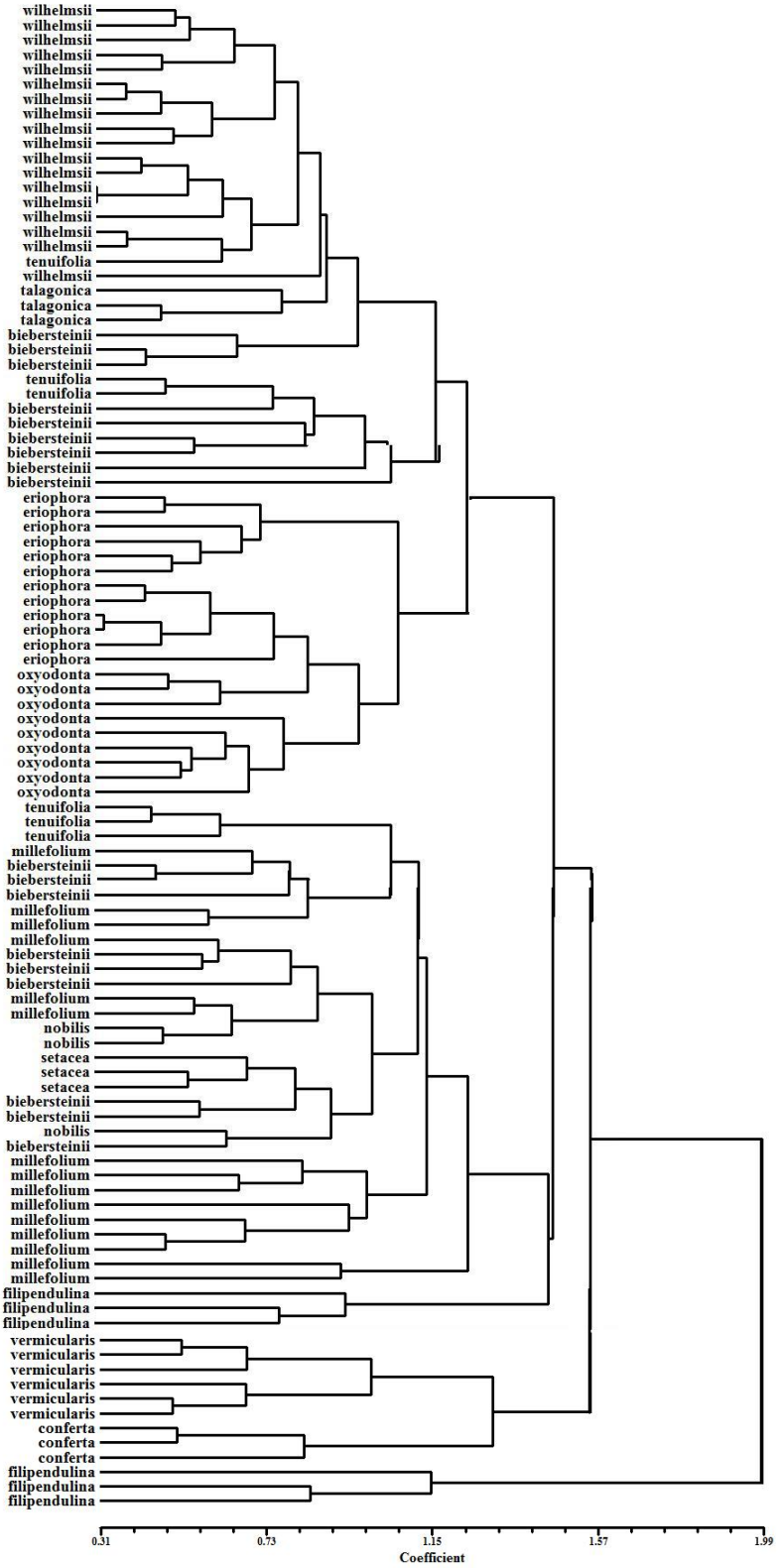

Fig. 1. UPGMA dendrogram of quantitative characters in *Achillea* species.

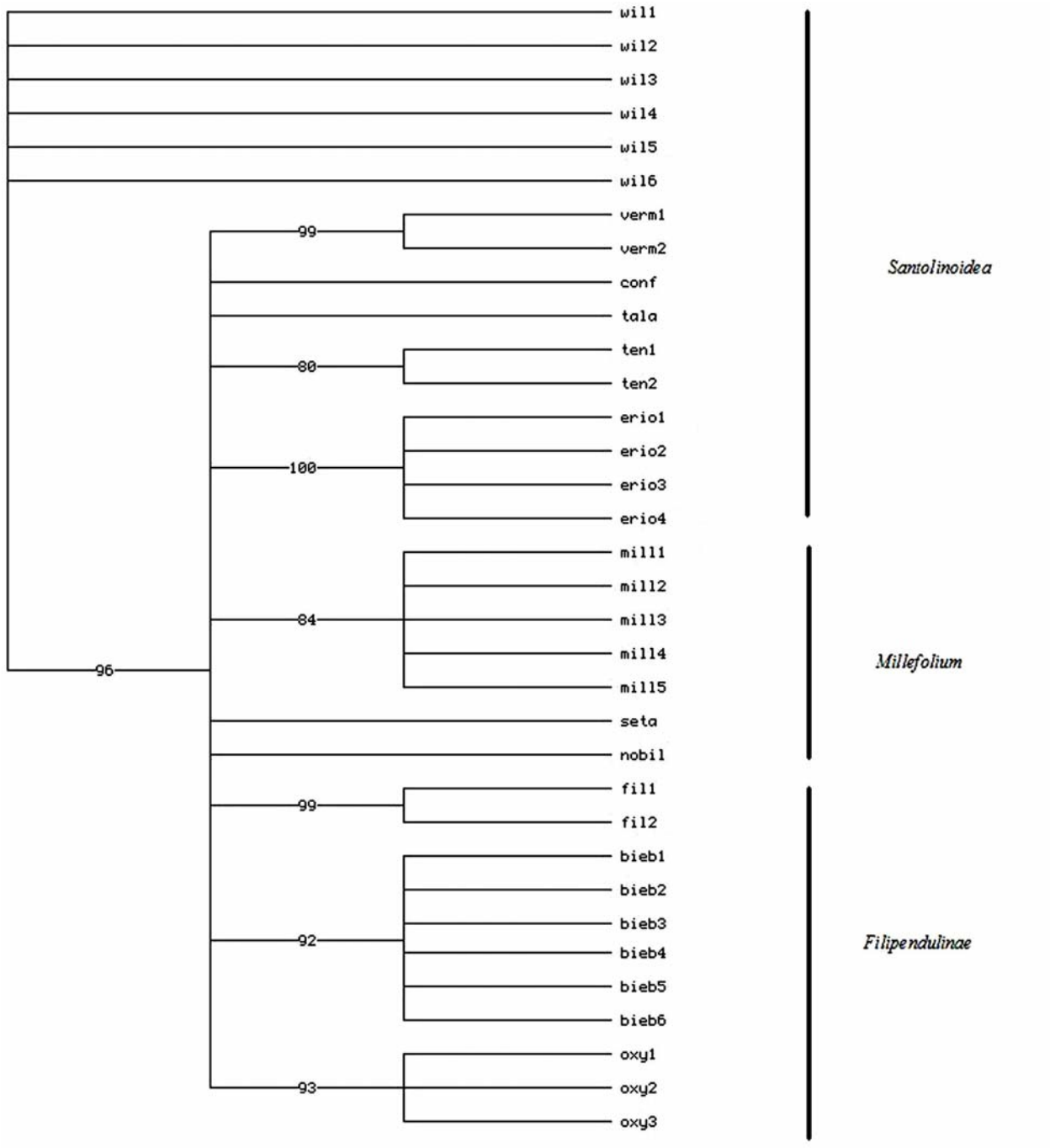

Fig. 2. NJ clustering of *Achillea* species based on all morphological characters. Values at the base of clades are bootstrap values. -Abbreviations: wil = *A. wilhemsii*, conf = *A. conferta* erio = *A. eriophora*, tala = *A. talagonica,* fil =*A. filipendulina,* nobil = *A. nobilis*, ten = *A. tenuifolia,* oxy = *A. oxyodonta*, seta = *A. setacea,* mill = *A. millefolium*, bieb = *A. biebersteinii*, ver = *A. vermicularis*.

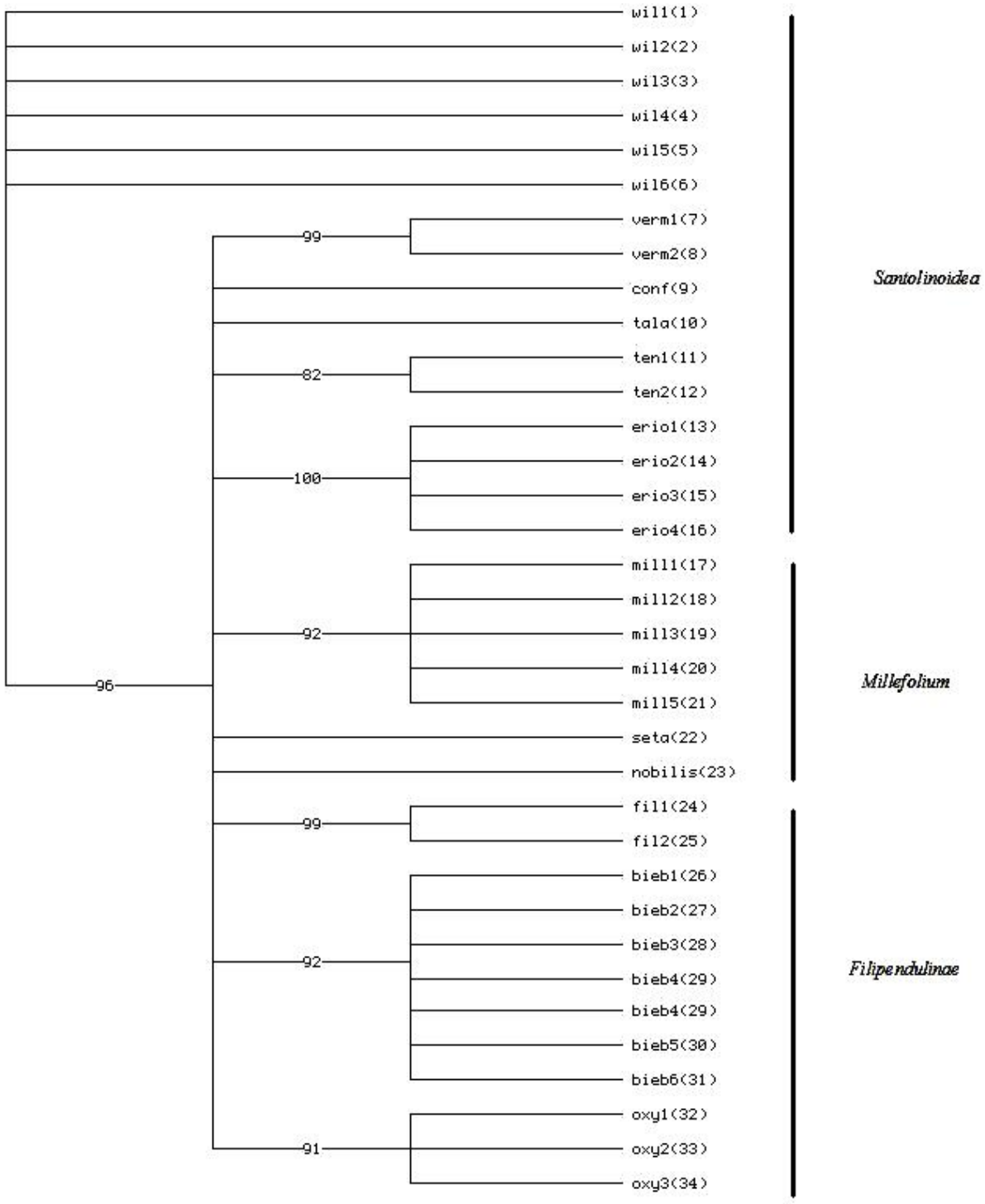

Fig. 3. Most parsimonious unrooted tree of *Achillea* species based on all morphological characters. Values at the base of clades are bootstrap values. -Abbreviations: wil = *A. wilhemsii*, conf = *A. conferta* erio = *A. eriophora*, tala= *A. talagonica,* fil =*A. filipendulina,* ten = *A. tenuifolia,* oxy = *A. oxyodonta*, seta = *A. setacea,* mill = *A. millefolium*, bieb = *A. biebersteinii*, ver = *A. vermicularis*.

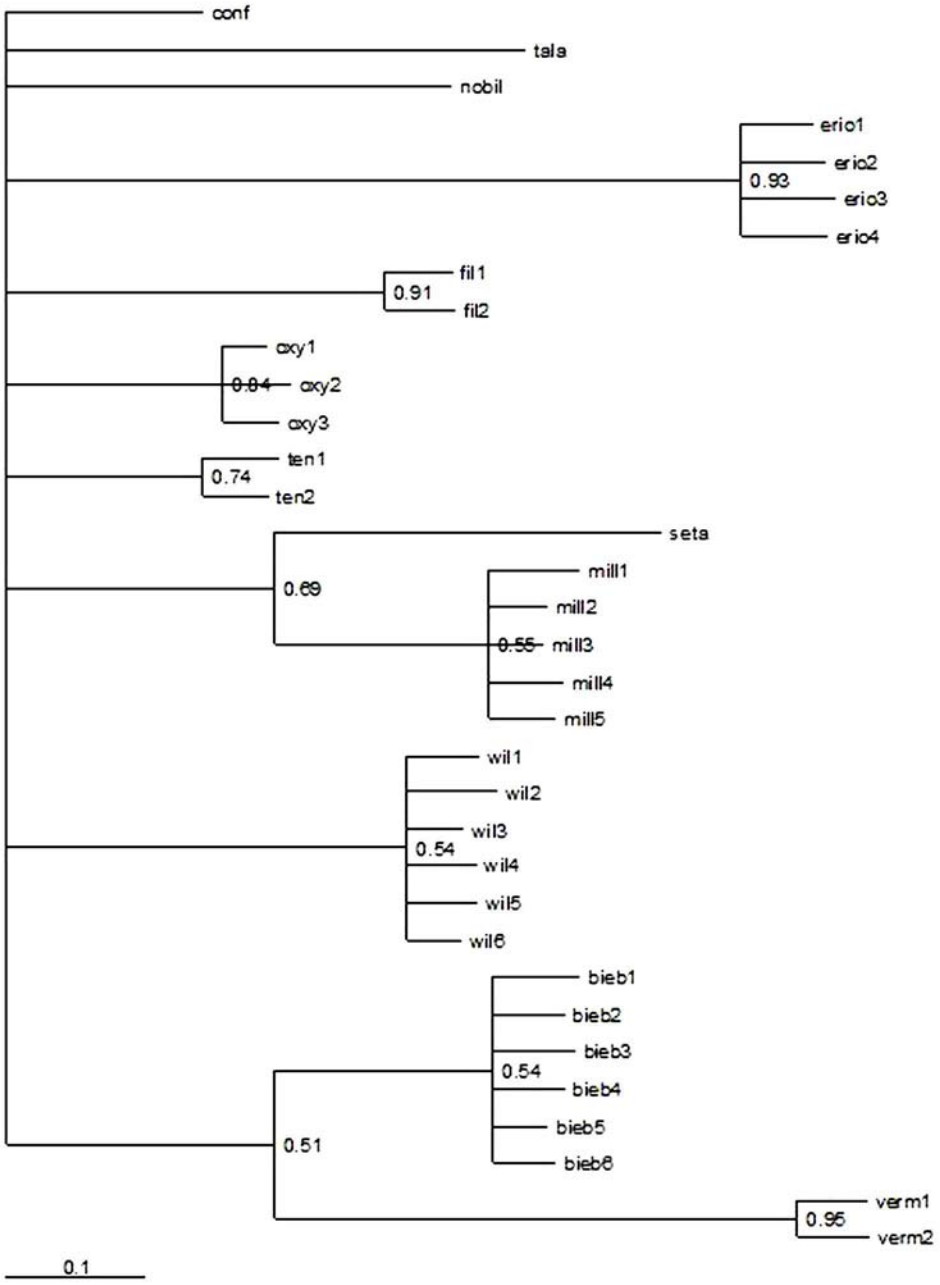

Fig. 4. Bayesian tree of *Achillea* species based on all morphological characters. Values at the base of clades are clade credibility values. -Abbreviations: wil = *A. wilhemsii*, conf = *A. conferta* erio = *A. eriophora*, tala = *A. talagonica,* fil = *A. filipendulina,* ten = *A. tenuifolia,* oxy = *A. oxyodonta*, seta = *A. setacea,* mill = *A. millefolium*, bieb = *A. Biebersteinii*, ver = *A. vermicularis*.

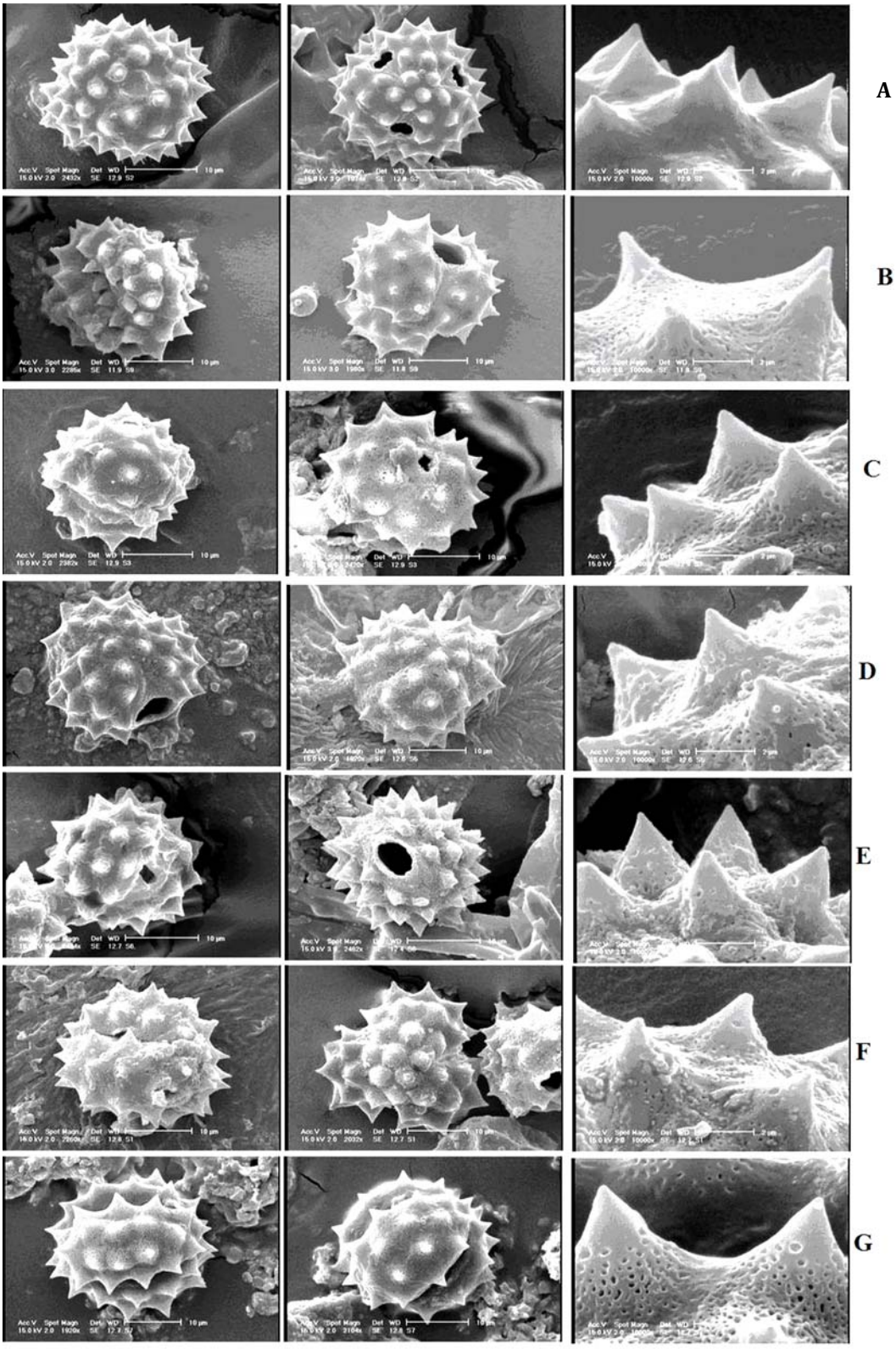

Fig. 5. Representative SEM pollen micrographs of *Achillea* species. -Abbreviations: A-G = *A. wilhelmsii*, *A. vermicularis*, *A. talagonica*, *A. tenuifolia* , *A. eriophora*, *A. millefolium* and *A. setacea* respectively.

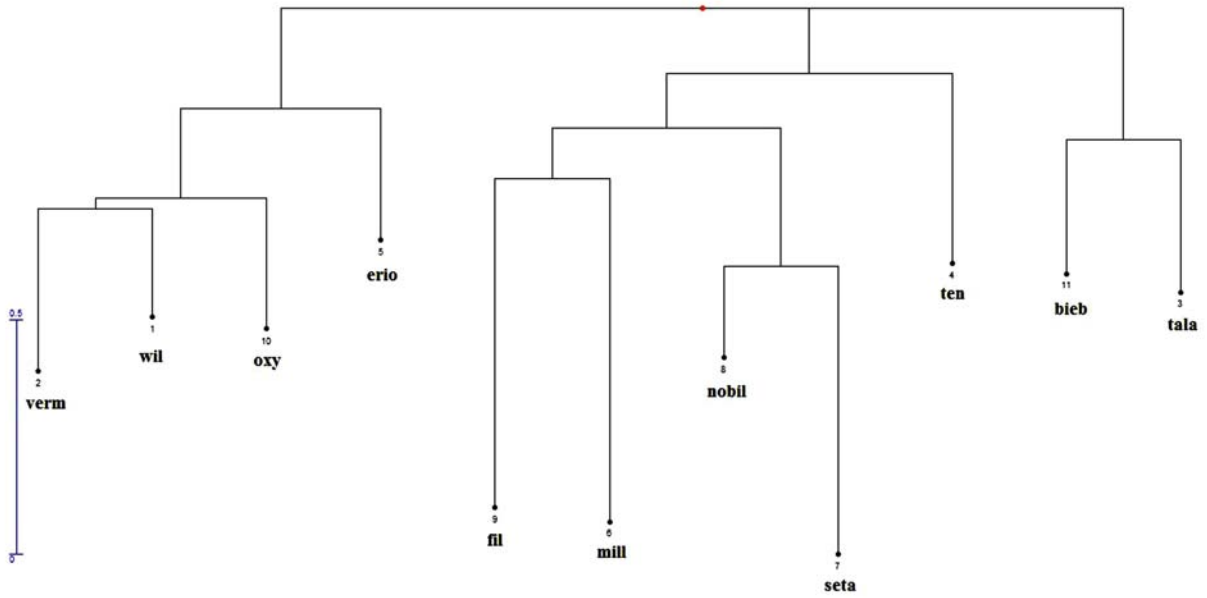

Fig. 6. NJ dendrogram of *Achillea* species based on pollen data. -Abbreviations: wil = *A. wilhemsii*, conf = *A. conferta* erio = *A. eriophora*, tala = *A. talagonica,* fil = *A. filipendulina,* ten = *A. tenuifolia,* oxy = *A. oxyodonta*, seta = *A. setacea,* mill = *A. millefolium*, bieb= *A. biebersteinii*, ver = *A. vermicularis*.

Palynological results obtained are in agreement with reports of Meo & Khan (2004) on *Achillea* species in Pakistan showing that pollen grains are usually isopolar, symmetrical, trionocolporate, non-lacunate and echinate in the studied species. The major palynological characters which proved useful to distinguish all the taxa of *Achillea* species are polar and equatorial diameter, spine length, number of spine rows between Colpi, exine width, pollen shape, P/E ratio, shape in polar and equatorial view, pollen class and aperture type. Exine thickness varied considerably among the species studied and suggested to be of value in the species taxonomy.

The results of clustering and ordination based on palynological characters also showed mixing of species from 3 sections of Flora Iranica and that the species relationship obtained differ from Flora Iranica taxonomic treatment. However such data may be used in the species identification. Yang & Ai (2002) studied pollen characteristics of 10 *Achillea* species and showed that the pollen grains are usually 3-colporate, subspheroidal. The exine ornamentation consisted of verrucate, spinulate and foveolate. But some differences in size, colpae and exine ornamentation were found. They concluded that the slight differences of the pollen morphology are useful to some extent for the classification of the ten species of *Achillea*. Akyalçın et al. (2007) reached the same conclusion while studying pollen morphology of *Achillea* species in Turkey. However, palynological characters are found to be useful in taxonomic treatment as Hall (1928) and Clark et al., (1980) and Pinar & Donmez (2000) used these features to distinguished some of the *Astereae* genera based on exine thickness, spine length, number of spine rows between colpi and distance between spines. Dawar et al. (2002) used pollen morphology in taxonomy of the genus *Inula* and showing its relationship with allied genera in *Compositae*. In general both morphological and palynological studies of *Achillea* species in Iran do not support taxonomic treatment of the genus and the three sections recognized in Flora Iranica.

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Table 4. *Pollen characters* in *Achillea* species studied.

	Species	Equatorial view (µm)	Polar view (µm)	P\E ratio	Exine thickness (µm)	No. of spine rows between colpi	Spine length (µm)
1	*A. wilhelmsii*	25	23.91	0.89	2.75	5	2.18
2	*A. vermicularis*	26.70	25	0.93	2.90	5	2.10
3	*A. talagonica*	21	22.50	1.07	2.80	4	2.51
4	*A. tenuifolia*	25.44	27.20	1.07	2.45	4	2.70
5	*A. eriophora*	24.78	22.67	0.91	2.56	4	2.63
6	*A. millefolim*	27.68	30.08	1.09	4.50	4	2.23
7	*A. setacea*	23.40	26.80	1.14	4.32	4	3.54
8	*A. nobilis*	22.90	25.30	1.11	4.05	4	2.75
9	*A. filipendulina*	25.34	30	1.18	4.13	3	2.30
10	*A. biebersteinii*	22.43	23	1.03	3.15	3	2.60
11	*A. oxyodonta*	26.50	25.33	0.95	2.65	5	2.50

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