



## Palynological study of *Ajania* and related genera (Asteraceae, Anthemideae)

JAUME PELLICER<sup>1</sup>, ORIANE HIDALGO<sup>2</sup>, SÒNIA GARCIA<sup>3</sup>, TERESA GARNATJE<sup>3</sup>, ALEKSANDR A. KOROBKOV<sup>4</sup>, JOAN VALLÈS<sup>1\*</sup> and JOAN MARTÍN<sup>1</sup>

<sup>1</sup>Laboratori de Botànica, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Joan XXIII s/n, 08028 Barcelona, Catalonia, Spain

<sup>2</sup>Plant Development and Evolution, Department of Environmental and Plant Biology, Ohio University, 500 Porter Hall, Athens, OH 45701, USA

<sup>3</sup>Institut Botànic de Barcelona (CSIC-ICUB), Passeig del Migdia s/n, Parc de Montjuïc, 08038 Barcelona, Catalonia, Spain

<sup>4</sup>Botanicheskii Institut im. V. L. Komarova, ul. Prof. Popova 2, Saint Petersburg 197376, Russia

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A morphometrical study of pollen grains using scanning electron microscopy was performed in seven genera belonging to subtribe Artemisiinae (Anthemideae). Forty-six populations representing 40 species were considered, mainly from the genus *Ajania* (31 populations studied of 25 species). This work also includes observations on the genera *Brachanthemum*, *Cancrinia*, *Crossostephium*, *Dendranthema*, *Elachanthemum*, *Hippolytia*, *Kaschgaria*, *Poljakovia* and *Stilpnolepis*. Most data presented here constitute the first pollen observation for some species and genera (*Cancrinia* and *Poljakovia*). Two different pollen exine ornamentations are confirmed for the tribe, *Anthemis*-type (echinate) and *Artemisia*-type (microechinate), a result consistent with previous studies. The *Artemisia*-type is exclusive to the subtribe, whereas the *Anthemis*-type is found present outside Artemisiinae, suggesting that it may represent the ancestral character state for the group. These pollen types appear to be clearly differentiated on the basis of their size and exine ornamentation. Their phylogenetic distribution in Artemisiinae also generally segregates them: the *Anthemis*-type is found in *Dendranthema* and allied genera, whereas the *Artemisia*-type occurs in *Artemisia* and closely related genera. However, we found some very rare exceptions to this trend (e.g. *Ajania junnanica*, *Elachanthemum* and *Stilpnolepis*), the possible origins of which are discussed. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, 161, 171–189.

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### INTRODUCTION

Pollen forms and structures in Asteraceae show great variation, as reflected in the numerous pollen types described for the family (Jeffrey, 2007). Pollen characters provide much taxonomically valuable information and have been commonly used as phylogenetic markers. This is the case in subtribe Artemisiinae Less., in which two pollen types were described on the basis of the exine ornamentation (Stix, 1960): the

*Anthemis*-type, with obvious spines (echinate), and the *Artemisia*-type, with spinules (microechinate). The exine ultrastructure has been studied for both pollen types (e.g. Skvarla & Turner, 1971 for *Anthemis* L.; Rowley, Claugher & Skvarla, 1999 and references therein, for *Artemisia* L.). These characters are useful, as a complement to external morphology, to separate some groups of genera (Skvarla *et al.*, 1977). However, because the ultrastructure is essentially uniform (Skvarla & Larson, 1965; Skvarla & Turner, 1966; Heywood & Humphries, 1977), the most distinctive trait is the surface ornamentation, i.e. the

\*Corresponding author. E-mail: joanvalles@ub.edu

occurrence of spines or spinules (Skvarla & Turner, 1966). Microechinate pollen was first reported by Wodehouse (1926) as restricted to a group of genera including *Artemisia* and some close relatives, which has been confirmed in many studies dealing with *Artemisia* spp. from different geographical origins (e.g. Monoszon, 1948, 1950a, b; Straka, 1952; Stix, 1960; Skvarla & Larson, 1965; Singh & Joshi, 1969; Praglowski, 1971; Vallès, Suárez & Seoane, 1987; Martín, Torrell & Vallès, 2001; Martín *et al.*, 2003; Grigoreva, Korobkov & Tokarev, 2009). Several further studies on pollen exine ornamentation and molecular phylogeny confirmed that each pollen type characterizes one of the main groups of Artemisiinae: *Dendranthema* (DC.) Des Moul. and relatives have the *Anthemis*-type, whereas *Artemisia* and allies show the *Artemisia*-type (Chen & Zhang, 1991; Rowley *et al.*, 1999; Martín *et al.*, 2001, 2003 for the pollen studies; Vallès *et al.*, 2003; Sanz *et al.*, 2008) for the phylogenetic analyses). Exceptions to this trend have been generally considered to be the result of taxonomic misplacement (Martín *et al.*, 2001, 2003). In this sense, pollen type has been used to confirm or justify the segregation of several genera from *Artemisia* and their placement in the *Dendranthema* group and vice versa. One such genus is *Ajania* Poljakov.

The Asian genus *Ajania* comprises c. 30–40 species, depending on the authors (Bremer & Humphries, 1993; Bremer, 1994; Kubitzki, 2007), with a large number of representatives in China and Japan and some in Afghanistan, Kazakhstan, Kyrgyzstan, Mongolia, Northern India, Russia and Tadjikistan. This genus was segregated from *Artemisia* by Poljakov (1955). Tzvelev (1961), in the *Flora of the USSR*, accepted the genus *Ajania* with c. 25 species, nine of which grow in the USSR, but considered, using pollen ornamentation as one of the main arguments, that Poljakov (1955) had erroneously combined into *Ajania* some species that should be maintained in *Artemisia*. Tzvelev (1961) pointed out that *Ajania* had evolved from ancestral taxa more closely related to *Dendranthema* and that the adaptation of *Ajania* to Middle Asian steppes and deserts gave rise to a strong resemblance to the representatives of *Artemisia* occupying these areas. In order to explain the similarities between the three genera, Bremer & Humphries (1993) assumed that independent lines have evolved from the same dendranthemoid ancestor. Three species were removed from *Ajania* to constitute a separate new genus, *Phaeostigma* Muldashev (Muldashev, 1982, 1983). This author justified this change based on pollen characters (microechinate pollen), among others, and also pointed out the affinities of this genus with *Artemisia*. He also used palynological

features for proposing the combination of *Ajania junnanica* Poljakov within *Artemisia*, because its pollen has ‘very small spines’ (Muldashev, 1983). Two species of *Ajania* and one of *Phaeostigma* have been recently studied from the palynological point of view (Martín *et al.*, 2001 for *Ajania fastigiata* (C.Winkl.) Poljakov and *A. fruticulosa* (Ledeb.) Poljakov; Martín *et al.*, 2003 for *Phaeostigma salicifolium* (Mattf.) Muldashev). Species of *Ajania* were found to have *Anthemis*-type pollen, which confirms their placement in the *Dendranthema* group, also supported by molecular phylogenetic analyses (Y. Masuda & K. Kondo, pers. comm.; Sanz *et al.* (2008). The representative of *Phaeostigma* included in the study had *Artemisia*-type pollen, a result congruent with the hypothesis of its close relationship with *Artemisia*. Because of their complex taxonomic history, with numerous relocations of species between the two main groups of Artemisiinae, *Ajania* and segregate genera represent a good group for addressing pollen studies in the subtribe.

The present paper aims to provide new pollen data for *Ajania* and some other representatives of Artemisiinae, including *Brachanthemum* DC., *Cancrinia* Kar. & Kir., *Crossostephium* Less., *Dendranthema*, *Elachanthemum* Y.Ling & Y.R.Ling, *Hippolytia* Poljakov, *Kaschgaria* Poljakov, *Poljakovia* Grubov & Filatova and *Stilpnolepis* Krasch. The specific objectives of this study are: (1) to increase the number of palynological data for Artemisiinae; (2) to improve the understanding of the characterization of the two pollen types found in the subtribe through the analysis of new and previous data from our team; (3) to discuss these findings in a phylogenetic framework with a view to contributing to a resolution of questions related to the systematic and phylogenetic relationships within the subtribe; and (4) to consider possible cause(s) for the transition from one pollen type to another.

## MATERIAL AND METHODS

### PLANT MATERIAL

Pollen grains from dried specimens of plants collected in the field and deposited in BCN (Universitat de Barcelona), HIMC (Inner Mongolia University, Hohhot) and LE (Botanicheskii Institut im. V.L. Komarova, Saint Petersburg) were used to carry out the study (Table 1). Observations using optical and scanning electron microscopy (SEM) were carried out in 46 populations of 40 species of the genera *Ajania* (31 populations of 25 species), *Brachanthemum* (four species), *Cancrinia* (two species), *Crossostephium* (one species), *Dendranthema* (two species), *Elachanthe-*

**Table 1.** Origin of the populations studied, with the indications of the herbaria where the voucher specimens are deposited

Taxa	Populations
<i>Ajania achilleoides</i> (Turcz.) Poljakov ex Grubov*	Mongolia, Ubsunur, 60 km SW Under-Khangai, Kheltguin-Ula mountains, 16.VIII.1979, Z. Kapamysheva (LE)
<i>A. achilleoides</i> (Turcz.) Poljakov ex Grubov*	Mongolia, Central Gobi, 16 km NE Erdene-Dalai, 4.IX.2004, Sh. Dariimaa, Sh. Tsooj, J. Vallès (BCN)
<i>A. achilleoides</i> (Turcz.) Poljakov ex Grubov*	Mongolia, Central Gobi, 46 km NE Erdene-Dalai, 4.IX.2004, Sh. Dariimaa, Sh. Tsooj, J. Vallès (BCN)
<i>A. aureoglobosa</i> (W.W.Sm. & Farr.) Muldashev	China, province of Gansu, near Liang Shui, 18.X.1914, E.N. Meyer (LE)
<i>A. fastigiata</i> (Winkl.) Poljakov*	China, autonomous region of Xingian-Uigur, Kashgar, 25 km SW Kiushisha, 1400 m, 19.X.1959, M. Petrov (LE)
<i>A. fruticulosa</i> (Ledeb.) Poljakov*	Kyrgyzstan, mountain pass in the Kurutag mountains, 16.XI.1957, A. Yunatov (LE)
<i>A. fruticulosa</i> (Ledeb.) Poljakov*	Mongolia, Southern Gobi, 10 km S Bulgan, Sh. Dariimaa, Sh. Tsooj, J. Vallès, E. Yatamsuren, 2.IX.2004 (BCN)
<i>A. fruticulosa</i> (Ledeb.) Poljakov*	Mongolia, Southern Gobi, 20 km SW Mandal Oboo, 4.IX.2004, Sh. Dariimaa, Sh. Tsooj, J. Vallès (BCN)
<i>A. gracilis</i> (Hook.f. & Thomson) Poljakov ex Tzvelev	Tadzhikistan, Pamiro-Alai, near Kirakul, 5.VIII.?, A. Kushakevich (LE)
<i>A. grubovii</i> Muldashev	Mongolia, Dzhungar Gobi, Mongolian Altai, 17.VIII.1979, V. Grubov (LE)
<i>A. junnanica</i> Poljakov	China, Northern Yunnan, Pe-Cong-Ching, 3200 m, 1909–1911, R. Maire (LE)
<i>A. khartensis</i> (Dunn) C.Shih	China, Gansu, 100 km SW Dunkhun, 2.VIII.1958, M. Petrov (LE)
<i>A. kokanica</i> (Krasch.) Tzvelev	Kyrgyzstan, Northern Alai, high river Shakhimaruan river, 12.VIII.1938, A. Mukhamedzhanov (LE)
<i>A. myriantha</i> (Franch.) Y.R.Ling ex C.Shih	China, Northern and Central Yunnan, mountains near Liao-Do, 2000 m, XI.1910, R. Maire (LE)
<i>A. nana</i> (Krasch.) Muldashev	China, Northern Szetschuan, between Epur and Kanguang, 19.X.1885, G.N. Potanin (LE)
<i>A. nematoloba</i> (Hand.-Mazz.) Ling ex C.Shih	Mongolia, Alaschan mountain, VIII.1880, N.M. Przewalski (LE)
<i>A. nubigena</i> (Wall.) C.Shih	Nepal, Bagmati zone, Kasuwa district, below Khanyyin, 3650 m, 22.IX.1966, D. Nicholson (LE)
<i>A. pacifica</i> (Nakai) K.Bremer & Humphries	Japan, Honshu prefecture, Chiba, 10 m, 1.XII.1973, M. Togashi (LE)
<i>A. pallasiana</i> (Fisch. ex Besser) Poljakov	China, Kheiluntszyn province, Yaohe district, Hualatszy, 10.IX.1950, Chang Kiang-Cheng (LE)
<i>Ajania parviflora</i> (Grun.) Ling	China, Inner Mongolia, Alxa province, road S128, km 102, near Suhait, sandy and stony soils, 6.IX.2007, J. Vallès, S.W. Zhao (BCN)
<i>A. potaninii</i> (Krasch.) Poljakov	China, Gansu, Fin-Ten-Lin mountain pass, 1885, G.N. Potanin (LE)
<i>A. przewalskii</i> Poljakov	Mongolia, Alaschan, 9.VIII.1880, N.M. Przewalski (LE)
<i>A. purpurea</i> C.Shih	China, Tibet, Yan-Uzi-Uzyan basin, Nru-Chu canyon, 25.VII.1900, V. Ladyguin (LE)

**Table 1.** *Continued*

Taxa	Populations
<i>A. remotipinna</i> (Hand.-Mazz.) Y.Ling & C.Shih	Mongolia, near Kalgans, 1870, A. Lomonosov (LE)
<i>A. roborowskii</i> Muldashev	China, Gansu, 25 km S Lanchisou, 12.VIII.1958, M. Petrov (LE)
<i>A. rupestris</i> (Matsum. & Koidz.) Muldashev*	Japan, Sirano-Asamajama, Happu-Giku, IX.1889, Tschonoski (LE)
<i>A. rupestris</i> (Matsum. & Koidz.) Muldashev*	Japan, Happu-Giku, IX.1889, Tschonoski (LE)
<i>A. scharnhorstii</i> (Regel & Schmalh.) Tzvelev*	China, Tian-Shan, Bogdo-Ola mountains, near Urumqi, 26.VIII.1908, G. Merzbacher (LE)
<i>A. scharnhorstii</i> (Regel & Schmalh.) Tzvelev*	China, Tian-Shan, Bogdo-Ola mountains, 29.VIII.1908, G. Merzbacher (LE)
<i>A. tibetica</i> (Hook.f. & Thomson) Tzvelev	China, Tibet, Peku lake, 4.650 m, 31.VIII.1991 (LE)
<i>A. trilobata</i> Poljakov	Kazakhstan, Semirschen region, Przhevalski district, canyon of river Karakol, 22.VII.1913, V. Saposhnikov (LE)
<i>Brachanthemum gobicum</i> Krasch.	Mongolia, Ubur-Khangai, Arms Bogd mountains, 31.VIII.2004, Sh. Dariimaa, Sh. Tsooj, J. Vallès (BCN)
<i>Brachanthemum kirghisorum</i> Krasch.	Kyrgyzstan, Alatau mountains, Issik-Kul lake basin, 15 km W of Kyzylty, 1650 m, 20.VII.1970, N.N. Izmailova, S.S. Ikonnikov, D.M. Ladugina (HIMC)
<i>Brachanthemum mongolorum</i> Grubov	Mongolia, Northern region, 15 km W Barun-Matad-Ula, 12.VIII.1989. Ch. Sanchir, V. Khramtsov (LE)
<i>Brachanthemum pulvinatum</i> (Hand.-Mazz.) C.Shih	China, 4.IX.1990 (HIMC)
<i>Cancrinia discoidea</i> (Ledeb.) Poljakov ex Tzvelev	Mongolia, Southern Gobi, 17 km NE Bulgan, 5.IX.1995, A. Bayandzag (BCN)
<i>Cancrinia maximowiczii</i> C.Winkl.	China, 21.VII.1980 (HIMC)
<i>Crossostephium chinense</i> (L.) Makino	China, Chzhchi province, Beijing surroundings, Pokhuashan mountains, 1850–1858, S.M. Vazilievskii (LE)
<i>Dendranthema mongolicum</i> (Y.R.Ling) Tzvelev	Mongolia, Arkhangai, mountain pass Sagan-Davaa, near Tsetserleg, 2200 m, 25.VIII.2004, Sh. Dariimaa, Sh. Tsooj, J. Vallès (BCN)
<i>Dendranthema zawadskii</i> (Herbich) Tzvelev	Mongolia, Bulgan, Khugunkhaan mountains, 2000 m, 25.VIII.2004, Sh. Dariimaa, Sh. Tsooj, J. Vallès (BCN)
<i>Elachanthemum intricatum</i> (Franch.) Y.Ling & Y.R.Ling	Mongolia, Suothern Gobi, Gobi Altai, near Gurvan Tes, 5.IX.1979, V.I. Grubov, A. Muldashev, Sh. Dariimaa (BCN)
<i>Hippolytia alashanensis</i> (Ling) C.Shih	China, Inner Mongolia, Alxa province, SW slopes of Helan Shan, Tonguan, 5.IX.2007, J. Vallès, S.W. Zhao (BCN)
<i>Hippolytia trifida</i> (Turcz.) Poljakov	China, 11.VIII.1994 (HIMC)
<i>Kaschgaria komarovii</i> (Krasch. & Rubtzov) Poljakov	Mongolia, Dzhungar Gobi, near Bulgan, 29.VII.1988, I.A. Gubanov, Sh. Dariimaa, R.V. Kamelin (BCN)
<i>Poljakovia falcatolobata</i> (Krasch.) Grubov & Filatova	China, Burkhan-Budda mountains, Khatu canyon, 25.VII.1911, V.N. Ladyguin (LE)
<i>Stilpnolepis centiflora</i> (Maxim.) Krasch.	China, 15.IX.1963 (HIMC)

Asterisks (\*) indicate different populations of the same species studied.

BCN, Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona; HIMC, Faculty of Life Sciences, Inner Mongolia University, Hohhot; LE, Botanicheskii Institut im. V.L. Komarova, Saint Petersburg.

*mum* (one species), *Hippolytia* (two species), *Kaschgaria* (one species), *Poljakovia* (one species) and *Stilpnolepis* (one species).

#### POLLEN OBSERVATIONS AND MEASUREMENTS

Pollen was obtained by dissecting dehydrated anthers in 96% ethanol. Samples were examined with a scanning electron microscope after acetolysis following Avetissian's (1950) micro-method. Observations were then carried out after coating with gold using a diode sputtering and a Hitachi 52300 scanning microscope at 15 kV. For biometrical measurements, pollen samples were acetolysed following the same method, mounted on glycerogelatine and sealed. Measurements were made using a Visopan apparatus (Reichert, Austria). For each specimen, 15 fully developed grains were measured, except in *Ajania aureoglobosa* (W.W.Sm. & Farr.) Muldashev (seven grains), *A. fruticulosa* (Ledeb.) Poljakov sample 57 (eight grains), *A. junnanica* Poljakov (10 grains) and *Crossostephium chinense* Merr. (five grains). The parameters considered, following Erdtman (1969), Faegri & Iversen (1975) and Reitsma (1970), were: polar diameter (P), equatorial diameter (E) and sphericity (P/E). For each, the arithmetic mean and standard deviation were calculated. In the case of pollen grains with spiny ornamentation, the height of the spine was also measured from the tip to the start of the multiperforate basement. The density of supra-rectal spines/spinules was calculated in the mesocolpium area by counting the number of spines/spinules in 25  $\mu\text{m}^2$  of the pollen surface. We also calculated an approximate pollen volume [V, calculated using the ellipsoid formula:  $V = 4/3\pi(1/2P)(1/2E)^2$ ] and counted the number of spines/spinules in 25  $\mu\text{m}^2$  of the pollen surface. The pollen terminology used follows Reitsma (1970).

#### STATISTICAL ANALYSES

A database grouping present and previous results (Martín *et al.*, 2001, 2003) in the whole subtribe Artemisiinae was constructed for comparative purposes between the different morphological traits of each pollen type (e.g. volume, spine height and spine density; Appendix). The pollen size measures being dependent on the preparation method (Reitsma, 1969), we restricted this database to the species processed with an identical protocol for allowing comparisons. StatGraphics Plus 5.1 (Statistical Graphic Corp.) was used to carry out the Kruskal–Wallis contrasts. This is a non-parametric test that does not involve any assumption about the frequency of distribution of the variables and therefore fits our data better. Some of the 76 representatives of Anthemideae

listed are not currently classified in Artemisiinae (as redefined by Oberprieler, Himmelreich & Vogt, 2007) and we have not included them for the statistical analyses.

#### MOLECULAR PHYLOGENY

Sequences for external (ETS) and internal (ITS) transcribed spacers from GenBank were analysed to provide a phylogenetic framework for discussing pollen type distribution and evolution in Artemisiinae. Representatives of the genera *Achillea*, *Lepidolopsis* and *Tanacetum* were chosen as outgroups for Artemisiinae on the basis of the analyses of Anthemideae of Oberprieler *et al.* (2007). Sequences were edited with BioEdit v7.0.9 (Ton Hall, Ibis Biosciences). The alignment was first performed using T-COFFEE as implemented by BioX 1.1b1 [E. Lagercrantz (<http://www.lagercrantz.name/software/biox/>)] and then this was manually revised in MacClade 4.08 (Maddison & Maddison, 2005). MrModeltest 2.2 (Nylander, 2004) was used to select the best-fit models of nucleotide substitution for our datasets. Bayesian inference analyses performed with MrBayes 3.1.1. (Huelsenbeck & Ronquist, 2001) were initiated with random starting trees and run for  $10^6$  generations. Four Markov chains were run simultaneously and trees were sampled every 100 generations, which resulted in 10 000 sampled trees. To ensure the Markov chains had become stable, log-likelihood values for sampling trees were plotted against generation time and those before stationarity were discarded as 'burn-in'. A majority-rule consensus tree was obtained with PAUP version 4.0b4a (Swofford, 1999). Posterior probability support (PP)  $\geq 95\%$  was considered statistically significant. We carried out separate and combined ETS and ITS analyses, restricting the dataset to individuals with both regions sequenced [ITS of *Phaeostigma salicifolium* AM774423 and EF577281, *P. variifolium* EF577283, and *Stilpnolepis centiflora* (Maxim.) Krasch. AY127695, AY127696 were consequently removed]. Clones of ETS for the same individual that grouped together in the separate analysis were combined in a consensus sequence and, if this was not the case, they were introduced separately in the combined dataset. In the same way, ETS and ITS sequences of inconsistent positioning in separate analyses were treated independently in the combined analysis. We also carried out independent ETS and ITS analyses involving the restricted taxonomic sampling of the combined dataset.

#### RESULTS

Pollen traits of the studied taxa are shown in Table 2, Fig. 1A–X and the Appendix. Results from statistical

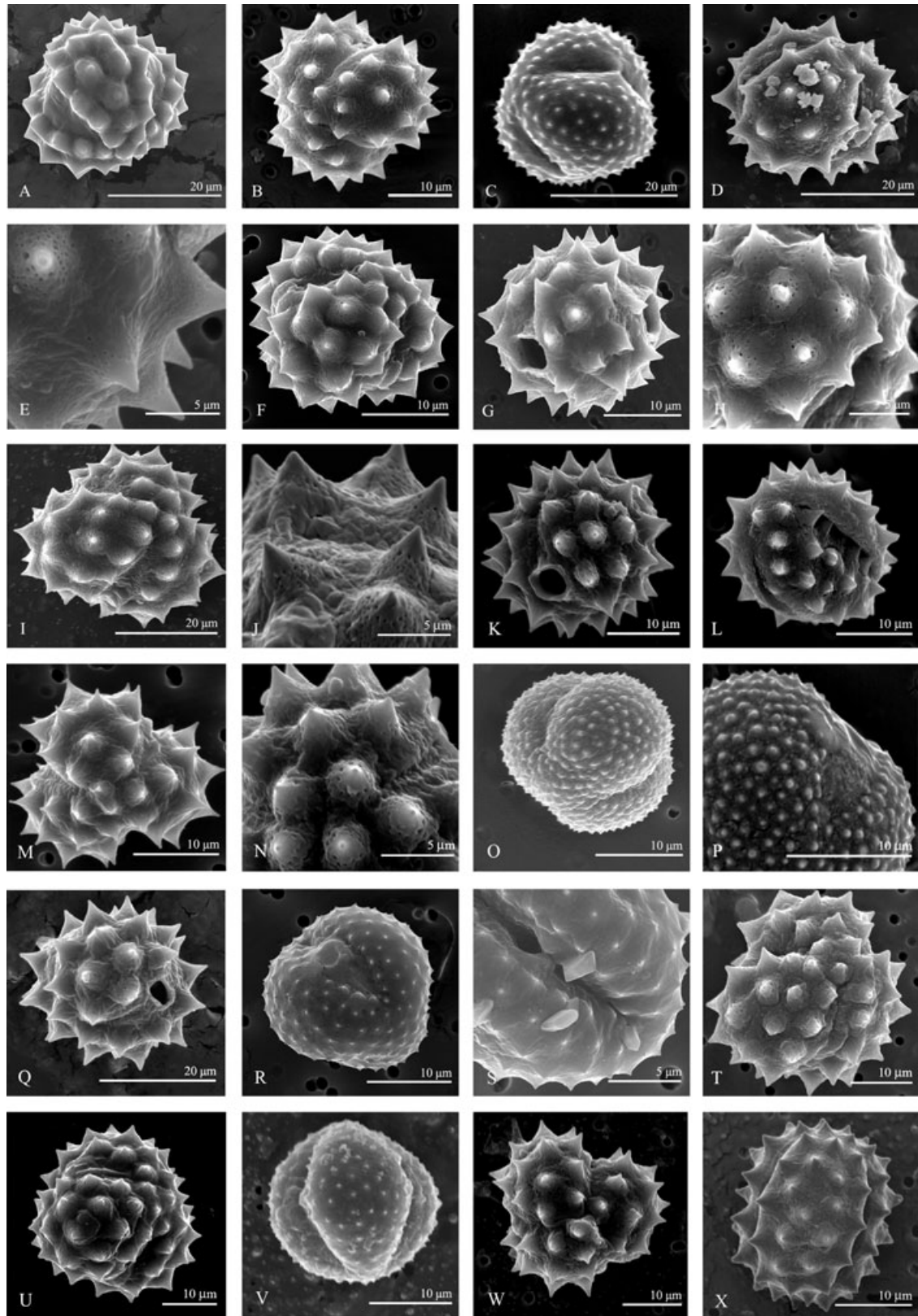
**Table 2.** Pollen characteristics of the taxa studied

Taxa	P (µm)	E (µm)	P/E (µm)	Pollen type	Spine height (µm)
<i>Ajania achilleoides</i> *	22.91–27.08 X = 24.92 (1.33)	20.83–27.08 X = 23.74 (1.63)	1.04	<i>Anthemis</i>	3.24–4.28 X = 3.60 (0.40)
<i>A. achilleoides</i> *	20.83–27.08 X = 22.91 (1.92)	17.70–25.00 X = 22.14 (1.69)	1.03	<i>Anthemis</i>	2.75–3.18 X = 2.92 (0.16)
<i>A. achilleoides</i> *	20.83–25.00 X = 22.91 (1.36)	18.75–22.91 X = 21.93 (1.27)	1.04	<i>Anthemis</i>	2.75–3.10 X = 2.85 (0.15)
<i>A. aureoglobosa</i>	19.79–25.00 X = 22.46 (1.88)	20.83–22.91 X = 21.72 (1.11)	1.03	<i>Anthemis</i>	2.75–3.10 X = 2.96 (0.17)
<i>A. fastigiata</i>	22.91–31.25 X = 26.31 (2.17)	22.91–31.25 X = 25.41 (1.92)	1.03	<i>Anthemis</i>	3.63–4.41 X = 3.83 (0.33)
<i>A. fruticulosa</i> *	20.83–33.33 X = 25.64 (4.41)	20.83–28.12 X = 23.34 (2.89)	1.09	<i>Anthemis</i>	3.76–4.15 X = 3.91 (0.14)
<i>A. fruticulosa</i> *	22.91–27.08 X = 25.27 (1.07)	22.91–29.16 X = 25.20 (1.58)	1.00	<i>Anthemis</i>	3.27–4.31 X = 3.75 (0.41)
<i>A. fruticulosa</i> *	20.83–29.16 X = 25.41 (2.18)	18.75–26.04 X = 23.81 (1.96)	1.06	<i>Anthemis</i>	2.59–4.41 X = 3.57 (0.74)
<i>A. gracilis</i>	21.87–28.12 X = 24.38 (1.73)	20.83–25.00 X = 22.65 (1.42)	1.07	<i>Anthemis</i>	1.98–2.84 X = 2.30 (0.34)
<i>A. grubovii</i>	25.00–29.16 X = 26.94 (1.51)	22.91–29.16 X = 25.20 (1.67)	1.06	<i>Anthemis</i>	1.72–2.06 X = 1.92 (0.12)
<i>A. junnanica</i>	14.58–20.83 X = 18.33 (2.31)	12.5–18.75 X = 16.45 (2.68)	1.11	<i>Artemisia</i>	–
<i>A. khartensis</i>	22.91–29.16 X = 24.85 (1.61)	20.83–27.08 X = 24.30 (1.79)	1.02	<i>Anthemis</i>	2.75–3.10 X = 2.92 (0.12)
<i>A. kokanica</i>	25.00–31.25 X = 26.73 (1.87)	22.91–27.08 X = 25.55 (1.17)	1.04	<i>Anthemis</i>	3.37–4.15 X = 3.68 (0.28)
<i>A. myriantha</i>	20.83–27.08 X = 23.88 (1.49)	20.83–26.04 X = 23.67 (1.39)	1.00	<i>Anthemis</i>	2.15–2.32 X = 2.21 (0.09)
<i>A. nana</i>	20.83–31.25 X = 24.51 (2.66)	20.83–26.04 X = 23.39 (1.46)	1.04	<i>Anthemis</i>	3.11–3.89 X = 3.39 (0.39)
<i>A. nematoloba</i>	14.58–23.95 X = 21.03 (2.36)	14.58–21.87 X = 19.64 (2.29)	1.07	<i>Anthemis</i>	2.06–2.15 X = 2.13 (0.04)
<i>A. nubigena</i>	25.00–29.16 X = 27.77 (1.28)	25.00–29.16 X = 27.14 (1.59)	1.02	<i>Anthemis</i>	3.10–4.31 X = 3.54 (0.45)
<i>A. pacifica</i>	35.41–41.66 X = 38.39 (2.11)	33.33–40.62 X = 34.05 (8.37)	1.12	<i>Anthemis</i>	3.89–4.93 X = 4.43 (0.43)
<i>A. pallasiana</i>	20.83–33.33 X = 27.56 (4.07)	20.83–33.33 X = 26.80 (3.57)	1.02	<i>Anthemis</i>	3.62–4.48 X = 4.03 (0.35)
<i>A. parviflora</i>	16.00–24.00 X = 19.72 (2.09)	18.00–22.00 X = 19.60 (1.20)	1.00	<i>Anthemis</i>	3.28–3.88 X = 3.67 (0.22)
<i>A. potaninii</i>	21.87–25.00 X = 23.18 (1.21)	18.75–25.00 X = 21.94 (2.06)	1.05	<i>Anthemis</i>	2.58–3.01 X = 2.84 (0.17)
<i>A. przewalskii</i>	22.91–25.00 X = 23.60 (0.85)	17.70–25.00 X = 22.28 (1.79)	1.05	<i>Anthemis</i>	3.11–3.89 X = 3.26 (0.34)
<i>A. purpurea</i>	25.00–29.16 X = 25.62 (1.23)	22.91–29.16 X = 24.64 (1.70)	1.03	<i>Anthemis</i>	2.58–3.10 X = 2.87 (0.22)
<i>A. remotipinna</i>	22.91–29.16 X = 25.62 (1.56)	20.83–28.12 X = 24.99 (1.71)	1.02	<i>Anthemis</i>	3.76–4.15 X = 3.96 (0.17)

Table 2. Continued

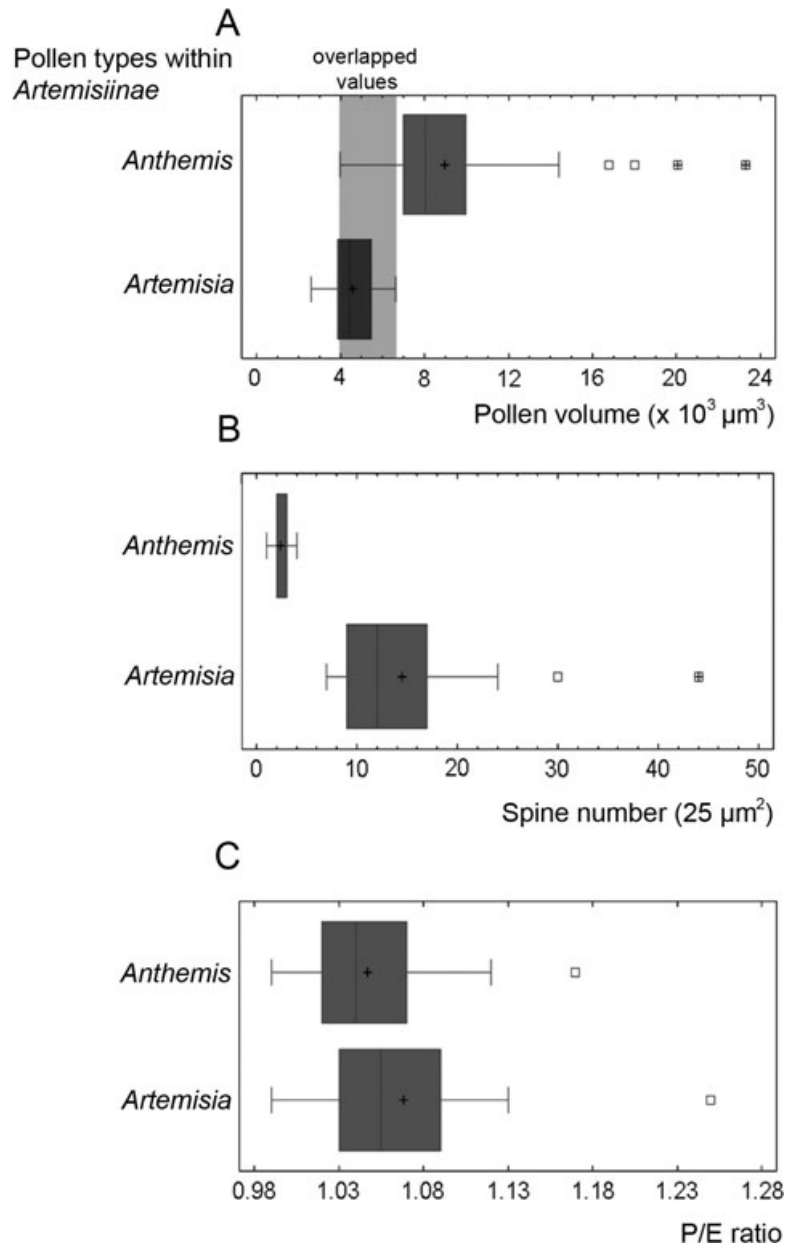
Taxa	P (µm)	E (µm)	P/E (µm)	Pollen type	Spine height (µm)
<i>A. roborowskii</i>	22.91–31.25 X = 26.31 (2.85)	18.75–31.25 X = 24.16 (3.03)	1.08	<i>Anthemis</i>	2.58–2.75 X = 2.63 (0.07)
<i>A. rupestris</i> *	22.91–28.12 X = 24.37 (1.61)	22.91–27.08 X = 24.02 (1.44)	1.01	<i>Anthemis</i>	3.01–3.62 X = 3.30 (0.29)
<i>A. rupestris</i> *	22.91–29.16 X = 26.31 (1.98)	20.83–29.16 X = 24.85 (2.29)	1.05	<i>Anthemis</i>	3.10–3.62 X = 3.37 (0.23)
<i>A. scharnhorstii</i> *	22.91–35.41 X = 26.38 (3.09)	20.83–27.08 X = 24.16 (2.16)	1.09	<i>Anthemis</i>	2.59–3.63 X = 3.21 (0.43)
<i>A. scharnhorstii</i> *	22.91–31.25 X = 26.31 (2.37)	20.83–29.16 X = 23.95 (2.55)	1.09	<i>Anthemis</i>	2.84–3.62 X = 3.08 (0.31)
<i>A. tibetica</i>	25.00–29.16 X = 25.69 (1.28)	20.83–27.08 X = 23.60 (1.70)	1.08	<i>Anthemis</i>	3.89–4.67 X = 4.30 (0.29)
<i>A. trilobata</i>	22.91–27.0 X = 25.20 (1.37)	22.91–26.04 X = 24.65 (1.01)	1.02	<i>Anthemis</i>	3.11–3.63 X = 3.31 (0.21)
<i>Brachanthemum gobicum</i>	33.33–37.5 X = 33.81 (1.17)	32.29–35.41 X = 33.67 (1.01)	1.00	<i>Anthemis</i>	3.62–4.56 X = 4.03 (0.41)
<i>B. kirghisorum</i>	24.80–34.00 X = 29.16 (2.55)	24.80–30.00 X = 27.56 (1.70)	1.05	<i>Anthemis</i>	3.2–4.02 X = 3.65 (0.32)
<i>B. mongolorum</i>	22.91–33.33 X = 29.02 (3.47)	22.91–31.25 X = 28.33 (3.03)	1.02	<i>Anthemis</i>	2.93–4.13 X = 3.56 (0.49)
<i>B. pulvinatum</i>	24.00–26.00 X = 24.9 (0.55)	22.00–25.33 X = 23.27 (0.84)	1.07	<i>Anthemis</i>	2.83–3.2 X = 2.99 (0.13)
<i>Cancrinia discoidea</i>	22.91–27.08 X = 24.16 (1.53)	20.83–25.00 X = 22.63 (1.54)	1.06	<i>Anthemis</i>	2.75–3.18 X = 3.01 (0.17)
<i>C. maximowiczii</i>	23.60–28.65 X = 25.98 (2.62)	20.00–25.07 X = 23.52 (1.56)	1.10	<i>Anthemis</i>	4.02–4.62 X = 4.26 (0.22)
<i>Crossostephium chinense</i>	19.48–27.08 X = 24.13 (3.18)	20.83–27.08 X = 22.87 (2.58)	1.05	<i>Artemisia</i>	–
<i>Dendranthema mongolicum</i>	31.25–35.41 X = 33.60 (0.99)	29.16–35.41 X = 32.01 (2.24)	1.04	<i>Anthemis</i>	4.15–5.71 X = 4.72 (0.64)
<i>D. zawadskii</i>	29.16–35.41 X = 32.42 (2.11)	27.08–33.33 X = 31.45 (2.01)	1.03	<i>Anthemis</i>	4.93–6.49 X = 5.50 (0.59)
<i>Elachanthemum intricatum</i>	22.91–25.00 X = 23.32 (0.95)	20.83–23.95 X = 22.63 (0.91)	1.03	<i>Artemisia</i>	–
<i>Hippolytia alashanensis</i>	24.00–28.80 X = 26.96 (1.55)	23.20–30.00 X = 26.6 (1.83)	1.01	<i>Anthemis</i>	3.73–4.17 X = 3.97 (0.16)
<i>H. trifida</i>	27.20–34.00 X = 30.94 (1.74)	24.80–34.00 X = 29.82 (2.41)	1.03	<i>Anthemis</i>	3.58–4.44 X = 4.07 (0.39)
<i>Kaschgaria komarovii</i>	20.83–22.91 X = 21.80 (0.99)	20.83–25.00 X = 21.94 (1.27)	0.99	<i>Artemisia</i>	–
<i>Poljakovia falcato lobata</i>	25.00–33.33 X = 29.64 (2.11)	20.83–33.33 X = 27.42 (3.96)	1.08	<i>Anthemis</i>	3.27–4.31 X = 3.75 (0.37)
<i>Stilpnolepis centiflora</i>	24.00–28.00 X = 26.00 (0.89)	22.00–26.00 X = 24.00 (1.26)	1.08	<i>Anthemis</i>	2.38–2.83 X = 2.58 (0.21)

Asterisks (\*) indicate different populations of the same species studied (presented in the same order as in Table 1). P, polar axis [range; X, mean values (standard deviation)]; E, equatorial axis [range; X, mean values (standard deviation)]; P/E, sphericity. Spine height: range; X, mean values (standard deviation).





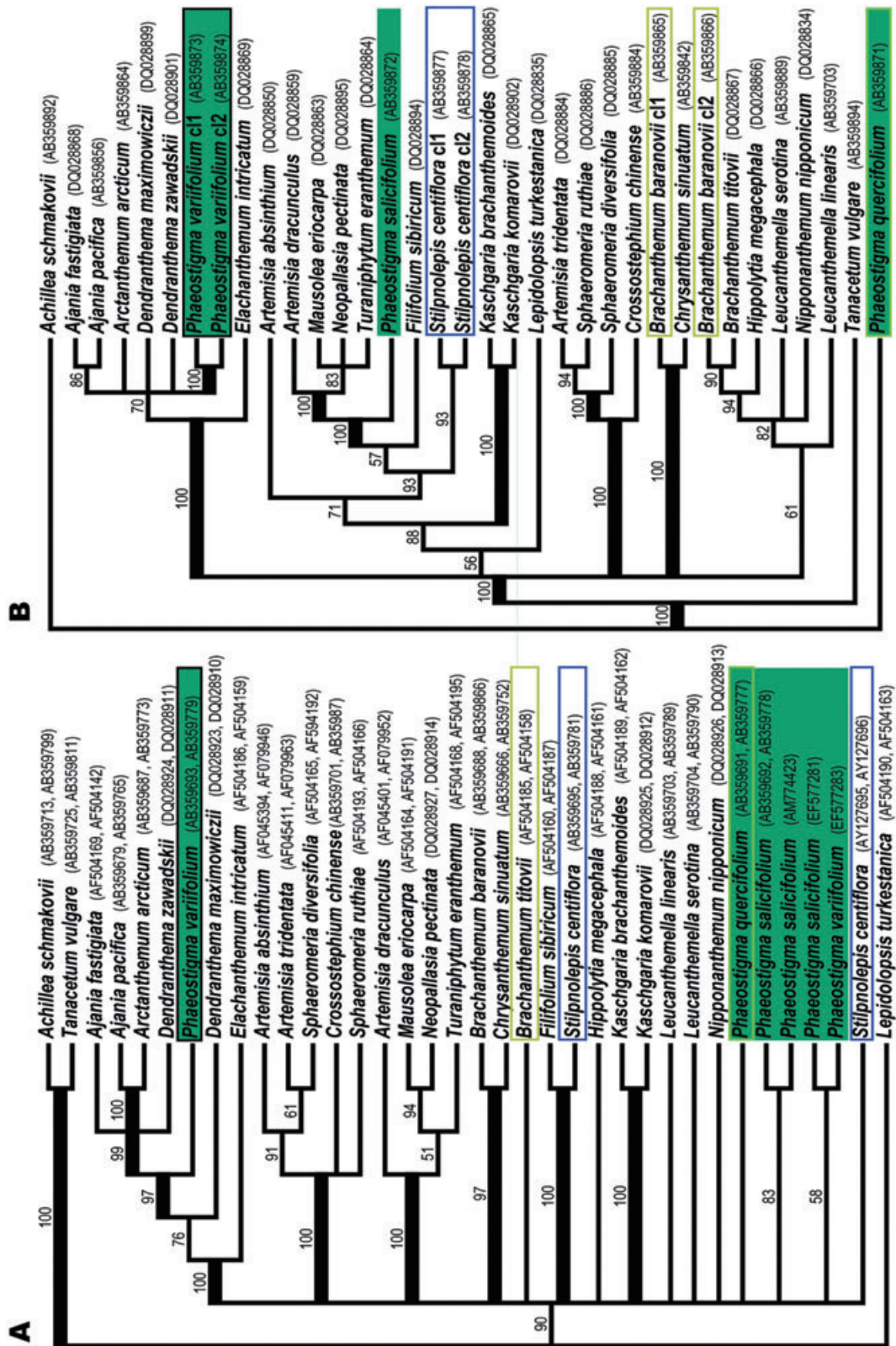
**Figure 1.** Pollen grains of some of the taxa studied at scanning electron microscopy (SEM). A, *Ajania fruticulosa*. B, *A. grubovii*. C, *A. junnanica*. D, *A. pacifica*. E, *A. pacifica* (exine detail from mesocolpium). F, *A. nematoloba*. G, *A. nubigena*. H, *A. roborowskii* (apocolpium). I, *Brachanthemum gobicum*. J, *B. gobicum* (exine detail from mesocolpium). K, *B. kirghisorum*. L, *B. pulvinatum*. M, *Cancrinia discoidea*. N, *C. maximowiczii* (exine detail from apocolpium). O, *Crossostephium chinense*. P, *C. chinense* (exine detail from apocolpium). Q, *Dendranthema zawadskii*. R, *Elachanthemum intricatum*. S, *E. intricatum* (exine detail, colpus). T, *Hippolytia alashanensis*. U, *H. trifida*. V, *Kaschgaria komarovii*. W, *Poljakovia falcatolobata*. X, *Stilpnolepis centiflora*. Scale bar, 5  $\mu\text{m}$ .

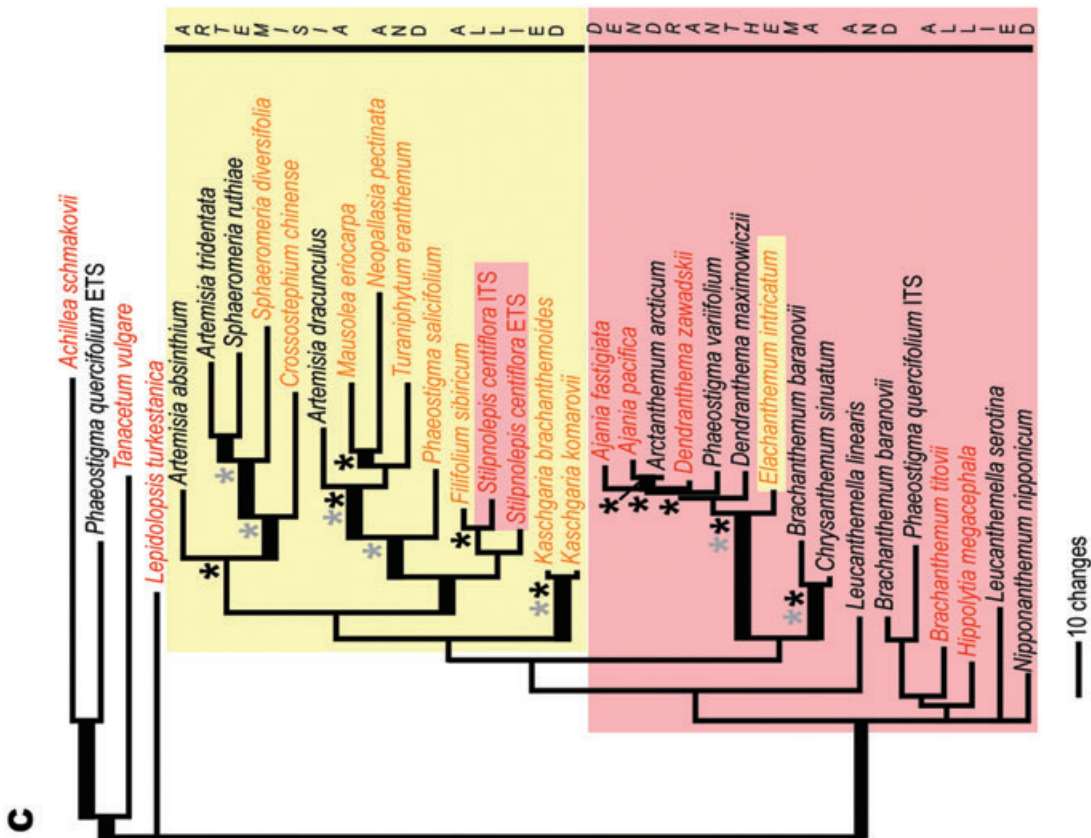


**Figure 2.** Box-and-whisker plots from statistical analyses of some pollen traits vs. pollen types. A, pollen volume. B, spine number. C, polar diameter/equatorial diameter (P/E ratio).

analyses are presented in Fig. 2A–C and from phylogenetic analyses in Fig. 3A–C. The studied pollen grains of Artemisiinae share the following features: they are 3-zonocolporate, isopolar and have radial

symmetry. The surface ornamentation is composed of supra-terctal spines or spinules. Consistently with the previous palynological works (Chen & Zhang, 1991; Martín *et al.*, 2001, 2003 and references therein), the





**Figure 3.** Bayesian phylogenetic inferences. Supported branches (PP  $\geq$  95%) are indicated in bold. PP values and GenBank accessions are provided for the first two trees. A, ITS majority rule consensus with SYM+G model (the GTR+G model was also selected by MrModeltest and gives similar results; data not shown). B, ETS majority rule consensus with GTR+G model. C, Combined ITS and ETS phylogram with GTR+I+G model (the GTR+G model, also selected by MrModeltest, gives comparable results; data not shown). Branches independently supported (PP  $\geq$  95%) by single ETS and ITS analyses involving the restricted taxonomic sampling of combined dataset are indicated on the combined tree with \* (grey) for ETS and \* (black) for ITS. Taxa with known pollen type are written in yellow (pale grey in the print version) for *Artemisia* and in red (dark grey in the print version) for *Anthemis*-type. ETS, external transcribed spacer; ITS, internal transcribed spacer; PP, posterior probability.

pollen observed in the present study can be assigned either to *Anthemis*-type or the *Artemisia*-type.

*ANTHEMIS* POLLEN TYPE (FIG. 1A, B, D–N, Q, T, U, W, X)

The shape is mainly spherical, but frequently slightly prolate and slightly oblate in some cases. Mean spine length ranges from  $1.92 \pm 0.12 \mu\text{m}$  (*Ajania grubovii* Muldashev) to  $5.50 \pm 0.50 \mu\text{m}$  (*Dendranthema zawadskii* (Herbich) Tzvelev), which corresponds to an echinate pollen. The spines are conical, with convex sides, gradually tapering into pointed tips. *Dendranthema* has larger spines than other genera of the group (4.5–5.5  $\mu\text{m}$ ), whereas the maximum spine length found in the remaining genera is 4.4  $\mu\text{m}$ . From one [e.g. *Ajania khartensis* (Dunn) C. Shih, *A. pacifica* (Nakai) K. Bremer & Humphries, *Brachanthemum gobicum* Krasch., *Dendranthema* spp.] to four [e.g. *Ajania aureoglobosa* (W.W. Sm. & Farr.) Muldashev, *A. fastigiata*, *A. fruticulosa*] ornamental elements (spines) are found per  $25 \mu\text{m}^2$  of pollen surface. Volumes vary between 3966.6 [*Ajania parviflora* (Grun.) Ling] and 23 305.1  $\mu\text{m}^3$  [*Ajania pacifica* (Nakai) K. Bremer & Humphries].

*ARTEMISIA* POLLEN TYPE (FIG. 1C, O, P, R, S, V)

The shape is spherical, although in some cases slightly prolate or oblate. The exine is microechinate, with spinules measuring  $< 1 \mu\text{m}$  in height. The spinules are conical, approximately as long as wide (at the base), with blunt tips. Density of ornamental elements range from seven [*Elachanthemum intricatum* (Franch.) Y. Ling & Y. R. Ling, *Phaeostigma salicifolium* (Appendix)] to 44 [*Vesicarpa potentilloides* Rydb. (Appendix)] per  $25 \mu\text{m}^2$  of pollen surface. Four species show a particularly high density of spinules ( $\geq 24$  spinules per  $25 \mu\text{m}^2$  of pollen surface): *Ajaniopsis penicilliformis* C. Shih, *Chamartemisia compacta* Rydb., *Sphaeromeria diversifolia* Rydb. and *Vesicarpa potentilloides* (Appendix). Volumes vary between 2597.1 (*Ajania junnanica*) to 6608.3  $\mu\text{m}^3$  (*Crossos-tephium chinense*).

GENUS *AJANIA* (FIG. 1A–G)

This genus has the general morphological traits described for the *Anthemis* pollen-type [with the exception of *A. junnanica* (Fig. 1C)]. The pollen shape is spheroidal, slightly prolate in most cases and sometimes slightly oblate. Sometimes perforations of the exine appear between the spines (= ornamental elements). The same structures were reported in *Artemisia* (Pragłowski, 1971; Vallès *et al.*, 1987) and they correspond to the microchannels described by

Rowley & Dahl (1977), Rowley, Dahl & Rowley (1981) and Rowley *et al.* (1999) in their ultrastructural study of the exines of *A. vulgaris* L. Pollen volumes vary 5.8-fold from 3966.6  $\mu\text{m}^3$  (*A. parviflora*) to 23 305.1  $\mu\text{m}^3$  (*A. pacifica*), but the shape is quite constant in the species of this genus studied (P/E ratio ranges from 1.00 to 1.12).

DISCUSSION

The comparison of the two pollen types highlights some strong differences. The mean of the *Anthemis* pollen-type volume ( $V_1$ ) is significantly larger (almost twice) than that of the *Artemisia*-type ( $V_2$ ) ( $V_1 = 8961.2 \mu\text{m}^3$ ;  $V_2 = 4574.6 \mu\text{m}^3$ ;  $P < 0.05$ ), with overlapping values between the volumes 3966.6 and 6608.3  $\mu\text{m}^3$  (Fig. 2A). The exine surface sculpture also clearly discriminates between these pollen types, the *Artemisia*-type having much smaller ornamentation elements than the *Anthemis*-type (Appendix) and significantly more abundant (the mean of ornamental elements found per  $25 \mu\text{m}^2$  of pollen surface is 2.36 for *Anthemis* and 14.52 for *Artemisia* pollen types;  $P < 0.05$ ; Fig. 2B). Both size and density of exine ornamentation thus show exclusive values for each pollen type. No difference was found in P/E ratio ( $P > 0.05$ ; Fig. 2C) and the shape of both pollen types is thus quite similar (the mean of P/E values is 1.04 for *Anthemis*, and 1.06 for *Artemisia* pollen types).

DISTRIBUTION OF THE POLLEN TYPES THROUGHOUT ARTEMISIINAE: THE SEGREGATION MOSTLY MAINTAINED

As expected, according to previous work (Sanz *et al.*, 2008), pollen types are segregated in the phylogenetic trees and characterize the two main groups of Artemisiinae, the *Artemisia* and *Dendranthema* groups (Fig. 3C). This confirms their value as phylogenetic markers in the tribe. However, some exceptions to this trend were found.

The molecular evidence places *Elachanthemum intricatum* in the *Dendranthema* group and *Stilpnolepis centiflora* in the *Artemisia* group, whereas these species both have the pollen type of the other group (Fig. 3C). Both belong to monotypic genera, segregated from *Artemisia* (Krascheninnikov, 1946 for *Stilpnolepis*; Ling & Ling, 1978 for *Elachanthemum*). Shih (1985) combined *Elachanthemum* spp. with *Stilpnolepis*. Ling (1987) argued against this, exine ornamentation being one of the most important differential traits; Kubitzki (2007) followed the same criterion as Ling and kept the genera separate, treating pollen type as a good taxonomic character. Apart from these systematic considerations, the lack of agreement of pollen type with phylogenetic placement

in those two genera could constitute the two first cases of reversal in pollen type reported for Artemisiinae. Nevertheless, to confirm the reversal event(s), it would be necessary to discard the hypothesis of pollen-type inheritance through hybridization for these species. The case of *Stilpnolepis* raises particular suspicion, because of its undetermined placement in previous ITS analyses (Watson *et al.*, 2002; Oberprieler *et al.*, 2007; Fig. 3A based on the same accessions AY127695, AY127696). This contrasts with the result involving different ITS accessions (AB359695, AB359781), which shows *Stilpnolepis* as sister to the genus *Filifolium* Kitam. with strong support (PP = 100%, Fig. 3A). Regarding *Elachanthemum*, ETS and ITS data do not provide any evidence of hybrid origin. Both regions strongly support the grouping of this taxon with *Dendranthema* and relatives (100% PP, Fig. 3A, B) in a clade with exclusively *Anthemis*-pollen species, *Elachanthemum* being the only exception (Fig. 3C). However, *Elachanthemum* shows an rDNA organization that is different from the rest of Artemisiinae. Most Artemisiinae have a linked rDNA type with 5S and 35S in the same unit (Garcia *et al.*, 2007, 2009), but the only confirmed exception found to this linkage in the subtribe is the case of *Elachanthemum* with the typical, separate arrangement of 5S and 35S found in most angiosperms. Other genera belonging to the same clade, such as *Ajania* or *Brachanthemum*, show the linked arrangement as found in *Artemisia*; nevertheless, results are still not conclusive for *Dendranthema*, in which it seems that linked and unlinked units may coexist in some species (Abd El-Twab & Kondo, 2006).

Our results also confirm the findings of Muldashev (1983) of an *Artemisia*-type pollen to *Ajania junnanica*, whereas the remaining species of *Ajania* have *Anthemis*-type pollen (Table 2, Appendix). In the molecular phylogenetic analysis, the sequenced *Ajania* group with *Dendranthema* and relatives, in accordance with their pollen affinities (Fig. 3C). No sequence data are available for *A. junnanica* and therefore the phylogenetic placement of this species has not yet been confirmed on a molecular basis. In fact, Muldashev (1983) suggested *A. junnanica* was certainly misplaced in *Ajania* and combined the species in the genus *Artemisia*, stating that it was 'absolutely clear' that, because of its pollen type, this taxon could not remain in *Ajania*. Not considering this trait, Ohashi & Yonekura (2004) combined *Ajania junnanica* in *Chrysanthemum* L., a genus with *Anthemis*-type pollen; those authors merged the complete genera *Ajania*, *Arctanthemum* (Tzvelev) Tzvelev, *Dendranthema* and *Phaeostigma* within *Chrysanthemum*. Bremer & Humphries (1993) also opted for the misplacement of *A. junnanica*, although in a slightly different way than Muldashev (1983). They consid-

ered *Ajania*, or part of the genus, as the sister group of *Artemisia* and allies (those having smooth or short-spined pollen).

*Ajania* shows some variability in pollen traits; it exhibits both pollen types (although the *Artemisia*-type is found only in one species of questioned taxonomic assignment to the genus), the greatest range of spine density (for the *Anthemis*-type) and the smallest and the largest pollen in the tribe. However, the other genera of Artemisiinae have not been as extensively sampled as *Ajania* in the present study and, consequently, we do not know if such diversity is exceptional or the rule in the tribe, or if it reflects the taxonomic heterogeneity of *Ajania*. It can, however, be stated that *Ajania* is basically a genus with *Anthemis*-type pollen grains.

Similar to *Ajania* in some morphological features, but with microechinate pollen grains as a distinctive trait, Shih (1978) described the monospecific genus *Ajaniopsis* and *Artemisia*-type pollen was confirmed in this taxon by Martín *et al.* (2001, 2003). Oberprieler *et al.* (2007) did not assign *Ajaniopsis* to a subtribe within Anthemideae because a molecular framework was lacking for this species, but suggested, on the basis of the results from Martín *et al.* (2003), that its pollen features clearly point to its inclusion in Artemisiinae.

This study also shows up several inconsistencies concerning the genus *Phaeostigma* (as stated in the Introduction, a new genus described in 1981 by Muldashev, made up of three species previously located in *Ajania*). Analysis of the ETS region groups *Phaeostigma quercifolium* (W.W.Sm.) Muldashev with *Achillea schmakovii* Kupr. (Fig. 3B, C) and the ITS region groups it among the early branching genera of Artemisiinae (Fig. 3A). Such a result may suggest a possible hybrid origin for this species, from two species belonging to different subtribes of Anthemideae. ETS firmly locates *Phaeostigma salicifolium* in a clade of the *Artemisia* group (PP = 100%, Fig. 3), in accordance with its *Artemisia*-type pollen (Martín *et al.*, 2003). These results for *P. salicifolium* agree with the assumption of a close relationship between *Phaeostigma* and *Artemisia* (Muldashev, 1982). However, this hypothesis is contradicted by the placement of *Phaeostigma varifolium* (Chang) Muldashev within the *Dendranthema* group, which is supported by both ETS and ITS markers (PP = 100%, Fig. 3). Therefore, the phylogenetic affinities of *Phaeostigma* remain unresolved and, furthermore, the monophyly of the genus could be questionable.

Our results highlight some inconsistencies between pollen types and taxonomic groups. However, evidence for possible pollen type reversals in Artemisiinae is still lacking, none of the cases considered above establishing such an event beyond doubt.

## EVOLUTIONARY TRENDS ON POLLEN FEATURES IN ARTEMISIINAE

The unsupported basal-most nodes of the ingroup impede the determination of the ancestral character state for the Artemisiinae pollen type (Fig. 3). Nevertheless, the fact that the species of the outgroup (and most of the tribe) show the *Anthemis* pollen type makes this the most likely option for the ancestral state, an assumption also supported by the palaeogeological record (Wang, 2004). According to this hypothesis, the main tendency in the subtribe would be toward the reduction of global size and size ornamentation of pollen.

Several factors implicated in pollen downsizing events are found in the literature, such as a shift to an annual life cycle (or more generally to shorter growth cycles), autogamy or an adaptation to extreme environmental conditions (Hidalgo *et al.*, 2008a, b and references therein). However, none of these factors seems to account for the pollen type distribution pattern observed in Artemisiinae. In fact, the characteristics shown by the two Artemisiinae pollen types fit with the two main pollination syndromes: (1) larger, heavily ornamented pollen grains, such as the *Anthemis*-type, with much pollen-kitt making the pollen sticky, being more likely related to entomophily; and (2) smaller (with also reduced size range variation), less ornamented pollen, as in the *Artemisia*-type, with almost no pollen-kitt making the pollen dry, more likely related to anemophily (Wodehouse, 1935; Friedman & Barrett, 2009). The pollination syndrome is also expressed in terms of floral and inflorescence features, with larger, showy structures found in insect pollinated plants and smaller, non-showy structures in wind pollinated plants (Friedman & Barrett, 2009). This trend agrees well in Artemisiinae with small, greenish or whitish capitula generally displayed by taxa with *Artemisia*-type pollen and radiate capitula (e.g. *Dendranthema*), coloured capitula (e.g. *Ajania pacifica*) or corymbose capitula (e.g. *Stilpnolepis*) in taxa showing *Anthemis*-type pollen. Therefore, a shift in pollination, from entomophily to anemophily may account for the change from *Anthemis* to *Artemisia* pollen type. Following this assumption, insect pollination would probably be the ancestral state in Artemisiinae, as it is for the whole of Asteraceae. The main apomorphy of the family, the capitulum, is basically designed to draw attention to the display, by making the flowers more noticeable to the pollinator. Asteraceae are mostly pollinated by animals and the few wind-pollinated representatives of the family are exceptions. These are the *Artemisia* group of our present study, the genus *Ambrosia* (Heliantheae) and some species of *Espeletia* (Millerieae/Heliantheae *s.l.*; Jeffrey, 2007).

There is, however, one species of Artemisiinae which has pollen and inflorescence characters pointing to different pollination syndromes, *Ajaniopsis penicilliformis*, with *Artemisia* pollen and showy capitula. In addition, several taxa have inflorescences not clearly attributable to one pollination type: *Crossostephium* and *Filifolium* (both with *Artemisia* pollen) and *Brachanthemum* and *Stilpnolepis* (both with *Anthemis* pollen). Such a pattern could indicate mixed pollination. Frequent insect visits have also been reported in different species of *Artemisia*, suggesting that entomophily could be involved to a certain degree, even in species showing the anemophilous syndrome (Garnock-Jones, 1986; Vallès, 1989). Some of these cases of incongruent pollen and inflorescence trends may also indicate that secondary shifts in pollination types are ongoing processes. This occurred in the genus *Espeletia*, another member of Asteraceae in which a shift from animal to wind pollination has taken place. In this case, the typical reduction of the spine size accompanying anemophily did not immediately follow the shift in pollination type, and was only observed in the more derived species (Rundel, Smith & Meinzer, 1994 and references therein).

Some other tendencies have been described in the group. One concerns polyploidy, occurring in *Ajania* and considered as one of the main evolutionary factors in plants (Otto & Whitton, 2000 and references therein). This maybe relevant in interpreting the data set analysed here, in that ploidy may express itself directly through pollen size (Muller, 1979; Julià & Martín, 1994). This trend cannot be confirmed, however, in *Ajania*. In fact, the largest and the smallest pollen were found in high polyploid species [*A. pacifica*,  $2n = 90$ , and *A. nematoloba* (Hand.-Mazz.) Ling ex C. Shih,  $2n = 72$ , respectively]. Nevertheless, the relationship between pollen size and ploidy is known to be easily overridden by other factors and is evolutionarily short-lived (Muller, 1979; Tate & Simpson, 2004). Another point concerns the group of taxa with the *Artemisia*-type pollen that have a particular high density of ornamental elements (see Results), which are all distributed in North America with the exception of *Ajaniopsis*. In fact, some of the species with large numbers of spinules (e.g. *Chamartemisia compacta*, *Vesicarpa potentilloides*) are nowadays labelled under a single genus, *Sphaeromeria*. Thus, the presence of a high density of spinules in the group might more likely reflect a close relationship rather than a parallel adaptation to particular environmental conditions. Additionally, pollen size, spine length and spine density are probably linked characters. As observed, the *Anthemis* pollen type is larger, with larger spines at a lower density than the *Artemisia* type and Wodehouse (1935)

already pointed out this relationship. In a recent article by Schols *et al.* (2005), a similar linkage was found between perforation size, perforation density and pollen size in *Dioscorea*: a high perforation density (restricted to some specific groups in this genus) was related to a smaller size and small and dense perforations.

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## APPENDIX

Species	Chrom. number	P min. (µm)	P max. (µm)	P (µm)	E min. (µm)	E max. (µm)	E (µm)	P/E	Pollen type	Spine height (µm)	No. of spines (25 µm <sup>2</sup> )	V (µm <sup>3</sup> )
<i>Ajania achilleoides</i>	18	22.91	27.08	24.90 ± 1.33	20.83	27.08	23.74 ± 1.63	1.04	Anthemis	3.60 ± 0.40	3	7 347.83
<i>Ajania achilleoides</i> 75	18	20.83	27.08	22.90 ± 1.92	17.70	25.00	19.64 ± 2.29	1.03	Anthemis	2.90 ± 0.16	3	4 625.06
<i>Ajania achilleoides</i> 76	18	20.83	25.00	22.91 ± 1.30	18.75	22.91	21.93 ± 1.20	1.04	Anthemis	2.80 ± 0.15	3	5 769.01
<i>Ajania aureoglobosa</i>	18	17.79	25.00	22.46 ± 1.88	20.83	22.91	21.70 ± 1.11	1.03	Anthemis	2.96 ± 0.10	4	5 537.68
<i>Ajania fastigiata</i>	18	22.91	31.25	26.31 ± 2.17	22.91	31.25	25.41 ± 1.92	1.03	Anthemis	3.83 ± 0.33	4	8 894.65
<b><i>Ajania fastigiata</i></b>	18	<b>20.00</b>	<b>25.00</b>	<b>22.60 ± 1.34</b>	<b>20.00</b>	<b>26.00</b>	<b>22.2 ± 1.46</b>	<b>1.02</b>	<b>Anthemis</b>	<b>2.90 ± 0.20</b>	<b>3</b>	<b>5 831.94</b>
<i>Ajania fruticulosa</i>	36	20.83	29.16	25.41 ± 2.18	18.75	26.04	23.81 ± 1.96	1.06	Anthemis	3.57 ± 0.70	2	7 542.62
<i>Ajania fruticulosa</i> 57	36	20.83	33.33	25.64 ± 4.41	20.83	28.12	23.34 ± 2.89	1.09	Anthemis	3.91 ± 0.10	4	7 313.38
<i>Ajania fruticulosa</i> 68	36	22.91	27.01	25.27 ± 1.07	22.91	29.16	25.20 ± 1.58	1.00	Anthemis	3.75 ± 0.41	2	8 202.43
<b><i>Ajania fruticulosa</i></b>	36	<b>20.00</b>	<b>25.00</b>	<b>22.6 ± 1.34</b>	<b>14.00</b>	<b>24.00</b>	<b>20.13 ± 2.62</b>	<b>1.12</b>	<b>Anthemis</b>	<b>2.90 ± 0.18</b>	<b>4</b>	<b>4 795.07</b>
<i>Ajania gracilis</i>		21.87	28.12	24.38 ± 1.73	20.83	25.00	22.65 ± 1.42	1.07	Anthemis	2.30 ± 0.34	3	6 548.91
<i>Ajania grubovii</i>		25	29.16	26.94 ± 1.51	22.91	29.16	25.20 ± 1.60	1.06	Anthemis	1.92 ± 0.12	3	8 957.72
<i>Ajania junnanica</i>		14.58	20.83	18.33 ± 2.31	12.50	18.75	16.45 ± 2.68	1.11	Artemisia		11	2 597.13
<i>Ajania khatanica</i>	54	22.91	29.16	24.85 ± 1.61	20.83	27.08	24.30 ± 1.79	1.02	Anthemis	2.92 ± 0.12	1	7 683.12
<i>Ajania myriantha</i>		25	31.25	26.73 ± 1.87	22.91	27.08	25.55 ± 1.17	1.04	Anthemis	3.68 ± 0.28	2	9 136.49
<i>Ajania nana</i>	18, 36	20.83	27.08	23.88 ± 1.40	20.83	26.04	23.67 ± 1.39	1.00	Anthemis	2.20 ± 0.09	3	7 005.34
<i>Ajania nematoloba</i>		20.83	31.25	24.51 ± 2.66	20.83	26.04	23.39 ± 1.46	1.04	Anthemis	3.39 ± 0.39	3	7 021.05
<i>Ajania nubigena</i>	72	14.58	23.95	21.03 ± 2.36	14.58	21.87	19.64 ± 2.29	1.07	Anthemis	2.10 ± 0.04	3	4 247.38
<i>Ajania pacifica</i>	90	35.41	41.66	38.39 ± 2.11	33.33	40.62	34.05 ± 8.37	1.12	Anthemis	3.54 ± 0.45	2	10 710.12
<i>Ajania pallasiana</i>	36	20.83	33.33	27.56 ± 4.07	20.83	33.33	26.80 ± 3.57	1.02	Anthemis	4.40 ± 0.43	1	23 305.10
<i>Ajania parvisflora</i> 47		16	24.00	19.72 ± 2.09	18.00	22.00	19.6 ± 1.20	1	Anthemis	4.03 ± 0.35	2	10 364.48
<i>Ajania potaninii</i>		21.87	25.00	23.18 ± 1.21	18.75	25.00	21.94 ± 2.06	1.05	Anthemis	3.67 ± 0.22	2	3 966.59
<i>Ajania przewalskii</i>	18, 36	22.91	25.00	23.60 ± 0.85	17.70	25.00	22.28 ± 1.79	1.05	Anthemis	2.84 ± 0.17	2	5 842.32
<i>Ajania cf. purpurea</i>		25	29.16	25.62 ± 1.23	22.91	29.16	24.64 ± 1.69	1.03	Anthemis	3.26 ± 0.34	3	6 133.96
<i>Ajania remotipinna</i>		22.91	29.16	25.62 ± 1.56	20.83	28.12	24.99 ± 1.71	1.02	Anthemis	2.87 ± 0.22	2	8 144.40
<i>Ajania roborowskii</i>		22.91	31.25	26.31 ± 2.85	18.75	31.25	24.16 ± 3.03	1.08	Anthemis	3.96 ± 0.17	2	8 377.42
<i>Ajania rupestris</i> HBB	18	22.91	28.12	24.37 ± 1.61	22.91	27.08	24.02 ± 1.44	1.01	Anthemis	2.63 ± 0.07	3	8 041.06
<i>Ajania rupestris</i> RUS	18	22.91	29.16	26.31 ± 1.98	20.83	29.16	24.85 ± 2.29	1.05	Anthemis	3.30 ± 0.29		7 362.07
<i>Ajania scharnhorstii</i> HBB	18	22.91	35.41	26.38 ± 3.09	20.83	27.08	24.16 ± 2.16	1.09	Anthemis	3.3 ± 0.23		8 506.92
<i>Ajania scharnhorstii</i> RUS	18	22.91	31.25	26.31 ± 2.37	20.83	29.16	23.95 ± 2.55	1.09	Anthemis	3.21 ± 0.43		8 062.45
<i>Ajania tibetica</i>	18	25	29.16	25.69 ± 1.28	20.83	27.08	23.60 ± 1.70	1.08	Anthemis	3.08 ± 0.31		7 901.88
<i>Ajania trilobata</i>		22.91	27.08	25.20 ± 1.37	22.91	26.04	24.65 ± 1.01	1.02	Anthemis	4.30 ± 0.29	2	7 941.81
<b><i>Ajaniopsis penicilliformis</i></b>	18	<b>19.00</b>	<b>26.00</b>	<b>22.88 ± 2.43</b>	<b>18.00</b>	<b>24.00</b>	<b>21.28 ± 1.74</b>	<b>1.04</b>	<b>Artemisia</b>	<b>3.31 ± 0.21</b>	<b>39</b>	<b>5 424.98</b>
<b><i>Arctanthemum hultenii</i></b>	<b>16, 32</b>	<b>24.00</b>	<b>34.00</b>	<b>29.32 ± 2.36</b>	<b>22.00</b>	<b>33.00</b>	<b>27.76 ± 2.54</b>	<b>1.05</b>	<b>Anthemis</b>	<b>3.80 ± 0.42</b>	<b>2</b>	<b>11 830.46</b>
<b><i>Artemisia vulgaris</i></b>	<b>16, 32</b>	<b>18.60</b>	<b>25.80</b>	<b>22.80 ± 0.49</b>	<b>21.40</b>	<b>25.80</b>	<b>21.40 ± 1.05</b>	<b>1.07</b>	<b>Artemisia</b>	<b>5.467.15</b>	<b>12</b>	<b>5 467.15</b>
<b><i>Artemisia incana</i></b>	<b>16</b>	<b>20.00</b>	<b>28.00</b>	<b>22.86 ± 1.96</b>	<b>19.00</b>	<b>25.00</b>	<b>21.66 ± 1.73</b>	<b>1.05</b>	<b>Artemisia</b>	<b>5.615.54</b>	<b>12</b>	<b>5 615.54</b>
<b><i>Artemisiastrum palmerii</i></b>	<b>18</b>	<b>18.00</b>	<b>24.00</b>	<b>20.84 ± 1.40</b>	<b>16.00</b>	<b>24.00</b>	<b>20.12 ± 1.14</b>	<b>1.03</b>	<b>Artemisia</b>	<b>4.417.25</b>	<b>17</b>	<b>4 417.25</b>

<b>18</b>	<b><i>Artemisiella stracheyi</i></b>	<b>20.00</b>	<b>28.00</b>	<b>24.04 ± 2.40</b>	<b>19.00</b>	<b>28.00</b>	<b>22.08 ± 2.27</b>	<b>1.09</b>	<b>Artemisia</b>	<b>8</b>	<b>6 136.65</b>
	<i>Brachanthemum gobicum</i>	33.33	37.50	33.81 ± 1.17	32.29	35.41	33.67 ± 1.01	1.00	<i>Anthemis</i>	1	20 069.20
	<i>Brachanthemum kirghissorum</i>	24.80	34.00	29.16 ± 2.55	24.80	30.00	27.56 ± 1.70	1.05	<i>Anthemis</i>	2	11 596.97
	<i>Brachanthemum mongolorum</i>	22.91	33.33	29.02 ± 3.47	22.91	31.25	28.33 ± 3.03	1.02	<i>Anthemis</i>	2	12 195.21
18	<i>Brachanthemum pulvinatum</i>	24.00	30.00	24.90 ± 0.55	22.00	25.33	23.27 ± 0.84	1.07	<i>Anthemis</i>	3	7 059.77
<b>18</b>	<b><i>Brachanthemum titovii</i></b>	<b>24.00</b>	<b>26.00</b>	<b>26.46 ± 1.92</b>	<b>22.00</b>	<b>28.00</b>	<b>25.26 ± 1.94</b>	<b>1.04</b>	<b>Artemisia</b>	<b>2</b>	<b>8 840.06</b>
14	<i>Cancerina discoidea</i>	22.91	27.08	24.16 ± 1.53	20.83	25.00	22.63 ± 1.54	1.06	<i>Anthemis</i>	2	6 478.35
	<i>Cancerina maximoviczii</i>	23.60	28.65	25.98 ± 2.62	20.00	25.07	23.52 ± 1.56	1.1	<i>Anthemis</i>	2	7 525.10
	<b><i>Chamartemisia compacta</i></b>	<b>18.00</b>	<b>24.00</b>	<b>19.88 ± 2.14</b>	<b>17.00</b>	<b>24.00</b>	<b>19.28 ± 1.84</b>	<b>1.03</b>	<b>Artemisia</b>	<b>30</b>	<b>3 869.27</b>
	<b><i>Crossostephium artemisioides</i></b>	<b>17.00</b>	<b>24.00</b>	<b>19.92 ± 1.29</b>	<b>16.00</b>	<b>22.00</b>	<b>18.56 ± 1.55</b>	<b>1.07</b>	<b>Artemisia</b>	<b>15</b>	<b>3 592.89</b>
18	<i>Crossostephium chinense</i>	19.48	27.08	24.13 ± 3.18	20.83	27.08	22.87 ± 2.58	1.05	<i>Artemisia</i>	13	6 608.28
<b>18, 36,</b>	<b><i>Dendranthema indicum</i></b>	<b>24.00</b>	<b>28.00</b>	<b>26.66 ± 2.30</b>	<b>23.00</b>	<b>26.00</b>	<b>24.33 ± 2.59</b>	<b>1.05</b>	<b>Artemisia</b>	<b>1</b>	<b>8 263.10</b>
54											
ca. 72	<i>Dendranthema mongolicum</i>	31.25	35.41	33.60 ± 0.99	29.16	35.41	32.01 ± 2.24	1.04	<i>Anthemis</i>	1	18 026.41
54	<i>Dendranthema zawadskii</i>	29.16	35.41	32.42 ± 2.11	27.08	33.33	31.45 ± 2.01	0.99	<i>Anthemis</i>	1	16 790.09
	<b><i>Elacanthemum imbricatum</i></b>	<b>16.00</b>	<b>22.00</b>	<b>19.84 ± 1.66</b>	<b>13.00</b>	<b>20.00</b>	<b>19.69 ± 2.09</b>	<b>1.12</b>	<b>Artemisia</b>	<b>10</b>	<b>4 027.46</b>
	<i>Elacanthemum imbricatum</i>	22.91	25.00	23.32 ± 0.95	20.83	23.95	22.63 ± 0.91	1.03	<i>Artemisia</i>	7	6 253.11
<b>18</b>	<b><i>Filifolium sibiricum</i></b>	<b>20.00</b>	<b>24.00</b>	<b>22.04 ± 1.58</b>	<b>16.00</b>	<b>24.00</b>	<b>20.48 ± 1.98</b>	<b>1.03</b>	<b>Artemisia</b>	<b>9</b>	<b>4 840.28</b>
	<i>Hippolytia alashanensis</i>	24.00	28.80	26.96 ± 1.55	23.20	30.00	26.60 ± 1.83	1.01	<i>Anthemis</i>	2	9 988.07
	<i>Hippolytia trifida</i>	27.20	34.00	30.94 ± 1.74	24.80	34.00	29.82 ± 2.41	1.03	<i>Anthemis</i>	1	14 405.69
	<b><i>Hippolytia megacephala</i></b>	<b>21.00</b>	<b>26.00</b>	<b>23.20 ± 1.25</b>	<b>16.00</b>	<b>24.00</b>	<b>19.80 ± 2.16</b>	<b>1.17</b>	<b>Artemisia</b>	<b>2</b>	<b>4 762.30</b>
	<b><i>Hulteniella integrifolia</i></b>	<b>24.00</b>	<b>34.00</b>	<b>20.78 ± 2.68</b>	<b>22.00</b>	<b>28.00</b>	<b>25.00 ± 1.49</b>	<b>1.11</b>	<b>Artemisia</b>	<b>2</b>	<b>6 800.24</b>
	<b><i>Kaschgaria komarovii</i></b>	<b>20.00</b>	<b>26.00</b>	<b>22.28 ± 1.61</b>	<b>18.00</b>	<b>24.00</b>	<b>21.40 ± 1.35</b>	<b>1.04</b>	<b>Artemisia</b>	<b>10</b>	<b>5 342.46</b>
	<i>Kaschgaria komarovii</i>	20.83	22.91	21.80 ± 0.99	20.83	35.00	21.94 ± 1.27	0.99	<i>Artemisia</i>	8	5 494.50
<b>36</b>	<b><i>Kaschgaria brachantemoides</i></b>	<b>20.00</b>	<b>26.00</b>	<b>23.46 ± 1.45</b>	<b>18.00</b>	<b>23.00</b>	<b>20.60 ± 1.49</b>	<b>1.13</b>	<b>Artemisia</b>	<b>9</b>	<b>5 212.68</b>
	<b><i>Lepidolopsis turkestanica</i></b>	<b>20.00</b>	<b>24.40</b>	<b>22.76 ± 1.21</b>	<b>17.00</b>	<b>24.00</b>	<b>20.93 ± 1.80</b>	<b>1.08</b>	<b>Artemisia</b>	<b>3</b>	<b>5 220.47</b>
<b>36</b>	<b><i>Mausolea ertocarpa</i></b>	<b>22.00</b>	<b>29.00</b>	<b>24.26 ± 1.76</b>	<b>14.00</b>	<b>23.00</b>	<b>19.40 ± 2.47</b>	<b>1.25</b>	<b>Artemisia</b>	<b>15</b>	<b>4 780.72</b>
<b>18, 36</b>	<b><i>Neopallasia pectinata</i></b>	<b>18.00</b>	<b>22.00</b>	<b>20.01 ± 1.95</b>	<b>17.00</b>	<b>21.00</b>	<b>20.00 ± 1.36</b>	<b>1.00</b>	<b>Artemisia</b>	<b>9</b>	<b>4 190.88</b>
	<b><i>Phaeostigma salicifolium</i></b>	<b>16.00</b>	<b>24.00</b>	<b>20.56 ± 1.83</b>	<b>13.00</b>	<b>22.00</b>	<b>19.12 ± 2.23</b>	<b>1.07</b>	<b>Artemisia</b>	<b>7</b>	<b>3 935.48</b>
	<b><i>Picrothamnus desertorum</i></b>	<b>18.00</b>	<b>24.00</b>	<b>21.20 ± 1.78</b>	<b>17.00</b>	<b>25.00</b>	<b>19.60 ± 1.57</b>	<b>1.08</b>	<b>Artemisia</b>	<b>17</b>	<b>4 264.29</b>
	<i>Poljakovia falcatotlobata</i>	25.00	33.33	29.64 ± 2.11	20.83	33.33	27.42 ± 3.96	1.08	<i>Anthemis</i>		11 668.41
	<b><i>Sphaeromeria diversifolia</i></b>	<b>17.30</b>	<b>19.40</b>	<b>18.82 ± 0.84</b>	<b>21.40</b>	<b>25.80</b>	<b>17.60 ± 1.17</b>	<b>1.06</b>	<b>Artemisia</b>	<b>24</b>	<b>3 052.41</b>
	<b><i>Stilpnolepis centiflora</i></b>	<b>27.00</b>	<b>34.00</b>	<b>29.28 ± 2.68</b>	<b>25.00</b>	<b>30.00</b>	<b>27.28 ± 1.52</b>	<b>1.07</b>	<b>Artemisia</b>	<b>2</b>	<b>11 409.28</b>
	<i>Stilpnolepis centiflora</i>	24.00	28.00	26.00 ± 0.89	22.00	26.00	24.00 ± 1.26	1.08	<i>Anthemis</i>	3	7 841.41
<b>18</b>	<b><i>Tanacetum vulgare</i></b>	<b>20.00</b>	<b>28.00</b>	<b>24.33 ± 2.14</b>	<b>18.00</b>	<b>26.00</b>	<b>22.13 ± 2.21</b>	<b>1.09</b>	<b>Artemisia</b>	<b>3</b>	<b>6 238.84</b>
	<b><i>Tridactylina kirilovii</i></b>	<b>24.00</b>	<b>30.00</b>	<b>26.08 ± 1.62</b>	<b>22.00</b>	<b>30.00</b>	<b>24.40 ± 1.85</b>	<b>1.06</b>	<b>Artemisia</b>	<b>2</b>	<b>8 129.91</b>
	<b><i>Turaniphytum condrongtoni</i></b>	<b>18.00</b>	<b>23.00</b>	<b>19.92 ± 1.16</b>	<b>17.00</b>	<b>22.00</b>	<b>18.80 ± 1.74</b>	<b>1.05</b>	<b>Artemisia</b>	<b>13</b>	<b>3 686.41</b>
<b>18</b>	<b><i>Turaniphytum eranthemum</i></b>	<b>16.00</b>	<b>22.00</b>	<b>18.80 ± 1.64</b>	<b>14.00</b>	<b>20.00</b>	<b>16.93 ± 1.98</b>	<b>1.11</b>	<b>Artemisia</b>	<b>17</b>	<b>2 821.44</b>
	<b><i>Vesicarpa potentilloides</i></b>	<b>19.00</b>	<b>22.00</b>	<b>20.84 ± 1.12</b>	<b>17.00</b>	<b>22.00</b>	<b>20.16 ± 1.59</b>	<b>1.04</b>	<b>Artemisia</b>	<b>44</b>	<b>4 434.83</b>

Data from species in bold have been extracted from Martín *et al.* (2001, 2003).