

Pollen morphology and its phylogenetic significance in tribe Sanguisorbeae (Rosaceae)

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Abstract Pollen grains of tribe Sanguisorbeae (Rosaceae, Rosoideae) were examined using scanning electron microscopy to identify useful characters, test taxonomic and phylogenetic hypotheses among genera, and elucidate pollen character evolution based on a molecular phylogeny. Aperture number, aperture structure, pollen shape, and exine sculpturing were variable within Sanguisorbeae and were used to delineate six pollen types. Four types (I–IV) were observed only in subtribe Sanguisorbinae whereas two types (V–VI) were found only in subtribe Agrimoniinae. Pollen grains of tribe Sanguisorbeae were generally subprolate to spheroidal in shape, had operculate or pont-operculate apertures, and had three apertures, except for *Margyricarpus* (tetraperturate). Exine sculpturing within Sanguisorbinae represented variations of striate, verrucate, rugulate, and perforate patterns often with microechinate sculpturing. Striate exine patterns and prolate shapes characterized the pollen of the Agrimoniinae, except for the microechinate-verrucate pattern and subprolate to spheroidal shapes observed in *Hagenia*. Pollen characters are most useful at the generic level and, when mapped on to a molecular phylogenetic tree of the tribe, are concordant with a monophyletic Agrimoniinae and a clade comprising *Margyricarpus* + *Acaena* + *Polylepis* + *Cliffortia* + *Sanguisorba* in the Sanguisorbinae. Outgroup

comparison indicated that operculate colpi, three apertures, and polymorphism for striate or microverrucate exines represented primitive states for tribe Sanguisorbeae.

Keywords Agrimoniinae · Palynology · Phylogeny · Sanguisorbeae · Sanguisorbinae

Introduction

Comparative studies of pollen morphology have provided useful characters for delimiting genera and species and resolving relationships in several lineages of Rosaceae (Fogle 1977a, b; Eide 1981; Hebda et al. 1991; Hebda and Chinnappa 1994; Pérez De Paz 2004). Using light microscopy (LM), Reitsma (1966) identified 11 pollen types among 18 European rosaceous genera based on variation in size, aperture, and exine morphology. Similarly, Naruhashi and Toyoshima (1979) differentiated pollen using LM characters among 32 Japanese genera. Within tribe Sanguisorbeae, Reitsma (1967) detected polymorphism in aperture number (3 or 6) and operculum presence in *Sanguisorba* L. s.l., although he could not resolve fine-scale exine sculpturing patterns.

Recent studies in the family using scanning electron microscopy (SEM) revealed a range of exine sculpturing patterns (Eide 1981; Hebda et al. 1988a, b, 1991; Hebda and Chinnappa 1990a, b) that were informative for resolving phylogenetic relationships (Wen and Nowicke 1999; Cooper et al. 2000) and for delimiting genera and/or species. Fogle (1977a, b) demonstrated species-specific variation in pollen size and exine sculpturing among the fruit-crop genera *Malus* Mill. (apples), *Prunus* L. (peaches and cherries), and *Pyrus* L. (pears). Maas (1977) observed that exine ridge (muris) width of striate pollen

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corresponded to ploidy levels among species of *Fragaria* L. (strawberry).

Previous SEM examinations of rosaceous pollen grains have generally sampled within geopolitical regions (Byatt 1976; Eide 1981; Hebda et al. 1991) and have not employed a clade-based sampling strategy. Eide (1981) demonstrated that 18 genera in northwest Europe were 3-colporate with or without an operculum and that each genus could be identified by exine sculpturing. In a series of investigations among genera in western Canada, Hebda et al. (1988a, b, 1991) and Hebda and Chinnappa (1990a, b) identified variation among exine sculpturing characters, which resulted in proposals to revise taxonomic and phylogenetic relationships. For example, Hebda and Chinnappa (1990a) postulated that *Aruncus* L., *Fragaria*, *Geum* L., *Potentilla* L., and *Sibbaldia* L. had microperforate pollen grains that were a distinct lineage from 14 genera with pollen exhibiting “microperforate/tectal ridges”. The five genera with microperforate pollen were treated previously in three tribes: Potentilleae (*Fragaria*, *Potentilla*), Dryadeae (*Geum*), and Spiraeaceae (*Aruncus*, *Sibbaldia*) (Schulze-Menz 1964; Kalkman 1988). Based on pollen morphology, Hebda and Chinnappa (1994) suggested an alternate arrangement of genera in tribes Dryadeae, Potentilleae, and Sanguisorbeae compared with that proposed by Kalkman (1988). They concluded that genera formerly placed in Dryadeae (*Coluria* R. Br., *Fallugia* Endl., *Geum*, *Orthurus* Juz., and *Waldsteinia* Willd.) with striate microperforate pollen should be transferred to the Potentilleae.

Among the 12–15 genera commonly recognized in the Sanguisorbeae (Hutchinson 1964; Kalkman 1988), only a few have had more than one species examined for variation in pollen morphology. Hebda and Chinnappa (1994) proposed that genera in the tribe sharing verrucate/rugulate/microverrucate exine sculpturing (*Acaena* Mutis ex L., *Cliffortia* L., *Hagenia* Moench, *Leucosidea* Eckl. & Zeyh., *Margyricarpus* Ruiz & Pav., *Polylepis* Ruiz & Pav.) represented a distinct evolutionary lineage from genera sharing striate/microperforate pollen (*Agrimonia* L., *Aremonia* Neck., *Spenceria* Trimen). In her LM and SEM examination of pollen variation among Macaronesian genera in the Sanguisorbeae, Pérez De Paz (2004) focused on species in subtribe Sanguisorbinae (eight of ten genera examined), but also examined *Hagenia* and *Leucosidea* from subtribe Agrimoniinae. She (Pérez De Paz 2004) found that the woody, dioecious genera *Bencomia* Webb & Berthel. and *Sarcopoterium* Spach (placed in the “*Sanguisorba* group” by Nordborg 1966) and two genera segregated from *Sanguisorba* (*Dendriopoterium* Svent. and *Marcetella* Svent.) shared microverrucate exine sculpturing. Pérez De Paz (2004) also portrayed representative pollen grains on an ITS-based phylogeny of Macaronesian species conducted by Helfgott et al. (2000).

We initiated our investigation of pollen morphology and its phylogenetic significance in the Sanguisorbeae by employing a broader sampling strategy especially of subtribe Agrimoniinae and by examining variation among pollen characters with reference to recent phylogenetic analyses of the family, subfamily, and tribe. Our primary objectives were to:

- 1 describe pollen morphology and identify useful pollen characters;
- 2 test taxonomic and phylogenetic hypotheses among genera; and
- 3 elucidate pollen character evolution by mapping states on to the consensus phylogenetic tree of Potter et al. (2007).

Materials and methods

Pollen grains from 25 species representing ten genera in Sanguisorbeae were sampled: *Acaena* (one species), *Agrimonia* (13), *Aremonia* (1), *Hagenia* (1), *Leucosidea* (1), *Margyricarpus* (1), *Polylepis* (2), *Poterium* (2), *Sanguisorba* (2) and *Spenceria* (1). Species from *Bencomia*, *Cliffortia*, *Dendriopoterium*, *Macetella*, *Poteridium* Spach, and *Sarcopoterium* in subtribe Sanguisorbinae were not sampled, although pollen data and SEM micrographs for these genera have been presented in Pérez De Paz (2004). *Alchemilla* L. was not included in this investigation, because phylogenetic investigations indicated that it was best placed within Potentilleae (Eriksson et al. 2003). To examine pollen morphology in relation to sister and out-group taxa, 13 representative species from *Dryas* L. (one species) and *Geum* (three species) in Dryadeae and *Fragaria* (two species) and *Potentilla* (seven species) in Potentilleae also were sampled. Specimen data for the species examined are presented in Table 1.

Pollen grains were removed from anthers on herbarium specimens, processed by acetolysis (Erdtman 1960), and prepared for examination with the SEM as reported by Chissoe et al. (1994, 1995), Chissoe and Skvarla (1996), and Skvarla et al. (2005). Samples were examined with a Jeol 880 SEM equipped with a lanthanum hexaboride gun. Digital images were captured and processed for publication using Adobe® Photoshop® 8.0. Description of pollen morphology followed Moore et al. (1991) and Punt et al. (1994).

Major morphological features of pollen grains were observed for each species and were analyzed to describe and categorize pollen types. To evaluate character evolution, pollen types and variable character states were mapped on to the strict consensus tree of 12 genera in tribe Sanguisorbeae provided in Potter et al. (2007). Except for

Table 1 Collection data of species examined in this study

<i>Acaena</i>	<i>Acaena elongata</i> L.; Costa Rica, R. Lent 136 (OKL)
<i>Agrimonia</i>	<i>Agrimonia bracteata</i> E. Mey. ex C.A. Mey; South Africa, R. Bayliss 3048 (MO). <i>A. coreana</i> Nakai; South Korea, B. Oh. 22372 (CUB). <i>A. eupatoria</i> L.; Germany, A. Polatschek <i>s.n.</i> (NY). <i>A. gryposepala</i> Wallr.; Canada, D. Ropke 523 (DAO). <i>A. incisa</i> Torr. & A. Gray; Florida, USA, W. Murrill <i>s.n.</i> (FLAS). <i>A. microcarpa</i> Wallr.; Florida, USA, West & Arnold <i>s.n.</i> (FLAS). <i>A. nipponica</i> Koidz.; Japan, Unknown <i>s.n.</i> (MO). <i>A. parviflora</i> Aiton; Connecticut, USA, L. Scerau <i>s.n.</i> (GH). <i>A. pilosa</i> Ledeb.; China, S. Lai S. & H. Shan <i>s.n.</i> (MO). <i>A. procera</i> Wallr.; Russia, A. Skvortsov <i>s.n.</i> (GH). <i>A. pubescens</i> Wallr.; USA, K.-S. Chung 163 (OKL). <i>A. rostellata</i> Wallr.; USA; K.-S. Chung 20 (OKL). <i>A. striata</i> Michx.; USA, B. Bell 279 (OKL)
<i>Aremonia</i>	<i>Aremonia agrimonoides</i> L.; Germany, Aellen <i>s.n.</i> (BRIT)
<i>Dryas</i>	<i>Dryas integrifolia</i> Vahl; Alaska, USA, R. Taylor 2745 (OKL)
<i>Fragaria</i>	<i>Fragaria moschata</i> Duchesne; Russia, B. Makapob <i>s.n.</i> (MO). <i>F. orientalis</i> Losinsk.; Russia, T. Buch & V. Yakubov <i>s.n.</i> (MO)
<i>Geum</i>	<i>Geum bulgaricum</i> Pančić; Bulgaria, P. Frost–Olsen 4721 (MO). <i>G. canadense</i> Jacq.; Oklahoma, USA, B. Hoagland 0252–97 (OKL). <i>G. urbanum</i> L.; Turkmen SSR, E. Maxmerob 175 (MO)
<i>Hagenia</i>	<i>Hagenia abyssinica</i> J. F. Gmel.; Ethiopia, W. de Wildecs. 9544 (MO)
<i>Leucosidea</i>	<i>Leucosidea sericea</i> Eckl. & Zeyh.; South Africa, M. Gafney 3 (MO)
<i>Margyricarpus</i>	<i>Margyricarpus pinnatus</i> (Lam.) Kuntze; Ecuador, W. Palacios & D. Rubio 7389 (MO)
<i>Polylepis</i>	<i>Polylepis racemosa</i> Ruiz & Pav.; Bolivia, M. Lewis 881014 (BRIT). <i>P. sericea</i> Wedd.; Ecuador, V. Zak & J. Jarmillo 2946 (BRIT)
<i>Potentilla</i>	<i>Potentilla anserina</i> L.; Alaska, USA, E. Crabb <i>s.n.</i> (OKL). <i>P. arguta</i> Pursh; Canada, W. Schofield 1280 (MO). <i>P. crantzii</i> (Crantz) G. Beck ex Fritch; Georgia, R. Gagnidze et al. 816 (MO). <i>P. fragarioides</i> L. var. <i>major</i> Maxim.; Japan, S. Tsugaru & T. Takahashi 6568 (MO). <i>P. fruticosa</i> L.; USA, M. Rohde 1114 (MO). <i>P. recta</i> L.; Oklahoma, USA, P. Nighswonger 1380 (OKL). <i>P. tridentata</i> Aiton; Minnesota, USA, S. Churchill 1214 (MO)
<i>Poterium</i>	<i>Poterium ancistroides</i> Desf.; Morocco, Unknown <i>s.n.</i> (MO). <i>P. minor</i> Scop.; France, F. Comte 2760 (MO)
<i>Sanguisorba</i>	<i>Sanguisorba canadensis</i> L.; Quebec, Canada, R. Clausen & H. Trapido 2783 (OKL). <i>S. officinalis</i> L.; China, T. Liu & Z. Zeng 4123 (MO)
<i>Spenceria</i>	<i>Spenceria ramalana</i> Trimen; China, J. Rock 4496 (GH)

Herbarium acronyms follow Index Herbariorum

Poteridium, pollen data was available for 11 genera analyzed by Potter et al. (2007) based on this investigation and Pérez De Paz (2004).

Results

Pollen morphology

Pollen grains examined from Sanguisorbeae, Dryadeae, and Potentilleae were radially symmetric, isopolar, colpate monads (Table 2; Figs. 1–30). Variation within Sanguisorbeae was observed in four characters (Figs. 1–18): aperture number (3, 4) and structure (operculate, pontoperculate), grain shape (spheroidal, subprolate, prolate), and exine sculpturing pattern (rugulate, verrucate, striate, perforate; often with microechinate ornamentation).

Pollen from nine of ten genera examined in Sanguisorbeae had three apertures (Figs. 4, 13, 16) except for *Margyricarpus* with four apertures (Figs. 1, 2). Our investigation showed three apparent apertures for *S. canadensis* L. and *S. officinalis* L. (Figs. 7, 8), which have been interpreted previously as 6-colporate (Nordborg 1966; Reitsma 1967; Naruhashi and Toyoshima 1979; Eide 1981; Hebda and

Chinnappa 1990b) or pseudo-6-colporate (Reitsma 1966). Our observations indicated the presence of tricolporate grains in *Sanguisorba*; each colpus had broad pontoperculate apertures resulting in polar views (Fig. 7) suggesting erroneously the presence of six colpi. Pollen from outgroup genera were uniformly tri-aperturate (Figs. 19, 22, 25, 28).

Pollen grain shapes were spheroidal in *Acaena*, *Polylepis*, *Margyricarpus*, and *Poterium*, and spheroidal to subprolate in *Hagenia* (Figs. 5, 14). Subprolate grains were observed in *Sanguisorba* whereas prolate grains characterized the pollen of *Agrimonia*, *Aremonia*, *Leucosidea*, and *Spenceria* (Figs. 2, 8, 17). Subprolate and spheroidal to subprolate grains were observed in outgroup genera (Figs. 20, 23, 26, 29).

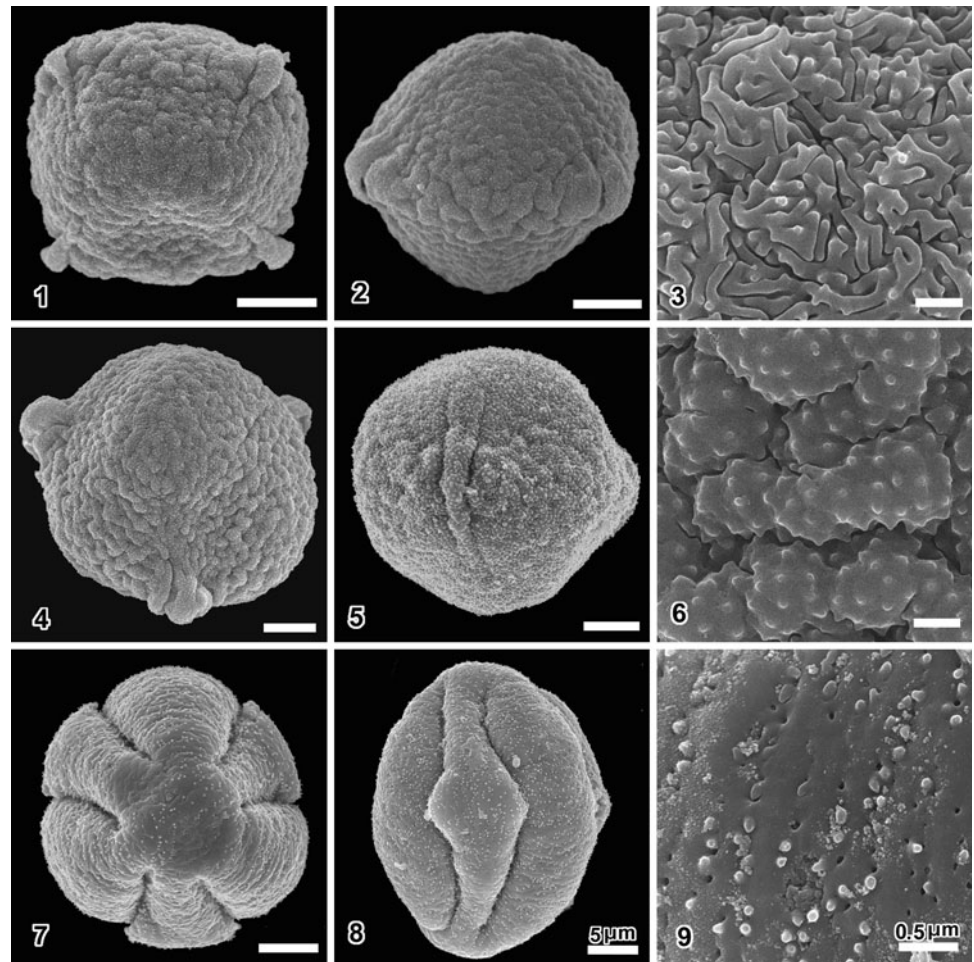
Two character states were observed for aperture structure:

- 1 operculate, with a distinctly delimited ectexinous/sexinous structure that covered part of an ectoaperture (Wodehouse 1935; Punt et al. 1994; Figs. 11, 14, 17) and
- 2 pontoperculate, characterized by an operculum that was not completely isolated from the remainder of the sexine (Punt et al. 1994; Figs. 2, 5, 8).

Table 2 Pollen morphological types in tribes Sanguisorbeae, Dryadeae, and Potentilleae

Pollen type	Aperture number	Pollen shape	Aperture structure	Exine sculpturing	Genus	Species examined	Figure
Tribe Sanguisorbeae							
Type I	4	Spheroidal	Pontoperculate	Rugulate	<i>Margyricarpus</i>	<i>M. pinnatus</i>	1–3
Type II	3	Spheroidal	Pontoperculate	Microechinate–Verrucate	<i>Acena</i> , <i>Polylepis</i>	<i>A. elongate</i> , <i>P. racemosa</i> , <i>P. sericea</i>	4–6
Type III	3	Subprolate	Broadly Pontoperulate	Microechinate–Perforate	<i>Sanguisorba</i>	<i>S. canadensis</i> , <i>S. officinalis</i>	7–9
Type IV	3	Spheroidal	Operculate	Microechinate–Striate (muri > groove)	<i>Poterium</i>	<i>P. ancistroides</i> , <i>P. minor</i>	10–12
Type V	3	Subprolate to spheroidal	Operculate	Microechinate–Verrucate	<i>Hagenia</i>	<i>H. abyssinica</i>	13–15
Type VI	3	Prolate	Operculate	Striate (muri > groove)	<i>Agrimonia</i> , <i>Aremonia</i> , <i>Leucosidea</i> , <i>Spenceria</i>	<i>A. bracteata</i> , <i>A. coreana</i> , <i>A. eupatoria</i> , <i>A. gryposepala</i> , <i>A. incisa</i> , <i>A. microcarpa</i> , <i>A. nipponica</i> , <i>A. parviflora</i> , <i>A. procera</i> , <i>A. pilosa</i> , <i>A. pubescens</i> , <i>A. rostellata</i> , <i>A. striata</i> , <i>Aremonia agrimonoides</i> , <i>L. sericea</i> , <i>S. ramalana</i>	16–18
Tribe Potentilleae							
Type VII	3	Subprolate	Operculate	Perforate–Striate (muri < groove)	<i>Fragaria</i> , <i>Potentilla</i>	<i>F. moschata</i> , <i>P. anserina</i> , <i>P. arguta</i> , <i>P. crantzii</i> , <i>P. fragarioides</i> , <i>P. fruticosa</i> , <i>P. tridentata</i> , <i>P. recta</i>	19–21
Type VIII	3	Subprolate to spheroidal	Operculate	Microverrucate	<i>Fragaria</i>	<i>F. orientalis</i>	22–24
Tribe Dryadeae							
Type IX	3	Subprolate to spheroidal	Uncovered	Perforate–Striate (muri < groove)	<i>Geum</i>	<i>G. bulgaricum</i> , <i>G. canadense</i> , <i>G. urbanum</i>	25–27
Type X	3	Spheroidal	Uncovered	Perforate–Striate (muri ≈ groove)	<i>Dryas</i>	<i>Dryas integrifolia</i>	28–30

Figs. 1–9 Scanning electron micrographs of pollen types I–III in tribe Sanguisorbeae. Pollen type I, 1–3, *Margyricarpus pinnatus*. 1 Polar view. 2 Equatorial view, spheroidal shape, colpus with a pontoperculum. 3 Rugulate exine sculpturing. Pollen type II, 4–6. 4 Polar view, *Polylepis racemosa*. 5 Equatorial view, spheroidal shape, colpus with a pontoperculum, *Acaena elongata*. 6 Microechinate–verrucate exine sculpturing, *P. racemosa*. Pollen type III, 7–9, *Sanguisorba canadensis*. 7 Polar view. 8 Equatorial view, subprolate shape, colpus with a broad pontoperculum. 9 Microechinate–perforate exine sculpturing. Scale bars 1, 2, 4, 5, 7, 8 = 5 μm ; 3, 6, 9 = 0.5 μm



Agrimonia, *Aremonia*, *Leucosidea*, *Spenceria*, *Hagenia*, and *Poterium* had pollen with operculate apertures whereas *Acaena*, *Margyricarpus*, *Polylepis*, and *Sanguisorba* had pontoperculate apertures. *Sanguisorba* had a broad pontoperculum (Figs. 7, 8), which was distinctive among other genera in the tribe. Among outgroups, pollen of the Potentilleae had operculate apertures whereas apertures of the Dryadeae were uncovered.

Exine sculpturing was the most variable and important character for delimiting pollen types. Pollen grains in the Sanguisorbeae represented two basic exine patterns, striate (Fig. 18) and non-striate variations encompassing rugulate, verrucate, and perforate sculpturing (Figs. 3, 6, 9). Microechinae were present also on the exines of several sculpturing patterns (Figs. 6, 9, 12, 15). Four of five sculpturing types in the tribe were observed in subtribe Sanguisorbinae (types I–IV), except for the unornamented striate grains (type VI) characteristic of subtribe Agrimoniinae (*Agrimonia*, *Aremonia*, *Leucosidea*, and *Spenceria*). Although *Hagenia* (type V) shared microechinate–verrucate exine sculpturing with type II grains (*Acaena*,

Polylepis), *Hagenia* had a unique combination of pollen shape, aperture structure, and exine sculpturing within the Sanguisorbeae.

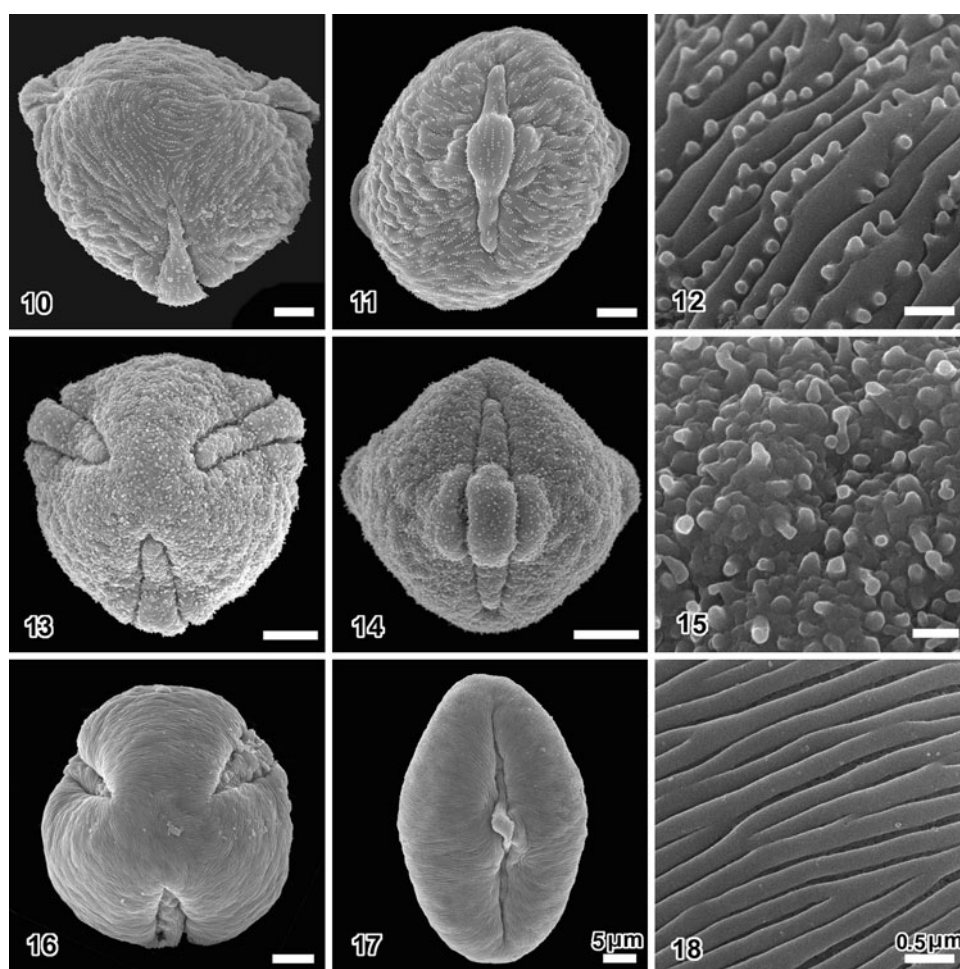
Pollen in the outgroups Dryadeae and Potentilleae also demonstrated a range of exine types based on perforate–striate and microverrucate sculpturing patterns (Figs. 21, 24, 27). There were three apertures in all outgroup taxa.

Pollen types

Using four variable characters, we differentiated six pollen types (I–VI) in Sanguisorbeae. Four types, I (*Margyricarpus*), III (*Sanguisorba*), IV (*Poterium*), and V (*Hagenia*), were confined to a single genus, whereas pollen types II and VI characterized pollen found in several genera. Four additional types (VII–X) were recognized among species representing the outgroup genera *Fragaria* (types VII, VIII), *Geum* (type IX), *Potentilla* (type VII), and *Dryas* (type X) (Table 2; Figs. 19–30).

Pollen type I was 4-colporate, quadrangular in polar view, and spheroidal in shape (Table 2; Figs. 1, 2).

Figs. 10–18 Scanning electron micrographs of pollen types IV–VI in tribe Sanguisorbeae. Pollen type IV pollen, **10–12**, *Poterium minor*. **10** Polar view. **11** Equatorial view, spheroidal shape, colpus with an operculum. **12** Microechinate–striate exine sculpturing with muri > grooves. Pollen type V, **13–15**, *Hagenia abyssinica*. **13** Polar view. **14** Equatorial view, subprolate to spheroidal shape, colpus with an operculum. **15** Microechinate–verrucate exine sculpturing. Pollen type VI, **16–18**, *Agrimonia*. **16** Polar view. **17** Equatorial view, prolate shape, colpus with an operculum, *A. eupatoria*. **18** Striate exine sculpturing with muri > grooves, *A. pilosa*. Scale bars **10**, **11**, **13**, **14**, **16**, **17** = 5 μm ; **12**, **15**, **18** = 0.5 μm



Apertures were covered by pontopercula and were slightly protruded. Exine sculpturing was rugulate. *Margyricarpus* was the only genus with this exine pattern.

Pollen type II was spheroidal in polar and equatorial views and had exine sculpturing described as microechinate–verrucate (Table 2; Figs. 4–6). Each of three apertures was covered by a pontoperculum and protruded. *Acaena* and *Polylepis* had this exine sculpturing.

Pollen type III was 3-colporate, subprolate, and had apertures that were broadly pontoperculate and not protruded (Table 2; Figs. 7, 8). Exine sculpturing was microechinate–perforate (Fig. 9). Colpori were long (ca. 80% of total pollen length) and wide (ca. 40% of circumference). Two species in *Sanguisorba* s. str., *S. canadensis* and *S. officinalis*, had pollen type III.

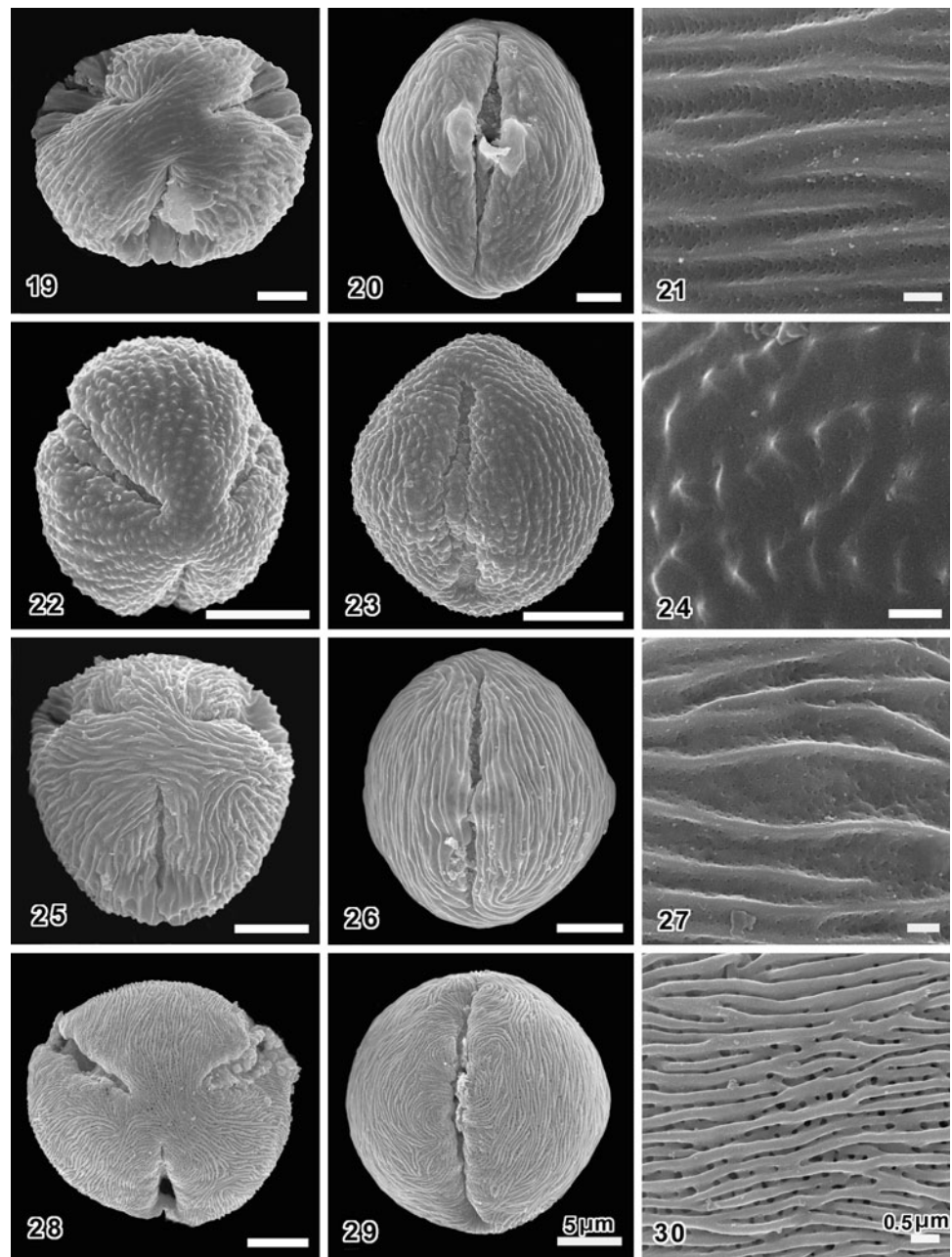
Pollen type IV was 3-colporate, spheroidal in shape, and had protruded apertures covered by opercula (Table 2; Figs. 10, 11). Exine sculpturing was striate and microechinate with nanopores (pores <0.5 μm diameter) among depressions (grooves) between striations (muri) (Fig. 12). Two species in *Poterium*, *P. minor* and *P. ancistroides*, had type IV pollen.

Pollen type V was 3-colporate, subprolate to spheroidal, and had protruded apertures covered by an operculum (Table 2; Figs. 13, 14). The exine sculpturing pattern was microechinate–verrucate (Fig. 15). Only the monotypic genus *Hagenia* had type V verrucate pollen.

Pollen type VI was 3-colporate, prolate in shape, and had operculate and unprotruded apertures (Table 2; Figs. 16, 17). Exine sculpturing was striate with the muri wider than the grooves (Fig. 12). This exine sculpturing pattern characterized the Agrimoniinae (*Agrimonia*, *Aremonia*, *Leucosidea*, *Spenceria*) except for the monotypic genus *Hagenia* (type V). In *Agrimonia pilosa* and *Aremonia agrimonoides*, nanopores were detected among depressions between striations (Fig. 18). In equatorial view, aperture length was approximately 80% of the pollen length (Fig. 17).

Pollen types VII to X (Table 2; Figs. 19–24) had operculate or uncovered apertures and had shapes ranging from spheroidal to subprolate. Exine sculpturing encompassed several patterns: perforate–striate with grooves wider than muri (types VII and IX, Figs. 21, 27), microverrucate (type VIII, Fig. 24), and perforate–striate with grooves of approximately equal width to muri (type X, Fig. 30).

Figs. 19–30 SEM photos of pollen types VII and VIII in tribe Potentilleae and pollen types IX and X in tribe Dryadeae. Pollen type VII, **19–21**, *Potentilla tridentata*. **19** Polar view. **20** Equatorial view, subprolate shape, colpus with an operculum. **21** Perforate–striate exine sculpturing with $\text{muri} < \text{grooves}$, *Potentilla anserina*. Pollen type VIII, **22–24** *Fragaria orientalis*. **22** Polar view. **23** Equatorial view, subprolate to spheroidal shape, colpus with an operculum. **24** Microverrucate exine sculpturing. Pollen type IX, **25–27**, *Geum canadense*. **25** Polar view. **26** Equatorial view, subprolate to spheroidal shape, colpus uncovered. **27** Perforate–striate exine sculpturing with $\text{muri} < \text{grooves}$. Pollen type X, **28–30**, *Dryas integrifolia*. **28** polar view **29** equatorial view, spheroidal shape, colpus uncovered. **30** Perforate–striate exine sculpturing with $\text{muri} \approx \text{grooves}$. Scale bars **19**, **20**, **22**, **23**, **25**, **26**, **28**, **29** = 5 μm ; **21**, **24**, **27**, **30** = 0.5 μm



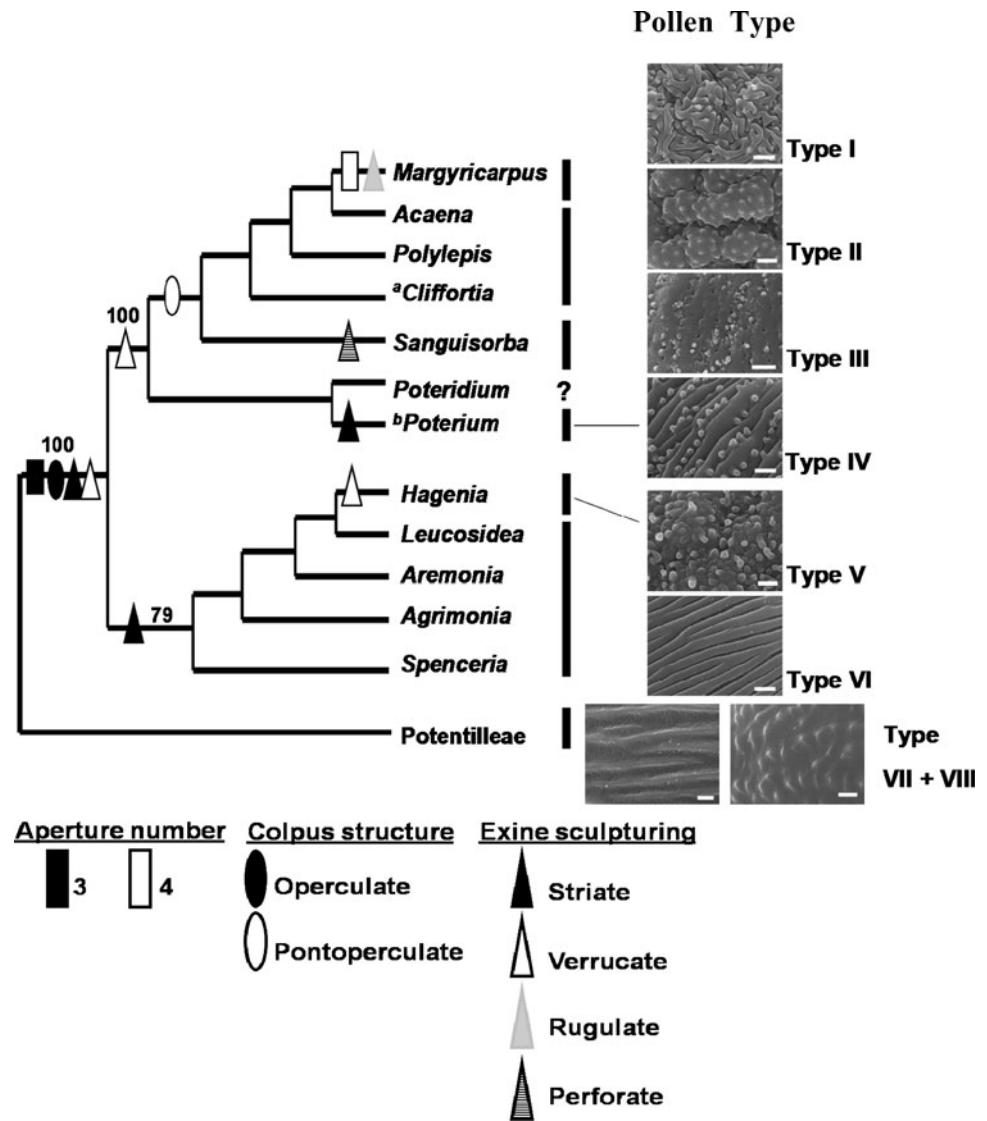
Discussion

Systematic significance

Our data corroborated previous studies (Reitsma 1966; Erdtman 1971; Pérez De Paz 2004) indicating that pollen grains in Sanguisorbeae were radially symmetric isopolar monads with tectate sexine. Aperture number and structure, grain shape, and exine sculpturing patterns were variable among genera (Table 2) and often served as diagnostic characters.

Apertures in the Sanguisorbeae were tricolporate or tetracolporate; tricolporate pollen was the most common aperture type and was found in all tribal genera except *Margyricarpus* (tetracolporate). The grains of *Sanguisorba canadensis* and *S. officinalis* were characterized previously as 6-colporate (Nordborg 1966; Hebda and Chinnappa 1990b; Li et al. 1999) or pseudo-6-colporate, because Reitsma (1966) interpreted colpi in three pairs with adjacent colpi sharing either an operculum (Nordborg 1966) or a pontoperculum (Punt et al. 1994). We propose that the pollen grains of *Sanguisorba* are tricolporate. Figure 8

Fig. 31 Phylogenetic tree adopted from Potter et al. (2007) with aperture number, colpus structure, and exine sculpturing patterns mapped and portrayed for tribe Sanguisorbeae. Bootstrap values are indicated. Pollen types and taxa represented: Type I. *Margyricarpus pinnatus*, type II. *Polylepis racemosa*, type III. *Sanguisorba canadensis*, type IV. *Sanguisorba minor*, type V. *Hagenia abyssinica*, type VI. *Agrimonia pilosa*, type VII. *Potentilla anserina*, type VIII. *Fragaria orientalis*. Scale bars 0.5 μm . ^aPollen data from Pérez De Paz 2004, ^b*Poterium minor* treated in Potter et al. 2007



portrays apertures with a broad pontoperculum running from pole to pole (Fig. 8), so that grains falsely appear “6-colporate” in polar view (Fig. 7). The pollen of *Sanguisorba* is distinctive with its broad pontoperculate apertures, but it exhibits characteristics found in tricolporate grains. *Bencomia*, *Cliffortia*, *Dendriopoterium*, *Marcatella*, and *Sarcopoterium* also are tricolporate (Pérez De Paz 2004).

Variable aperture numbers have been reported in the literature. Tricolporate and hexacolporate grains were reported in *Sanguisorba officinalis* by Nordborg (1966); tetracolporate grains, which characterized the pollen of *Margyricarpus*, were observed rarely in *Acaena*, *Bencomia*, *Dendriopoterium*, and *Marcatella* by Pérez De Paz (2004). *Sanguisorba officinalis* encompasses several named species, more than one ploidy level ($4x$, $8x$), and the greatest geographic range of any “species” or species complex in *Sanguisorba* s. lat. (Nordborg 1966).

A correlation between variation in pollen aperture number and ploidy level has been suggested by Erdtman (1963), but it is not known whether the “hexacolporate” grains reported for *S. officinalis* were a false identification of tricolporate grains or whether they represented six colpi associated with polyploidy. Species that exhibited pollen grains with an extra colporus in *Acaena*, *Bencomia*, *Dendriopoterium*, and *Marcatella* were homoploid with $2n = 4x = 28$ (Pérez De Paz 2004). We did not observe heterogeneity in aperture number within pollen samples.

Spheroidal, subprolate, and prolate grains are observed in Sanguisorbeae and represent important characters differentiating pollen of types I to IV from the subprolate or prolate grains characteristic of types V and VI, respectively. Pollen shapes for types I through V representing those found in subtribe Sanguisorbinae and in the genus *Hagenia* should be assessed with caution, because grain

shape was variable within samples and is known to be affected by extraction and preparation methods (Hebda et al. 1988b; Moore et al. 1991). Type VI pollen was consistently prolate, was found in four of five genera in Agrimoniinae, and can serve as a diagnostic character for *Agrimonia*, *Aremonia*, *Leucosidea*, and *Spenceria*. Pérez De Paz (2004) reported spheroidal grains among seven of nine genera she investigated in Sanguisorbinae, but observed subprolate grains in *Hagenia* (P/E ratio 1.12) and prolate grains in *Leucosidea* (P/E ratio 1.5) within the Agrimoniinae.

Uncovered colpi were observed in the Dryadeae and those associated with a pontoperculum and an operculum were found in the Sanguisorbeae and Potentilleae. Our results are concordant with those of Pérez De Paz (2004) who recorded operculate and pontoperculate grains in the Sanguisorbinae. We detected only operculate grains in the Agrimoniinae, which agrees with the findings of Reitsma (1966), Naruhashi and Toyoshima (1979), and Hebda et al. (1988b), but conflicts with the report of pontoperculate grains for *Hagenia* (Pérez De Paz 2004). Because an operculum is segregated from the sexine by thin membranes, it can be lost easily during preparation unlike a pontoperculum, which is linked to the sexine at the ends of the aperture. Both uncovered (types VII and VIII) and operculate (types IX and X) colpi were observed among outgroups by us and others (Hebda et al. 1988b).

Exine sculpturing is the most variable character in the Sanguisorbeae with at least five states observed. Striate sculpturing characterized types IV and VI (Figs. 12, 18), and four variations of non-striate sculpturing were found in types I, II, III, and V. Pores with diameters less than 0.5 μm (nanopores), were observed in types IV and VI. The exine sculpturing patterns of *Bencomia*, *Dendriopterium*, *Marcetella*, and *Sarcopoterium* were described by Pérez De Paz (2004) and matched our type IV for *Poterium*. In the generic summary of Potter et al. (2007), *Bencomia*, *Dendriopterium*, *Marcetella*, and *Sarcopoterium* were submerged within *Poterium*.

Our study and that of Pérez De Paz (2004) indicated that variation among pollen characters was greater in the Sanguisorbinae (four pollen types) than the Agrimoniinae (two pollen types). Our results also are consistent with the taxonomic synopsis presented in Potter et al. (2007) that does not support a broad concept of *Sanguisorba* (Nordborg 1966, 1967; Kalkman 2004), but does support circumscription of the genus *Poterium* to include *Bencomia*, *Dendriopterium*, *Marcetella*, and *Sarcopoterium*. Generic delimitation within subtribe Sanguisorbinae requires additional study of molecular, morphological, and chromosomal variation among species of *Sanguisorba* s. lat. and *Poterium* s. lat.

Phylogenetic utility and character evolution

Pollen characters are most useful phylogenetically at the generic and subtribal level. Although tribe Sanguisorbeae and subtribes Sanguisorbinae and Agrimoniinae were resolved with high support in molecular phylogenetic studies (Morgan et al. 1994; Potter 2003; Potter et al. 2007), we observed no synapomorphic pollen characters that define the tribe or subtribes. Previous studies (Hebda and Chinnappa 1990a, 1994; Pérez De Paz 2004) did not find pollen characters diagnostic at the tribal level, even though Hebda and Chinnappa (1990a, 1994) proposed revisions to tribal boundaries in Dryadeae and Potentilleae based on pollen characters. Pollen characters of tribe Sanguisorbeae observed in this study and in Pérez De Paz (2004) were polymorphic and were observed in genera representing several tribes in the Rosoideae. Outgroup comparisons indicated that operculate colpi, three apertures, and polymorphism for striate and/or various types of nonstriate exines were primitive states for tribe Sanguisorbeae.

Unique combinations of pollen characters characterize several genera and groups of genera in the Sanguisorbeae. Based on our study and that of Pérez De Paz (2004), a strongly supported clade (Potter et al. 2007) comprising *Margyricarpus* + *Acaena* + *Polylepis* + *Cliffortia* + *Sanguisorba* (100% Bayesian clade credibility) is characterized also by spheroidal or subprolate shape, nonstriate exines, and a pontoperculate aperture structure within the tribe. In subtribe Agrimoniinae, our data indicate that a well-supported clade comprising *Agrimonia* + *Aremonia* + *Leucosidea* + *Spenceria* (+*Hagenia*) (Potter et al. 2007; Chung 2008) shared prolate grain shape, operculate aperture structures, and smooth striate grains. Although *Hagenia* shared operculate apertures and had high support for inclusion in the Agrimoniinae clade in analyses by Potter et al. (2007) and Chung (2008), *H. abyssinica* differed from other subtribal genera in subprolate grain shape and microechinate–verrucate sculpturing. Both of these characters were observed in subtribe Sanguisorbinae and were more consistent with a basal phylogenetic placement within the Agrimoniinae rather than the derived position depicted in Fig. 31.

We did not resolve any clear-cut patterns of character evolution in the tribe. Because of variation in shape, aperture number and type, and exine sculpturing in pollen of the Rosoideae, pollen characters were most useful as lineage markers among groups of genera and when mapped on to the backbone of a well-supported molecular phylogeny, for example that of Potter et al. (2007). Pollen characters seem to be most useful for reconstructing phylogenetic relationships in the Rosoideae only when used as part of a broad total-evidence approach.

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