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A SYSTEMATIC STUDY OF GENUS *AGRIMONIA* (ROSACEAE)

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By

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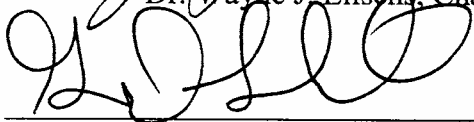
A SYSTEMATIC STUDY OF GENUS *AGRIMONIA* (ROSACEAE)

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BOTANY AND MICROBIOLOGY


BY



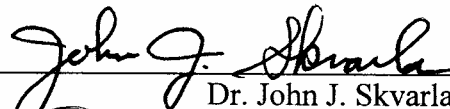
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ABSTRACT OF DISSERTATION

First, pollen grains of tribe Sanguisorbeae (Rosaceae, Rosoideae) were examined using scanning electron microscopy in order to identify useful characters in the tribe, test taxonomic and phylogenetic hypotheses among genera, and elucidate pollen character evolution. Aperture number, aperture structure, equatorial shape, and exine sculpturing characters grouped the pollen grains into six types. Microverrucate and striate exine sculpturing patterns support a monophyly of Sanguisorbinae and Agrimoniinae respectively. In Agrimoniinae, evolutionary directions of elliptic to circular equatorial shapes and striate to microverrucate sculpturing are apparent and distinguish *Hagenia* from the other genera. Pontoperculate apertures are found only in Sanguisorbinae and support close relationships of *Acaena*, *Cliffortia*, *Margyricarpus*, and *Polylepis*. In chapter 2, phylogeny and biogeography of Agrimoniinae composed of four monotypic endemics (*Aremonia*, *Hagenia*, *Leucosidea*, and *Spenceria*) and a worldwide genus (*Agrimonia*) are constructed from nuclear and plastid sequences (GBSSI-1, *trnL-trnF*, *trnS-trnG-trnG*). All nucleotide data support the monophyly of Agrimoniinae with a basal group of the Asian monotypic *Spenceria* ($2n=2x=14$), the sister relationship of the African genera *Hagenia* and *Leucosidea*, and the monophyly of *Agrimonia* ($2n=4x$, $6x$, and $8x$) + *Aremonia* ($2n=6x$). In *Agrimonia*, nuclear data define a lineage of Asian and American tetraploids (*Agrimonia coreana*, *A. microcarpa*, *A. parviflora*, and *A. rostellata*). These results suggest that the most recent ancestor of the subtribe distributed in Asia (tethyan) and paleo-migration might have occurred between the Northern and Southern Hemispheres and that polyploidy is

a major evolutionary process in Agrimoniinae and *Agrimonia*. Lastly, the genus *Agrimonia* is characterized as a monophyletic group of 19 species defined by two unique characters, interrupted pinnately compound leaves and bristly epicalyx segments. Species occur primarily in temperate regions throughout North America, Central America, the West Indies, southern South America, Europe, Asia, and southern Africa. Descriptions, nomenclatural histories, distribution maps, and keys are presented to 19 species and three varieties of *A. pilosa*. Neotypes of *A. hirsuta* and *A. nipponica*, an isolectotype of *A. pilosa*, and a lectotype of *A. villosa* are designated. The evolution of polyploidy and 12 morphological characters is evaluated within a phylogenetic framework.

CHAPTER 1

CHUNG, SKVARLA, AND ELISENS – POLLEN MORPHOLOGY OF TRIBE SANGUISORBEEAE (ROSACEAE)

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 in order to identify useful characters in the tribe, test taxonomic and phylogenetic hypotheses among genera, and elucidate pollen character evolution based on a molecular phylogeny. Aperture number, aperture structure, equatorial shape, and exine sculpturing characters grouped the pollen grains into six types. Four types characterized the subtribe Sanguisorbinae and two types described the subtribe Agrimoniinae. Microverrucate and striate exine sculpturing patterns support a monophyly of these subtribes. In Agrimoniinae, evolutionary directions of elliptic to circular equatorial shapes and striate to microverrucate sculpturing are apparent and distinguish *Hagenia* from the other genera.

Sanguisorbinae exhibited greater pollen morphological diversity, demonstrating diverse character states of aperture number and structure. The three and six apertures of *Sanguisorba* s.l. are either operculate or uncovered. Pontoperculate apertures are found only in Sanguisorbinae and support close relationships of *Acaena*, *Cliffortia*, *Margyricarpus*, and *Polylepis*. In Dryadeae colporate apertures are uncovered while in Potentilleae they are operculate.

Keywords: Agrimoniinae, Palynology, Phylogeny, Sanguisorbeae, Sanguisorbinae.

INTRODUCTION

Comparative studies of pollen morphology have provided useful information for delimitating genera and species in several lineages of Rosaceae (Fogle 1977a, 1977b; Eide 1981; Hebda et al. 1991; Hebda and Chinnappa 1994; Pérez de Paz 2004). Using light microscopy (LM), Reitsma (1966) identified 11 pollen types based on variation in size, aperture, and exine morphology among 18 European rosaceous genera. 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 the genus *Sanguisorba*, although he could not resolve fine-scale exine sculpturing patterns.

Recent studies in the family using scanning electron microscopy (SEM) demonstrated that a range of exine sculpturing patterns was present (e.g. Eide 1981; Hebda et al. 1988a, 1988b; Hebda and Chinnappa 1990a, 1990b; Hebda et al. 1991) and that they have been informative for understanding phylogenetic relationships (Wen and Nowicke 1999; Cooper et al. 2000) and for delimiting genera and/or species. Fogle (1977a, 1977b) demonstrated species-specific diversity in pollen size and exine sculpturing among the rosaceous fruit-crop genera *Malus* (apples), *Prunus* (peaches and cherries), and *Pyrus* (pears). Maas (1977) indicated that width variation of exine ridges (muri) of striate sculpturing in *Fragaria* (strawberry) species corresponded to ploidy levels.

Previous SEM examinations of rosaceous pollen grains were limited to taxa in particular geopolitical areas (e.g. Byatt 1976; Eide 1981; Hebda et al. 1991). 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 rosaceous genera in western Canada, Hebda, Chinnappa, and Smith (1988a, 1988b, 1991) and Hebda and Chinnappa (1990a, 1990b) identified significant diversity of exine sculpturing characters, which resulted in proposals to revise taxonomic and phylogenetic relationships. For example, Hebda and Chinnappa (1990a) postulated that the genera *Aruncus*, *Fragaria*, *Geum*, *Potentilla*, and *Sibbaldia* with microperforate pollen grains were a distinct lineage from 14 genera sharing microperforate/ tectal ridges. These genera were previously treated in three tribes: Potentilleae (*Fragaria*, *Potentilla*), Dryadeae (*Geum*), and Spiraeae (*Aruncus*, *Sibbaldia*) (Schulze-Menz 1964; Kalkman 1988). Based on pollen morphology, Hebda and Chinnappa (1994) suggested an alternate arrangement of genera in Dryadeae, Potentilleae, and Sanguisorbeae compared to that proposed by Kalkman (1988). They concluded that genera formerly placed in Dryadeae (*Coluria*, *Fallugia*, *Geum*, *Orthurus*, and *Waldsteinia*) with striate microperforate pollen should be transferred to Potentilleae.

Among the 12 to 15 genera commonly recognized in Sanguisorbeae (Hutchinson 1964; Kalkman 1988), only a few have been examined palynologically. Hebda and Chinnappa (1994) proposed that genera sharing verrucate/rugulate/microverrucate exine sculpturing (*Acaena*, *Cliffortia*, *Hagenia*,

Leucosidea, *Margyricarpus*, *Polylepis*) represented a distinct evolutionary lineage from genera sharing striate/microperforate pollen (*Agrimonia*, *Aremonia*, *Spenceria*). Pérez de Paz (2004) examined Macaronesian genera in Sanguisorbeae and found that the woody, dioecious genera *Bencomia* and *Sarcopoterium* and two genera segregated from *Sanguisorba* (*Dendriopoterium*, *Marcetella*) shared microverrucate exine sculpturing that agreed with results from a molecular phylogenetic study of these genera (Helfgott et al. 2000).

We initiated the present study because pollen morphology in the tribe Sanguisorbeae has been sampled incompletely, and because molecular phylogenetic relationships in the tribe and family were sufficiently resolved to provide a phylogenetic framework for the tribe (Potter et al. 2007). 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 based on the consensus phylogenetic topology presented in Potter et al. (2007).

MATERIALS AND METHODS

Pollen grains from 25 species representing ten genera in Sanguisorbeae were sampled: *Acaena* (1 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*, and *Sarcopoterium* in subtribe Sanguisorbinae were not sampled although pollen data for all of these genera have been presented in Pérez de Paz (2004). *Alchemilla* also was not included in this investigation based on phylogenetic investigations indicating that it is best placed within Potentilleae (Eriksson et al. 2003). To examine pollen morphology in relation to sister and outgroup taxa, 13 representative species from *Dryas* (1 species) and *Geum* (3 species) in Dryadeae and *Fragaria* (2 species) and *Potentilla* (7 species) in Potentilleae also were sampled. Specimen data is 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 prepared by Chissoe et al. (1994), Chissoe et al. (1995), Chissoe and Skvarla (1996), and Skvarla et al. (2005). Samples were examined with a JEOL 880 SEM equipped with a lanthanum hexaboride gun, and digital images were captured. Description of pollen morphology followed Moore et al. (1991) and Punt et al. (1994).

Major morphological features 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 onto the strict consensus tree

provided in Potter et al. (2007), which included all genera examined by us except *Poteridium*.

RESULTS

Pollen morphology -- Pollen grains examined from Sanguisorbeae, Dryadeae, and Potentilleae were radially symmetric isopolar colpate monads (Table 2; Figs. 1-31). Variation within Sanguisorbeae was observed in four characters (Figs. 1-18): aperture number (3, 4, 6) and structure (operculate, pontoperculate, uncovered), equatorial shape (circular, elliptic), and exine sculpturing pattern (microverrucate, striate etc.).

Pollen from nine of ten genera examined in Sanguisorbeae has three apertures (Figs. 4, 13, 16) except for *Margyricarpus* (four apertures; Figs. 1, 2) and *Sanguisorba* s.l., which is polymorphic for three (Figs. 10, 11) or six apertures (Figs. 7, 8). Our investigations showed six apparent apertures for *S. canadensis* and *S. officinalis* (Figs. 7, 8), which were characterized previously as 6-colpate (Nordborg 1966; Reitsma 1967; Naruhashi and Toyoshima 1979; Eide 1981; Hebda and Chinnappa 1990b) or pseudo-6-colpate (Reitsma 1966), because ectoapertures are not positioned opposite one another. Pollen from outgroup genera were uniformly tri-aperture (Figs. 19, 22, 25, 28).

Equatorial shapes were circular in *Acaena*, *Polylepis*, and *Hagenia* (Figs. 5, 14), whereas elliptic grains characterized the pollen of *Agrimonia*, *Aremonia*, *Leucosidea*, *Margyricarpus*, *Sanguisorba*, and *Spenceria* (Figs. 2, 8, 17). Elliptic grains were observed in outgroup genera (Figs. 20, 23, 26), except for the circular grains of *Dryas integrifolia* (Fig. 29).

Three character states were observed for the aperture structure 1) an operculum (a distinctly delimited ectexinous/ sexinous structure which covers part of an

ectoaperture as described by Wodehouse 1935 and Punt et al. 1994; Figs. 11, 14, 17), 2) a pontopericulum (an operculum that has not completely isolated the remainder of the sexine; Figs. 2, 5; Punt et al. 1994), and 3) an uncovering (viz., a lack of both an operculum and a pontopericulum; Fig. 8). *Agrimonia*, *Aremonia*, *Leucosidea*, and *Spenceria* had an operculate aperture, whereas *Acaena*, *Margyricarpus*, and *Polylepis* had apertures with pontopercula. Pollen grains of *Hagenia* and *Poterium* were operculate, whereas *Sanguisorba* was uncovered. Potentilleae had operculate apertures while Dryadeae had only uncovered apertures.

Exine sculpturing was the most variable and important character for delimiting pollen types. Most pollen grains represented variations on two basic exine patterns, microverrucate (Fig. 15) and striate (Fig. 18). Some species exhibit more than one exine pattern. For example, the pollen of *Poterium ancistroides* and *P. minor* combined features of both basic patterns and was described as microverrucate-striate (Fig. 12).

Except for the microverrucate pollen of *Hagenia*, four genera in Agrimoniinae, *Agrimonia*, *Aremonia*, *Leucosidea*, and *Spenceria*, exhibited striate sculpturing. Sanguisorbinae genera had microverrucate pollen in combination with other exine patterns such as verrucate (*Acaena*, *Polylepis*; Fig. 6), reticulate (*Margyricarpus*; Fig. 3), microperforate (*Sanguisorba canadensis* and *S. officinalis*; Fig. 9), or striate (*Poterium ancistroides*, *P. minor*; Fig. 12).

Outgroup pollen observed in Dryadeae and Potentilleae also demonstrated a range of exine types based on striate and microverrucate sculpturing in combination

with microperforate (Figs. 21, 24, 27) and perforate (Fig. 30) patterns. Apertures numbered three in the all outgroup taxa. In Dryadeae colporate apertures are uncovered while in Potentilleae they are operculate.

Pollen types -- Using the aforementioned characters, we describe six pollen types (I-VI) in Sanguisorbeae. Four types, I (*Margyricarpus*), III (*Sanguisorba*), IV (*Poterium*), and V (*Hagenia*), are confined to a single genus, whereas pollen types II and VI characterize pollen found in several genera. Four additional types (VII-X) are also 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 is 4-colporate, quadrangular in polar view, and elliptic in equatorial view (Table 2; Figs. 1, 2). Apertures are covered by pontopercula and are slightly protruded. Exine sculpturing is microverrucate and reticulate. *Margyricarpus* is the only genus with this type.

Pollen type II is circular in polar and equatorial views and have microverrucate, verrucate, and nanoporate (viz., less than 0.5 μm diameter; Fig. 6) exine sculpturing patterns (Table 2; Figs. 4-6). Each of three apertures is covered by a pontoperculum and protruded. *Acaena* and *Polylepis* have this type.

Pollen type III is 6-colporate, circular in polar view, elliptic in equatorial view, and has apertures that are neither covered nor protruded (Table 2; Figs. 7, 8). Exine sculpturing is microverrucate and microperforate (Fig. 9). Uncovered Colpori are long

(ca. 80% of total pollen length in equatorial view). Two species in *Sanguisorba*, *S. canadensis* and *S. officinalis* have pollen type III.

Pollen type IV is 3-colporate, circular in polar view, elliptic in equatorial view, and have protruded apertures covered by opercula (Table 2; Figs. 10, 11). Exine sculpturing is striate and microverrucate with nanopores among depressions (lumina) between striate (muri) (width: muri > lumina; Fig. 12). Two species in *Poterium*, *P. minor* and *P. ancistroides*, have pollen type IV.

Pollen type V is 3-colporate, circular in polar and equatorial views, and have apertures covered by an operculum and protruded (Table 2; Figs. 13, 14). The exine sculpturing pattern is microverrucate with nanopores (Fig. 15). Only the monotypic genus *Hagenia* has pollen type V.

Pollen type VI is 3-colporate, circular in polar view, elliptic in equatorial view, and have operculate and unprotruded apertures (Table 2; Figs. 16, 17). Exine sculpturing is striate (muri > lumina; Fig. 12). This pollen type characterizes Agrimoniinae genera (*Agrimonia*, *Aremonia*, *Leucosidea*, *Spenceria*) except for the monotypic genus *Hagenia* (type V). In *Agrimonia pilosa* and *Aremonia agrimonoides*, nanopores are detected among depressions between striate (Fig. 18). In equatorial view, aperture length is about 80% of the pollen length (Fig. 17).

Pollen types VII to X (Table 2; Figs. 19-24) have operculate or uncovered apertures, elliptic or circular equatorial shapes, and exine sculpturing patterns that were microperforate-striate (types VII, muri < lumina; Fig. 21), microverrucate-

microperforate (type VIII, Fig. 24), microperforate-striate (type IX, $\text{muri} < \text{lumina}$; Fig. 27) or perforate-striate (type X, $\text{muri} \approx \text{lumina}$; Fig. 30).

DISCUSSION

Pollen morphology and its systematic significance in tribe Sanguisorbeae -- Our data and previous studies (Reitsma 1966; Erdtman 1971; Pérez de Paz 2004) indicate that pollen grains in Sanguisorbeae are radially symmetric isopolar monads with colporate apertures and a tectate sexine. Aperture number and structure, grain shape, and exine sculpturing patterns are variable among genera (Table 2).

Apertures in the Sanguisorbeae number three, four, or six - tricolporate, tetracolporate, or hexacolporate, respectively. Tricolporate pollen is the most common aperture type and is found in all tribal genera except *Margyricarpus* (tetracolporate) and *Sanguisorba* s.l., which has both tricolporate and hexacolporate grains (Nordborg 1966). The ‘hexacolporate’ grains of *Sanguisorba canadensis* and *S. officinalis* were characterized as 6-colporate by Hebda and Chinnappa (1990b) and Li et al. (1999), but were described as pseudo-6-colporate by Reitsma (1966), because the colpi occur in three pairs with two adjacent colpi sharing either an operculum (Nordborg 1966) or a pontoperculum (Punt et al. 1994). *Bencomia*, *Cliffortia*, *Dendriopoterium*, *Marcatella*, *Sarcopoterium* also are tricolporate (Pérez de Paz 2004).

Aperture number is generally consistent at the generic or infrageneric level. Tri- and hexacolporate grains have been observed in *Sanguisorba officinalis* (Nordborg 1966) and tetracolporate grains were rarely observed in the normally tricolporate genera *Acaena*, *Bencomia*, *Dendriopoterium*, and *Marcatella* by Pérez de Paz (2004). *Sanguisorba officinalis* encompasses several named species, more than one ploidy level, and the greatest geographic range of any ‘species’ in *Sanguisorba* s.l.

(Nordborg 1966). A correlation between variation in pollen aperture number and ploidy level has been suggested by Erdtman (1963), but among species of *Sanguisorba* and *Poterium*, ploidy levels are not consistent with number of apertures. Both the tricolporate *P. minor* and hexacolporate *S. officinalis* exhibit tetraploid ($2n=28=4x$) and octoploid ($2n=56=8x$) races, and the tricolporate *P. ancistroides* has only tetraploid races ($2n=28=4x$) (Mishima et al. 2002). Species that exhibited pollen grains with an extra colporus in *Acaena*, *Bencomia*, *Dendriopoterium*, and *Marcatella* were homoploid with $2n=28=4x$ (Pérez de Paz 2004).

Both circular and elliptic grains are observed in Sanguisorbeae and represent an important character differentiating circular pollen of types II and V from the elliptic grains observed in types I, III, IV, and VI. Pollen shapes should be used as characters with caution because grain shape can vary considerably within species and can be affected by extraction and preparation methods (Hebda et al. 1988b; Moore et al. 1991). Type VI pollen is elliptic and found in four of five genera in Agrimoniinae, but we observed both circular and elliptic grains in Sanguisorbinae. Pérez de Paz (2004) observed only circular grains in the eight genera she investigated in Sanguisorbinae, but noted mildly elliptic grains in *Hagenia* (P/E ratio 1.12) and prolate grains in *Leucosidea* (P/E ratio 1.5), the only genera she investigated in Agrimoniinae.

Uncovered colpi and those associated with a pontopericulum and an operculum were observed in the Sanguisorbeae. Although Pérez de Paz (2004) recorded only operculate and pontoperculate grains in eight genera of the Sanguisorbinae, we observed uncovered colpi in two species of *Sanguisorba* s. str. Additionally,

whereas we detected only operculate grains in Agrimoniinae, Pérez de Paz (2004) recorded pontoperculate grains for *Hagenia* (our type V). Because an operculum is segregated from the sexine and has thin membranes, it can be easily lost during preparation unlike a pontoperculum, which is linked to the sexine at the ends of the aperture. Within the Agrimoniinae, conflicting observations of operculate and pontoperculate grains were recorded for *Hagenia* (Pérez de Paz 2004) and *Agrimonia* (Reitsma 1966; Naruhashi and Toyoshima 1979; Hebda et al. 1988b). Both uncovered and operculate colpi were observed among outgroup types VII and VIII (Figs. 20, 23) and recorded by others (Hebda et al. 1988b).

Exine sculpturing is the most variable character in the Sanguisorbeae with at least five states observed. Striate sculpturing characterizes pollen types IV and VI (Figs. 12, 18), and variations (type I-III, V) and combinations (type IV) of microverrucate sculpturing delimit the other types. Nanopores are observed in types IV-VI. Exine sculpturing of *Bencomia*, *Dendriopoterium*, *Marcetella*, and *Sarcopoterium* described by Pérez de Paz (2004) belongs to type IV, which is shared by *Poterium*. It supports generic treatment of Potter et al. (2007) that those genera are submerged within *Poterium*.

Variation among pollen characters is greater in Sanguisorbinae with four pollen types identified among ten genera sampled in this study and the five sampled by Pérez de Paz (2004). Variation is greatest in *Sanguisorba* s.l. in aperture number and structure, and exine sculpturing. Our data and that of Potter et al. (2007) do not support a broad concept of *Sanguisorba* (Nordborg 1966, 1967; Kalkman 2004), but

agree well with segregation of the genus *Poterium*. Pollen grains of *Bencomia*, *Dendriopoterium*, *Marcetella*, and *Sarcopoterium* examined in Pérez de Paz (2004) exhibit microverrucate-striate exine sculpturing which is observed in type IV pollen, supporting *Poterium* s.l. as proposed (Potter et al. 2007). Additional study of molecular, morphological, and chromosomal variation among species in *Sanguisorba* s.l. seems warranted.

Phylogenetic utility of pollen characters -- Exine sculpture of microverrucate and striate patterns strongly supports monophyly of Sanguisorbinae and Agrimoniinae respectively (Fig. 31). Exine and shape characters indicate an evolutionary distinctiveness of *Hagenia* in Agrimoniinae, and four apomorphic characters in Sanguisorbeae are found.

Our data and that of Potter et al. (2007) contrast with the hypothesis of Hebda and Chinnappa (1994) that define the woody southern hemisphere genera in Sanguisorbeae as a distinct evolutionary lineage based on exine sculpturing. Woody southern hemisphere genera sharing microverrucate sculpturing are categorized into three different types based on aperture structure and equatorial shape: elliptic grains with pontoperculate apertures are characterized in type I (*Margyricarpus*), circular pollen grains with pontoperculate apertures define types II (*Acaena*, *Cliffortia*, *Polylepis*), and type V (*Hagenia*) is distinguished by circular grains with operculate apertures (Fig. 31). Although exine sculpturing has been useful for defining lineages (Cooper et al. 2000), our results demonstrate that a combination of pollen characters should be considered when attempting to resolve phylogenetic relationships.

In both subtribes, genera sharing microverrucate sculpturing are woody, derived and occur in the Southern hemisphere. The results support the hypotheses of Bramwell (1978) on relationships among pollen morphology, growth forms, and geographic distribution. In Sanguisorbinae, woody African and South American *Acaena*, *Cliffortia*, *Margyricarpus*, and *Polylepis* sharing microverrucate sculpturing are derived from the herbaceous Eurasian genus *Sanguisorba* (only *Poterium*) of striate sculpturing with an additional microverrucate exine pattern. In Agrimoniinae, the microverrucate exine of an African tree *Hagenia* is derived from herbaceous Eurasian *Agrimonia*, *Aremonia*, and *Spenceria*. Shrubby southeastern African endemic *Leucosidea* is the exception for this pattern.

In Agrimoniinae, pollen characters indicate phylogenetic particularity of *Hagenia*. Equatorial shape and exine sculpturing characters of *Hagenia* are unique; they are also distinct from the other subtribal genera in stigma morphology and unisexual flowers in panicles (Kalkman 2004). Dioecious flowers easily distinguish *Hagenia* from a woody African endemic *Leucosidea*, and the distinct exine sculpturing is thought to be related to wind pollination (Linder and Midgley 1996; Edlund et al. 2004).

Potter et al. (2007) indicated that Potentilleae is sister to Sanguisorbeae, and Colurieae (*Geum*) is basal to Sanguisorbeae and Potentilleae in subfamily Rosoideae, whereas *Dryas* belongs to newly defined subfamily Dryadoideae and is relatively distant phylogenetically. Distinct exine of *Dryas* with perforate sculpturing supports their proposed subfamilial system. Our data also shows that *Geum* shares pollen

characters with *Potentilla* and *Fragaria* except aperture structure, supporting Hebda and Chinnappa (1990b) and Potter et al. (2007). Based on pollen characters of *Potentilla* and *Fragaria*, outgroup pollen is characterized with tri-colporate, operculate, elliptic grains with striate or microverrucate exine sculpturing (Fig. 31).

Four apomorphic characters in the tribe are found: tetra-/ pontoperculate and hexa-/ uncovered apertures, circular equatorial shape, and a microverrucate exine pattern (Fig. 31). In Sanguisorbinae, tetra-/ pontoperculate apertures are apomorphic for Type I (*Margyricarpus*), hexa-/ uncovered aperture characters are apomorphies for *Sanguisorba*, and *Poterium* exhibits an apomorphy of an additional striate to microverrucate exine. Pontoperculate apertures support types I and II, observed only in derived genera *Acaena*, *Cliffortia*, *Margyricarpus*, and *Polylepis* (Figs. 2, 5), and type II defined by circular pollen grains of pontoperculate colpi and microverrucate sculpturing well supports a close relationship among *Acaena*, *Cliffortia*, and *Polylepis* (Figs. 5, 31). A tri- and operculate aperture, elliptic equatorial shape, and striate exine pattern are plesiomorphies in Agrimoniinae except *Hagenia*.

The three pollen characters (i.e. aperture number and structure and exine sculpturing) have evolved independently once or more times within the tribe (Fig. 31). Exine sculpturing has evolved a distinct lineage of *Hagenia* (type V) in Agrimoniinae. In Sanguisorbinae, aperture structure has been derived to uncovered apertures for *Sanguisorba* (type III) and to pontoperculate apertures for *Acaena*, *Cliffortia*, *Margyricarpus*, and *Polylepis* (types I and II), and an aperture number has been

evolved to six for *Sanguisorba* s. str. (type III) and to four for *Margyricarpus* (type I), and an additional striate exine sculpturing is gained for *Poterium* (type IV).

In Agrimoniinae, evolutionary modifications from elliptic pollen with striate sculpture to circular pollen grain with microverrucate sculpture are well demonstrated (Figs. 14, 15, 17, 18). Elliptic pollen grains of basal genera (*Agrimonia*, *Aremonia*, *Spenceria*, and *Leucosidea*) possess a plesiomorphy of striate exine sculpturing although a width ratio of muri and lumina differs from outgroup taxa (Figs. 18, 21). A derived genus *Hagenia* demonstrates apomorphic character states of circular shape and microverrucate exine sculpturing. Sanguisorbinae also exhibits an elliptic to circular shape except *Margyricarpus*. Pollen of *Margyricarpus* s.l., *Sanguisorba* s.l., *Poteridium*, and *Poterium* s.l. (Kalkman 2004; Potter et al. 2007) should be further investigated to better understand diverse evolutionary patterns of pollen characters in Sanguisorbinae.

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TABLE 1. Collection data of species examined in this study.

Species	Location	Collector	Herbarium
<i>Acaena elongata</i> L.	Costa Rica	R. W. Lent 136	OKL
<i>Agrimonia bracteata</i> E. Mey. ex C.A. Mey.	South Africa	R. D. A. Bayliss 3048	MO
<i>Agrimonia coreana</i> Nakai	South Korea	Oh. B.-H. et al. 22372	CBN
<i>Agrimonia eupatoria</i> L.	Germany	A. Polatschek <i>s.n.</i>	NY
<i>Agrimonia gryposepala</i> Wallr.	Canada	D. Ropke 523	DAO
<i>Agrimonia incisa</i> Torr. & A. Gray	FL, USA	W. A. Murrill <i>s.n.</i>	FLAS
<i>Agrimonia microcarpa</i> Wallr.	FL, USA	West & Arnold <i>s.n.</i>	FLSA
<i>Agrimonia nipponica</i> Koidz.	Japan	Unknown <i>s.n.</i>	MO
<i>Agrimonia parviflora</i> Aiton	CT, USA	L. W. Scerau <i>s.n.</i>	GH
<i>Agrimonia pilosa</i> Ledeb.	China	Lai S. & Shan H. R. <i>s.n.</i>	MO
<i>Agrimonia procera</i> Wallr.	Russia	A. K. Skvortsov <i>s.n.</i>	GH
<i>Agrimonia pubescens</i> Wallr.	USA	Chung KS 163	OKL
<i>Agrimonia rostellata</i> Wallr.	USA	Chung KS 20	OKL
<i>Agrimonia striata</i> Michx.	USA	B. Bell 279	OKL
<i>Aremonia agrimonoides</i> L.	Germany	Aellen <i>s.n.</i>	BRIT
<i>Dryas integrifolia</i> Vahl	Alaska, USA	R. J. Taylor 2745	OKL
<i>Fragaria moschata</i> Duchesne	Russia	B. Makapob <i>s.n.</i>	MO
<i>Fragaria orientalis</i> Losinsk.	Russia	T. Buch & V. Yakubov <i>s.n.</i>	MO
<i>Geum bulgaricum</i> Panc	Bulgaria	P. Frost-Olsen 4721	MO
<i>Geum canadense</i> Jacq.	OK, USA	B. Hoagland 0252-97	OKL
<i>Geum urbanum</i> L.	Turkmen SSR	Maxmerob, E. 175	MO
<i>Hagenia abyssinica</i> J. F. Gmel.	Ethiopia	W. de Wilde c. s. 9544	MO
<i>Leucosidea sericea</i> Eckl. & Zeyh.	South Africa	M. A. Gafney 3	MO
<i>Margyricarpus pinnatus</i> (Lam.) Kuntze	Ecuador	W. Palacios & D. Rubio 7389	MO

TABLE 1. (cont.)

<i>Polylepis racemosa</i> Ruiz & Pav.	Bolivia	M. Lewis 881014	BRIT
<i>Polylepis sericea</i> Wedd.	Ecuador	V. Zak & J. Jarmillo 2946	BRIT
<i>Potentilla anserine</i> L.	Alaska, USA	E. D. Crabb <i>s.n.</i>	OKL
<i>Potentilla arguta</i> Pursh	Canada	W. B. Schofield 1280	MO
<i>Potentilla cranizii</i> (Crantz) G. Beck ex Fritch	Georgia	Gagnidze R. et al. 816	MO
<i>Potentilla fragarioides</i> L. var. <i>major</i> Maxim.	Japan	S. Tsugaru & T. Takahashi 6568	MO
<i>Potentilla fruticosa</i> L.	USA	M. C. Rohde 1114	MO
<i>Potentilla recta</i> L.	OK, USA	P. Nighswonger 1380	OKL
<i>Potentilla tridentate</i> Aiton	MN, USA	S. Churchill 1214	MO
<i>Poterium ancistroides</i> Desf.	Morocco	Unknown <i>s.n.</i>	MO
<i>Poterium minor</i> Scop.	France	F. Comte 2760	MO
<i>Sanguisorba canadensis</i> L.	Quebec, Canada	R. T. Clausen & H. Trapido 2783	OKL
<i>Sanguisorba officinalis</i> L.	China	T. Liu & Z. Zeng 4123	MO
<i>Spenceria ramalana</i> Trimen	China	J. F. Rock 4496	GH

TABLE 2. Pollen morphological types in tribes Sanguisorbeae, Dryadeae, and Potentilleae.

Pollen type	Aperture number	Equatorial shape	Aperture structure	Exine sculpturing	Genus	Species examined	Figure
Tribe Sanguisorbeae							
Type I	4	Elliptic	Pontoperculate	Microverrucate-Reticulate	<i>Margyricarpus</i>	<i>M. pinnatus</i>	1, 2, 3
Type II	3	Circular	Pontoperculate	Microverrucate-Verrucate	<i>Acaena</i> <i>Polytepis</i>	<i>A. elongate</i> , <i>P. racemosa</i> , <i>P. sericea</i>	4, 5, 6
Type III	6	Elliptic	Uncovered	Microverrucate-Microperforate	<i>Sanguisorba</i>	<i>S. canadensis</i> , <i>S. officinalis</i>	7, 8, 9
Type IV	3	Elliptic	Operculate	Microverrucate-Striate (muri > lumina)	<i>Poterium</i>	<i>P. ancistroides</i> , <i>P. minor</i>	10, 11, 12
Type V	3	Circular	Operculate	Microverrucate	<i>Hagenia</i>	<i>H. abyssinica</i>	13, 14, 15
Type VI	3	Elliptic	Operculate	Striate (muri > lumina)	<i>Agrimonia</i> <i>Arenonia</i> <i>Leucosidea</i> <i>Spenceria</i>	<i>A. bracteata</i> , <i>A. coreana</i> , <i>A. eupatorioides</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>Arenonia agrimonoides</i> , <i>L. sericea</i> , <i>S. ramalana</i>	16, 17, 18

TABLE 2. (cont.)

Tribe Potentilleae							
Type VII	3	Elliptic	Operculate	Microperforate-Striate (muri < lumina)	<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. rufo-rosea</i> , <i>P. tridentata</i> , <i>P. recta</i> <i>F. orientalis</i>	19, 20, 21
Type VIII	3	Elliptic	Operculate	Microverrucate- Microperforate	<i>Fragaria</i>	<i>F. orientalis</i>	22, 23, 24
Tribe Dryadeae							
Type IX	3	Elliptic	Uncovered	Microperforate-Striate (muri < lumina)	<i>Gewm</i>	<i>G. bulgaricum</i> , <i>G. canadense</i> , <i>G. urbanum</i>	25, 26, 27
Type X	3	Circular	Uncovered	Perforate-Striate (muri \approx lumina)	<i>Dryas</i>	<i>Dryas integrifolia</i>	28, 29, 30

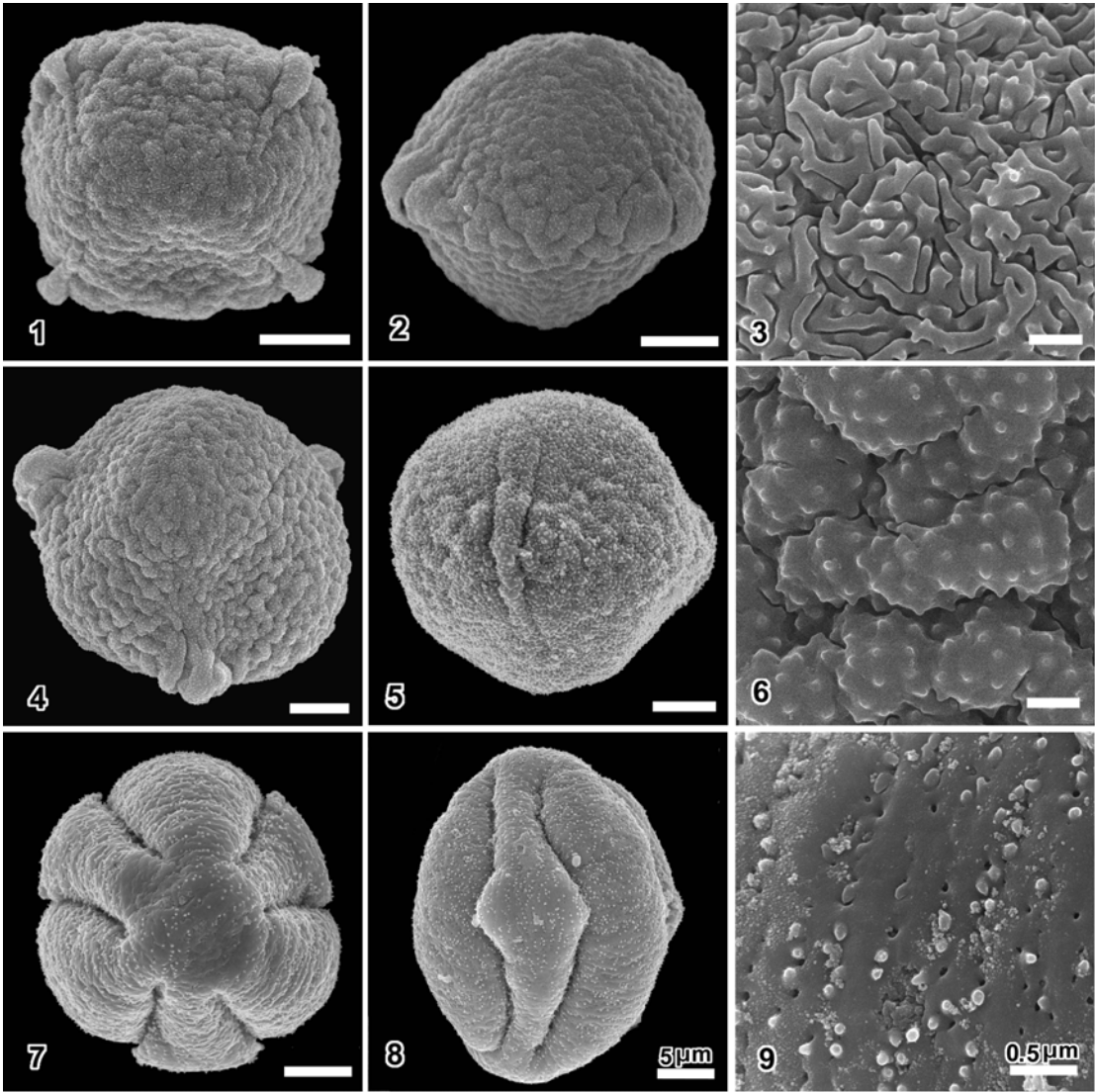
Figs. 1-9. Scanning electron micrographs of pollen types I-III in Sanguisorbeae.

Pollen type I, 1-3, *Margyricarpus pinnatus*. 1. polar view. 2. equatorial view, elliptic shape, colpus with a pontoperculum. 3. microverrucate-reticulate exine sculpturing.

Pollen type II, 4-6. 4. polar view, *Polylepis racemosa*. 5. equatorial view, circular shape, colpus with a pontoperculum, *Acaena elongata*. 6. microverrucate-verrucate exine sculpturing, *P. racemosa*.

Pollen type III, 7-9, *Sanguisorba canadensis*. 7.

polar view. 8. equatorial view, elliptic shape, colpus uncovered. 9. microverrucate-microperforate exine sculpturing. Scale bars: 1, 2, 4, 5, 7, 8 = 5 μm ; 3, 6, 9 = 0.5 μm .

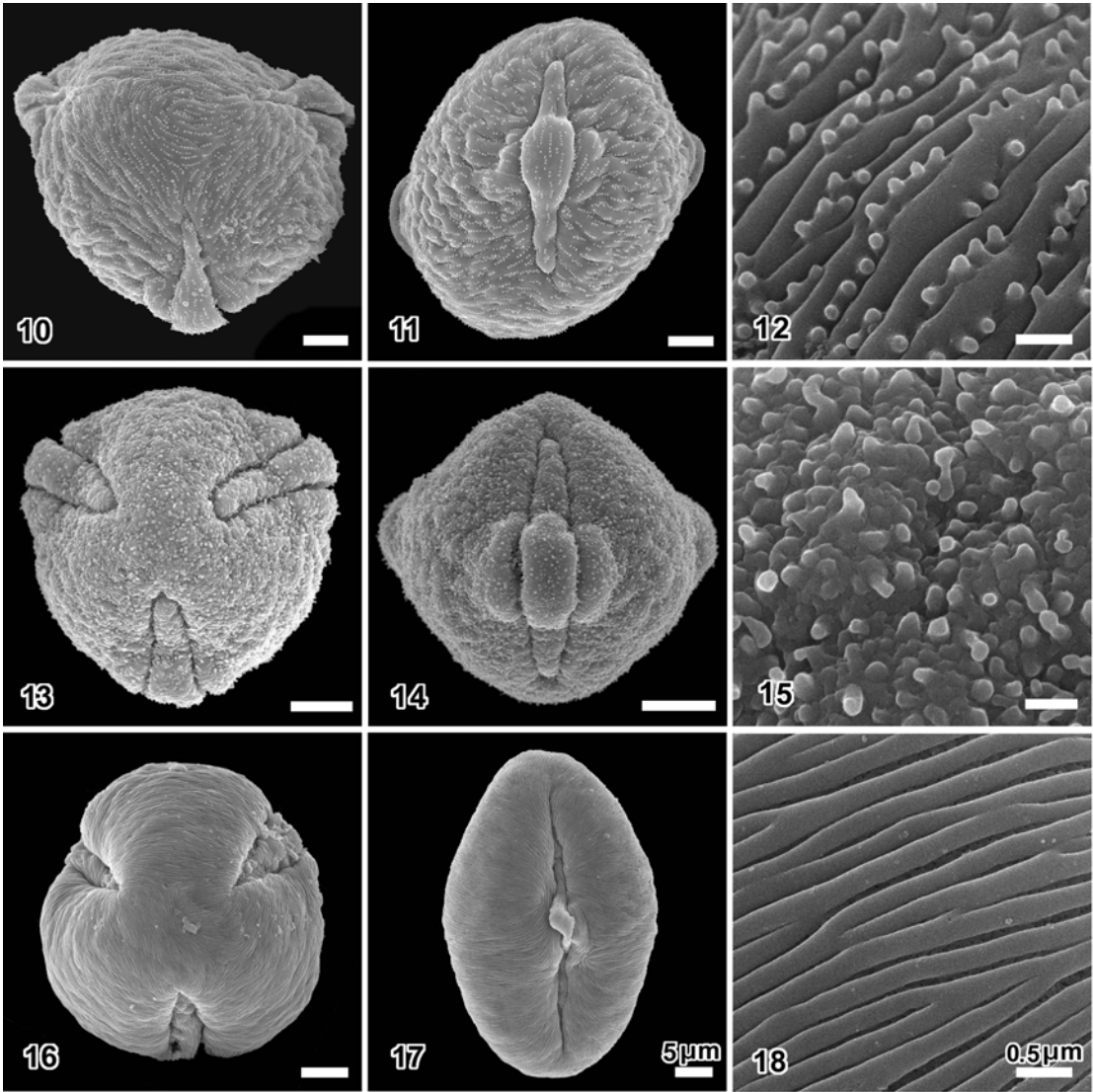


Figs. 10-18. Scanning electron micrographs of pollen types IV-VI in Sanguisorbeae.

Pollen type IV pollen, 10-12, *Poterium minor*. 10. polar view. 11. equatorial view, elliptic shape, colpus with an operculum. 12. microverrucate-striate exine sculpturing.

Pollen type V, 13-15, *Hagenia abyssinica*. 13. polar view. 14. equatorial view, circular shape, colpus with an operculum. 15. microverrucate exine sculpturing.

Pollen type VI, 16-18, *Agrimonia*. 16. polar view. 17. equatorial view, elliptic shape, colpus with an operculum, *A. eupatoria*. 18. striate exine sculpturing, *A. pilosa*. Scale bars: 10, 11, 13, 14, 16, 17 = 5 μm ; 12, 15, 18 = 0.5 μm .



Figs. 19-30. SEM photos of pollen types VII and VIII in Potentilleae and pollen types IX and X in Dryadeae. Pollen type VII, 19-21, *Potentilla tridentate*. 19. polar view. 20. equatorial view, elliptic shape, colpus with an operculum. 21. microperforate-striate exine sculpturing, *Potentilla anserine*. Pollen type VIII, 22-24. *Fragaria orientalis*. 22. polar view. 23. equatorial view, elliptic shape, colpus with an operculum. 24. microverrucate-microperforate exine sculpturing. Pollen type IX, 25-27, *Geum canadense*. 25. polar view. 26. equatorial view, elliptic shape, colpus uncovered. 27. microperforate-striate exine sculpturing. Pollen type X, 28-30, *Dryas integrifolia*. 28. polar view. 29. equatorial view, circular shape, colpus uncovered. 30. perforate-striate exine sculpturing. Scale bars: 19, 20, 22, 23, 25, 26, 28, 29 = 5 μm ; 21, 24, 27, 30 = 0.5 μm .

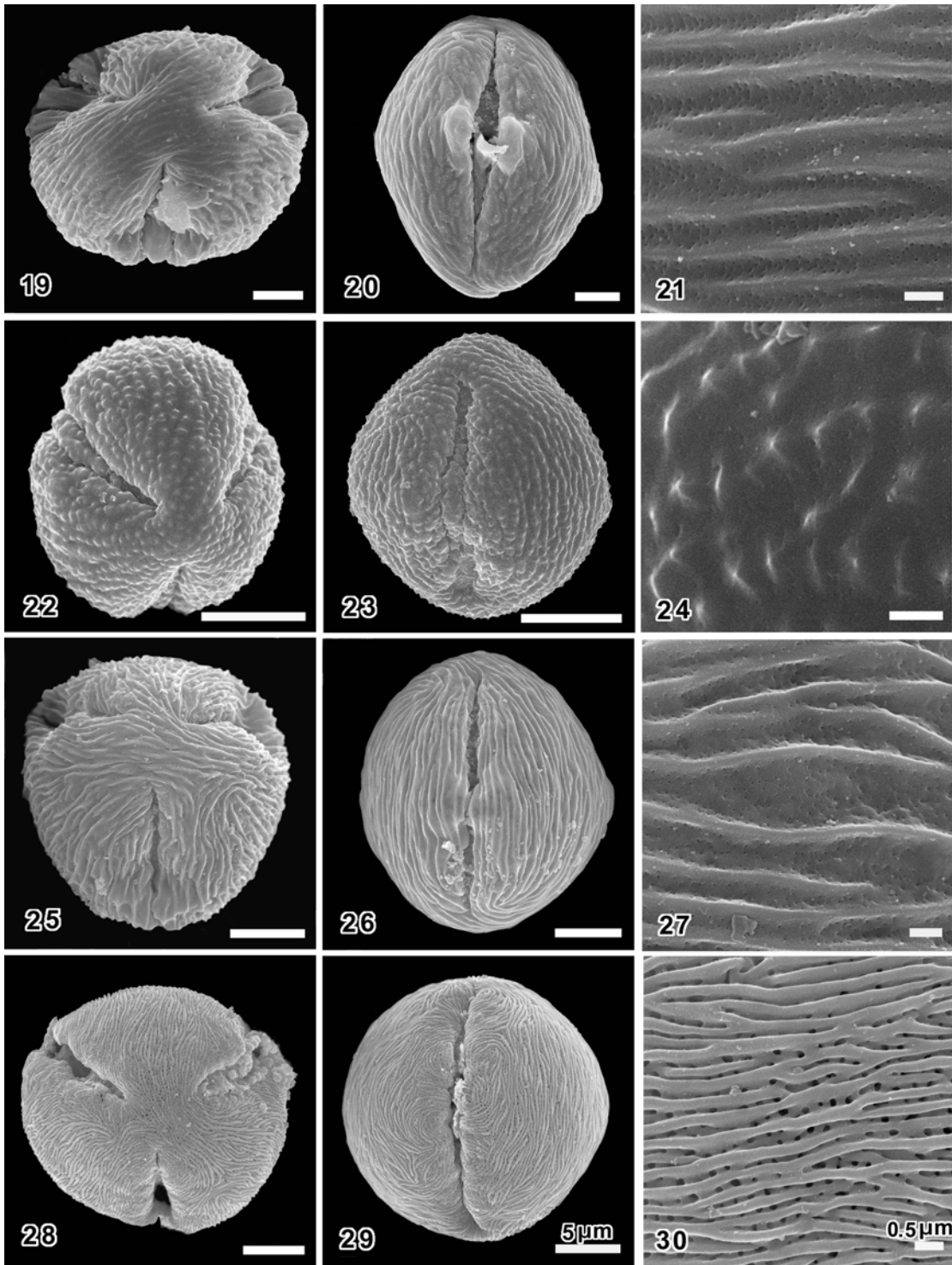
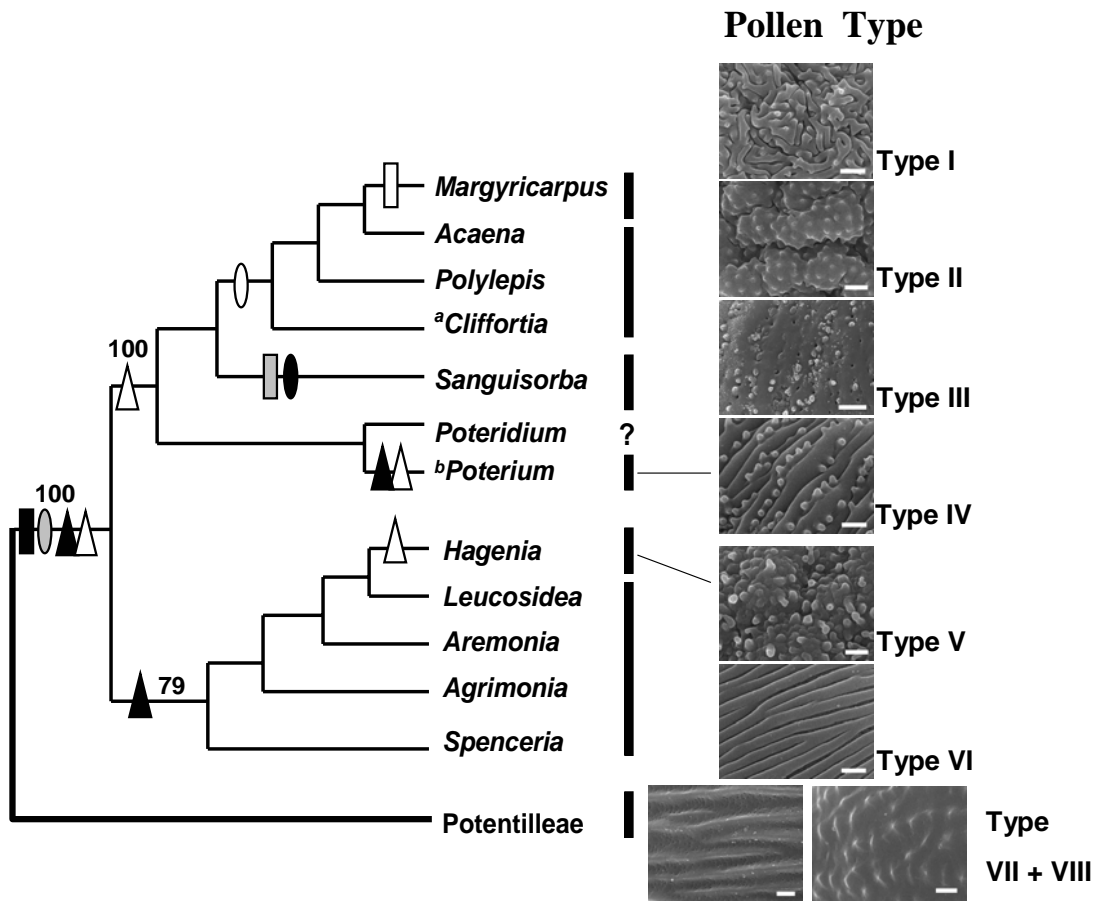


Fig. 31. Phylogenetic tree adopted from Potter et al. 2007 with aperture number, colpus structure, and exine sculpturing patterns mapped and portrayed for Sanguisorbeae. Bootstrap values are indicated. Pollen types and taxa represented: Type I. *Margyricarpus pinnatus*, type II. *Polylepis racemosa*, 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.



CHAPTER 2

CHUNG AND ELISENS – PHYLOGENY AND BIOGEOGRAPHY OF SUBTRIBE AGRIMONIINAE

Phylogeny and Biogeography of Subtribe Agrimoniinae (Rosaceae) using Nuclear and Chloroplast DNA Sequence Data

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ABSTRACT

Phylogeny and biogeography of subtribe Agrimoniinae (Rosoidae) composed of four monotypic endemics (*Aremonia*, *Hagenia*, *Leucosidea*, and *Spenceria*) and a worldwide genus (*Agrimonia*) are constructed from nuclear and plastid sequences (GBSSI-1, *trnL-trnF*, *trnS-trnG-trnG*). All nucleotide data support the monophyly of subtribe Agrimoniinae with a basal group of the Asian monotypic *Spenceria* ($2n=2x=14$), the sister relationship of the African genera *Hagenia* and *Leucosidea*, and the monophyly of *Agrimonia* ($2n=4x$, $6x$, and $8x$) + *Aremonia* ($2n=6x$), a European monotypic genus. In *Agrimonia*, nuclear data define a lineage of Asian and American tetraploids (*Agrimonia coreana*, *A. microcarpa*, *A. parviflora*, and *A. rostellata*). These results hypothesize that the most recent ancestor of the subtribe distributed in Asia (tethyan) and paleo-migration might have occurred between the Northern and Southern Hemispheres. Furthermore, the results strongly suggest that polyploidy is a major evolutionary process in Agrimoniinae and *Agrimonia*, demonstrating the strong correlations between polyploidy and geographic distributions.

Keywords: Agrimoniinae, *Agrimonia*, biogeography, GBSSI-1, polyploidy, *trnL-trnF*, *trnS-trnG*.

INTRODUCTION

Subtribe Agrimoniinae (Rosaceae, subfamily Rosoideae) represents an excellent group to test hypotheses regarding polyploid and morphological evolution and to investigate biogeographic patterns and endemism. The subtribe comprises four monotypic, narrowly endemic genera and one speciose and widely distributed genus: *Aremonia* (1 species, southern Europe), *Hagenia* (1 species, east Africa), *Leucosidea* (1 species, southern Africa), *Spenceria* (1 species, central Asia), and *Agrimonia* (19 species, Eurasia and South America) (Fig. 1A). Most species and genera in the subtribe occur in regions with temperate climates and exhibit variation in growth form (herbaceous, woody), involucre presence, inflorescence type (racemes, panicles), floral structure (bisexual, unisexual), ploidy level ($2x$, $4x$, $6x$, $8x$), and geographic distribution (Table 1; Hutchinson 1964; Kalkman 2004).

The subtribe has chromosome counts representing four ploidy levels based on $x=7$. Similar to cytogeographic patterns observed in many lineages (Stebbins 1971, Tiffney and Manchester 2001; Scheen et al. 2004; Adams and Wendel 2005; Felberf and Ranker 2007), geographic ranges are associated with ploidy level in subtribe Agrimoniinae. *Spenceria* is a narrow endemic and the only known diploid ($2n=14=2x$) in the subtribe; it has been hypothesized to be the most primitive genus (Takahashi et al. 1996; Ikeda et al. 2006). Intermediate geographic ranges characterize the hexaploid species ($2n=42=6x$) found in *Hagenia* and *Aremonia*. The chromosome number for *Leucosidea* is unknown, although its range is intermediate in area. The ranges of species of *Agrimonia* in North America, Europe, and Asia, are

more narrow for tetraploid species ($2n=28=4x$) compared to the greater geographic range for octoploid species ($2n=56=8x$) (Fig. 1B). Based on morphological, biogeographic, and chromosomal data, hybrid and polyploid modes of speciation have been proposed by Iwatsubo et al. (1993) and Ikeda et al. (2006) in subtribe Agrimoniinae. These mechanisms of speciation are common in the Rosaceae and have been documented in several genera (Morgan 1994; Alice and Campbell 1999; Potter et al. 2000; Smedmark 2003).

Previous phylogenetic investigations of tribe Sanguisorbeae and subtribe Agrimoniinae were conducted at a broad scale within the Rosaceae (e.g. Eriksson et al. 2003; Potter et al. 2007). These investigations included seven genera from subtribe Sanguisorbinae and five genera from subtribe Agrimoniinae: the monotypic genera *Aremonia*, *Hagenia*, *Leucosidea* and *Spenceria*, but only one species from *Agrimonia*. Within subtribe Agrimoniinae, Eriksson et al. (2003) and Potter et al. (2007) did not use more than one plastid or nuclear genetic region per species. Although the monophyly of tribe Sanguisorbeae and subtribes Sanguisorbinae and Agrimoniinae were supported (Eriksson et al. 2003, Potter et al. 2007), the monophyly of the genus *Agrimonia* has not been confirmed and phylogenetic relationships among genera have not been investigated with increased sampling within *Agrimonia*.

The most recent and comprehensive classification of *Agrimonia* was proposed by Skalický (1970, 1971, 1973). Based on flower and fruit characters as well as geographic distribution, he proposed five series and seven subseries in the genus. Many of his series encompass a wide range of morphological variation. Skalický

(1971) also postulated evolutionary relationships among the series. He proposed that series *Parviflorae* was basal in the genus and that series *Pilosae* and *Eupatorriae* were the most derived. Skalický's (1970, 1971, and 1973) hypotheses on classification and phylogeny of *Agrimonia* have not been tested in a phylogenetic framework.

To elucidate phylogenetic relationships among genera and species in subtribe Agrimoniinae, we analyzed DNA sequence variation from two plastid markers (*trnL-trnL-trnF* and *trnS-trnG-trnG*) and two nuclear regions (nrITS and GBSSI-1). The primary objectives of our investigations were to: 1) test the monophyly of subtribe Agrimoniinae and the genus *Agrimonia*, 2) determine phylogenetic relationships among genera and whether the central Asian endemic *Spenceria* ($2n=14=2x$) is basal in the subtribe, 3) resolve the major lineages within *Agrimonia* and test Skalický's (1970, 1971, 1973) hypotheses of species and series relationships, and 4) elucidate the pattern of polyploid and morphological evolution and biogeographic relationships in the subtribe.

MATERIALS AND METHODS

Sampling design and outgroup selection

We conducted phylogenetic analyses using the DNAs from 44 accessions of 17 species representing all five genera in subtribe Agrimoniinae (Appendix 2-1), and sequences archived in GENBANK (<http://www.ncbi.nlm.nih.gov>) for five species in five genera from subtribe Sanguisorbinae and 13 species representing 12 outgroup genera (Appendix 2-2). Thirteen of 19 species of *Agrimonia* were sampled; accessions sampled per species ranged from two to five and covered the geographic range of several species. Outgroups were selected from phylogenetic relationships presented in Eriksson et al. (2003) and Potter et al. (2007) that indicated genera in tribes Potentilleae and Colurieae were strongly supported as appropriate outgroups.

Fresh materials for DNA extractions were obtained from field-collected material, and plants propagated from seed, root cuttings, or living material (supplied by G. J. Kline). Voucher specimens were deposited in the Robert Bebb Herbarium (OKL) at the University of Oklahoma. Dry materials for DNA extractions were sampled from herbarium specimens loaned from the following herbaria: A, CBU, DAO, MEXU, MO, MU, SUCH, and TEX.

DNA amplification and sequencing

Based on our pilot studies and previous reports documenting levels of variation suitable for inter- and intrageneric investigations (e.g., Potter et al. 2002; Eriksson et al. 2003;), we selected two plastid and two nuclear genic regions. Primer sequences for the four genic regions are listed in Table 2 and primer locations are depicted in

Figure 2. The two regions of the plastid genome selected were the *trnL*^{UAA} intron + *trnL*^{UAA}–*trnF*^{GAA} intergenic spacer and the *trnS*^{GCU}–*trnG*^{UUC} intergenic spacer + the *trnG*^{UUC} intron (hereafter *trnL-trnF* and *trnS-trnG-trnG*, respectively). These regions have provided useful data at the generic level in several families including the Rosaceae (Shaw and Small 2004; Shaw et al. 2005; Bortiri et al. 2006).

We selected two nuclear regions for sequencing, the nrDNA nuclear ribosomal repeat (ITS1 + 5.8S coding region + ITS2) and the 5' end of a granule-bound starch synthase gene (GBSSI-1 or *waxy*). The ITS regions of nrDNA have been used widely for elucidating phylogenetic relationships among species in the Rosaceae (Alice and Campbell 1999; Helfgott et al. 2000; Bortiri et al. 2006). Two new ITS primers were designed for this investigation (AgITSr and ITSr2). The single or low copy number GBSSI has been investigated and used widely for phylogenetic analyses in the Rosaceae (Bailey and Doyle 1999; Smedmark et al. 2003; Potter et al. 2007). Two copies of GBSSI (-1 and -2) are reported in Rosaceae subfamily Rosoideae (Mason-Gamer et al. 1998); primers specific for GBSSI-1 in the Rosoideae were used in this study (Table 2, Evans et al. 2000). GBSSI-1 has several introns; we targeted four introns between exons 3 to 7 for analysis (Fig. 2). The long fourth intron has considerable variation in subfamily Rosoideae and has been useful at the generic and infrageneric level (Evans et al. 2000).

Each region was amplified via polymerase chain reaction (PCR) of total DNA isolated from fresh and/or dried leaves using the CTAB method (Doyle and Doyle 1987). Primer sequences, PCR conditions, and sequencing procedures for each region

followed Taberlet et al. (1991), Evans et al. (2000), Urbatsch (2000), and Shaw et al. (2005) with minor modifications. PCR-amplified DNAs were checked for band size and number in agarose gels; suitable bands were purified using gel-cutting techniques (Invitrogen, PureLink™ Quick Gel Extraction Kit, Catalog no. K2100-12) before sequence reactions were initiated. Sequencing was performed using the BigDye Terminator kit (Applied Biosystems, Foster City, California) on an Applied Biosystems 3130xl automated sequencer at the Zoology Multi-user molecular laboratory of the University of Oklahoma. Confirmation of targeted genetic regions was conducted via BLAST searches of homologous nucleotide sequences (Altschul et al. 1997). Nucleotide sequences from multiple primers of each accession were aligned initially using CodonCode Aligner (CodonCode Corporation, Dedham, Massachusetts) followed by alignment using ClustalX (Thompson et al. 1997); final alignments were made manually in MacClade version 3.07 (Maddison and Maddison 1997) as needed. Ambiguous sequences were excluded from analyses.

Phylogenetic analyses

Separate phylogenetic analyses were conducted for each genic partition using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian analyses. MP analyses were conducted using PAUP* version 4.0b10 (Swofford 2003) with 100 (MP) heuristic searches with random sequence addition, tree-bisection reconnection (TBR) branch swapping, collapsing zero-length branches, and equal weighting and unordering of all characters. Gaps were treated as missing data, and multiple character states at a site were interpreted as uncertainty. ML analyses also were conducted with

PAUP* and utilized similar parameters as MP analyses except for 10 heuristic searches. Partitioned analyses also were conducted for combined plastid and nuclear data with and without indels. Indels were coded using the simple method (Simmons and Ochoterena 2000) employed in FastGap 1.0.8. (Borchsenius 2007). Clade support was estimated with bootstrap analyses (Felsenstein 1985) conducted with 500 pseudoreplicates for nuclear data and 100 pseudoreplicates for cpDNA data.

Bayesian phylogenetic analyses were performed using MrBayes v.3.0 (Huelsenbeck and Ronquist 2001). Markov Chain Monte Carlo simulations were implemented using 1,000,000 generations sampled every 100 generations. The first 2500 trees (25%) were discarded in the 'burning-in' phase and the remaining 7500 trees used to estimate Bayesian posterior probabilities. Alternative 'burning-in' numbers and higher generations were simulated to see whether tree topology and/or branch support values could be improved. Posterior probability values derived from Bayesian inference served as a measure of support (Hall 2001; Huelsenbeck and Ronquist 2001). This process was repeated with an additional 10,000 generations until topologies and posterior probabilities were stabilized. Separate and combined datasets were analyzed with and without indels.

Concordance of nuclear and plastid datasets was assessed using the Incongruence Length Difference (ILD) test of Farris et al. (1994) using $P > 0.05$ as the criterion for combined analysis.

RESULTS

Characteristics of cpDNA sequence data

trnL-trnL-trnF -- PCR amplification with primers c and d produced a single band in agarose gel electrophoresis. By two forward (primers c and e) and reverse primers (primers d and f), purified PCR products were sequenced (Fig. 2A). The size of the sequence is ca. 910-913 bp (*A. parviflora* 910 bp ~ *Hagenia abyssinica* 913 bp), all species have an A-tail (10-12 bp long) in a ca. 315 bp region and a T-tail (9-11 bp long) in a ca. 805 bp region (Fig. 2A) with variation in length. Insertions and/or deletions are detected in a ca. 700 bp region. The final alignment of *trnL-trnF* data consists of 1079 sites. 88 characters are parsimony-informative (8.15 %), and within ingroup 1.39% of total characters is parsimony-informative (Table 3).

trnS-trnG-trnG -- Primers *trnG* and *trnS* were used for amplification of the regions in PCR reactions and produced a strong band in 1.5 % agarose electrophoresis. The sequences determined by two forward and one reverse primer (Fig. 2B) range ca. 1400 bp. Because of the proximal ends of the primers (Oh and Potter 2003) and unavailability of close outgroup data, the exact length of the regions are uncertain. All ingroup taxa have two T-tails (10-18 bp long) between ca. 1030 and ca. 1100 bp regions with variation in length (Fig. 2B). Insertions and/or deletions are detected in ca. 800 bp and 1120 bp regions. The finalized alignment of 1598 bp was analyzed. Within ingroup, 160 characters are parsimony-informative (1.81%) (Table 3).

Characteristics of nDNA sequence data

nrITS -- Using primers ITS-I and ITS4, the regions were amplified in PCR reactions, and then purified PCR products were sequenced with internal primers ITS2, ITS3, AgITSr, and ITSr2 (Fig. 2C). The size of the whole nrITS regions (ITS1, 5.8S, and ITS2) is ca. 630 (*Hagenia abyssinica* 609bp ~ *Agrimonia parviflora* 660bp). The deletion of 23 bp is detected in *Hagenia* and *Leucosidea*. Variance in ingroup is very high, nearly 20% and, variation among accessions is often higher than among species. In addition, ILD test of combined data with GBSSI-1 shows high incongruence ($P = 0.0302$). For these reasons, further phylogenetic analyses of nrITS data are suspended.

The examination of the sequence chromatograms was conducted to confirm the presence of incomplete homogenization. One of the most intriguing features of nrITS is that sites of homogenization in action can be detected in recent polyploids (Kovarik et al. 2005; Soltis et al. 2008). *Agrimonia nipponica* and *A. pubescens* exhibited several polymorphic sites. However, because the patterns and sites were not consistent among accessions within the species, it cannot be determined as incomplete homogenization. In addition, the species showing polymorphic sites are tetraploids, which can be considered as diploids in *Agrimonia*.

GBSSI-1 -- Using primers 3F and 7R, from 3rd exon to 7th exon including four intron regions (from 3rd to 6th) were amplified in PCR reactions, and then with primers 3F, 3R, and 7R, purified PCR products were sequenced (Fig. 2D). Searches of nucleotide sequences from each accession using BLAST (Altschul et al. 1997) showed high homology with GBSSI-1 nucleotide sequences from *Geum reptans*

(AJ534187). Including outgroup, the 913 bp aligned were analyzed. 15.9% of characters is parsimony-informative with outgroup, and within ingroup, 9.2% of characters is parsimonious-informative. The combined dataset with indels improves consistency index (CI), retention index (RI), as well as tree resolution (Table 3).

Combined datasets of cpDNA and/or nDNA with and/or without indels

ILD (Farris et al. 1994) tests were conducted prior to the phylogenetic analyses for all combined datasets: 1) *trnL-trnF* + *trnS-trnG-trnG*, 2) two cpDNA regions + their indels, 3) GIBSSI-1 + its indels, and 4) combined all cpDNA + combined GIBSSI-1. All combined datasets showed high congruence ($P \approx 1$), except the combined data of all cpDNA and GIBSSI-1 data, which were largely incongruent ($P = 0.0625$). In all datasets, the inclusion of indels increases not only variable characters but also parsimonious informative characters (Table 3). In particular, indels in the GIBSSI-1 data are greatly informative (Fig. 3).

Phylogenetic trees from plastid data

In all the separate and combined datasets, the analyses indicate the monophyly of the subtribe with a strong support (bootstrap=100%, posterior probabilities=100%), having *Spenceria* as a basal group, and *trnL-trnF* data shows the sister relationship between *Hagenia* and *Leucosidea* (Figs. 4, 5). All the data also place *Aremonia* within an *Agrimonia* clade with a high support (bootstrap=100%, posterior probabilities=100%), but a clade where *Aremonia* is placed is largely unresolved. The parsimony and Bayesian analyses of *trnL-trnF* data including all the genera in tribe Sanguisorbeae as in Potter et al. 2007 also supported a monophyly of Agrimoniinae

with a basal group of *Spenceria* (trees not shown; 427 steps, CI=0.8337, RI=0.8774), and topologies of ingroup taxa are the same as Fig. 4A. For *Agrimonia*, the separate *trnS-trnG-trnG* and combined data of two cpDNA regions with and without indels (Figs. 4B, 5) make the clade with *Agrimonia incisa*, *A. parviflora*, *A. rostellata*, and *A. microcarpa* although the *trnL-trnF* data forms the polytomies of them (Fig. 4A).

Phylogeny from nuclear data

GBSSI-1 datasets both without and with indels in all analyses indicate that the subtribe is monophyletic (bootstrap=99%, posterior probabilities=100%) and *Aremonia* is nested within *Agrimonia* (bootstrap=99%, posterior probabilities=100%). *Spenceria* is basal in the subtribe, and *Hagenia* and *Leucosidea* are sister, having a sister relationship to *Spenceria*. The clade of *A. coreana*, *A. microcarpa*, *A. parviflora*, and *A. rostellata* is basal in the *Agrimonia+Aremonia* clade (Fig. 6). Instead of *A. incisa* (a member of the basal clade in *Agrimonia+Aremonia* clade in the plastid trees), *A. coreana* is a member of the basal clade (bootstrap=94%, posterior probabilities=100%). *Agrimonia striata* and *A. pilosa* are sister to each other although support values are relatively low and placements of the clade are uncertain (Fig. 6, bootstrap=55%, posterior probabilities=88%).

DISCUSSION

Phylogenetic utility of genetic markers

As many previous studies have shown (e.g. Bailey and Doyle 1999; Oh and Potter 2005), nDNA markers are phylogenetically more informative than cpDNA regions (Table 4), and the GBSSI-1 shows a lower homoplasy level than cpDNA makers. In particular, CI and RI values in ingroup of GBSSI-1 are very high with and without indels, indicating low homoplasy of the sequences. In cpDNA makers, the *trnS-trnG-trnG* regions exhibit higher divergence than the *trnL-trnF* maker. These are consistent with previous observations (e.g. Shaw et al. 2005; Shaw et al. 2007).

The nrITS regions are too ambiguous to be analyzed. Uncertainty on interpreting nrITS data has been detected in many flowering plants (Álvarez and Wendel 2003) including subfamily Rosoideae (e.g. Eriksson 1998; Kerr 2004). For example, with the nrITS data alone, *Sanguisorbeae* appeared polyphyletic nested within *Potentilla* although the monophyly of *Sanguisorbeae* was confirmed by combined nuclear and cpDNA data (Eriksson et al. 2003). This inconsistent result may be caused by complex evolutionary history of the given taxa, reflecting diverse evolutionary consequences in different genetic regions. Diverse evolutionary rates of nrITS within ingroup and with outgroup taxa are too great to allow confident alignment and rooting (Álvarez and Wendel 2003). The phylogenies of polyploid taxa have been misinterpreted by nrITS data (Soltis et al. 2008). The low-copy nuclear gene GBSSI-1 is utilized due to ambiguity of nrITS, and this region provides

significant information. The low-copy nuclear genes can play a critical role for the phylogenetic investigations of polyploid taxa (Álvarez and Wendel 2003).

In our separate analyses of the cpDNA and nDNA partitions, strong topological conflicts are detected as shown in many flowering plants (e.g. Smedmark and Eriksson 2002; Eriksson et al. 2003; Morgan 2003). For example, incongruence of species within the basal clades of the *Agrimonia*+*Aremonia* is significant between two partitions. The cpDNA datasets form the clade with only American tetraploids (*A. incisa*, *A. microcarpa*, *A. parviflora*, and *A. rostellata*), whereas the GBSSI-1 datasets produce the clade of an Asian tetraploid (*A. coreana*) and three American tetraploids (*A. microcarpa*, *A. parviflora*, and *A. rostellata*), placing *A. incisa* as a basal group to the unresolved clade of American and Eurasian species. Potential causes of the conflicts on the topologies are discussed in the following section.

Phylogeny of Agrimoniinae

Chloroplast and nuclear DNA results strongly support the monophyly of Agrimoniinae and agree with previous hypotheses (Eriksson et al. 2003; Potter et al. 2007), and they also provide great support for the monophyly of *Agrimonia*+*Aremonia*. In general, the plastid and nuclear data result in congruence on the phylogenetic relationships among the genera: 1) Asian endemic *Spenceria* is basal in the subtribe, 2) the African endemic *Hagenia*+*Leucosidea* (although tree topologies differ in two partitions) clade is sister to *Spenceria*, and 3) tetraploid *Agrimonia* species are basal in the *Agrimonia*+*Aremonia* clade.

The Asian endemic diploid is basal in the subtribe and sister to the African genera (Fig. 7). *Spenceria* is strictly endemic to Southwest China and Bhutan in the Hengduan mountain range, a well known hotspot of biodiversity (Nie et al. 2002; Ikeda et al 2006). In this region, over 20% of plants are endemic and diploid species are predominant with only 22% of the polyploid frequency, suggesting that polyploidy may have played a minor role in evolution and biodiversity (Nie et al. 2002; Nie et al. 2005). A series of floristic investigations of this region has been conducted (e.g. Biodiversity of the Hengduan Mountains and adjacent area of south-central China), but phylogeny of the endemic taxa has not been elucidated. Our results suggest that *Spenceria* is an ancient group in the subtribe and has a close relationship with the African lineage.

The cpDNA and nDNA provide strong support for the basal group consisting of only tetraploid species in the *Agrimonia+Aremonia*, although the members of species in the clades and relationships among those species are incongruent between cpDNA and nDNA data (Figs. 5, 6). These tetraploid species are geographically restricted only in either eastern North America (*Agrimonia incisa*, *A. parviflora*, and *A. rostellata*) or eastern Asia (*A. coreana*). Morphological distinction of *A. coreana* from the other Asian species also supports this result. In eastern Asia, *A. coreana* and *A. nipponica* share geographic areas, but they can be easily distinguished by fruit shape and size, flowering time, and ecological habitats. *A. coreana* is considered to be an ancient group in the *Agrimonia+Aremonia* lineage.

Phylogeny of *Agrimonia* (Testing Skalický hypotheses)

Because the species in *Agrimonia* are largely unresolved, it is hard to define all major lineages in the genus and test Skalický's hypotheses. However, based on a basal lineage in the genus and some resolved relationships among species, Skalický's hypotheses on classification are not supported although some of his phylogenetic hypotheses on a species level can be accepted. The closely related species *Agrimonia parviflora*, *A. microcarpa*, and *A. rostellata* were divided into two series in Skalický's classification: *Tuberosae* (*Agrimonia microcarpa*, *A. pubescens*, and *A. rostellata*) and *Parviflorae* (*A. incisa* and *A. parviflora*). The GBSSI-1 evidences reject his hypothesis on the recent divergence of *A. coreana*, but the results prove the hypothesis on early divergence of *A. parviflora* (by the cpDNA and nDNA) and *A. incisa* (only by cpDNA).

One of the interesting results is that most broadly distributed Eurasian (*Agrimonia pilosa*) and North American (*A. striata*) octoploids form a clade although support values are not significant (Fig. 6). Morphological variation within the species and similarity between the two species has caused complex taxonomies and difficulty on species delimitation (e.g. Nakai 1933; Rydberg 1913; Kline and Sørensen 2000). In addition, *A. pilosa* has even been considered as a variety of *A. striata* (annotated specimens by Rumjantsev, per. obs.). Our GBSSI-1 results suggest that the similar morphology between the two species can be homologous.

The relationships among the rest of Asian, European, and American species are largely unresolved. Combined cpDNA data support a close relationship between

Agrimonia gryposepala (American 8x) and *A. pubescens* (American 4x) and between *A. eupatoria* (European 4x) and *A. procera* (European 8x) (Fig. 5), but the placements of the species are not consistent with the nuclear data (Fig. 7). The polytomies are thought to be caused by lack of informative data, known as a soft polytomy (Salemi and Vandamme 2003). Therefore, more gene sampling is required to resolve phylogenetic relationships among the species.

All data strongly support the monophyly of *Agrimonia* and *Aremonia*, suggesting a hybrid origin of *Aremonia* (Fig. 7). *Aremonia agrimonoides* (European endemic 6x) shares geographic regions with *Agrimonia repens* (European 4x) and is well adapted to high elevation. Character losses following hybridity might explain morphological variation between the two genera. Recent molecular evidence shows that hybridity and polyploidy can promote an adaptation (Hegarty and Hiscock 2004).

We propose that *Aremonia agrimonoides* (L.) Neck. should be included in *Agrimonia* as originally proposed by Wallroth 1842 because all cpDNA and nDNA datasets indicate the monophyly of *Agrimonia+Aremonia* with strong support, and morphological characters also supports the monophyly of the group. Both genera have interrupted pinnate compound leaves, and papillae sculpturing of seed coat is newly reported here as a synapomorphy for the *Agrimonia+Aremonia* (Fig. 2B in Chapter 3).

Conflicts between plastid and nuclear sequence data

The most significant incongruence between cpDNA and nDNA is the difference on members of species in basal clades in the *Agrimonia+Aremonia* (Figs. 5, 6). The basal clade by the nDNA data has the Asian tetraploid species *Agrimonia*

coreana instead of the American tetraploid species *A. incisa* in the cpDNA. Under the assumption of nDNA-supported relationships (Fig. 7), the occurrence of introgression from *A. incisa* can be postulated, or *A. incisa* may maintain the ancestral cpDNA haplotype shared with the other American tetraploids, known as lineage sorting (Doyle 1992) or deep coalescence (Maddison and Maddison 1997).

Agrimonia is thought to evolve via hybrid and polyploidy, and conflicting topologies among markers suggest complex evolutionary events in *Agrimonia*. We presume that this group has diverse polyploids not only in polyploid levels but also in ages and types of polyploidy (e.g. neopolyploids vs. older polyploids and/or autopolyploids vs. allopolyploids). The rates of polyploidy also have an association with reproductive systems: polyploids self-fertilize more than diploids (Barringer 2007). In *Agrimonia*, hybrid and polyploidy seem to have played a major role in speciation and evolution, and incongruence on topologies among markers may have resulted from a series of hybrid and polyploid events and consequences of them. We will attempt to explain the conflicting patterns when more data, especially from additional nuclear genes, are analyzed.

Polyploidy

Fig. 7 demonstrates that a minimum of three polyploid events are required for the current polyploid levels of each terminal taxon: one in the *Hagenia+Leucosidea*, one for the *Agrimonia+Aremonia* clade, and more recently one for the unresolved *Agrimonia* clade. The ploidy level of *Leucosidea* has not been confirmed, but because chromosome number of the genus is at least more than 22 (per. obs.), we postulate

polyploidy for the *Hagenia+Leucosidea* clade. Placements of the first two polyploid events are congruent to where the homoplasious evolution of four morphological characters may have taken place (Fig. 2A in chapter 3), suggesting that morphological character evolution might have followed polyploidy.

Polyploidy has played an important role in the evolution of Agrimoniinae, demonstrating a strong association with geographic distribution patterns and morphological character evolution. Polyploidy has impacted gene formation and modification and promoted adaptive evolution and speciation over evolutionary time (Rieseberg et al. 2003; Adams and Wendel 2005). More detailed genetic and phylogenetic investigations are required to test hypotheses on association between polyploidy and morphological character evolution in the subtribe.

Biogeography

The placement of *Spenceria* (Central Asia) as a basal group in Agrimoniinae suggests that the subtribe may have derived from Tethyan (Fig. 7). *Spenceria* occurs in Eastern Asiatic region in boreal floral subkingdom and Irano-Turanian region in Tethyan subkingdom (Takhtajan 1986), and *Agrimonia+Aremonia* occurs in both floristic regions, having broader distribution. *Hagenia+Leucosidea* is thought to be an ancient lineage derived from Tethyan in an early evolutionary period.

As a most parsimonious explanation, a most common ancestor of the subtribe is likely to be in Asia, and three migration events to Africa, Eastern North America, and Europe can be postulated. In particular, a most common ancestor might have broadly distributed throughout the Tethyan regions. The migration event to Africa is

thought to have taken place in the early evolutionary time of the subtribe. Estimated time for the migration is ca. 40 Million years ago (Mya) with a consideration of estimated origin of Rosaceae (Morgan et al. 1994), and here we refer to it as a paleo-migration. The following migration events to Eastern North America and to Europe are more probable by an assistance of bristly epicalyxes on fruits of *Agrimonia*.

The basal group consisting of North American tetraploids (*A. parviflora*, *A. rostellata*, and *A. microcarpa*) and an Asian tetraploid species (*A. coreana*) occur strictly in eastern parts of the continents. It might have been caused by geological events such as repeated glaciations in the continents in diverse time periods (Tiffney and Manchester 2001; Donoghue and Smith 2004). These tetraploids may have been distributed wider before, and dispersed by the Bering land bridge (BLB), which has played an important role for dispersal and/or as a physical barrier between the continents prior to either allopatric or sympatric speciation (Tiffney and Manchester 2001).

The circumboreal distributions of octoploids in the three continents are congruent and support a complex and dynamic biogeographic history of the Northern Hemisphere (Tiffney 1985; Wen 1999; Xiang and Soltis 2001). Although relationships among the Asian, European, and North American species are largely unresolved, the clade is well supported (Figs. 5A, 6). When early divergence of the African and the *Agrimonia*+*Aremonia* lineages is proved, migration events can be hypothesized not only via the BLB but also by the North Atlantic land bridge which was available ca. 40 Mya (Tiffney and Manchester 2001, Scheen 2004).

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TABLE 1. Character differences among genera in subtribe Agrimoniinae.

Genus (species)	Habit	Ploidy level	Pollen shape/ Exine pattern	Inflorescence type	Flower sexuality	Involucre presence	Geographic distribution
<i>Agrimonia</i> (19)	Herb	4x, 6x, 8x	Elliptic/ Striate	Raceme	Bisexual	Absent	Temperate N. & S. America, Eurasia, Central Europe
<i>Aremonia</i> (1)	Herb	6x	Elliptic/ Striate	Raceme	Bisexual	Present	Central Europe
<i>Hagenia</i> (1)	Tree	6x (?)	Circular/ Microverrucate	Panicle	Unisexual /Dioecious	Absent	Central Africa
<i>Leucosidea</i> (1)	Shrub	?	Elliptic/ Striate	Raceme	Bisexual	Absent	Southern Africa
<i>Spenceria</i> (1)	Herb	2x	Elliptic/ Striate	Raceme	Bisexual	Present	Central Asia

TABLE 2. Primer sequences used in phylogenetic analyses of tribe Sanguisorbeae and subtribe Agrimoniinae.

Locus	Primer	Sequence	Source
<i>trnL-trnL-trnF</i>	c B49317	CGA AAT CGG TAG ACG CTA CG	Taberlet et al. 1991
	d A49855	GGG GAT AGA GGG ACT TGA AC	Taberlet et al. 1991
	e B49873	GGT TCA AGT CCC TCT ATC CC	Taberlet et al. 1991
	f A50272	ATT GAA CTG GTG ACA CGA G	Taberlet et al. 1991
<i>trnS-trnG-trnG</i>	<i>trnS</i>	AGA TAG GGA TTC GAC CCT CGG T	Shaw and Small 2004
	<i>3trnG</i>	GTA GCG GGA ATC GAA CCC GCA TC	Shaw and Small 2004
	<i>5trnG2S:</i>	TTT TAC CAC TAA ACT ATA CCC GC	Shaw et al. 2005
	<i>5trnG2G:</i>	GCG GGT ATA GTT TAG TGG TAA AA	Shaw et al. 2005
nrITS	ITS-I	RTC CAC TGA ACC TTA TCA TTT AG	Urbatsch 2000
	ITS 2	GCT GCG TTC TTC ATC GAT GC	White et al. 1990
	ITS 3	GCA TCG ATG AAG AAC GCA GC	White et al. 1990
	ITS 4	TCC TCC GCT TAT TGA TAT GC	White et al. 1990
	AgITSr	TAA ATT CAG GTA ACC CCG	This study
	ITSr2	AAT TCA CGC CGG TGT TCG	This study
GBSSI-1	3F	TAC AAA CGA GGG GTT GAT CG	Evans et al. 2000
	3R	AGA CGG AGA TTC TAC CTG GCA T	This study
	7R	CCT TGG TAA GCA ATG TTG TG	Evans et al. 2000

Note R=A/G; Y=C/T

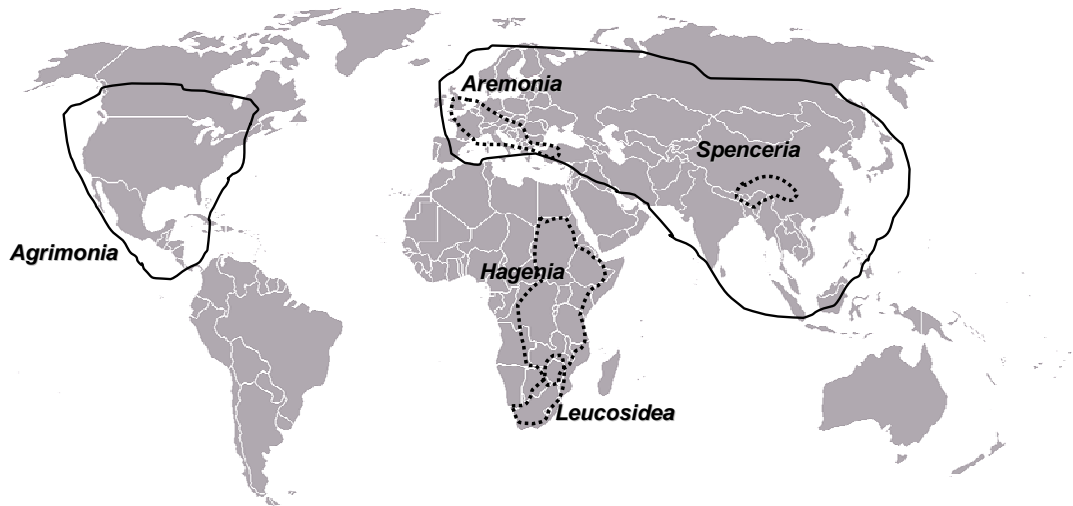
TABLE 3. Characteristics of cpDNA and nuclear GBSSI-1 regions sequenced in phylogenetic analyses of tribe Sanguisorbeae and subtribe Agrimoniinae.

Comparisons among ingroup taxa are indicated in parentheses.

DNA regions		number of characters	number of variable characters	number of informative characters	% of informative characters
cpDNA	<i>trnL-trnL-trnF</i>	1079	233 (71)	88 (15)	8.15 (1.39)
	<i>trnS-trnG-trnG</i>	1598	322 (119)	160 (29)	10.01 (1.81)
	<u>Combined</u>				
	without indels	2677	545 (190)	248 (44)	9.26 (1.64)
	with indels	1875	742 (251)	345 (66)	12.00 (2.30)
Nuclear GBSSI-1	without indels	913	382 (162)	145 (84)	15.9 (9.2)
	with indels	1008	476 (205)	179 (100)	17.8 (9.9)

FIG. 1. Geographic distribution of subtribe Agrimoniinae. A. Range of five genera in the subtribe, showing the four monotypic genera and regional endemics *Aremonia*, *Hagenia*, *Leucosidea*, and *Spenceria*. B. Geographic range of 13 tetraploid and octoploid species in genus *Agrimonia*, showing narrower range of tetraploids.

A.



B.

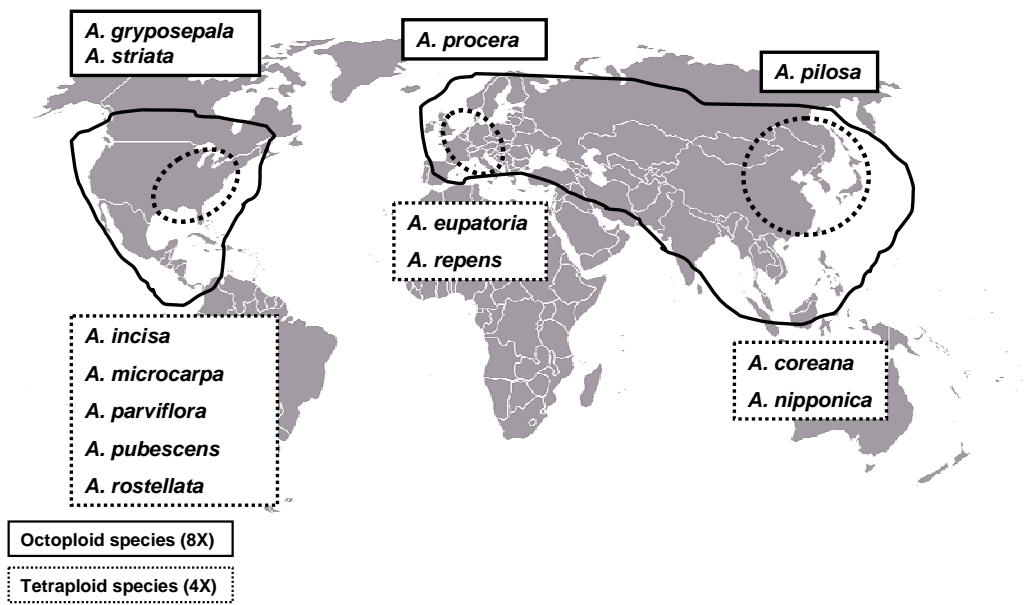


FIG. 2. Primer locations and DNA regions sequenced in phylogenetic study of tribe Sanguisorbeae and subtribe Agrimoniinae. A. *trnL-trnL-trnF* intron and spacer. B. *trnS-trnG-trnG* spacer and intron. C. nrITS spacers. D. GBSSI-1 exons 3-7, 4 and introns. Open boxes represent coding regions and lines represent noncoding regions. The arrows indicate proximal primer annealing sites. Striped circles indicate locations of T-tails (horizontal) and an A-tail (vertical).

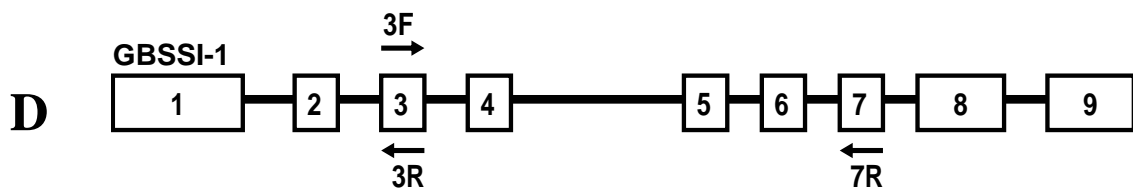
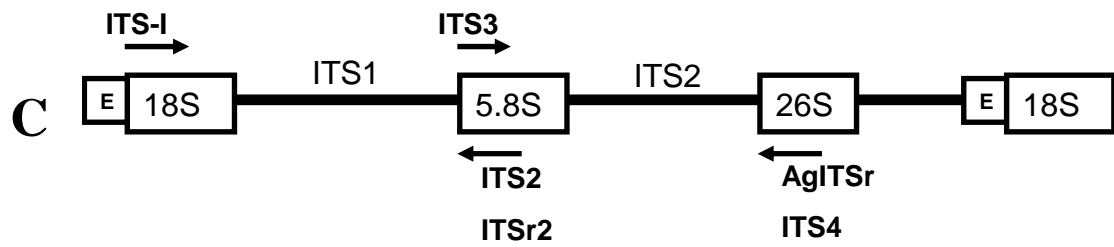
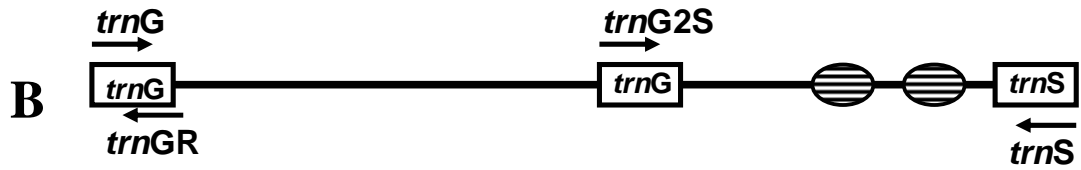
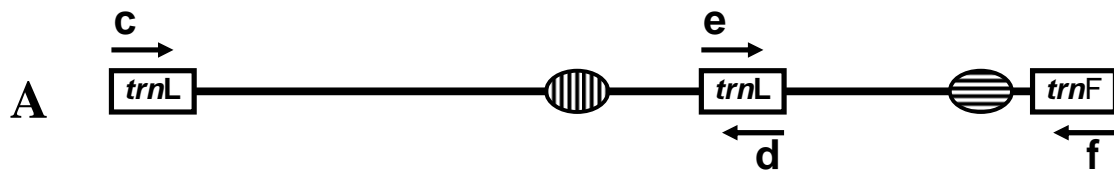
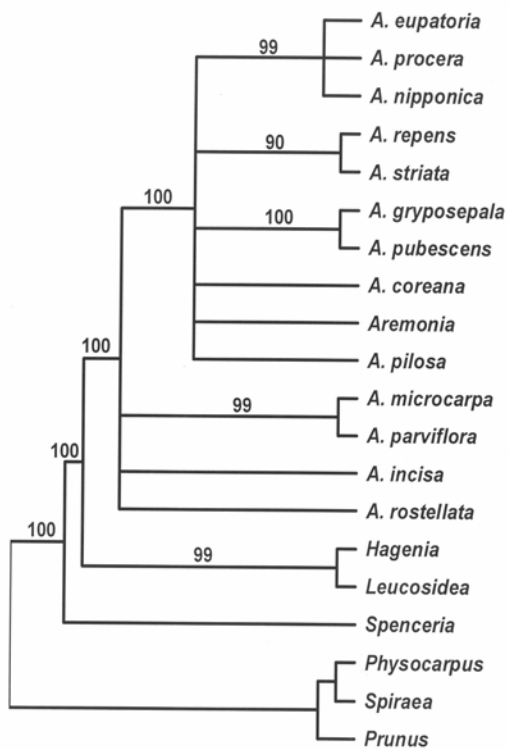


FIG. 3. Insertions and deletions in GBSSI-1.

	560	570	580	590	600
<i>Agrimonia coreana</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAA----	-----	-----GCC
<i>A. eupatoria</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. gryposepala</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. incisa</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. microcarpa</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAA----	-----	-----GCC
<i>A. nipponica</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. parviflora</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAA----	-----	-----GCC
<i>A. pilosa</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. procera</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. pubescens</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. repens</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>A. rostellata</i>	TGGTTGAAAAG	GGCAGCTTTC	GCTGAA----	-----	-----GCC
<i>A. striata</i>	TGGTTGAAAAG	GGCATCTTTC	GCTGAAGAGC	TTG-----A	GATCTTTGCC
<i>Aremonia</i>	TGGTTGAAAAG	GGCAGCTTTC	ACTGAAGAGC	TTG-----A	GATCTTTGCC
<i>Hagenia</i>	-----	-----TTGC	ATTCAAGTGA	TTT-----AA	CATCTTTGCC
<i>Leucosidea</i>	-----	-----TTGC	ATTCAAGTGA	TTT-----AA	CATCTTTGCC
<i>Spenceria</i>	TGGTCAAAAAG	GGCATCTTTT	GCTGAAGAGA	TTGCATTTAA	GATCGTTGGC
<i>Geum</i>	TGGTTAAGTA	GGC----TTC	AGGGTCAAAT	TTGCCTTTTA	CATCTCTGGT
<i>Rosa</i>	TGGTCGAAAAG	GGCAGCTTTC	GCTGAAGAGA	TTGCCTTGTG	GATTTTTGCC
<i>Rubus</i>	TGGTTTAAAAG	GGCAGCTTTC	GTAGAAG---	-----	-----

FIG. 4. Phylogenetic trees from Bayesian analyses of each cpDNA region without indels. Bayesian posterior probabilities (%) are shown. A. *trnL-trnL-trnF*. B. *trnS-trnG-trnG*.

**A. *trnL-trnF*
no indels**



**B. *trnS-trnG-trnG*
no indels**

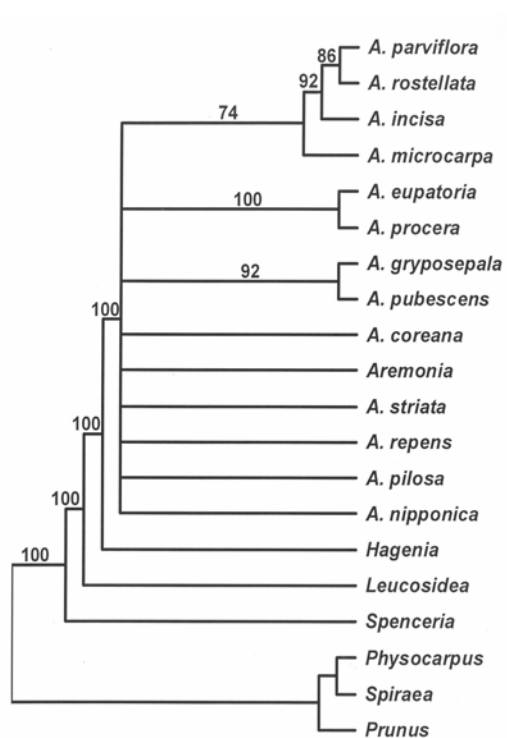
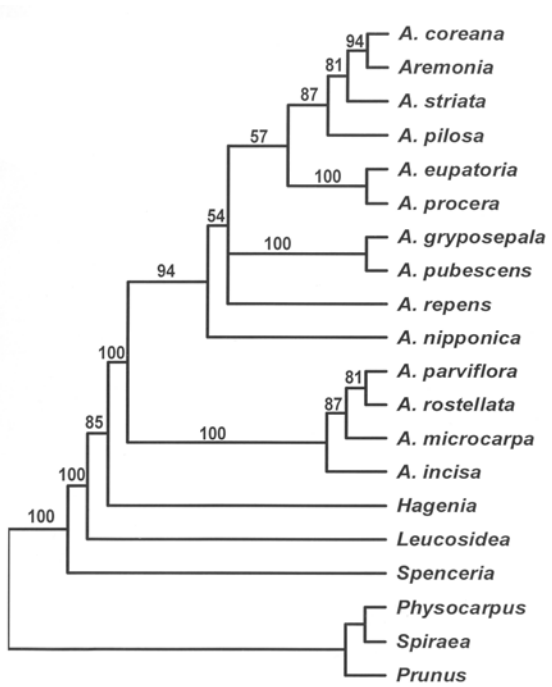


FIG. 5. Phylogenetic trees from Bayesian and Maximum Parsimony (MP) analyses of combined cpDNA data with indels. A. Bayesian analysis with posterior probabilities (%). B. Strict consensus tree from four most parsimonious MP trees with bootstrap values (%); tree length = 919, CI = 0.8901, RI = 0.8513.

**A. Bayesian cpDNA
+ indels**



**B. MP strict consensus cpDNA
+ indels**

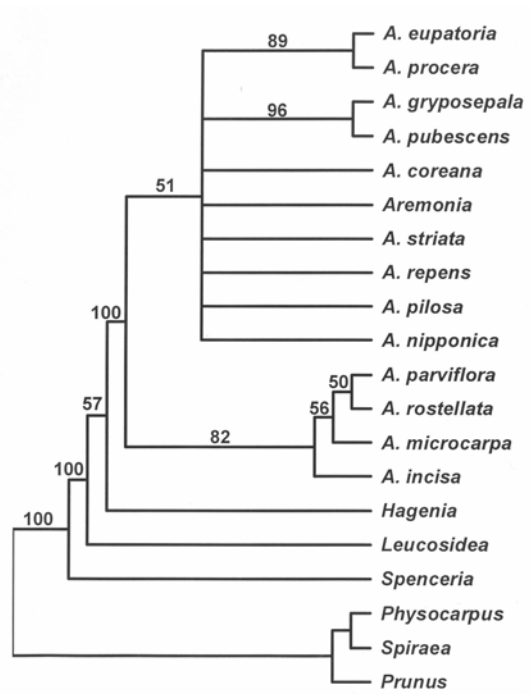
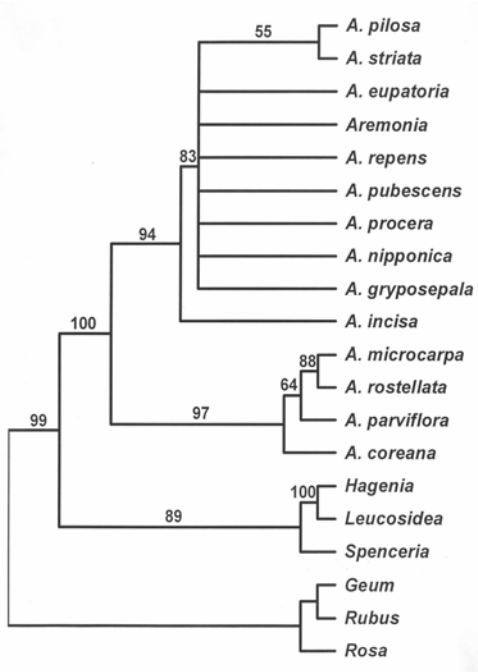


FIG. 6. Comparison of phylogenetic trees from Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian analyses of GBSSI-1 data with indels included. A. Strict consensus tree of MP and ML analyses that had concordant topologies. Bootstrap values (%) are indicated (tree length = 645, CI = 0.8760, RI = 0.7849). B. Bayesian tree with posterior probabilities (%) shown.

A. GBSSI-1 MP and ML



B. GBSSI-1 Bayesian

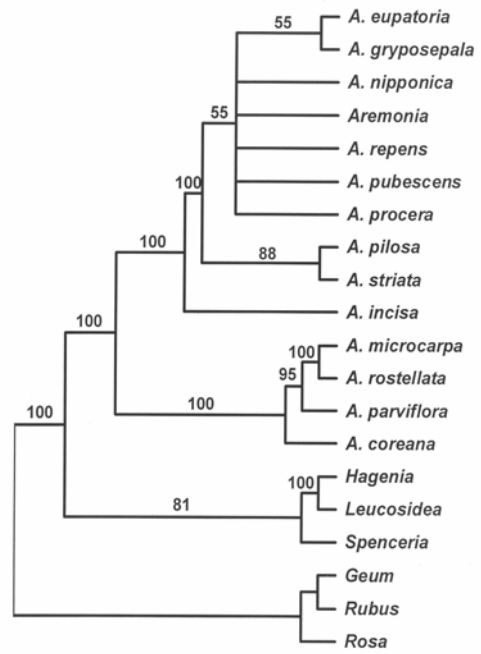
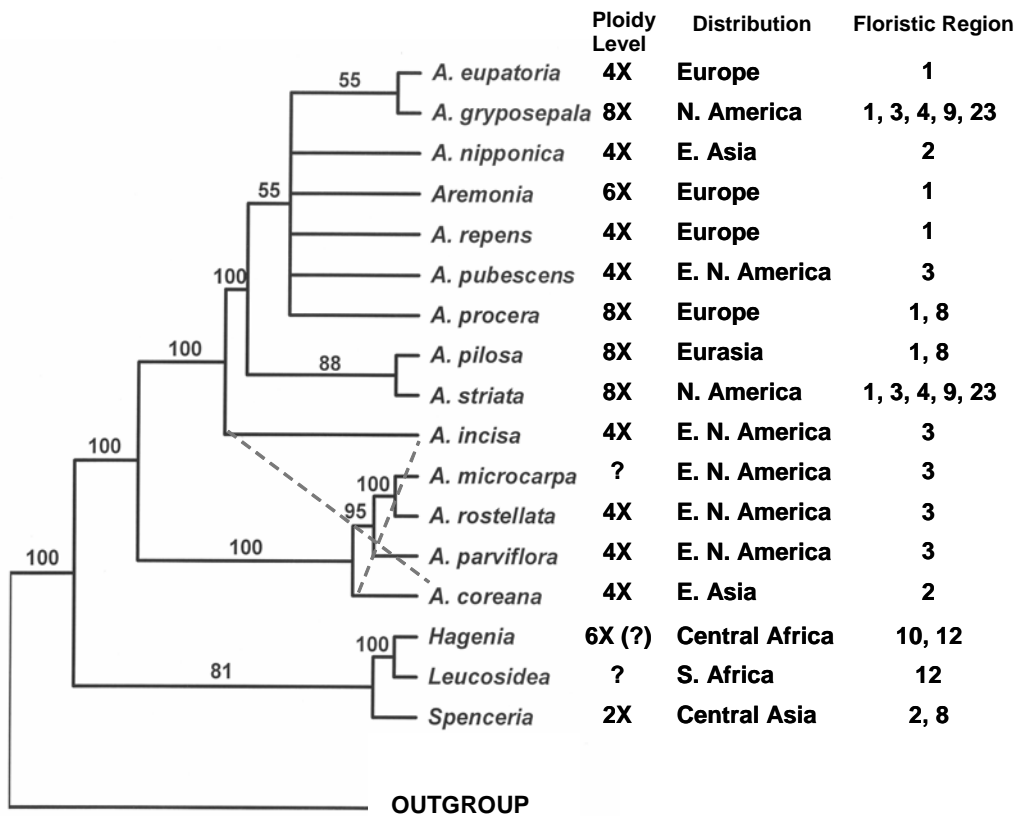


FIG. 7. Ploidy levels, geographic distribution, and floristic regions of species and genera in subtribe Agrimoniinae indicated on Bayesian GBSSI-1 tree with Posterior Probabilities. Topological conflict with *A. incisa* and *A. coreana* on Bayesian cpDNA tree is indicated by dashed lines.

Floristic regions are those of Takhtajan (1986): 1. Circumboreal, 2. Eastern Asiatic, 3. North American Atlantic, 4. Rocky Mountain, 8. Irano-Turanian, 9. Madrean, 10. Guineo-Congolian, 12. Sudano-Zambezian, 23. Caribbean.



CHAPTER 3

CHUNG AND ELISENS: SYSTEMATICS OF GENUS *AGRIMONIA*

Taxonomic Monograph of genus *Agrimonia* (Rosaceae)

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ABSTRACT

The genus *Agrimonia* (Rosaceae, Rosoideae, Sanguisorbeae) is characterized as a monophyletic group of 19 species defined by two unique characters, interrupted pinnately compound leaves and bristly epicalyx segments. Species occur primarily in temperate regions throughout North America, Central America, the West Indies, southern South America, Europe, Asia, and southern Africa. Descriptions, nomenclatural histories, distribution maps, and keys are presented to 19 species and three varieties of *A. pilosa*. Neotypes of *A. hirsuta* and *A. nipponica*, an isolectotype of *A. pilosa*, and a lectotype of *A. villosa* are designated. Characters most useful for delineating species and varieties include apical leaflets, interstitial leaflets, epicalyx segments, and bristly epicalyx segments, and fruiting hypanthia. The evolution of polyploidy and 12 morphological characters is evaluated within a phylogenetic framework.

INTRODUCTION

The genus *Agrimonia* L. comprises 15 to 25 species distributed mainly in temperate regions throughout North America, Central America, the West Indies, southern South America, Europe, Asia, and the southern Africa (Hutchinson 1964; Skalický 1973; Robertson 1974; Kalkman 2004). Because previous taxonomic treatments have been presented primarily as regional investigations or conducted in a floristic context (e.g. U.S.S.R. - Juzepczuk 1941; Japan - Naruhashi 2001; China - Li et al. 2003; North America - Kline & Sørensen in press), a range of specific and infraspecific concepts and an incomplete view of taxonomic and biogeographic relationships have been proposed. We present a taxonomic treatment dealing with all species worldwide; we recognize 19 species including one species, *A. pilosa*, with three varieties.

Species included in the genus *Agrimonia* are herbaceous perennials that share two characters unique in tribe Sanguisorbeae, interrupted pinnately compound leaves and fruits armed with bristles (Rydberg 1913; Robertson 1974; Kalkman 2004). The base chromosome number is $x=7$ (Darlington and Wylie 1955; Skalický 1968), and a range of ploidy levels representing naturally occurring tetraploids ($2n=4x=28$) and octoploids ($2n=8x=56$) has been observed within the genus. Natural interspecific hybrids with intermediate ploidy levels ($2n=6x=42$) also have been reported in *Agrimonia* (Hara and Kurosawa 1968; Murata and Umemoto 1983; Stace 1991; Iwatsubo et al. 1993). Wallroth (1842) and Skalický (1962) pointed out that hybrid cultivars greatly complicated our understanding of species boundaries within

Agrimonia. Additionally, the European species *A. procera*, *A. odorata*, and *A. repens* were misidentified commonly as *A. eupatoria*, which further complicated the taxonomic history of the genus.

Several species in *Agrimonia* have been used for medicinal and horticultural purposes. *Agrimonia eupatoria*, the most common species of *Agrimonia* found in Europe, was used in folk medicine as a hemostatic agent, tonic for asthenia, astringent for diarrhea, and a diuretic; it also was grown commonly in gardens (Miller 1840; Bilia et al. 1993). *Agrimonia eupatoria* was reported initially in North America in the 19th century but was introduced most likely as a medicinal and gardening herb (Kline and Sørensen 1990; Wessel and Thieret 2000). In China, *A. pilosa* is a well known traditional medicinal herb (Liou 1959; Yu and Li 1977). Li (2005) revealed that three common Chinese medicinal herbs including *A. pilosa* showed anti-HSV-1 activity contributed from polyphenolic compounds.

The taxonomic history of *Agrimonia* is remarkably complicated. Although 19 species are recognized here, Wallroth (1842) published a monograph of *Agrimonia* with 14 species, whereas Bentham and Hooker (1865) and Hutchinson (1964) recognized 21 and 25 species, respectively. More than 80 species and varieties have been named, and many species, subspecies, and varieties were described with minimal protologues (e.g. Michaux 1803; Muhlenberg 1813; Barton 1815; Nakai 1933; Hara and Kurosawa 1968; Murata and Umemoto, 1983). For example, Michaux (1803) named *A. eupatoria hirsuta* with a bare protologue as “rough-haired”; it is synonymous to *A. gryposepala* (Kline and Sørensen 2000). When *A. pumila* was

described (Muhlenberg 1813), its diagnosis was given as “yellow, little” and its distribution as “Mississippi”.

Some broadly distributed and morphologically variable species such as *A. pilosa* and *A. eupatoria* also have complicated taxonomic histories (e.g. Wallroth 1842; Nakai 1933; Juzepczuk 1941; Skalický 1962; Hara and Kurosawa 1968; Murata and Umemoto 1983; Naruhashi and Seo 1996). Under *A. eupatoria*, Muhlenberg (1813) described *A. eupatoria glabra*, Hooker (1832) considered *A. parviflora* a variety of *A. eupatoria*, and Wallroth (1842) later described *A. eupatoria* α *rotundifolia* and *A. eupatoria* β *longifolia*. *Agrimonia nepalensis* and *A. viscidula* have been treated as subspecies or varieties of *A. eupatoria* and *A. pilosa* (Kuntze 1891; Komarov 1903; Murata and Umemoto 1983).

Even within the same geographic areas, conflicting taxonomic treatments were proposed because of broad morphological variation and diverse interpretations (e.g. Juzepczuk 1941; Hara and Kurosawa 1968; Naruhashi 2001; Li 2003). In North America, Bicknell (1896) recognized seven species, whereas Rydberg (1913) acknowledged 12 species. Both authors agreed only on their treatments of *A. incisa*, *A. striata*, and *A. parviflora*. Kline and Sørensen (2008) provided a taxonomic revision of eight species in North and Central America. Skalický (1968) recognized *A. eupatoria*, *A. pilosa*, *A. procera*, and *A. repens* in Europe, but earlier (Skalický 1962) he reported six European species, including *A. grandis* and *A. asiatica*.

Infrageneric treatments within *Agrimonia* were presented by Wallroth (1842), Juzepczuk (1941), and Skalický (1970, 1971, 1973). Wallroth (1842) proposed two

sections based on the number of epicalyx segments and two subsections based on orientation of epicalyx segments in fruit; his section *Orthoctenium* included only *A. agrimonoides*. In a series of publications, Skalický (1970, 1971, 1973) categorized 17 species in five series and four subseries, which were expanded from Juzepczuk (1941) who delineated three series with seven Russian species. More recent taxonomic revisions were restricted regionally. Fuks (1987) revised species in Brazil, and Chung and Kim (2000) treated Korean species. Taxonomic revisions of Kline and Sørensen (2000) and Wessel and Thieret (2000) in North America were limited to species in Illinois and Kentucky, respectively. The taxonomic treatment of Kline and Sørensen (2008) provided the most comprehensive revision of North and Central American species. In this paper, a comprehensive taxonomic monograph of genus *Agrimonia* is presented, which includes submergence of *Aremonia* in *Agrimonia*.

MATERIALS AND METHODS

The data presented here were obtained primarily from herbarium specimens, the authors' field collections, and living plants grown at the OU Botany greenhouse propagated from seed, living plants, and root cuttings. Specimens were loaned from several herbaria: A, BM, BRIT, CAN, CBU, DAO, FLAS, GA, MBK, MEXU, MO, MU, NY, OKL, PH, SNUA, SUCH, TEX, TI, VDB and XAL. Observations and field collections of plants in natural populations were conducted in Japan and Korea (2007) and the eastern and southern USA (2006 to 2008). All specimens examined are cited for species and varieties, unless more than 200 herbarium specimens were studied for a taxon, in which case representative specimens throughout the range of the species are cited. For mapping, an ArcMapGIS software was utilized.

Character evolution was evaluated based on the Bayesian phylogenetic tree constructed from nuclear GBSSI-1 sequence data within subtribe Agrimoniinae (Chung and Elisens, unpublished; chapter 2). Twelve morphological characters (Table 1) were mapped directly onto the phylogenetic tree and evaluated. Scanning Electron microscopy (SEM) of pollen (Chung et al. unpublished; chapter 1) and seed coats (Chung et al. unpublished) of the five genera in subtribe Agrimoniinae were examined using JEOL 880 Scanning Electron Microscopy (SEM) at the Samuel Roberts Noble Electron Microscopy Laboratory in the University of Oklahoma. Chromosome numbers was examined using 0.002M 8-hydroxyquinoline treatment of roots. Fixation and staining were followed Iwatsubo et al. (1993).

MORPHOLOGY

Roots

All species have well developed fibrous roots. Four North American species have fibrous roots with tuber-like swellings: *A. incisa*, *A. microcarpa*, *A. pubescens*, and *A. rostellata*. Skalický (1971) categorized species bearing tuber-like swellings in series *Tuberosae* except *A. incisa*. Roots with tuber-like swellings produce young shoots in spring; they may be an adaptation to a dry environment (Singhurst 1996; MacRoberts and MacRoberts 1997).

Stems

Agrimonia agrimonoides has aerial stems producing only inflorescences, whereas all other species have well developed aerial stems that produce both inflorescences and leaves. Three species, *A. eupatoria* (Europe), *A. bracteata* (southern Africa), and *A. nipponica* (Asia), have short internodes (1-2 cm) in the lower stems, so that the basal leaves appear like rosettes when in flower. All species have well-developed rhizomes that produce young shoots in fall.

Leaves

All species have interrupted pinnately compound leaves, which is a diagnostic character for the genus. Terms for leaflets used here are modified from Fajardo et al. (2008) (Fig. 1A). Terminal leaflets are the largest, and sizes and numbers of lateral leaflets vary among species. Interstitial leaflets are placed between lateral leaflets and also vary in size and number. Leaflet shape, number, and margins have been used widely for species delimitation (e.g. Nakai 1933; Chung and Kim 2000; Kline and

Sørensen 2000). The number of lateral leaflets in mid-caulis used in descriptions was counted from live plants and herbarium specimens with either flowers or fruits.

Vestiture of abaxial surface is also variable among species with tomentose, villose, and/or glandular trichomes observed.

Stipules

Stipules are adnate to lower (proximal) parts of petioles. The distal free portions of stipules are falcate and/or flabellate. Shape and size of stipules are variable among species and provide diagnostic characters for species delimitation.

Inflorescences

Racemes are developed generally on the terminal parts of stems, although some species have axillary racemes. Flower number and density on an inflorescence vary among species. For calculation of flower density, the number of flowers was determined per inflorescence at 5 cm from the first formed flowers.

Bracteoles

Two types of bracteoles are found in *Agrimonia*, pedicel bracteoles and hypanthium bracteoles (Fig. 1B). The pedicel bracteole is attached at the base of the pedicels and is either entire or trifid. Hypanthium bracteoles number 1 or 2, are attached at the base of hypanthia, and have margins that are either toothed or entire. Only *A. agrimonoides* has one hypanthium bracteole. Hypanthium bracteoles have been referred to previously as an involucre (Hutchinson 1964; Kalkman 2004).

Epicalyx segments

The epicalyx is positioned below the calyx (Fig. 1B) and is divided distally into several segments. *Agrimonia agrimonoides* has 5 triangular epicalyx segments (1-2 mm), which are antepetalous in a whorl alternate with the whorl of triangular calyx segments. All other species have numerous (>20) epicalyx segments, 1 to 3 mm long, that become hooked bristle-like structures (bristly epicalyx segments hereafter) elongating to 2-6 mm in fruit (Fig. 1C). The orientation of epicalyx segments in fruit is variable among species. The bristly epicalyx serves apparently as an aid in epizooic fruit dispersal (MacRoberts and MacRoberts 1997; Wessel and Thieret 2000).

Flowers

The perianth is 5-merous with five sepals and five light yellow to golden yellow petals that vary in size and shape. Flower size varies from 5 to 14 mm in diameter (petal to petal). Stamen number ranges from 4 to 28. There are two pistils per flower in all species, and no gynoecial characters have been useful for species delimitation.

Pollen

All species exhibit monad, isopolar, radially symmetric, and tricolporate pollen grains. They are subcircular or circular in polar view and prolate in equatorial view. The pollen grains are 35.7-52.5 μm in polar axes and 28.6-42.1 μm in equatorial diameters (Eide 1981). The exine is tectate with striate, and muri are dense, straight, long, arranged perpendicular to the colpus. Columellae are densely arranged.

Fruits

Achenes are enclosed in hardened hypanthia (fruiting hypanthia hereafter; Fig. 1C), which were referred to as “fruits” in previous studies (e.g. Hutchinson 1964; MacRoberts and MacRoberts 1997; Kalkman 2004). Size, shape, and vestiture of fruiting hypanthia have provided diagnostic characters for species and series delimitations (e.g. Skalický 1962; Skalický 1971; Chung and Kim 2000; Kline and Sørensen 2008).

Seeds

Seeds are ovate to depressed ovate in side view and circular in top view, and size varies in 1.3-3.4 x 1.1-3.2 mm. Seed coats are papillate, which supports a submergence of *Aremonia* to *Agrimonia* (Fig. 2B).

MORPHOLOGICAL CHARACTER EVOLUTION

Twelve morphological characters of systematic significance in subtribe Agrimoniinae and *Agrimonia* were mapped onto the phylogenetic tree from a Bayesian analysis of nuclear GBSSI-1 data (Fig. 2A) to test hypotheses of character evolution (Table 1). Subtribe Agrimoniinae comprises *Agrimonia* (including *Aremonia*) and three monotypic genera *Hagenia*, *Leucosidea*, and *Spenceria*. Because *Spenceria ramalana* is the most basal genus in our analyses and in those of Potter et al. (2007), the character states of *Spenceria* were postulated as plesiomorphic.

Two unique character states, epicalyx segments >20 and papillate seed coats (characters 7, 11), support the *Agrimonia* + *Aremonia* clade, whereas woody growth form (character 1) is unique to the [African] *Hagenia* + *Leucosidea* clade (Fig. 2A). Absence of caulescent leaves and interstitial leaflets, presence of an involucre, and entire leaf margins (characters 2, 6, 8, 9; Fig. 2A) are homoplasious for the *Agrimonia* + *Aremonia* and the *Hagenia* + *Leucosidea* lineages. Our results also indicate that five epicalyx segments and the absence of caulescent leaves and involucres (characters 2, 6, 7; Fig. 2A) represent reversals (loss) for *Aremonia*. The monotypic African endemic genus *Hagenia* has no fewer than five unique characters: paniculate inflorescences, unisexual flowers, spatulate stigmas, unhardened hypathia in fruit and microverrucate pollen sculpturing pattern. Two characters not depicted on Fig. 2A, interrupted pinnately compound leaves and bristly epicalyx segments, delineate and are invariant for subtribe Agrimoniinae.

CHROMOSOME NUMBERS, POLYPLOIDY, AND HYBRIDIZATION

All chromosome numbers in *Agrimonia* and subtribe Agrimoniinae are based on $x = 7$ (Table 2) as in tribe Sanguisorbeae (Stace 1991; Iwatsubo et al. 1993). Within *Agrimonia*, tetraploids ($2n=4x=28$), hexaploids ($2n=6x=42$), and octoploids ($2n=8x=56$) have been reported. Because the lowest ploidy levels reported among species of *Agrimonia* are tetraploids, the lineage appears to have an ancient origin where diploids have gone extinct. Hybridization associated with polyploidy has been hypothesized for facilitating speciation within the genus and subtribe (Iwatsubo et al. 1993; Takahashi et al. 1996). Conflicts between phylogenetic trees derived from plastid and nuclear nucleotide sequence data also suggest reticulate evolution within *Agrimonia* (Chung and Elisens, unpublished; chapter 2). Tetraploid and octoploid species occur in Asia, Europe, and North America, with the most widespread species having an $8x$ chromosomal complement.

Based on karyotype analyses, Iwatsubo et al. (1993) confirmed the hybrid origin of the Asian *Agrimonia x nipponica-pilosa* between *A. nipponica* and *A. pilosa* var. *japonica*, which was first proposed by Murata and Umemoto (1968). Iwatsubo et al. (1993) also proposed that *A. pilosa* var. *japonica* is of allopolyploid origin rather than an autopolyploid derivative of either *A. coreana* or *A. nipponica*. Naturally occurring hybrids have been reported in Europe, such as *A. x schultzi-broeskensis* and *A. eupatoria x A. procera* ($2n=42$), but they are sterile (Medd 1955; Skalický 1962). The European hybrids have intermediate morphological characters and are found in areas of sympatry between progenitor species. The hexaploid chromosome

complement of the central European endemic *A. agrimonoides* (= *Aremonia*) suggests hybridization coupled with polyploidy (Chung and Elisens, unpublished; chapter 2).

No purported natural hybrids have been reported among North American species.

TAXONOMIC TREATMENTS

Agrimonia L. Sp. Pl. 448. 1753. Gen. Pl. ed. 5: 206.—TYPE: *Agrimonia eupatoria* L.

Aremonia Neck. Elem. 100. 1790.—TYPE: *Aremonia agrimonoides* (L.) Neck.

Amonia Nestl. Mon. Potent. 17. 1816.—TYPE: *Amonia agrimonoides* (L.)

Nestl.

Spallanzania Pollini Hort. Veron. Pl. Nov. 10 1816.—TYPE: *Spallanzania agrimonoides* (L.) Pollini.

Herbaceous perennials to 150 cm. Roots fibrous with or without tuber-like swellings. Rhizomes present. Stems with one or two types, shortened vegetative and erect elongated reproductive stems or with both vegetative and reproductive stems erect and elongated, un-branched or branched above, vestiture hirsute, pilose, or villose. Leaves caulescent or basal in shortened stems, rosettes absent or present in flower, alternate, interrupted pinnately compound; stipules foliaceous, persistent, adnate to lower parts of petioles, free portions flabelliform and/or falcate, obliquely and horizontally spreading, evenly or unevenly toothed, dentate, or incised, 1-4 × 2-4 cm; petioles 0.5-3 cm long; blades obovate or obtrullate, leaflets 3-35 in mid-caulis, sessile; terminal leaflets lanceolate, oblong, elliptic, obovate to obovate, apex obtuse, acute, or acuminate, base cuneate or widely cuneate, margins dentate, incised, or serrate; lateral leaflets in 1-8 pairs, margins dentate, incised, or serrate; interstitial leaflets among lateral leaflets in 1-3 pairs, elliptic or obovate, margins entire or dentate, 0.4-1.5 cm long. Inflorescences racemes, branched or non-branched, with 3-

100 flowers, 5-40 cm long. Flowers perfect, 5-merous, 7-15 mm diameter, pedicels terete, 0.8-5 mm long, bracts 1, simple or trifid, toothed; pedicel bracteoles 1, entire or trifid, attached basally; hypanthium bracteoles (involucre) 1 or 2, entire or toothed, attached basally; hypanthia obconical, long obconical, turbinate or hemispheric, vestiture glandular, hirsute, pilose, or villose; epicalyx segments 5 to 50, persistent, triangular or cylindrical, 1-2 mm, apices inflexed. Sepals 5, persistent, triangular, acute, glandular and pubescent; petals 5, light yellow to golden yellow, oblong to obovate, apices rounded, 3.5-6 × 1.6-2.5 mm; stamens 4-28, persistent, stylopodia present; filaments light yellow, basifixed; anthers yellow, capitate, dehiscence longitudinal; pistils 2, persistent, simple, free; ovaries pendulous, locules 1, placentation basal; styles 2, pale yellow, filiform, 2-3.5 mm; stigmas reniform, dilated. Achenes light brown, 1 or 2 enclosed in a hardened hypanthium, testa membranous; fruiting hypanthia obconical, long obconical, turbinate, or hemispheric, woody, deeply or shallowly sulcate, bristly epicalyx segments numerous or lacking, ascending, spreading and/or deflexed, hooked attached on upper edge, 3-11 × 2-11 mm, often deflexed at maturity.

Flowering from May to September. Base chromosome number $x=7$. 19 species. Temperate regions throughout North America, Central America, West Indies, and South America (Argentina and Brazil), Europe, Asia, and southern Africa (South Africa and Zimbabwe).

KEY TO THE SPECIES OF AGRIMONIA

1. Leaves caulescent and basal. Involucres present. Epicalyx segments 5.

1. *Agrimonia agrimonoides*.

1. Leaves caulescent only. Involucres absent. Epicalyx segments 20-50.

2. Lateral leaflets 1-3 pairs in mid-caulis.

3. Vestiture of abaxial surface of leaflets densely glandular and tomentose.

4. Stamens 14-18. Stipules flabellate.

3. *A. coreana*.

4. Stamens 5-10. Stipules falcate.

5. Fruiting hypanthia hemispheric; sulci shallow. Roots without tuber-like

swellings. Asia.

10. *A. nipponica*.

5. Fruiting hypanthia turbinate; sulci deep. Roots with tuber-like swellings.

North America.

9. *A. microcarpa*.

3. Vestiture of abaxial surface of leaflets scattered glandular or pubescent.

6. Hypanthia hemispheric; vestiture glandular only. Roots with tuber-like

swellings.

17. *A. rostellata*.

6. Hypanthia turbinate; vestiture glandular and pubescent. Roots without tuber-like swellings.

7. Flowers 8-10 mm in diameter. Length of fruiting hypanthia 5.2-8 mm;

epicalyx segments ascending and spreading.

6. *A. gryposepala*.

7. Flowers 6-7 mm in diameter. Length of fruiting hypanthia 3-4.5 mm;

epicalyx segments ascending only.

13. *A. pringlei*.

2. Lateral leaflets 4-8 pairs in mid-caulis.

8. Lateral leaflets 4 pairs in mid-caulis; smaller than terminal leaflets.
9. Vestiture of abaxial surface of leaflets densely glandular and tomentose.
10. Stamens 18-24. **16. *A. repens*.**
10. Stamens 8-16.
11. Flowers 8-11 mm in diameter. Length of fruiting hypanthia 6-11 mm.
 Roots without tuber-like swellings.
12. Stamens 10-16. Pedicel bracteoles longer than flowers; epicalyx segments
 deflexed in flower. South Africa. **2. *A. bracteata*.**
12. Stamens 8. Pedicel bracteoles as long as flowers; epicalyx segments
 ascending in flower. Eurasia. **4. *A. eupatoria*.**
11. Flowers 5.5-7 mm in diameter. Length of fruiting hypanthia 3.8-5 mm long.
 Roots with tuber-like swellings.
13. Stamens 5. Leaflet margins incised. Hypanthia hemispheric; vestiture
 glandular only; sulci shallow in fruit. **8. *A. incisa*.**
13. Stamens 7-12. Leaflet margins dentate to serrate. Hypanthia turbinate;
 vestiture glandular and pubescent; sulci deep in fruit. **15. *A. pubescent*.**
9. Vestiture of abaxial surface of leaflets scattered glandular or pilose.
14. Epicalyx segments deflexed in flower. Length of fruiting hypanthia 7-11 mm.
14. *A. procera*.
14. Epicalyx segments ascending in flower. Length of fruiting hypanthia 4.5-6 mm.
15. Leaflet margins incised. **5. *A. gorovoi*.**
15. Leaflet margins dentate or serrate.

16. Length of hypanthia in fruit equal to epicalyx segments. Terminal leaflet apices acute. Eurasia. **12. *A. pilosa*.**
16. Length of hypanthia in fruit greater than epicalyx segments. Terminal leaflet apices acuminate. North America. **18. *A. striata*.**
8. Lateral leaflets 6-11 pairs in mid-caulis; as large as terminal leaflets.
17. Hypanthia hemispheric; vestiture densely glandular and villose. Vestiture of stems and leaves glandular and villose. **19. *A. villosa*.**
17. Hypanthia turbinate; vestiture glandular or hirsute. Vestiture of stems and leaves hirsute.
18. Terminal leaflets lanceolate to narrow elliptic. Vestiture of hypanthia glandular only; sulci deep in fruit. Stipules falcate; margins unevenly toothed. **11. *A. parviflora*.**
18. Terminal leaflets elliptic to obovate. Vestiture of hypanthia glandular and hirsute; sulci shallow in fruit. Stipules flabellate-falcate; margins evenly toothed. **7. *A. hirsuta*.**

1. *Agrimonia agrimonoides* L., Sp. Pl. 1:448. 1753. *Aremonia Agrimonoides* (L.)

DC. Prod. ii. 588. 1825.—TYPE: Italy. “Habitat in Italiae nemoribus umbrosis humentibus”, Herb. *Linn. No.* 628.3 (lectotype, designated by Theurillat in Cafferty & Jarvis (ed.), 2002, LINN, photo!).

Herbaceous perennials to 30-80 cm. Roots without tuber-like swellings. Stems of two types, shortened vegetative and erect elongated reproductive, simple, pilose. Leaves caulescent and basal; compound leaves obovate; stipules ovate, apices acute, entire, $0.8-1.2 \times 0.2-0.4$ cm; leaflets 3-5; terminal leaflets ovate to widely oblong, apex obtuse, base widely cuneate, margins dentate, $3.0-5.0 \times 2.8-4.3$ cm, adaxial surface scattered pubescent, abaxial surface densely tomentose; 1st lateral leaflets as large as terminal leaflets, 2nd lateral leaflets smaller than 1st leaflets by 0.8-1.2 cm long; 1st and 2nd interstitial leaflets of 1 pair each. Inflorescences loose (3-5 flowers/5cm-inflorescence), 1-5 flowers per inflorescence; flowers 8-11 mm in diameter; pedicels 3-5 mm long; pedicel bracteoles deeply trifid; hypanthium bracteoles 1 (involucre), incised; hypanthia obconical, densely pilose; sepals glandular and pilose, 1.5-2 mm long; epicalyx segments 5, alternate to sepals, triangular, 1-2 mm long; petals yellow, ovate, $3.8-4.5 \times 1.8-2.1$ mm; stamens 5-10. Achenes 1; fruiting hypanthia globose, vestitue glandular and pilose, shallowly sulcate, (3.66-) 5.86 (-6.78) \times (2.68-) 4.18 (-4.84) mm.— Chromosome number. $2n = 42$ (Baltisberger 1991).

Flowering from April to June.

Distribution (Fig. 3). Austria, Czech Republic, Germany, Italy, Republic of Slovenia, and Romania; also recorded from Albania, Bulgaria, England, Federal Republic of Yugoslavia, Greece, Hungary, Switzerland, and Turkey (European part) (Skalický 1968); open areas in deciduous or mixed woods; 400-1000 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-1.

The presence of basal leaves, involucre, and 5 epicalyx segments easily distinguishes *A. agrimonoides* from other species in *Agrimonia*, and indicates why several authors have segregated the species in the monotypic genus *Aremonia* (Skalický 1962; Kalkman 2004; Potter et al. 2007). We submerge *Aremonia* within *Agrimonia*, because shared papillate seed coats (Fig. 2B) and molecular phylogenetic analyses based on variation in plastid and nuclear sequences (Chung and Elisens, unpublished, chapter 2) support the monophyly of *Agrimonia* + *Aremonia* and the nesting of *A. agrimonoides* within *Agrimonia*. Further genetic investigation is required to test the hypothesis of hybrid origin suggested by its hexaploid chromosome number and topological position in nuclear and plastid-derived phylogenetic trees.

2. *Agrimonia bracteata* E. Mey. ex C. A. Mey., Bull. Acad. Sci. St. Petesb. 10: 349.

1842.—TYPE: without specific locality ‘Africa on the cover’, *unspecified s.n.*

(lectotype, designated by Skalický, 1963: L, photo!).

Herbaceous perennials 70-150 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, simple or branched above, spreading pilose.

Leaves caulescent; rosettes present in flower; compound leaves obovate; stipules flabelliform-falcate, obliquely or horizontally spreading, unevenly toothed, 1.2-1.5 × 0.9-1.1 cm; leaflets 7-9; terminal leaflets oblong-elliptic, rhombic-elliptic to obovate,

apex obtuse, base widely cuneate, margins dentate-serrate, 3.0-8.0 × 1.3-4.2 cm, adaxial surface scattered pubescent, abaxial surface densely glandular and tomentose; 1st lateral leaflets smaller than terminal leaflets by 1 cm long, 2nd lateral leaflets smaller than 1st lateral leaflets by 0.8-1.1 cm long; 1st interstitial leaflets 3 pairs with 2 different sizes, middle ones largest 0.8-1 cm long, 2nd interstitial leaflets usually 1 pair. Inflorescences loose (3-5 flowers/5cm-inflorescence); flowers 10-13 mm in diameter; pedicel 2-3 mm long; pedicel bracteoles trifid, middle fids twice longer than flowers; hypanthium bracteoles 2, toothed; hypanthia turbinate, hirsute; epicalyx segments numerous, cylindrical, apices inflexed, deflexed, 1.3-2.2 mm long; petals yellow to golden yellow, oblong, 4.8-5.2 × 2.1-2.4 mm; stamens 10-16. Achenes 1 or rarely 2; fruiting hypanthia turbinate, vestiture glandular and pilose, deeply sulcate, bristly epicalyx segments numerous, ascending, deflexed, and spreading on upper edge (8.68-) 10.42 (-11.03) × (7.68-) 10.02 (-10.89) mm.

Chromosome number unknown.

Flowering from November to February.

Distribution (Fig. 4). South Africa (Eastern Cape, Mpumalanga, and Western Cape) and Zimbabwe (Manicaland); dry grassland; 1700-1900 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-2.

Agrimonia bracteata is endemic to southern Africa. Pedicel bracteoles longer than flowers and comparatively large flowers distinguish *A. bracteata* from other

species. This species also attains greater height compared to most other species. The species has not been included in phylogenetic analyses.

3. ***Agrimonia coreana*** Nakai, Koryo Shikenrin Ippan: 83. 1932. *Agrimonia pilosa*

Ledeb. *coreana* (Nakai) Liou & Chen., Fl. Pl. Medic. Chin. Bor.-Or. 94.

1959.—TYPE: North Korea. “in monte Kongôsan” *T. Nakai no. 5526* (syntype: TI!); South Korea. “Tange montium Chiisan” *T. Nakai s.n.*, “monte Hakuyozan” *T. Nakai no. 11395* (syntype: TI!); North Korea. Kangwan-do: Diamond (Geumgang) Mountain, 13 Aug 1916, *T. Nakai no. 55262* (lectotype, designated by M. Kitagawa, 1963: TI!).

Agrimonia pilosa Ledeb. var. *simplex* T. Shimizu, Acta Phytotax. Geobot. 17: 87, f. 2.

1958.—TYPE: Japan. Honshu: Iwate, Mt. Ureira, Shimoheigun, 11 Aug, *T. Shimizu no. 741* (holotype: KYO, photo!).

Agrimonia tokatiensis Ko. Ito, J. Geobot. 9: 69–70. photo 1. 1961.—TYPE: Japan.

Hokkaido: Tokati, Tiyoda, Ikeda Town, Aug. 8 1959, *K. Ito s.n.* (holotype: SAP, photo!).

Herbaceous perennials 30-100 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, simple or branched rarely above, slim, pubescent. Leaves caulescent; compound leaves obtrullate; stipules flabelliform, horizontally spreading, evenly toothed, 0.9-1.2 × 0.7-0.9 cm; leaflets 3-5; terminal leaflets oblong-elliptic, rhombic-elliptic to obovate, apex obtuse, base cuneate, margins dentate-

serrate, 3.0-6.0 × 1.3-3.2 cm, adaxial surface scattered pubescent, abaxial surface densely glandular and tomentose; 1st lateral leaflets smaller than terminal leaflets by ca. 1cm long, 2nd lateral leaflets as large as one third of 1st lateral leaflets; 1st interstitial leaflets 2 (3) pairs with 2 different sizes, second pair (or middle) ones largest ca. 0.5 cm long, 2nd interstitial leaflets usually 1 pair. Inflorescences loose (3-5 flowers/5cm-inflorescence), no more than 20 flowers per plant; flowers 0.9-11 mm in diameter; pedicel 1-2 mm long; pedicle bracteoles 1, deeply tri-fid; hypanthium bracteoles 2, deeply toothed; hypanthia turbinate, densely pubescent; epicalyx segments numerous in multiple rows, persistent, cylindrical, apices inflexed, ascending, 1-2 mm long; petals yellow, oblong, 4.2-4.6 × 1.6-1.9 mm; stamens 15-28. Achenes 1 or rarely 2; fruiting hypanthia turbinate, vestiture glandular and pubescent, deeply sulcate, bristly epicalyx segments numerous, ascending and spreading on upper edge (5.66-) 6.07 (-6.46) × (4.25-) 4.63 (-5.11) mm.— Chromosome number. $2n = 28$ (Iwatsubo et al. 1993).

Additional illustration. Chung & Kim (2000: 325, fig.3).

Flowering from June to July.

Distribution (Fig. 5). China (Beijing, Hebei, Heilongjiang, Neimenggu Zizhigu, Jilin, Liaoning, Shandong, and Zhejiang), Japan (Hokkaido, Honshu, and Kyushu), North Korea, South Korea, Russia (Aga Buryatia, Amur, Buryatia, Chita, Evenk, Irkutsk, Jewish, Khabarovsk, Magadan, Primorskiy, Sakha (Yakutia), Taymyr (Dolgano-Nenet), and Ust-Orde Buryat); mixed woods; 600-1200 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-3.

Agrimonia coreana is easily distinguished from other Asian species *A. pilosa* and *A. nipponica* by flabellate stipules, inflorescences with a low density of flowers (loose), large fruits, and flowering time in early summer. Because of a delay in typification (Kitagawa 1963), the identity and taxonomic history of the species has been confused and complicated. *Agrimonia velutina*, treated usually as a synonym of *A. coreana* (Hara and Kurosawa 1968); Lee 1996; Naruhashi 2001), has not been entered into synonymy until a type has been examined. Karyotype analysis (Iwatsubo 1993) and GBSSI-1 sequence data (chapter 2) suggest that *A. coreana* is not closely related to other Asian species.

4. *Agrimonia eupatoria* L., Sp. Pl. 1:448. 1753.—TYPE: “Habitat in Europae pratis apricis argillaceis.” Herb. *Linn. no. 628.1* (lectotype, designated by Skalický, 1973: LINN, photo!).

Agrimonia asiatica Juz., Sor. Rast. SSSR 3: 138.1934. *Agrimonia eupatoria* Juz. subsp. *asiatica* Skalický, Feddes Report. 79: 35. 1968.—TYPE: Prope Orenburg (Čkalov, opp. ad pedem montium uralensium) *J. Schell a. 1848* (lectotype. designated by Skalický, 1968: LE).

Agrimonia eupatoria L. var. *villosa* Skalický, Feddes Report. 79: 36. 1968.—TYPE: Persia (Iran) Khorasan: in valle fluvii Atrek inter Shirwan et Budjnurd, *K. H. Rechinger no. 1798* (holotype: W).

Herbaceous perennials 30-80 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, branched rarely above, spreading pubescent; rosettes present in flower. Leaves caulescent; compound leaves obovate; stipules flabelliform-falcate, obliquely or horizontally spreading, incised, 1.3-1.5 × 0.7-0.9 cm; leaflets 7-9; terminal leaflets elliptic, apex obtuse, base cuneate, margins dentate, 2.7-3.8 × 1.5-2.2 cm, adaxial surface scattered pubescent, abaxial surface scattered glandular and densely tomentose; 1st lateral leaflets smaller than terminal leaflets by 0.4-0.6 cm long, 2nd lateral leaflets smaller than 1st lateral leaflets by 0.6-0.9 cm long; 1st interstitial leaflets 3 pairs with 2 different sizes, middle ones largest 0.4-0.7 cm long, 2nd interstitial leaflets usually 2 or 3 pairs. Inflorescences loose (ca. 11 flowers/5cm-inflorescence); flowers 11-13 mm across; pedicel 1-3 mm long; pedicel bracteoles 1, deeply tri-fid; hypanthium bracteoles 2, toothed; hypanthia obconical, pubescent and glandular; epicalyx segments numerous, persistent, cylindrical, apices inflexed, 0.8-1.2 mm long; petals yellow to golden yellow, elliptic, 5-6 × 2.3-2.6 mm; stamens 8. Achenes 1 or 2; fruiting hypanthia long obconical, vestiture glandular and pubescent, deeply and broadly sulcate, bristly epicalyx segments numerous, ascending and spreading on upper edge (6.46-) 7.35 (-8.27) × (4.02-) 5.06 (-6.23) mm.—
Chromosome number. $2n = 28$ (Hollingsworth et al. 1992).

Additional illustration. Ross-Craig (1956: plate 9).

Flowering from May to September.

Distribution (Fig. 6). Albania, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France (incl. Corsica), Georgia, Germany, Greece, Hungary, Iran (Kartli), Ireland, Italy (incl. Sicily), Netherlands, Norway, Poland, Portugal (incl. Azores), Republic of Armenia, Romania, Russia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom, and Yugoslavia; open woods; 400-1200 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-4.

Long obconical fruiting hypanthia easily distinguish *A. eupatoria* from other species. Previously segregated varieties of *Agrimonia eupatoria* based on leaf margin and leaf vestiture characters are submerged into the species, because of continuous patterns of morphological variation observed among flower and fruit characters used to differentiate varieties (Skalický 1962). In order to delineate geographic distribution clearly, additional specimens especially from Russia need to be investigated.

5. *Agrimonia gorovoi* Rumjantsev, Bot. Zhurn. 72(9): 1258. 1987.— TYPE: Russia.

Primorskij: Chassan Distr. as septentrionem 1km a lacu Talmi, pratum gramino-so-variiherbosum, 20 Aug. 1986, *P.G. Gorovoi & S. Rumjantsev s.n.* (holotype: MHA; isotypes: A! LE MW VLA).

Herbaceous perennials 40-150 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, usually branched above, horizontal spreading pilose (3 mm). Leaves caulescent; stipules flabelliform-falcate, horizontally spreading, deeply incised, 1.8-2 × 1.3-1.5 cm; leaflets 7-9; terminal leaflets obovate,

apex obtuse, base cuneate, margins deeply incised, $4.3-4.8 \times 1.8-2.0$ cm, adaxial surface scattered pubescent, abaxial surface glandular and pubescent; 1st lateral leaflets smaller than terminal leaflets by 1 cm long, 2nd lateral leaflets smaller than 1st lateral leaflets by 0.8-1 cm long; 1st interstitial leaflets 3 pairs with 2 different sizes, middle ones largest 0.8-1 cm long, 2nd interstitial leaflets usually 1 pair. Inflorescences loose (5-9 flowers/5cm-inflorescence); flowers 10-12 mm in diameter; pedicel 2.5-3 mm long; pedicel bracteoles 1, deeply tri-fid, hypanthium bracteoles 2, tri-fid; hypanthia obconical, glandular and pubescent; epicalyx segments numerous, persistent, cylindrical, apices inflexed, ascending, 0.8-1 mm long; petals yellow, obovate, $4.8-5 \times 2.1-2.4$ mm; stamens 10. Achenes 1 or rarely 2; fruiting hypanthia long obconical or obconical, vestiture glandular and pilose, broadly sulcate, bristly epicalyx segments numerous ascending on upper edge ($4.38-$) 5.23 (-5.67) \times ($3.12-$) 3.78 (-4.01) mm.

Chromosome number unknown.

Additional illustration. Rumjantsev (1987: 1259. figs. 1-6).

Flowering in August.

Distribution (Fig. 7). Russia (Primorsky province) and South Korea (Kangwon province); boggy meadows; 800-1000 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-5.

Agrimonia gorovoi occurs only in boggy meadows near the ocean in extreme SE Russia and Korea. The species is characterized by a stamen number of 10, well-developed rhizomes, and incised leaf margins.

6. *Agrimonia gryposepala* Wallr., Beitr. Bot. (Wallr.) 1: 49. 1842. *Eupatorium gryposepalum* (Wallr.) Lunell, Am. Midland. Nat. 5: 237. 1918.—TYPE: United States. Pennsylvania: in graminosis, Aug. 1824, E. Poeppig s.n. (lectotype, designated by Kline & Sørensen, 1990: W; isolectotypes: HAL MO! PR W).

Agrimonia parviflora Ait. var. *macrocarpa* Focke., Bot. Gaz. 16 (1): 3. 1891. *A. macrocarpa* (Focke ex Donn. Sm.) Rydb., N. Amer. Fl. 22(5):392. 1913.—TYPE: Guatemala. Alta Verapaz: Cobanm 4300 pp, Feb 1889, H. von Türckheim 1409 (holotype: BREM; isotype: US, photo!).

Herbaceous perennials 55-100 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, branched above, scattered glandular and pubescent. Leaves caulescent; compound leaves obtrullate; stipules falcate, obliquely spreading, toothed, 1.5-1.7 × 1.1-1.3 cm; leaflets 5; terminal leaflets obovate, apex obtuse, base widely cuneate, margins dentate, 5.5-7.9 × 2.3-3.4 cm, adaxial surface scattered pubescent, abaxial surface glandular and pubescent; 1st lateral leaflets slightly smaller than terminal leaflets by 0.8-1 cm, 2nd lateral leaflets as large as a half of terminal leaflets; 1st interstitial leaflets 2 or 3 pairs with 2 different sizes, middle ones largest

0.5-1.1 cm long, 2nd interstitial leaflets 0 or 1 pair. Inflorescences loose (5-8 flowers/5 cm-inflorescence), no longer than 20 cm; flowers 0.8-10 mm in diameter; pedicel 1-5 mm long; pedicle bracteoles 1, deeply tri-fid, hypanthium bracteoles 2, entire or toothed; hypanthia obconical, glandular and pubescent; epicalyx segments numerous, persistent, cylindrical, apices inflexed, deflexed, 1-2 mm long; petals yellow, oblong, 3.6-4 × 1.3-1.6 mm; stamens 4-9. Achenes 1 or rarely 2; fruiting hypanthia turbinate, vesture scattered glandular and pubescent, deeply sulcate, bristly epicalyx segments numerous, ascending and spreading on upper edge (6.04-) 6.90 (-7.35) × (7.03-) 8.23 (-8.98) mm.— Chromosome number. $2n = 56$ (Brittan 1953).

Additional illustrations. Britton & Brown (1913: 266, fig. 2267).

Flowering from June to September.

Distribution (Fig. 8). Canada (British Columbia, Manitoba, New Brunswick, Nova Scotia, Ontario, Ottawa, Prince Edward Island, Quebec, and Saskatchewan,), Guatemala (Alta Verapaz, Chimahenahgo, El Quiche, and Heuhuetenahge), Mexico (Chiapas and Coahuil), and United States; also recorded from Guatemala (Baja Verapaz adn Quezaltenango, San Marcos, and Suchitepequez), Mexico (Nuevo Leon) (Kline & Sorenson 2008); deciduous or mixed deciduous woods; 300-1300 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-6.

Agrimonia gryposepala is distinguished easily from other species by large flowers and fruiting hypanthia with spreading bristly epicalyx segments. Leaf margins

and shapes are variable, but flower and fruit characters are consistent and can be used to delineate the species consistently. Loose inflorescences characterized by low density of flowers are shared with *A. rostellata*, but apices of flower buds are acute in *A. gryposepala* and round in *A. rostellata*.

7. Agrimonia hirsuta Bong. ex C. A. Mey., Ann. Sci. Nat., Bot. 18:378

1842.—TYPE: ‘Acaena n sp.’ ‘Antonina Fm? I Paulo’ *Weir 449* (neotype, here designated: BM!).

Herbaceous perennial herbs 80-120 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, simple or branched above, spreading hirsute. Leaves caulescent; compound leaves obovate; stipules flabelliform-falcate, obliquely or horizontally spreading, evenly dentate, 2.6-2.9 × 1.5-1.7 cm; leaflets 9-11; terminal leaflets elliptic to obovate, apex obtuse, base cuneate, margins dentate-serrate, 3.0-4.8 × 0.9-1.3 cm, adaxial surface hirsute, abaxial surface glandular and tomentose; 1st and 2nd lateral leaflets as large as terminal leaflets; 1st interstitial leaflets 4-5 pairs with 3-4 different sizes, upper most ones largest 0.6-1.2 cm long, 2nd interstitial leaflets usually 3-4 pairs. Inflorescences dense (13-15 flowers/5cm-inflorescence); flowers 5-8 mm in diameter; pedicel 1-2 mm long; pedicel bracteoles 1, deeply tri-fid; hypanthium bracteoles 2, toothed; hypanthia obconical, glandular or hirsute; epicalyx segments numerous, persistent, cylindrical, apices inflexed, ascending, ca. 1mm long; petals yellow, oblong, 3.1-3.4 × 1.6-1.8 mm; stamens 4-10. Achenes 1 or rarely 2; fruiting hypanthia obconical, vestiture glandular or pubescent, shallowly sulcate,

bristly epicalyx segments ascending on upper edge (3.38-) 4.25 (-4.48) × (2.76-) 3.98 (-4.21) mm.

Additional illustration. Fuks (1987: 81, fig. 4).

Flowering in January and February.

Distribution (Fig. 9). Brazil (Parana and Santa Catarina); also recorded from Brazil (Rio Grande do Sul) (Fuks 1987); open woods; 900-1000 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix. 3-7.

Agrimonia hirsuta has been reported only from Brazil. Large stipules and dense inflorescences characterize the species. A neotype is here newly designated.

8. *Agrimonia incisa* Torr. & A. Gray, Fl. N. Amer.: 431. 1840. *Agrimonia parviflora*

(Aiton) β *incisa* (Torr. & A. Gray) Alph. Wood, Amer. Bot. Fl.: 108.

1870.—TYPE: United States. Florida: Tampa Bay, 1834, *Burrows s.n.*

(syntype: NY!); South Carolina: Santee River, *unspecified s.n.* (syntype: NY!);

Alabama: without specific locality, [before 1840]. *Dr. H. Gates s.n.* (lectotype, designated by Kline and Sorensen, 2008: NY!).

Herbaceous perennials 30-100 cm. Roots with tuber-like swellings. Stems vegetative and reproductive, erect, simple or rarely branched above, spreading pubescent. Leaves caulescent; compound leaves obovate; stipules flabelliform-falcate,

obliquely spreading, incised, 1.2-1.8 × 0.6-1.2 cm; leaflets (5) 7-9; terminal leaflets narrowly elliptic to elliptic, apex obtuse, base widely cuneate, margins incised, 2.5-3.5 × 0.9-1.5 cm, adaxial surface pubescent, abaxial surface glandular and tomentose; 1st and 2nd lateral leaflets as large as terminal leaflets or slightly smaller than terminal leaflets by 1.7-2mm long; 1st interstitial leaflets 2 or 3 pairs with 2 different sizes. . Inflorescences elongate, loose (4-5 flowers/5 cm-inflorescence); flowers 6-9 mm in diameter; pedicel 1-2 mm long; pedicle bracteoles 1, tri-fid; hypanthium bracteoles 2, entire or toothed; hypanthia hemispheric, glandular only; epicalyx segments numerous, persistent, cylindrical, apices inflexed, ascending 1-1.3 mm long; petals light yellow, oblong, 3.2-3.5 × 1.5-1.7 mm; stamens 5. Achenes 1 or rarely 2; fruiting hypanthia hemispheric to long obconical, vestiture densely glandular, shallowly sulcate, bristly epicalyx segments numerous ascending on upper edge (4.53-) 5.01 (-5.86) × (2.76-) 3.27 (-3.95) mm. Chromosome number. $2n = 28$.

Flowering from June to October.

Distribution (Fig. 10). United States (Florida, Georgia, Mississippi, South Carolina, and Texas); also recorded from Alabama, Missouri, and Louisiana (Kline and Sorensen 2008); open deciduous, mixed or pine woods; sandy soil; 200-400 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-8.

Agrimonia incisa is distinctive among American species and is characterized by incised leaf and stipule margins and long hypanthia with glandular vestiture. It occurs in the southeastern USA from South Carolina to east Texas, where it is

classified as a rare species by the Texas Natural Heritage Program (Singhurst 1996). Phylogenetic analyses of cpDNA sequences suggest that *A. incisa* is closely related to the tetraploids *A. rostellata*, *A. parviflora*, and *A. microcarpa* (chapter 2).

9. Agrimonia microcarpa Wallr., Beitr. Bot. (Wallr.)1: 39–40. 1842. nom. cons.

prop. C. Kline and P. Sørensen, 2007.—TYPE: United States. South Carolina: Cherokee Co., on the access rd. to Kings Cr. At the int[ersection], floodplain of Kings Cr., 11 Oct 1993, *D. kennemore, Jr. 1174* (*typ. con. prop.*, designated by Kline and Sørensen, 2007: USCH!).

Agrimonia pumila Muhl. ex E. P. Bicknell. Bull. Torrey Bot. Club 23: 514-151.

1896.—TYPE: United States. Florida: without specific locality. [1878-1895], *Chapman s.n.* (lectotype, designated by Kline and Sørensen, 2008: NY!).

Herbaceous perennials 30-80 cm. Roots with tuber-like swellings. Stems vegetative and reproductive, erect, simple or branched above, slim, spreading pubescent. Leaves caulescent; compound leaves obtrullate; stipules falcate-flabelliform, obliquely or horizontally spreading, dentate-serrate, 1.3-1.5 × 0.2-0.5 cm, ; leaflets 3-5; terminal leaflets obovate to rhombic-obovate, apex obtuse, base widely cuneate, margins dentate-crenate, 3.2-4.7 × 1.3-1.9 cm, adaxial surface scattered pubescent, abaxial surface densely glandular and tomentose; 1st lateral leaflets smaller than terminal leaflets by 1cm long, 2nd lateral leaflets smaller than 1st lateral leaflets by 0.8-1cm long; 1st interstitial leaflets 1 pair (occasionally 2 pairs), 2nd interstitial

leaflets absent. Inflorescences loose (3-5 flowers/5cm-inflorescence); flowers 6-9 mm in diameter; pedicel 1-2 mm long; pedicle bracteoles 1, tri-fid, hypanthium bracteoles 2, entire; hypanthia turbinate, scattered glandular and pubescent; epicalyx segments numerous, persistent, cylindrical, apices inflexed, ascending, 0.8-1.2 mm long; petals yellow, oblong, 3.2-3.5 × 1.5-1.8 mm; stamens 5-9. Achenes 1 or rarely 2; fruiting hypanthia turbinate, vestiture densely glandular and pubescent, deeply sulcate, bristly epicalyx segments numerous, ascending and spreading on upper edge (4.29-) 4.55 (-4.73) × (4.30-) 4.55 (-4.98) mm.

Chromosome number unknown.

Additional illustration. Britton & Brown (1913: 267, fig. 2269).

Flowering from July to October.

Distribution (Fig. 11). United States (Alabama, Connecticut, Florida, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia; also recorded from Arkansas, New Jersey, and Pennsylvania in United States (Kline and Sørensen 2008); open deciduous woods; 600 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix. 3-9.

Agrimonia microcarpa is characterized by a small number of lateral leaflets and loose inflorescences. Phylogenetic analyses suggest that it is closely related to *A. rostellata*, which has a similar geographic distribution and shares roots with tuber-like swellings (chapter 2).

10. *Agrimonia nipponica* Koidz., Bot. Mag. Tokyo 44: 104. 1930; Kitagawa, J. Jap. Bot. 38: 305, 1963; Wild Fl. Jap. 1981; Ohwi and Kitagawa, New Fl. Jap. 860, 1983. *Agrimonia pilosa* Ledeb. var. *nipponica* (Koidz.) Kitamura, Col. III. Herb. Pl. Jap. 122, 1961; Park, Herb. Pl. Kor. Dicot. 217. 1974. *Agrimonia pilosa* Koidz. fo. *nipponica* (Koidz.) Ohwi, Fl. Jap. 650, 1953.—TYPE: Japan. Yokohama, ‘Japonia Yokohama’ 1862, *Maxomowiz s.n.* (neotype, here designated: L, photo!).

Agrimonia nipponica Koidz. var. *occidentalis* Skalický, Fl. Cambodge, Los & Vietnam 6: 135. 1968. *Agrimonia pilosa* Ledeb. var. *occidentalis* (Skalický) Z. Wei & Y. B. Chang, Fl. Zhejiang, 3: 237. 1993.—TYPE: Chine, Kouang Si, Wait Sap District, Tong Shan, en fleurs, Sept. 1933. *W. T. Tsang 22749* (holotype: P).

Agrimonia viscidula Bunge var. *japonica* Miq., Ann. Mus. Bot. Lugduno-Batavi 3: 38. 1867.—TYPE: Japan. Nagasaki, 1862, *Oldham, R. 225* (holotype: L, photo!).

Herbaceous perennials 30-80 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, branched above, scattered pubescent. Leaves caulescent; compound leaves obtrullate; stipules falcate, obliquely or horizontally spreading, evenly toothed, 1.0-1.2 × 0.7-1.0 cm; leaflets 3-5; terminal leaflets oblong-elliptic to elliptic, apex obtuse, base cuneate, margins dentate, 3.0-6.0 × 1.3-3.2 cm, adaxial surface scattered pubescent, abaxial surface glandular and densely tomentose;

1st lateral leaflets smaller than terminal leaflets by 0.7-1 cm long, 2nd lateral leaflets as large as one third of 1st lateral leaflets; 1st interstitial leaflets 3 pairs with 2 different sizes, middle ones largest 0.4-0.6 cm long, 2nd interstitial leaflets usually 1 pair. Inflorescences dense (15 flowers/5 cm inflorescence); flowers 4.5-7 mm in diameter; pedicel 1-2 mm long; pedicel bracteoles 1, deeply tri-lobed, hypanthium bracteoles 2, entire or toothed; hypanthia hemispheric, scattered glandular and pubescent; epicalyx segments numerous in multiple rows, persistent, cylindrical, apices inflexed, ascending, 1-2 mm long; petals yellow, elliptic, 2-2.3 × 1-1.2 mm; stamens 5-12. Achenes 1 or rarely 2; fruiting hypanthia hemispheric, vestiture scattered glandular and pubescent, shallowly sulcate, bristly epicalyx segments numerous, ascending and spreading on upper edge (3.22-) 3.57 (-3.84) × (1.96-) 2.21 (-2.36) mm.— Chromosome number. $2n = 28$ (Iwatsubo et al. 1993).

Additional illustration. Chung & Kim (2000: 327, fig. 4).

Flowering from July to October.

Distribution (Fig. 12). China (Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Jianyang, Kwangxi, Shangdong, Sichuan, Yunnan, and Zhenjiang), Japan (Chubu, Chugoku, Hondo, Kansai, Kanto, Kyushu, Shinkoku, Tohoku, Toyama, and Tokoyama), and South Korea (Chungcheongbuk-do, Chungcheongnam-do, Gangwon-do, Gyeonggi-do, and Jeju-do, and Jellanam-do); open woods; 400-800 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-10.

Agrimonia nipponica is distinguished from other species by small flowers and fruiting hypanthia. Variability in the shape of compound leaves has resulted in several varieties here reduced to synonymy, because variation patterns were detected within populations and even within individual specimens.

11. *Agrimonia parviflora* Aiton, Hortus Kew II: 130. 1789. *Eupatoria parvilorum* (Aiton) Nieuwland, Am. Midland. Nat. 4:71. 1915.—TYPE: Cultivated native of North America, [1766], *Herbarium Miller s.n.*, annotated “*Agrimonia parviflora* mss.” in Solander’s hand (lectotype, designated by Kline and Sørensen, 2008: BM!).

Agrimonia sauveolens Pursh, Fl. Amer. Sept. (Pursh) 1:336. 1814.—TYPE: United States. Virginia: Giles Co., Salt Pound Mountain, 1806, *F. Pursh s.n.* (lectotype, designated by Kline and Sørensen, 2008: PH!).

Agrimonia serrifolia Wallr., Beitr. Bot. (Wallr.)1:40. 1842.—TYPE: United States. Without specific locality, [1833-1834], *F. Beyrich s.n.* (lectotype, designated by Kline and Sørensen, 2008: PR).

Agrimonia polyphylla Urban, Symb. Antill. (Urban) 7:227. 1912.—TYPE: Dominican Republic. La Vega. Prope Constanza, 1Alt. 190m, Jul 1910, *H. von Türckheim* 3243 (lectotype, designated by V. Skalický, 1970: W; isolectotypes: BM! NY!).

Herbaceous perennials 50-150 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, usually branched, densely spreading pubescent. Leaves caulescent; compound leaves obovate; stipules falcate, obliquely spreading, unevenly toothed, $2.3-3.2 \times 1.3-1.6$ cm; leaflets 9-13; terminal leaflets narrow elliptic to lanceolate, apex acute, base cuneate, margins serrate, $3.5-6.4 \times 0.8-1.7$ cm, adaxial surface glabrous or scattered pubescent, abaxial surface scattered glandular and pubescent; 1st lateral leaflets slightly smaller than terminal leaflets, 2nd lateral leaflets as large as terminal leaflets; 1st interstitial leaflets 3 or 4 (5) pairs with 3 or 4 different sizes, the first or second pairs largest 0.5-1 cm long. Inflorescences elongate, branched, very dense (14-16 flowers/5 cm inflorescence); flowers 6-8 mm in diameter; pedicel 1.5-2.2 mm long; pedicel bracteoles 1, deeply tri-fid, hypanthium bracteoles 2, tri-fid or toothed; hypanthia turbinate, scattered glandular and pubescent; epicalyx segments numerous, persistent, cylindrical, apices inflexed, ascending, 1-1.3 mm long; petals yellow, oblong, $2.3-2.5 \times 1.0-1.4$ mm; stamens 7-11. Achenes 1 or rarely 2; fruiting hypanthia turbinate, vestiture scattered glandular and pubescent, deeply sulcate, bristly epicalyx segments numerous, ascending and spreading on upper edge $(3.7-4.41 (-4.73) \times (2.41-3.36 (-4.57))$ mm.— Chromosome number. $2n = 28$ (Hara and Kurosawa 1968).

Additional illustrations. Britton & Brown (1913: 268, fig. 2272); Wessel & Thieret (2000: 148, fig. 1).

Flowering from July to September.

Distribution (Fig. 13). Brazil (Parana), Canada (Ontario), Dominica Republic (San Juan and Santo Domingo), Mexico, and United States (mainly Eastern); also recorded from Argentina (Jujuy), Brazil (Minas Gerais, Santa Catarina, and Sao Pulo) (Fuks 1987); deciduous and mixed deciduous woods, in wet places, also pine woods (in Dominican Republic); river banks, edges of lakes or river; 400-800 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-11.

Agrimonia parviflora is distinguished from other species by numerous lateral leaflets that are equal in length to terminal leaflets. The species exhibits the most disjunct distribution patterns in the genus, because it occurs in eastern North America, the Caribbean island of Hispaniola, and Brazil and Argentina in South America. Additional study of South American specimens is required to refine its geographic range (Fig. 13). Phylogenetic analyses of nuclear and plastid sequence data (chapter 2) from West Indian and South American specimens are needed to investigate a putative close relationship among *A. parviflora*, *A. rostellata*, and *A. microcarpa*.

12. *Agrimonia pilosa* Ledeb., Ind. Sem. Horti Dorpat. Suppl. 1823.—TYPE: Altai.

without specific locality, 1832, *Ledebour s.n.* (lectotype: PRC; isoelectotype, here designated: GH!).

Herbaceous perennials 50-150 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, branched above, pubescent. Leaves caulescent;

compound leaves obovate; stipules falcate or flabellate-flacate, obliquely or horizontally spreading, entire, unevenly toothed, or dentate, 1.1-1.2 × 0.8-1.0 cm; leaflets 7-9; terminal leaflets elliptic to obovate, apex acute, base cuneate, margins dentate-serrate, 2.3-6.0 × 1.6-4.2 cm, adaxial surface scattered pubescent, abaxial surface scattered glandular and pubescent; 1st lateral leaflets smaller than terminal leaflets by 1cm long, 2nd lateral leaflets as large as one third of 1st lateral leaflets; 1st interstitial leaflets 3 pairs with 2 different sizes, middle ones largest 0.8-1.0 cm long, 2nd interstitial leaflets usually 1 or 2 pair(s). Inflorescences dense (8-10 flowers/5cm-inflorescence); flowers 8-12mm in diameter; pedicel 1-2 mm long; pedicle bracteoles 1, deeply tri-fid, hypanthium bracteoles 2, deeply toothed; hypanthia turbinate, pubescent; epicalyx segments numerous in multiple rows, persistent, cylindrical, apices inflexed, ascending, 1-2 mm long; petals yellow to golden yellow, obovate 4.5-4.7 × 2.1-2.3 mm; stamens 5-18. Achenes 1 or rarely 2; fruiting hypanthia turbinate, scattered glandular and pubescent, deeply sulcate, bristly epicalyx segments numerous ascending and converging on upper edge (5.66-) 6.07 (-6.46) × (4.25-) 4.63 (-5.11) mm.—Chromosome number. $2n = 56$ (Semereko 1990; Stepanov and Muratova 1992).

Agrimonia pilosa exhibits the broadest geographic distribution and extent of morphological variation of any species in *Agrimonia*. Consequently, there have been many varieties and segregate species named by previous workers. *A. pilosa* var. *succapitata* can be easily distinguished by not elongated inflorescences, and *A. pilosa* var. *zeylanica* is characterized with 5 stamens. Chromosome counting and genetic investigation of the varieties need to be conducted.

KEY TO VARIETIES OF *AGRIMONIA PILOSA*

1. Stipules falcate; margins entire or toothed. var. *pilosa*
1. Stipules flabellate-falcate; margins dentate.
2. Inflorescences non-elongated; 1.5-6.5 cm long in fruit. Stamens 12-18. var. *succapitata*
2. Inflorescences elongated; 15-30 cm long in fruit. Stamens 5. var. *zeylanica*

12. a. *Agrimonia pilosa* Ledeb. var. *pilosa*

Agrimonia pilosa Ledeb. subsp. *japonica* (Miq.) Hara, J. Jap. Bot. 43: 398.

1968.—TYPE: Japan, Nagasaki, *Oldham 225* (holotype: U).

Agrimonia nepalensis D. Don *obovata* Skalický, Fl. Cambodge, Laos & Vietnam 135.

1968.—TYPE: China, Yunnan, near Ta Pin Tze, *Delavang 473* (holotype: P).

Agrimonia viscidula Bunge, Mem. Sav. Itr. St. Petersb. ii. 100 1831. *Agrimonia*

viscidular Bunge var. *japonica* Miq., Ann. Mus. Bot. Lugduno-Botavum 3: 38.

1867.—TYPE: China, near Beijing, *Bunge s.n.* (holotype: Herbarium).

Terminal leaflets elliptic to obovate. Stipules falcate, entire or unevenly toothed. Inflorescences elongated and branched.

Additional illustration. Chung & Kim (2000: 324, fig. 2).

Distribution (Fig. 14). Bhutan, China (Anhui, Beijing, Gansu, Guangdong, Guangxizhuangzu, Guizhou, Hebei, Heilongjiang, Henan, Hubei, Huan, Jiangsu, Jiangxi, Jilin, Mongolia, Neimenggu, Shaanxi, Shandong, Shanxi, Xizang, Yunan, and Zhejiang), Czech Republic, Finland, India, Italy, Japan (Hokkaido, Honshu, Ryukyu

Is., Shikoku, and Yamanashi), Laos, Myanmar, Nepal, North Korea, Norway, Poland, Romania, Russia, South Korea, Sweden, Thailand, and Vietnam; edges of mixed forests, along trails; 200-1400 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-12.

12b. *Agrimonia pilosa* Ledeb. var. *succapitata* Naruh., J. Phytogeogr. Taxon 44: 82.

1996.— TYPE: Japan. Toyama: Minotani, Jyohana-machi, Higashi-tonamigun, edge of forest along road, 24 Sep 1996, N. *Naruhashi* & M. *Seo* 96092402 (holotype: KANA; isotypes: B, GH!, MAK, OSA, PE, TI, TNS, TUS).

Terminal leaflets elliptic to obovate. Inflorescences not elongate (*succapitata*), 1.5-6.5 cm long. Stamens 12-18.

Flowering from August to September.

Distribution (Fig. 14). Japan (Toyama); edges of mixed forests; 600m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-12.

12c. *Agrimonia pilosa* Ledeb. var. *zeylanica* (Moon ex Hook. f.) K. M. Purohit & Panigrahi, J. Jap. Bot. 58(10): 294. 1983. *Agrimonia zeylanica* Moon, Cat. Pl. Ceylon.

37. 1824.—TYPE: Sri Lanka (Ceylon), central province, at an elevation of 5-7000ft, *Thwaites* C. P. 2769 (holotype: BM!).

Stems densely pubescent. Leaf margins crenate-dentate. Stipules flabellate-falcate, evenly dentate. Stamens 5.

Flowering in August.

Distribution (Fig. 14). Sri Lanka; also recorded from Myanmar (Wu et al. 2003) and India (Sikkim and Meghalaya) (Purohit and Panigrahi 1983); edges of forest; 1000-2100 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-12.

13. *Agrimonia pringlei* Rydb., N. Amer. Fl. 22(5): 395. 1913. —TYPE: Mexico. Vera Cruz de Ignacio de la Liave. Near Jalapa 30 Nov 1903, *C. G. Pringle 11876* (holotype: GH!; isotypes: VT NY!).

Herbaceous perennials 40-100 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, simple or branched above, pubescent. Leaves caulescent; compound leaves obtrullate; stipules falcate, obliquely or horizontally spreading, toothed, 0.6-0.8 × 0.3-0.6 cm; leaflets 3-5; terminal leaflets elliptic obovate, apex obtuse, base cuneate, margins dentate, 2.4-4.8 × 1.4-1.6 cm, adaxial surface scattered pubescent, abaxial surface scattered glandular and pubescent; 1st lateral leaflets smaller than terminal leaflets by 0.5-0.7 cm long, 2nd lateral leaflets smaller than primary lateral leaflets by ca. 1 cm long; 1st interstitial leaflets 1 rarely 2 pairs with 1 or 2 different sizes, upper most ones largest 0.3-0.5 cm long, 2nd interstitial

leaflets usually 1 pair. Inflorescences loose (5-7 flowers/5cm-inflorescence); flowers 5-8 mm in diameter; pedicel 1-2 mm long; pedicle bracteoles 1 tri-fid, hypanthium bracteoles 2, entire or toothed; hypanthia turbinate, glandular; epicalyx segments numerous, persistent, cylindrical, apices inflexed, ascending, 1-1.2 mm long; petals light yellow, oblong, 1.8-2.1 × 1.1-1.3 mm; stamens 9. Achenes 1 or rarely 2; fruiting hypanthia turbinate, vestiture glandular only, shallowly and broadly sulcate, bristly epicalyx segments numerous, ascending on upper edge, (3.91-) 4.35 (-4.68) × (2.27-) 2.96 (-3.88) mm.

Chromosome number unknown.

Flowering from August to October.

Distribution (Fig. 15). Mexico (Puebla, Tamaulipas, and Veracruz); also recorded from Hidalgo, Queretago, and San Luis Potosi in Mexico (Kline and Sørensen 2008); deciduous or mixed deciduous woods; 900-1500 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-13.

Agrimonia pringlei is endemic to Mexico and characterized by a small number of lateral leaflets and loose inflorescences with small number of flowers.

14. *Agrimonia procera* Wallr., Erst. Beitr. Fl. Hercyn.: 573. 1840.—TYPE: 'Hort.

Reg. Paris.' *unspecified s.n.* (holotype: BM!).

Herbaceous perennials 80-140 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, branched above, spreading pubescent. Leaves caulescent; compound leaves obovate; stipules flabelliform, obliquely or horizontally spreading, deeply dentate, 2.5-3.2 × 1.5-1.7 cm; leaflets 7-9; terminal leaflets elliptic, ovate to obovate, apex obtuse, base cuneate, margins deeply dentate-serrate, 3.0-7.0 × 1.3-3.2 cm, adaxial surface scattered pubescent, abaxial surface glandular and pubescent; 1st lateral leaflets smaller than terminal leaflets by 0.8-1 cm long, 2nd lateral leaflets smaller than 1st lateral leaflets by 1-1.5 cm long; 1st interstitial leaflets 3 or 4 pairs with 2 different sizes, second pair ones largest 0.9-1.3 cm long, 2nd interstitial leaflets usually 3 pairs with 2 sizes. Inflorescences dense (15 flowers/5 cm-inflorescence); flowers 11-13 mm in diameter; pedicel 1-3 mm long; pedicle bracteoles deeply tri-fid, hypanthium bracteoles 2, entire or toothed; hypanthia long obconical, glandular and pubescent; epicalyx segments numerous, persistent, cylindrical, apices inflexed, ascending and deflexed, 2.0-2.3 mm long; petals golden yellow, oblong, 4.8-5.4 × 2.4-2.6 mm; stamens 10. Achenes 2 or rarely 1; fruiting hypanthia long obconical, vestiture glandular and pubescent, shallowly sulcate, bristly epicalyx segments numerous, ascending, deflexed, and spreading on upper edge, (7.86-) 9.22 (-10.23) × (7.13-) 9.02 (-11.56) mm.— Chromosome number. $2n = 56$ (Hollingsworth et al. 1992).

Additional illustration. Ross-Craig (1956: plate 10).

Flowering from June to July.

Distribution (Fig. 16). Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy (incl. Sicily), Netherlands, Norway, Poland, Portugal, Romania, Russia, Spain, Sweden, Switzerland, United Kingdom, and Yugoslavia; open woods; 400-1200 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-14.

Agrimonia procera has been popular as gardening and medicinal plant in Europe, and produces sterile F₁'s when it hybridizes with *A. eupatoria* (Skalický 1962). It is distinguished from *A. eupatoria* by deflexed epicalyx segments and large fruiting hypanthia with shallow sulci. Phylogenetic analyses of the cpDNA regions *trnL-trnF* and *trnS-trnG-trnG* suggest a sister relationship to *A. eupatoria*, but nuclear sequence data (GBSSI-1) do not resolve phylogeny relationships with other species (chapter 2).

15. *Agrimonia pubescens* Wallr., Beitr. Bot. (Wallr.) 1: 45-46. 1842.—TYPE: United States. New York: Albany Co., Clarksville, in dry woods, 1 Sept. 1931, *H. D. House no. 18907* (neotype, designated by V. Skalický, 1973: PRC; isoneotypes: NYS TEX!).

Agrimonia eupatoria γ *mollis* Torr. & A. Gray, Fl. N. Amer. 1:431. 1840. *A. mollis* (Torr. & A. Gray) Britton, Bull. Torrey Bot. Club 19: 221-222. 1892. *Eupatoria molle* (Torr. & A. Gray) Nieuwland, Am. Midland. Nat. 4:71. 1915.—TYPE: United States. Red River [1831-1834], *Dr. Z. Pitcher s.n.* (lectotype, designated here: NY!).

Agrimonia mollis var. *bicknellii* Kearney, Bull. Torrey Bot. Club 24: 565. 1897.

Agrimonia bicknellii (Kearney) Rydb., N. Amer. Fl. 22(5): 294. 1913.— TYPE:
United States. Tennessee: Wolf Cr., 23 Aug 1897, *T. H. Kearney, Jr.* 691
(holotype: OS; isotypes: MO! NY! US).

Herbaceous perennials 50-80 cm. Roots with tuber-like swellings. Stems vegetative and reproductive, erect, usually branched, densely pubescent. Leaves caulescent; compound leaves obovate; stipules falcate, obliquely spreading, toothed, 1.8-2.0 × 0.7-0.9 cm; leaflets 7-9; terminal leaflets elliptic to obovate, apex acute, base widely cuneate, margins serrate, 3.8-6.0 × 0.8-2.3 cm, adaxial surface scattered pubescent, abaxial surface scattered glandular and tomentose; 1st lateral leaflets smaller than terminal leaflets by 1.3-1.5 cm, 2nd lateral leaflets smaller than terminal leaflets by 2.0-2.2 cm, 3rd lateral leaflets less than one third of terminal leaflets; 1st interstitial leaflets 1 or 2 pairs with 1 or 2 different sizes, upper most pairs largest 0.4-0.6 cm long. Inflorescences elongate, branched, dense (8-12 flowers/5 cm-inflorescence); flowers 6-8 mm in diameter; pedicel 1.2-1.4 mm long; pedicle bracteoles 1, tri-fid, hypanthium bracteoles 2, shallowly tri-fid; hypanthia turbinate, scattered glandular and pubescent; epicalyx segments numerous, apices inflexed, ascending, 1-1.2 mm long; petals yellow, oblong, 2.3-2.5 × 1.0-1.2 mm; stamens 5-13. Achenes 1 or rarely 2; fruiting hypanthia turbinate, vestiture scattered glandular and pubescent, deeply sulcate, bristly epicalyx segments numerous, ascending and

spreading on upper edge (4.22-) 5.05 (-5.59) × (4.32-) 5.06 (-5.43) mm.—

Chromosome number. $2n = 28$ (Haruhashi and Iwatsubo per. comm.).

Additional illustration. Britton & Brown (1913: 267, fig. 2270).

Flowering from July to September.

Distribution (Fig. 17). Canada (Ontario) and United States (mainly Eastern); open places in deciduous and mixed deciduous woods; 600-1300m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-15.

Agrimonia pubescens can be characterized within the genus by a distinctive hypanthium shape and hypanthium vestiture. It has been confused with *A. microcarpa* because of similarity of leaf morphology. Kline and Sørensen (2008) suggested a further investigation of morphological variation to confirm status of three varieties.

16. *Agrimonia repens* L., Syst. Nat., ed. 10. 2: 1046. 1759.—TYPE: “Hibita [in Oriente.] Sp. Pl., ed. 2, 1:643 (1762)” Herb. *Linn. No. 628.2* (lectotype, designated by Skalický, 1973: LINN, photo!).

Herbaceous perennials 80-140 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, branched above, spreading pubescent. Leaves caulescent; compound leaves obovate; stipules flabelliform, obliquely or horizontally spreading, deeply dentate, 2.5-2.8 × 1.2-1.5 cm.; leaflets 7-9; terminal leaflets elliptic

obovate, apex obtuse, base cuneate, margin dentate-serrate, 3.0-5.9 × 1.3-2.0 cm, adaxial surface scattered pubescent, abaxial surface glandular and pubescent; 1st lateral leaflets smaller than terminal leaflets by 0.6-0.8 cm long, 2nd lateral leaflets as large as 1st lateral leaflets; 1st interstitial leaflets 3 or 4 pairs with 3 different sizes, second pair ones largest 0.5-0.7 cm long, 2nd interstitial leaflets usually 3 pairs with 2 sizes. Inflorescences dense (16-20 flowers/5cm-inflorescence); flowers 12-14 mm in diameter; pedicel 1-3 mm long; pedicle bracteoles deeply tri-fid longer than flowers, hypanthium bracteoles 2 or 3, entire; hypanthia long obconical, glandular and hirsute; epicalyx segments numerous, persistent, cylindrical, apices inflexed, deflexed, 1.5-2mm long; petals yellow to golden yellow, obovate, 4.8-5.5 × 3.0-3.4 mm long; stamens 18-22. Achenes 2 or rarely 1; fruiting hypanthia obconical, vestiture glandular and pilose, deeply sulcate on the middle, bristly epicalyx segments numerous, ascending, deflexed, and spreading on upper edge, (8.14-) 9.08 (-10.15) × (8.36-) 9.15 (-9.15) mm.— Chromosome number. $2n = 28$ (Medd 1955).

Flowering from June to July.

Distribution (Fig. 18). Austria, Belgium, Czech Republic, Germany, Italy, Romania, and Turkey; mixed woods; 400-1200 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-16.

Agrimonia repens is easily distinguished from other species by its large flowers with large number of stamens and fruiting hypanthia with deflexed bristly epicalyx segments.

17. Agrimonia rostellata Wallr., Beitr. Bot. (Wallr.) 1: 42–43. 1842. *Eupatorium*

rostellatum (Wallr.) Nieuwland, Am. Midland. Nat. 4: 71. 1915.—TYPE:

United States. Illinois: Pope Co., Shawnee National Forest, Bell Smith Springs picnic ground, 3 Aug 1998, *G. Kline 358D* (neotype, designated by Kline and Sørensen, 2008: DEK; isoneotypes: CAS F MO! NY!).

Herbaceous perennials 30-80 cm. Roots with tuber-like swellings. Stems vegetative and reproductive, erect, somewhat zig-zag (geniculate), usually branched, scattered pubescent or glabrous. Leaves caulescent; compound leaves obtrullate; stipules falcate, obliquely spreading, unevenly toothed, 0.5-0.8 × 0.4-0.9 cm; leaflets 3-5; terminal leaflets elliptic to rhombic-obovate, apex obtuse, base widely cuneate, margins dentate, 3.1-7.4 × 1.4 × 4.4 cm, adaxial surface scattered pilose, abaxial surface scattered glandular; 1st leaflets as large as terminal leaflets, 2nd lateral leaflets smaller than a half of 1st lateral leaflets; 1st interstitial leaflets 1 pair, 2nd interstitial leaflets 0. Inflorescences loose (4-6 flowers/5 cm-inflorescence), no longer than 15 cm long; flowers 6-8 mm in diameter; pedicels ca. 1 mm long; pedicel bracteoles 1, entire, toothed, or tri-fid; hypanthium bracteoles 2, entire or toothed; hypanthia hemispheric, densely glandular; epicalyx segments numerous, cylindrical, apices inflexed, ascending, 0.8-1.2 mm long; petals light yellow, oblong, 2.3-2.5 × 1.2-1.4 mm; stamens 5-10. Achenes usually 1; fruiting hypanthia hemispheric, vestiture

glandular, shallowly sulcate, bristly epicalyx segments numerous, ascending on upper edge (3.92-) 4.42 (-5.48) × (2.34-) 3.39 (-4.31) mm.— Chromosome number. $2n = 28$.

Additional illustration. Britton & Brown (1913: 267, fig. 2268).

Flowering from June to October.

Distribution (Fig. 19). United States (mainly Eastern); also recorded from Delaware, District of Columbia, Michigan, and New Jersey (Kline and Sorensen 2008); mesic deciduous forest to dry pine woods to 1100 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-17.

Agrimonia rostellata is easily distinguished by the small size of its flowers and fruiting hypanthia. Fewer pairs of lateral and interstitial leaflets also characterize the species. Phylogenetic analyses of both nuclear (GBSSI-1) and plastid (*trnL-trnF*) sequences suggest a close relationship with the eastern North American tetraploid species *A. parviflora* and *A. microcarpa* (chapter 2).

18. *Agrimonia striata* Michx., Fl. Bor.-Amer. 1:287. 1803.—TYPE: Canada.

[QUEBEC]: Chicoutimi, [1793], left plant on sheet, *A. michaux s.n.* (lectotype, designated by Kline and Sørensen, 2008: P-MICH; photo GH!).

Agrimonia brittoniana E. P. Bicknell, Bull. Torrey Bot. Club 23: 510, 517, pl. 282.

1896.—TYPE: United States. Maine: York Harbor, 12 Aug 1894, *E. P. Bicknell s.n.* (holotype: NY photo!; isotype: NY!).

Agrimonia striata Michx. var. *campanulata* Fernald, *Rhodora* 40 (477): 333-334.

1938.—TYPE: United States. Arizona: Cochise Co., Ramsey Cañon, Huachua Mts., 29 Sep 1929, *M. Jones 24920* (holotype: GH!).

Herbaceous perennials 55-130 cm. Roots with tuber-like swellings. Stems vegetative and reproductive, erect, base woody, usually branched, spreading pubescent. Leaves caulescent; compound leaves obovate; stipules falcate, obliquely or horizontally spreading, entire or unevenly toothed, 2.4-2.6 × 1.2-1.4 cm; leaflets (5) 7-9; terminal leaflets elliptic, rhombic-elliptic to obovate, apex acuminate, base widely cuneate, margins serrate, 5.5-9.2 × 2.4-3.7 cm, adaxial surface scattered pubescent, abaxial surface scattered glandular and pubescent; 1st lateral leaflets smaller than terminal leaflets by 0.8-1cm long, 2nd lateral leaflets smaller than 1st lateral leaflets by 0.8-1cm long; 1st interstitial leaflets 3 pairs with 2 different sizes, middle ones largest 0.7-1.2 cm long, 2nd interstitial leaflets usually 1-3 pairs. Inflorescences dense (10-12 flowers/5cm-inflorescence); flowers 8-12 mm in diameter; pedicel short, 1-3 mm long; pedicle bracteoles 1, tri-fid; hypanthium bracteoles 2, toothed; hypanthia obconical, hirsute; epicalyx segments numerous, cylindrical, apices inflexed, ascending 1.6-2mm long; petals light yellow, oblong, 4.1-4.3 × 1.8-2.2 mm; stamens 6-12. Achenes 1 or rarely 2; fruiting hypanthia obconical, vestiture glandular on furrow, pilose and glandular on ridges, deeply sulcate, bristly epicalyx segments numerous, ascending and converging on upper edge (5.71-) 6.18 (-6.70) × (42.80-)

3.04 (-3.23) mm.— Chromosome number. $2n = 56$ (Probatov and Sokolovskaya 1995).

Additional illustration. Britton & Brown (1913: 268, fig. 2271).

Flowering from July to September.

Distribution (Fig. 20). Canada (Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Ottawa, Prince Edward Island, Quebec, and Saskatchewan), Mexico (Chihuahua, Hidalgo, Sinaloa, and Sonora), and United States; also recorded from Mexico (Distrito Federal and Tamaulipas) (Kline and Sørensen 2008); open places in mixed forests, edges of woods; 1200-3700 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-18.

Agrimonia striata is distinguished from other species by dense inflorescences, obconic fruiting hypanthia with deep sulci. Variation in leaf shape and margin is great, but inflorescence and fruit characters clearly define the species clearly.

Phylogenetic analyses of nuclear GBSSI-1 sequences suggest that *A. striata* is sister to *A. pilosa* that occurs throughout Eurasia (chapter 2).

19. *Agrimonia villosa* Cham. et Schldl., *Linnaea* 2:27. 1827.—TYPE: Brazil. Rio de Janeiro [from 1815 to 1817], *F. Sellow* (lectotype, here designated, LE).

Herbaceous perennials 70-100 cm. Roots without tuber-like swellings. Stems vegetative and reproductive, erect, branched above, densely silky pubescent and glandular. Leaves caulescent; compound leaves obovate; stipules flabelliform-falcate, obliquely spreading, evenly dentate, 3.1-3.4 × 1.4-1.6 cm; leaflets 9-11; terminal leaflets elliptic to obovate, apex obtuse, base widely cuneate, margins dentate, 3.5-4 × 1.5-1.8 cm, adaxial surface densely villose, abaxial surface densely glandular and villose; 1st and 2nd lateral leaflets as large as terminal leaflets; 1st interstitial leaflets 3 pairs with 2 different sizes, upper most ones largest 0.5-0.7 cm long, 2nd interstitial leaflets usually 2 pairs with 2 different sizes. Inflorescences dense (15-18 flowers/5cm-inflorescence); flowers 7-10 mm in diameter; pedicel 1.6-2 mm long; pedicle bracteoles 1, tri-fid; hypanthium bracteoles 2, entire or toothed; hypanthia obconical, glandular and villose; epicalyx segments numerous, cylindrical, apices inflexed, ascending, 1-1.2 mm long; petals yellow, oblong, 4.0-4.3 × 1.8-2.1 mm; stamens 9. Achenes 1 or rarely 2; fruiting hypanthia long hemispheric, vestiture glandular and long pilose, shallowly sulcate, bristly epicalyx segments numerous, ascending on upper edge (2.8-) 3.2 (-3.5) × (1.8-) 2.0 (-2.4) mm.

Additional illustration. Fuks (1987: 80, fig. 3).

Flowering from January to March.

Distribution (Fig. 21). Brazil (Parana, Rio Grande do Sul, and Santa Catarina); also recorded from Sao Paulo in Brazil (Fuks 1987); open places; 600-1300 m.

REPRESENTATIVE SPECIMENS EXAMINED. see Appendix 3-19.

Agrimonia villosa is characterized by dense inflorescences and villose vestiture on stems and leaves. It is only found in Brazil. The relationship between *A. villosa* and the eastern North American species *A. parviflora* requires investigation because the species are closely related in molecular phylogenetic trees.

DOUBTFUL AND EXCLUDED NAMES

- Agrimonia ceylanica* Wight, Icon. Pl. Ind. Orient (Wight). t. 224. 1839. A type specimen needs to be investigated.
- Agrimonia euaptoria* var. *glabra* Barton, Fl. Philadelph. Prodr.: 53. 1815, nomen nudum.
- Agrimonia eupatoria* var. *glabra* Mulh., Cat. Pl. Amer. Sept.: 47. 1813, nomen nudum.
- Agrimonia eupatoria* var. *hirsuta* Barton, Fl. Philadelph. Prodr.: 53. 1815, nomen nudum.
- Agrimonia eupatoria* var. *hirsuta* Muhl., Cat. Pl. Amer. Sept.: 47. 1813, nomen nudum.
- Agrimonia grandis* Andrz. ex C. A. Mey., Fl. URSS 6: 166-169, fig. 32, 1954. A type needs to be examined
- Agrimonia lanata* N. Wallich, Numer. List: 709. 1828, nomen nudum.
- Agrimonia nepalensis* D. Don, Prodr. Fl. Nepal. 229. 1825 —TYPE: Nepal, *Hamilton* s.n. (holotype; BM). An examination of type specimen is required to determine if it is a synonym of *A. pilosa* var. *pilosa*.
- Agrimonia pumila* Mulh. ex Raf., Fl. Ludov. : 134. 1817, nomen nudum.

Agrimonia pumila Mulh., Cat. Pl. Amer. Sept.: 47. 1813, nomen nudum.

Agrimonia sororia Fisch. & C. A. Mey. Bull. Sc. Acad. Petersb. X: 344. 1842. It might be a synonym of *A. eupatoria*, but a type specimen examination has not been examined.

Agrimonia trifolia W. Young, Cat. Arbr. Amer.: 28. 1783, nomen nudum.

Agrimonia velutina Juzepczuk, Fl. URSS 10: 636. 1941. Although the name has been treated as a synonym of *A. coreana* by many authors (e.g. Lee 1996; Naruhashi 2001; Li et al. 2003), the application of the name is in doubt until the type is examined.

Agrimonia x nipponica-pilosa Murata, Acta Phytotax. Geobot. 34: 176.1983. —TYPE: Japan, Kyushu, Kagoshima, Iwatski, Fujita, *Ueda et Nishida 168* (holotype: KYO). The type needs to be examined.

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TABLE 1. Morphological characters evaluated in this study.

Character	State	
1. Growth form	0 = herb	1 = tree or shrub
2. Caulescent leaves	0 = absent	1 = present
3. Inflorescence type	0 = raceme	1 = panicle
4. Flower type	0 = bisexual	1 = unisexual
5. Stigma type	0 = smooth	1 = spathulate
6. Involucre	0 = present	1 = absent
7. Epicalyx number	0 = 5	0 = >20
8. Interstitial leaflets	0 = absent	1 = present
9. Leaf margin	0 = entire	1 = toothed
10. Hardened hypanthium in fruit	0 = present	1 = absent
11. Seed coat sculpturing	0 = smooth	1 = papillate
12. Pollen sculpturing	0 = striate	1 = microverrucate

TABLE 2. Chromosome numbers and their sources in subtribe Agrimoniinae.

Taxon	2n	Locality	Source
<i>Agrimonia coreana</i> Nakai	28	Japan	Iwatsubo et al. 1993
<i>A. eupatoria</i> L.	28	Ireland	Hollingsworth et al. 1992
<i>A. grandis</i> Andr. ex C. A. Mey.	42	Unknown	Murín 1997
<i>A. gryposepala</i> Wallr.	56	Unknown	Brittan 1953
<i>A. incisa</i> Torr. & A. Gray	28	USA	This study (Chung KS TX3, OKL)
<i>A. japonica</i> (Miq.) Koidz.	56	Japan	Iwatsubo et al. 1993
<i>A. nipponica</i> Koidz.	28	Japan	Iwatsubo et al. 1993
<i>A. parviflora</i> Sol. in Aiton	28	USA	Haruhashi and Iwatsubo (per. comm.)
	28	USA	Hara and Kurosawa 1968
<i>A. pilosa</i> Ledeb.	56	Russia	Semereko 1990
	56	Russia	Stepanov and Muratova 1992
<i>A. pilosa</i> var. <i>japonica</i> (Miq.) Nakai	56	Japan	Probatova et al. 1989
<i>A. pilosa</i> var. <i>nepalensis</i> D. Don	56	Nepal	Hara and Kurosawa 1968
<i>A. procera</i> Wallr.	56	Ireland	Hollingsworth et al. 1992
<i>A. pubescens</i> Wallr.	28	USA	Haruhashi and Iwatsubo (per. comm.)
	28	USA	This study (Chung KS19, OKL)
<i>A. repens</i> L.	28	Europe	Medd 1955
<i>A. rostellata</i> Wallr.	28	USA	This study (Chung KS29, OK)
<i>A. x nipponica-pilosa</i> Murata	42	Japan	Iwatsubo et al. 1993
<i>A. striata</i> Michx.	56	Unknown	Probatov and Sokolovskaya 1995
	56	USA	Haruhashi and Iwatsubo (per. comm.)
	56	Unknown	Brittan 1953
<i>Aremonia agrimonoides</i> (L.) Neck.	42	Greece	Baltisberger 1991
(= <i>Agrimonia agrimonoides</i> L.)	42	Slovak Republic	Murín and Majovsky 1987
<i>Hagenia abyssinica</i> J. F. Gmel.	40 (?)	Ethiopia	Feyissa 2006
<i>Spenceria ramalana</i> Triemn	14	China	Takahashi et al. 1996

FIG. 1. Important leaf and floral characters of *Agrimonia*. A. Interrupted pinnately compound leaf: a. terminal leaflet, b-d. lateral leaflets (b, 1st lateral leaflet; c, 2nd lateral leaflet; d, 3rd lateral leaflet); e-f. interstitial leaflets (e, 1st interstitial leaflets; f, 2nd interstitial leaflet); g. stipule. B. Flower: 1. pedicle bracteole; 2. pedicel; 3. hypanthium bracteole; 4. epicalyx segments; 5. hypanthium. C. Fruiting hypanthium: 4. epicalyx segments.

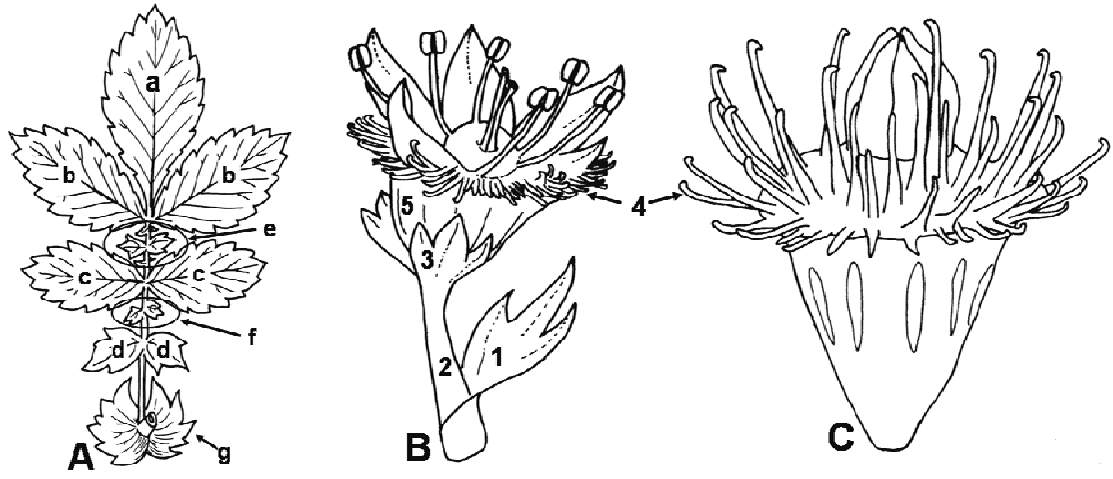
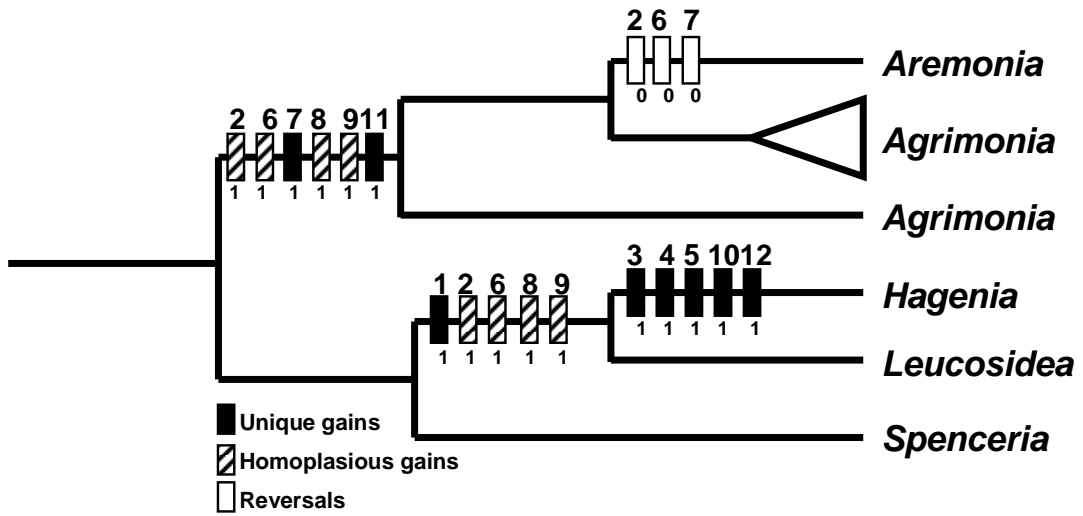
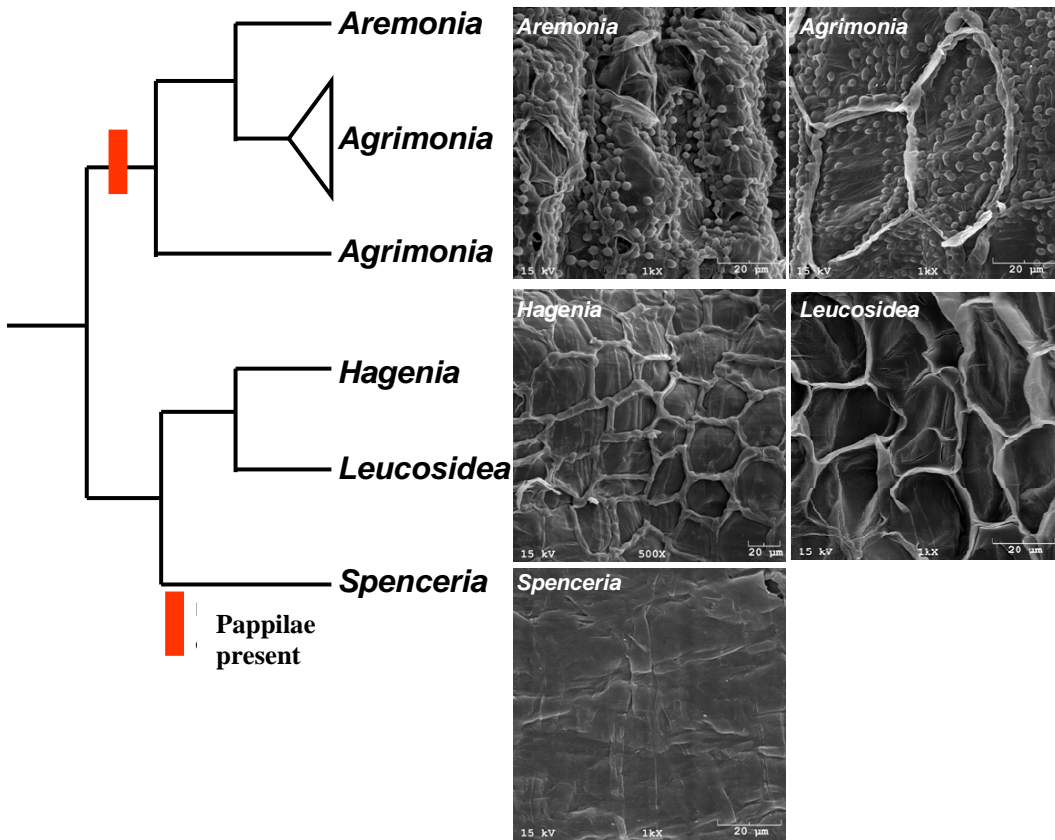


FIG. 2. Morphological character evolution in subtribe Agrimoniinae inferred by mapping characters onto simplified Bayesian phylogenetic trees derived from variation at the nuclear GBSSI-1 locus. A. Inferred changes of 12 morphological characters presented in Table 1. Boxes represent character reversals (= a loss) and unique and homoplasious gains. Numbers above boxes represent character number in Table 1. B. Seed coat evolution in subtribe Agrimoniinae. Monophyly of *Agrimonia* + *Aremonia* supported by papillate sculpturing on seed coats. SEM micrographs of seed coats: *Agrimonia coreana* (Chung 2007-5, OKL), *Aremonia agrimonoides* (G. Gigo s.n., DAO), *Leucosidea sericea* (Balkwill & Balkwill 9343, MO), *Hagenia abyssinica* (J. W. Ash 1388, MO), and *Spenceria ramalana* (C. Schneider 3316, A).

A



B



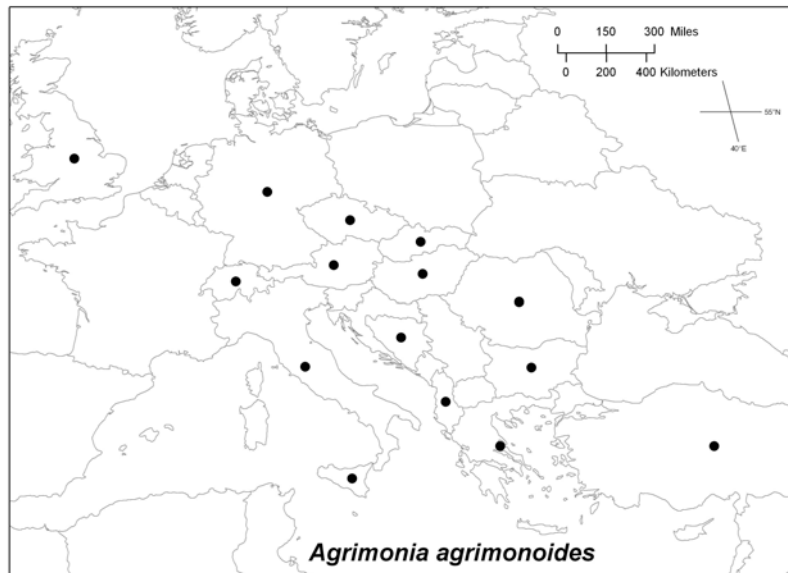


FIG. 3. The distribution of *Agrimonia agrimonoides*.

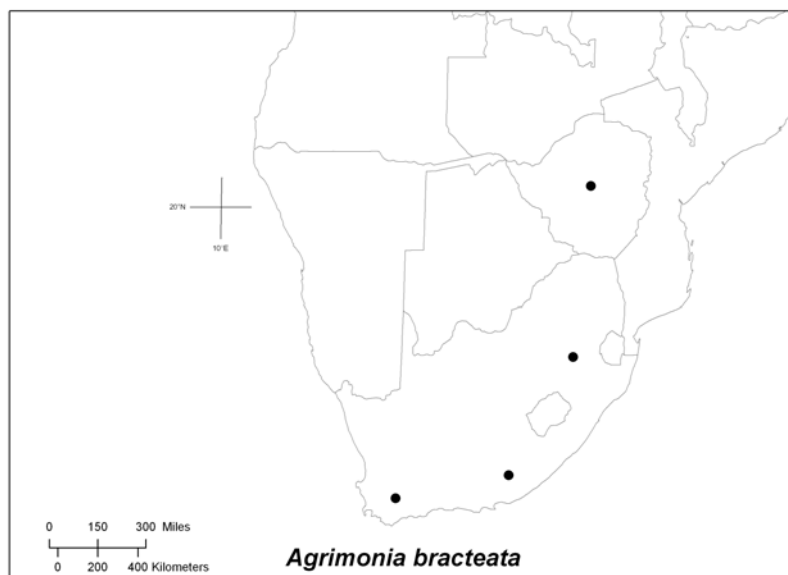


FIG. 4. The distribution of *A. bracteata*.

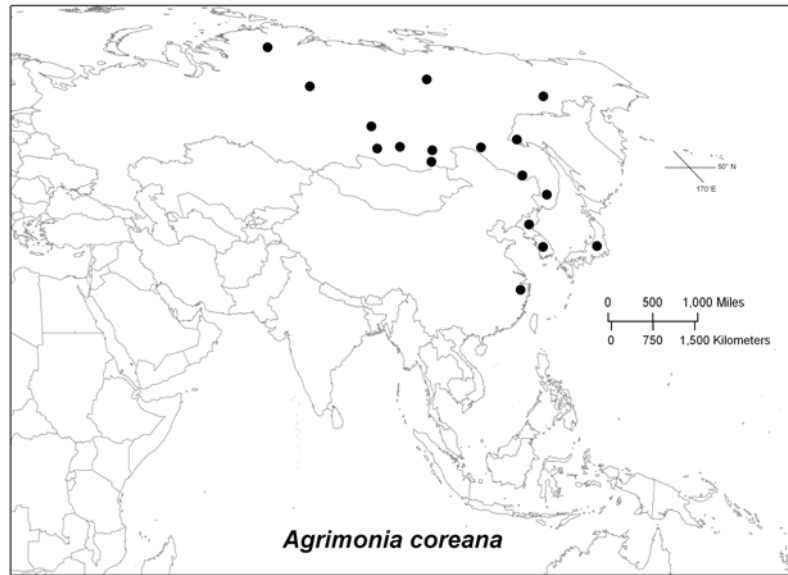


FIG. 5. The distribution of *A. coreana*.

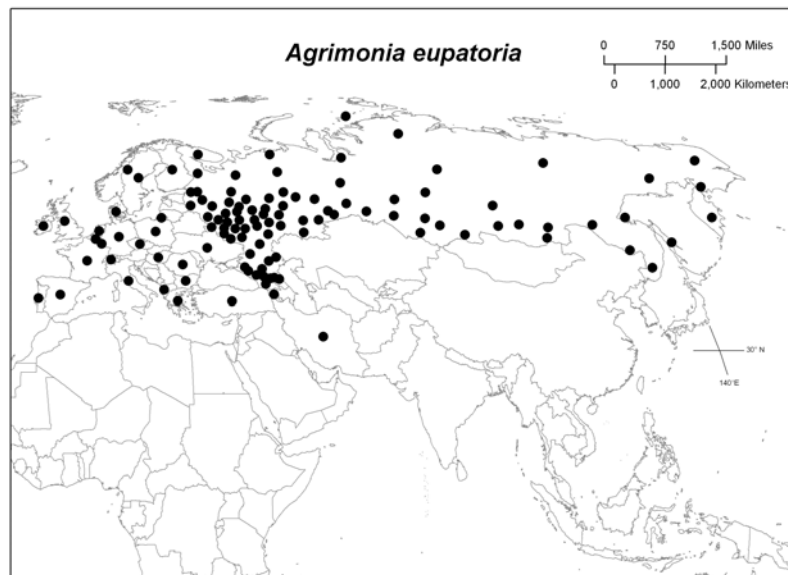


FIG. 6. The distribution of *A. eupatoria*.

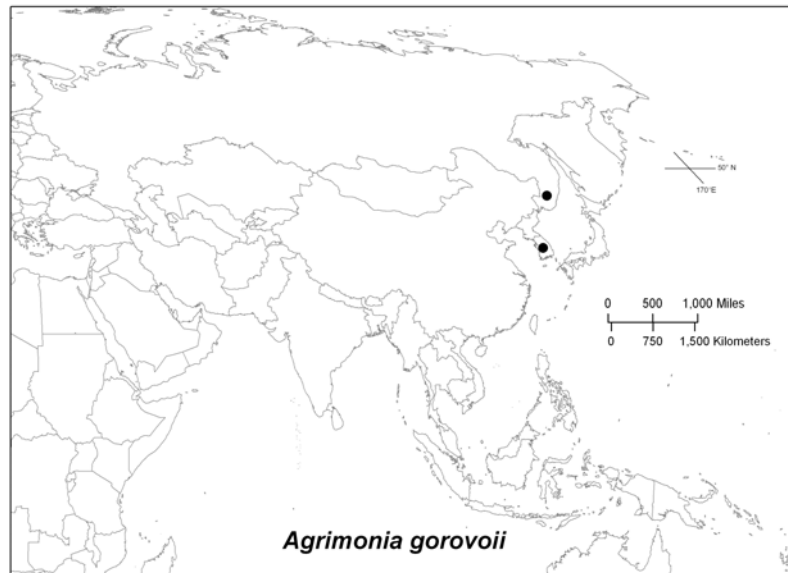


FIG. 7. The distribution of *A. gorovoi*.

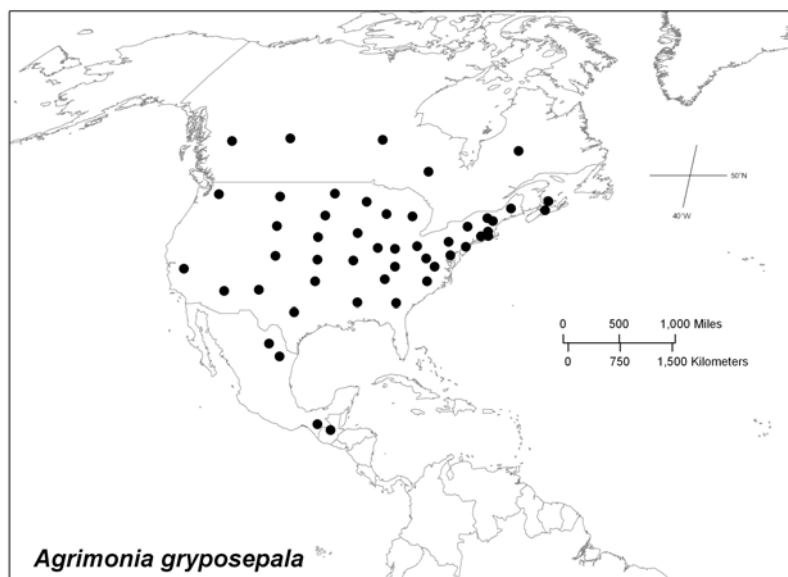


FIG. 8. The distribution of *A. gryposepala*.

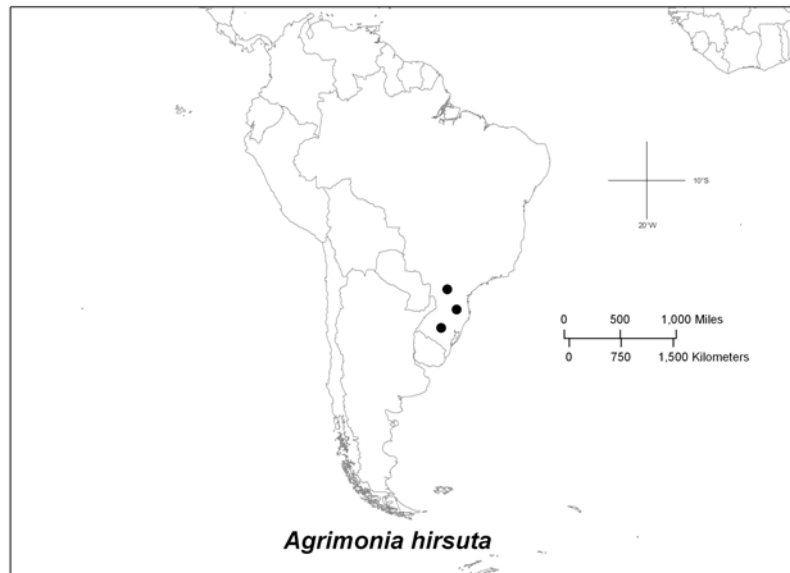


FIG. 9. The distribution of *A. hirsuta*.

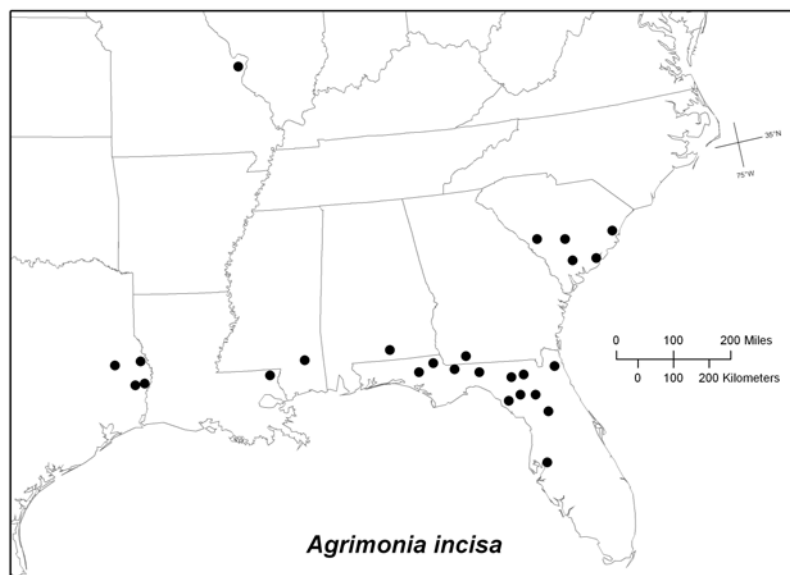


FIG. 10. The distribution of *A. incisa*.

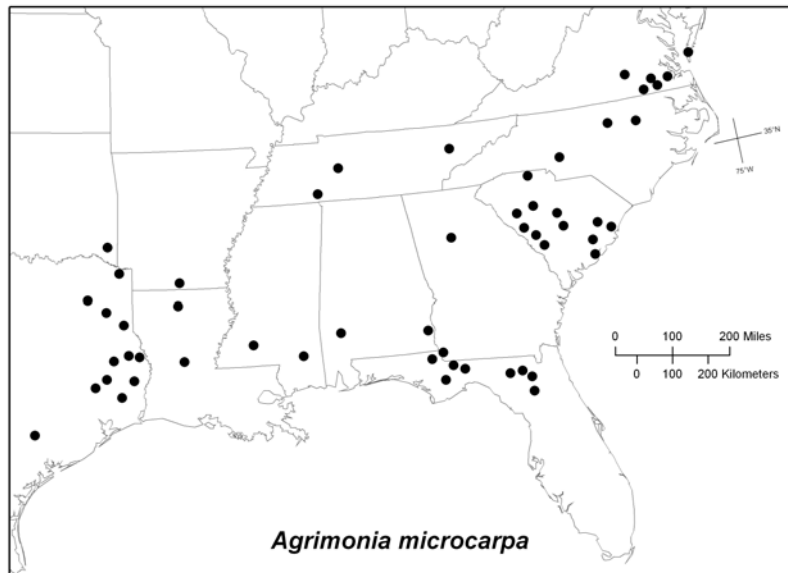


FIG. 11. The distribution of *A. microcarpa*.

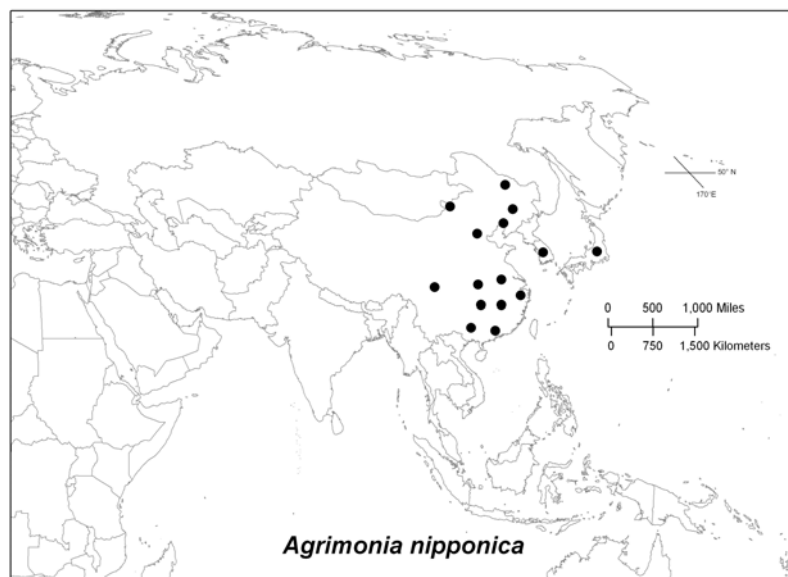


FIG. 12. The distribution of *A. nipponica*.

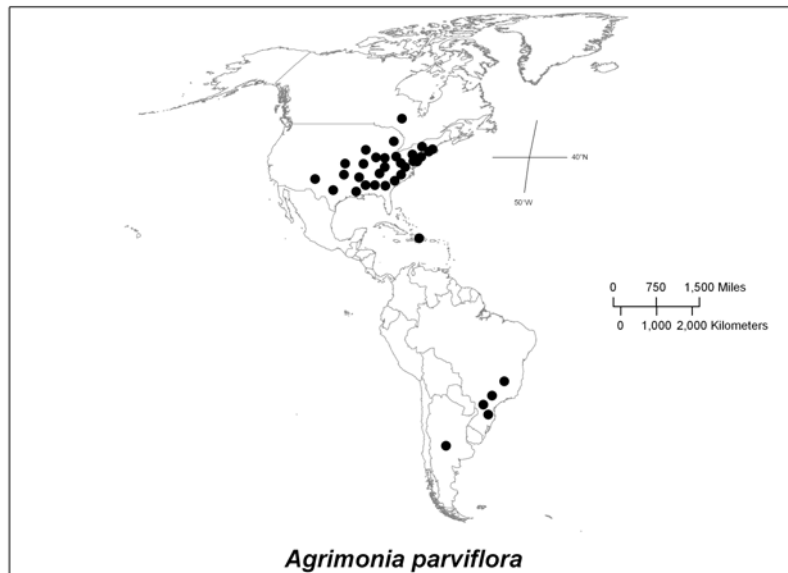


FIG. 13. The distribution of *A. parviflora*.

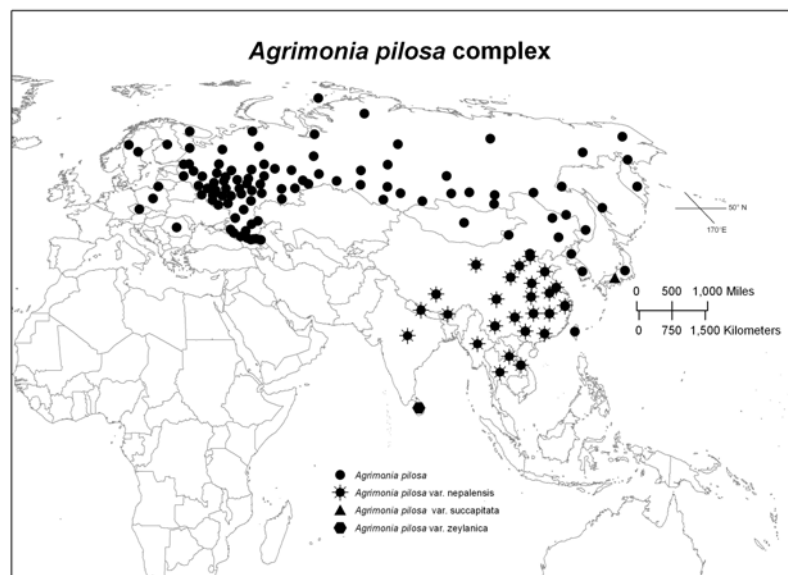


FIG. 14. The distribution of *A. pilosa*.

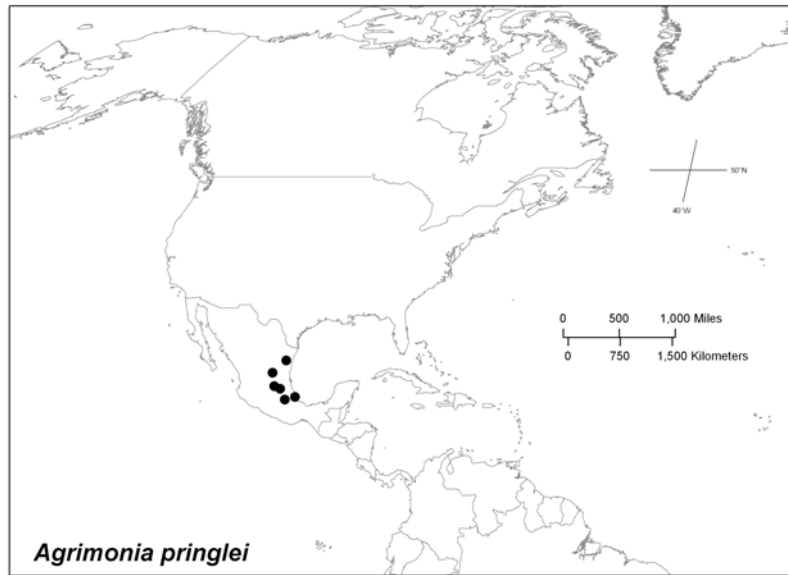


FIG. 15. The distribution of *A. pringlei*.

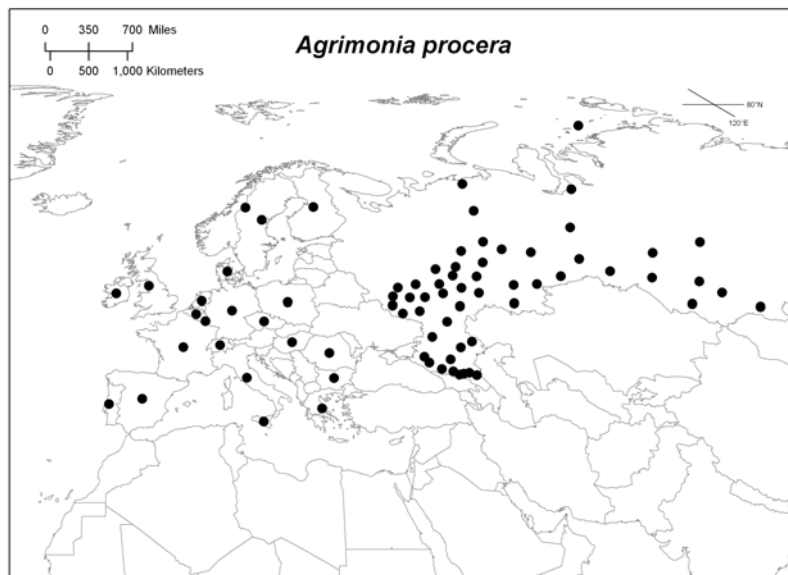


FIG. 16. The distribution of *A. procera*.

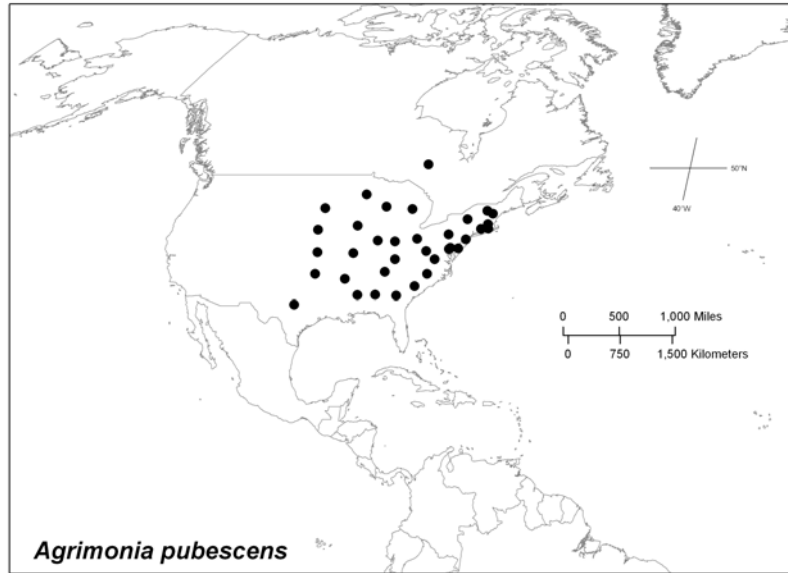


FIG. 17. The distribution of *A. pubescens*.

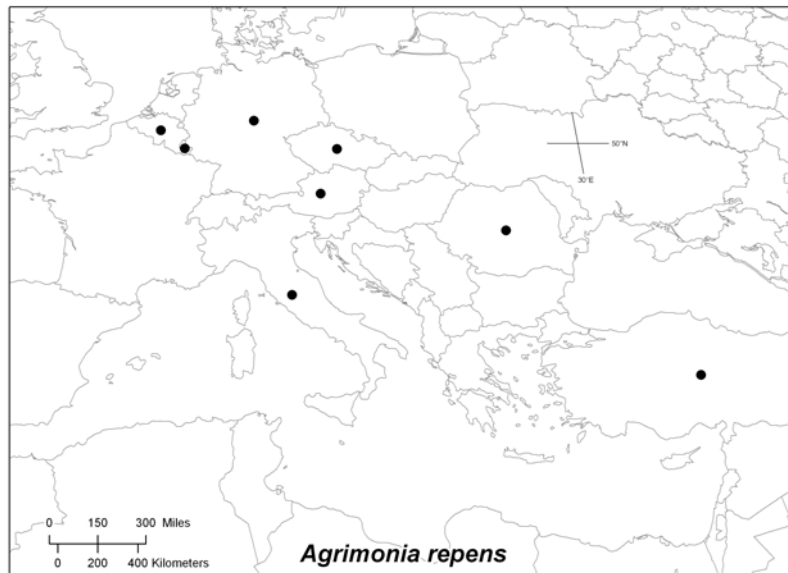


FIG. 18. The distribution of *A. repens*.

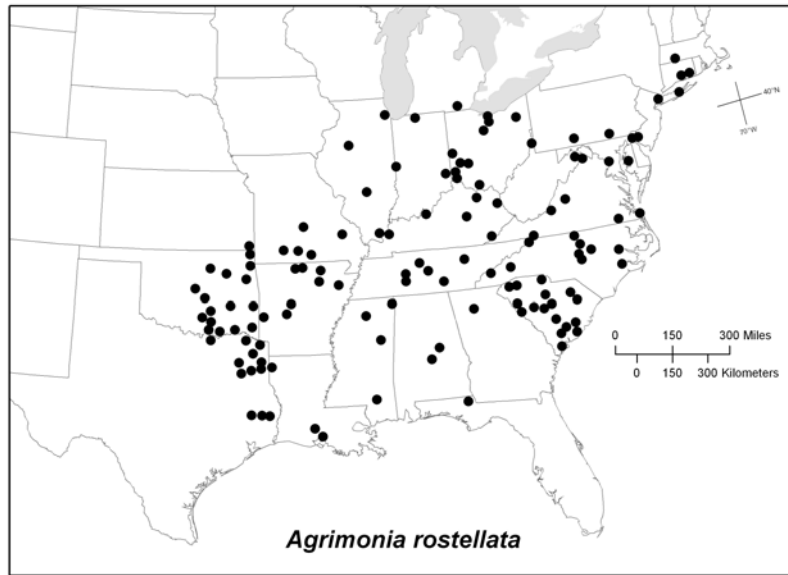


FIG. 19. The distribution of *A. rostellata*.

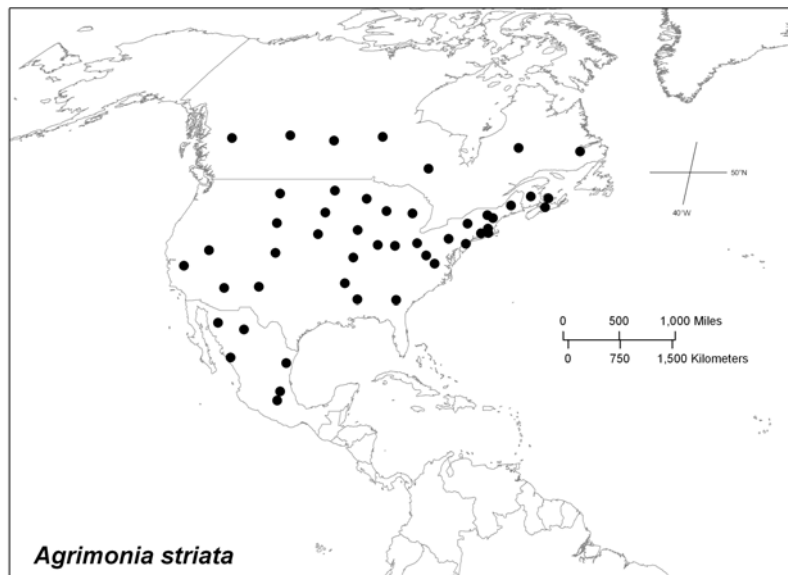


FIG. 20. The distribution of *A. striata*.

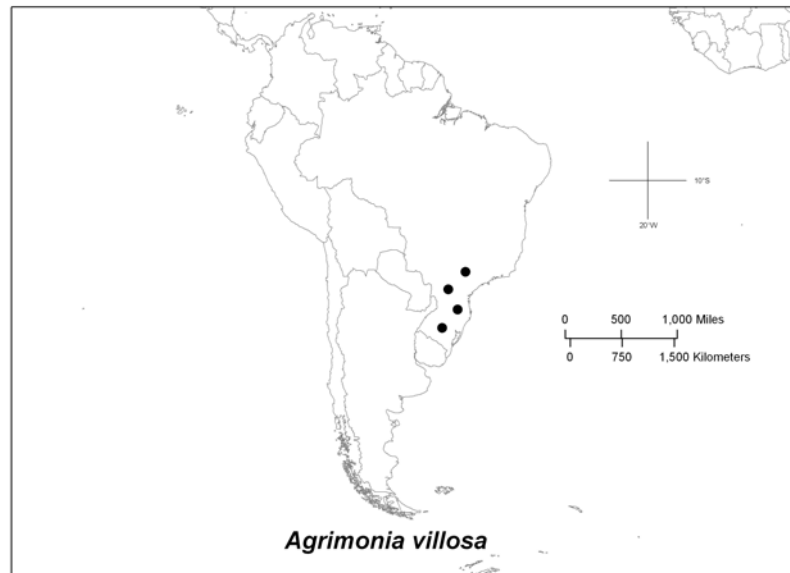


FIG. 21. The distribution of *A. villosa*.

APPENDIX 2-1. Collection data for genera and species used in phylogenetic analyses of tribe Sanguisorbeae and subtribe Agrimoniinae.

Agrimonia coreana Nakai — accession 1, Oh et al. 050805-128 (CBU), Chungchungbukdo, Korea; accession 2, Oh et al. 050805-174 (CBU), Chungchungbukdo, Korea; accession 3, Chung 2007-16 (OKL), Kangwondo, Korea; accession 4, Chung 2007-2 (OKL), Kanagawa, Japan. *A. eupatoria* L. — accession 1, Dale McNeal 378 (MO), Republic of Armenia; accession 2, Unspecified *s.n.* (TEX), Canary Islands, accession 3, M. Merello et al. 2035 (MO), Georgia. *A. gryposepala* Wallr. — accession 1, M. A. Vincent 10769 (MU), Wisconsin, USA; accession 2, G. M. Keleher 458 (DAO), Manitoba, Canada; accession 3, Unspecified *s.n.* (MEXU), Chiapas, Mexico; accession 4, Chung 38 (OKL), Tennessee, USA; accession 5, Chung 39 (OKL), Tennessee, USA. *A. incisa* Torr. & A. Gay — accession 1, Chung 2007-TEX3 (OKL), Texas, USA; accession 2, S. L. Orzell & E. L. Bridges 18075 (TEX), Florida, USA; accession 3, Chung 2007-TEX1 (OKL), Texas, USA. *A. microcarpa* Wallr. — accession 1, C. A. Allen et al. PKL0697 (OKL), Louisiana, USA; accession 2, Nelson 26673 (SUCH), South Carolina, USA. *A. nipponica* Koidz. — accession 1, Chung 2007-9 (OKL), Kangwondo, Korea; accession 2, Chung chungpyeng 3 (OKL), Kangwondo, Korea; accession 3, T. Iwasaki 212 (MO), Tohoku, Japan. *A. parviflora* Aiton — accession 1, Chung 31 (OKL), Oklahoma, USA; accession 2, Chung 40 (OKL), Tennessee, USA. *A. pilosa* Ledeb. — accession 1, J. Zuo 0970 (MO), Hunan, China; accession 2, T. Z. Liu 217 (MO), Mongolia, China; accession 3, Chung 2007-4 (OKL), Kanagawa, Japan; accession 4, Chung 2007-7 (OKL), Kanagawa, Japan; accession 5, Chung 2007-1 (OKL), Chungchungbukdo, Korea. *A. procera* Wallr. — accession 1, Chung 14 (OKL), Russia; accession 2, Chung 12 (OKL), Unknown (Moscow). *A. pubescens* Wallr. — accession 1, Chung 17 (OKL), Pennsylvania, USA; accession 2, Chung 19 (OKL), Pennsylvania, USA; accession 3, N. McCarty & A. Buthod OOL509 (OKL), Oklahoma, USA; accession 4, Chung 18 (OKL), Pennsylvania, USA; accession 5, Chung 21 (OKL), Missouri, USA. *A. repens* L. —

accession 1, Chung 11 (OKL), Unknown (KEW); accession 2, Chung 10 (OKL), Unknown (KEW). *A. rostellata* Wallr. — accession 1, Chung 20 (OKL), PA, USA; accession 2, Elisens 1102 (OKL), Maryland, USA; accession 3, Elisens 1108 (OKL), Maryland, USA. *A. striata* Michx. — accession 1, Elisens 1164 (OKL), New York, USA; accession 2, R. S. Felger 92-952 (MEXU), Sonora, Mexico; accession 3, V. E. McNeilus 97-517 (TEX), Minnesota, USA; accession 4, Chung 1 (OKL), Vermont, USA; accession 5, Chung 2 (OKL), Colorado, USA. *Aremonia agrimonoides* (L.) Neck. — M. Paun & G. Popescu *s.n.* (DAO), Wallachia, Romania. *Hagenia abyssinica* J. F. Gmel. — L.. Festo & R. Langson 4535 9 (MO), Iringa, Tanzania. *Leucosidea sericea* Eckl. & Zeyh. — Chung L2 (OKL), Unknown (Seeds). *Spenceria ramalana* Trimen — D. E. Boufford et al. 31247 (A), Xizang (Tibet), China.

APPENDIX 2-2. GenBank accession numbers of sequences of outgroup taxa used in phylogenetic analyses of tribe Sanguisorbeae and subtribe Agrimoniinae.

Taxon	GenBank accession numbers		
	<i>trnL-trnL-trnF</i>	<i>trnS-trnG-trnG</i>	GIBSSI-1
<i>Acaena cylindristacha</i>	AJ512775		
<i>Alchemila aplina</i>	AJ512217		
<i>Chamaespilus alpina</i>	DQ863227		AF500404
<i>Fragaria vesca</i>	AJ512232		
<i>Geum reptans</i>	AJ297337		AJ534187
<i>Oemleria cerasiformis</i>		AY871263	
<i>Physocarpus opulifolius</i>	AY555417	AY500742	
<i>Polylepis tarapacana</i>	AJ512773		
<i>Potentilla reptans</i>	AJ512241		
<i>Poteridium annua</i>	AY634766		
<i>Poterium minor</i>	AJ634772		
<i>Prunus caroliniana</i>		AY500741	
<i>Prunus virginiana</i>	AF348561	AY500739	
<i>Rosa multiflora</i>	AY634764		AF285993
<i>Rubus odoratus</i>	EF055334		AF285994
<i>Sanguisorba officinalis</i>	AJ416465		
<i>Spiraea cantoniensis</i>	DQ897578	AY871264	
<i>Tetraglochin cristatum</i>	AJ512777		

APPENDIX 3-1. Representative Specimens Examined of *Agrimonia agrimonoides*.

AUSTRIA. Carinthia: Villach, H. Schack s.n. (SMU). **CZECH REPUBLIC. Moravian-Silesian Region:** Vsetin, G. Rican 806 (DAO). **GERMANY. Baden-Wuttemberg:** Waldshut district, P. Aellen s.n. (SMU). **ITALY. Veneto:** Verona, G. Rigo s.n. (DAO). **REPUBLIC OF SLOVENIA. Styria:** Neuhaus, M. Heider 757 (DAO). **ROMANIA. Sud-Vest:** Mehedinto Co., M. Paun & H. Popescu 965 (DAO).

APPENDIX 3-2. Representative Specimens Examined of *Agrimonia bracteata*. **SOUTH AFRICA. Eastern Cape:** Komgha, R. D. A. Batliss 3048 (MO), **Mpumalanga:** Lothair, G. Germishuizen 3914 (MO). **Western Cape:** Cape, R. D. A. Bayliss 3048 (MO). **ZIMBABWE. Manicaland:** Inyanga, N. C. Case 8262 (MO), Inyanga, T. F. Ngoni 541 (MO).

APPENDIX 3-3. Representative Specimens Examined of *Agrimonia coreana*. **CHINA. Manhuria:** Lao-dun region, D. Livinov 1755 (GH). **JAPAN. Tohoku:** Iwate, M. Takahashi 9685 (TI). Miyagi, Y. Tateichi et al. 15195 (GH). **NORTH KOREA. Hamgyengbukdo,** without specific locality, T. Nakai 6218 (TI). **Kangwondo:** Kumgang Mt. T. Nakai 5526 (TI). **SOUTH KOREA. Kangwondo:** Youngnong, Oh et al. 050805-175 (CBU). Mt. Geumsu, Oh et al. 050805-125 (CBU). **Chulanamdo:** Mt. Baekunsa, T. B. Lee s.n. (SNUA). Mt. Backyang, T. Nakai 6218 (TI). Mt. Jili, T. Nakai s.n. (TI). **Chungchendbukdo:** Mt. Walak, Oh et al. 050805-174 (CBU).

APPENDIX 3-4. Representative Specimens Examined of *Agrimonia eupatoria*.

ALGERIA. without specific locality, A. Faure s.n. (NY). **ARMENIA. Kotayk:** Tsakhkadzor, D. McNeal 378 (MO). **AUSTRIA. Burgenland:** Oberwart, F. Hopflinger s.n. (TEX). **Styria:** without specific locality, P. Troyer 23960 (SMU). Without specific locality, Holuby et al. 2419 (GH). Belgium. Hainaut: without specific locality, R. Germain 8835 (DAO). **CAPE VERDE.** Praia, V. Hedrick 3 (SMU). **CROATIA.** Without specific locality, J. Ball 1890 (GA). **DENMARK. Aarhus:** Lisberg, K. Larsen 166 (SMU). **Sjalland:** without specific locality, H. Mortensen s.n. (DAO). **Zealand:** Stampen, J. Svendsen 488 (NY). **ENGLAND. Buckinghamshire:** Aston Clinton, J. E. Dandy 933 (DAO). **North Yorkshire:** Harrogate and Knaresboro, T. W. Edmondson 4905 (NY). Skipton, T. H. Holmes 3908 (NY). **Oxfordshire:** without specific locality, C. E. Hufford 17633 (NY). **Salisbury:** without specific locality, A. S. Pease 8288 (GH). **Surrey:** without specific locality, E. T. & H. N. Moldenke 9231 (NY). **West Yorkshire:** Bradford, T. W. Edmondson 6465 (NY). Long Preston, L. Rotheray 2937 (NY). **FINLAND.** Aboensis, H. Lindberg 272 (DAO). Ahvenanma, M. V. Kurki s.n. (DAO). Alandia, Brando, U. Laine & J. Virtanen s.n. (DAO). Hermala, Storon, P. H. Lindberg s.n. (DAO). Kaldo, Nauvo, B. Saxen s.n. (DAO). Karjala, A. J. Hunskonen s.n. (DAO). Sortavala, Mt. Rauskunvuori, A. J. Huuskonen s.n. (DAO). **FRANCE.** Aquitanine, Bordeaux, C. J. Pitard s.n. (NY). Cote-Or, P. A. Genty 3761 (GH). Etoutteville, I. Tidestrom 13867 (NY). Loire Infereure, Verbon, B. J. Peter s.n. (DAO). Sicily, M. D. Babington s.n. (NY). Versailles, E. M. Round s.n. (NY). **GEORGIA. Samtskhe-Javakheti:** Borijomi, 41 45 31 N, 43 31 17 E, M. Merello et al. 2035 (MO). Greater Caucasus, Gagnidze R. & Nakhurtsrishvili G. 831 (MO). **Kvemo Kartli:** Tsagreri, Gagnidze R. et al. 2788 (MO). **Megrelia:** without specific locality, I. Mandenova et al. s.n. (MO). **GERMANY. Bavaria:** UTM 6938300, 5508500, R. Spellenberg 11126 (NY). **Schleswig-Holstein:** Buchen, S. Jeppesen & K. Larsen 36 (SMU). **Thuringia:** Weimar, s.n (GA). Without specific locality, J. Butler 354 (NY). Without specific locality, W. A. Kellermann s.n. (SMU). Without specific locality, H. Eggers 17703 (SMU). **INDIA. Manipur:** Ukhrul, F. Kingdon-Ward 17742 (NY). **IRAN. Guilan:** without specific locality, N.

Lindsay 1025 (MO). **ITALY.** Rome, *s.n.* (SMU). Sicily, Palermo, *R. 126* (NY). Tuscany, *M. L. Forasassi s.n.* (SMU). Without specific locality, *G. R. Toni s.n.* (NY). **NETHERLANDS.** **South Holland:** Valkenburg, *L. N. Wend s.n.* (NY). **Utrecht:** Rijsenburg, *R. C. Bakhuizen s.n.* (SMU). Without specific locality, *Brink Jr. s.n.* (TEX). Without specific locality, *A. H. Someren s.n.* (TEX). **NORWAY.** **Bergen:** Sogne Fjord, *A. W. Trethow s.n.* (DAO). **POLAND.** **Lesser Poland:** Miechow, *A. Jasiewicz & M. Sychowa 229* (GA). **Masovian:** Warsaw, *D. Uroblewska s.n.* (DAO). Without specific locality, T. Tacik et al. *s.n.* (NY). **PORTUGAL.** Azores: without specific locality, C. S. Brown 84 (MO), without specific locality, Wm. Trelease 268 (MO). without specific locality, C. S. Brown 84 (GH). Without specific locality, M. Liha 1673 (DAO). **ROMANIA.** **Oltenia:** Dolj, *D. M. Cirtu 966* (DAO). **RUSSIA.** Mt. Koltza, O. Degener 33934 (NY). **SCOTLAND.** **Fife:** Burntisland, *J. Lamond & P. Woods 3137* (DAO). **SWEDEN.** **Ostergotland:** Omberg, *Gunnel & B. Peterson s.n.* (MO). Without specific locality, *G. Kjellmert s.n.* (NY). **Sodermanland:** Without specific locality, *G. Samuelsson 1025* (DAO). Without specific locality, *D. Aspund s.n.* (GH). **Stockholm:** Stockholm, *s.n.* (NY). **Uppland:** Bonkyrka, *H. Smith 190* (NY). Gamla Uppasala, *C. M. Norman s.n.* (NY). Gryta Parish, *C. G. Alm & H. Smith 637* (DAO). Without specific locality, *G. A. Ringsells s.n.* (GH). **SWITZERLAND.** **Bern:** Walperswil, E. Berger 3913 (TEX). without specific locality, *E. Berger s.n.* (NY). **SYRIA.** Lebanon, Flesen bei Ghazir, ca. 400m, *P. Busujan 234* (NY). **UKRAINE.** **Donetsk:** Novoazovsjiensis, *G. Kuznetzova s.n.* (NY). **Lviv:** Zolochiv, *S. Ziman s.n.* (MO). **WALES.** **Monmouthshire:** Peterston Wentlooge, *S. G. Harrison 62* (SMU). Without specific locality, *W. C. Lane s.n.* (GH).

APPENDIX 3-5. Representative Specimens Examined of *Agrimonia gorovoi*. **RUSSIA.** **Primorskij:** Chassan, *P. G. Gorovoi & S. D. Rumjantsev* (A). **SOUTH KOREA.** **Kangwondo:** Daekwanlueng, *T. B. Lee 1495* (SNUA).

APPENDIX 3-6. Representative Specimens Examined of *Agrimonia gryposepala*. **CANADA.** **British Columbia:** Cheam Lake, *V. J. Krajina s.n.* (DAO), Downie Creek, 51 30 N, 118 25W, *V. J. Krajina s.n.* (DAO). **Manitoba:** Adam lake, Turtle Mt. 29 02 N, 100 04 W, *G. M. Keleher 458* (DAO). **New Brunswick:** Kings Co., *M. L. Fernald 1939* (GH), Madawaska Co., *P. R. Robert & N. Bateman 64-2986* (DAO). **Nova Scotia:** Colchester Co., *E. C. Smith 18963a* (DAO), Cumberland Co., *W. B. Schofield 5348* (DAO), Digby Co., *C. H. Bissell et al. 21643* (GH), Granville Co., *M. L. Fernald & N. C. Fassett 24037* (CAN), Inverness Co., *E. C. Smith et al. 4814* (CAN), King Co., *H. P. Bell & A. L. Gorham s.n.* (CAN). **Ontario:** Algoma district, *L. Jenkins 8841* (DAO), Britannia Co., *Macoun 59898* (CAN), Byron Co., *A. A. Wood s.n.* (DAO), Carleton Co., *K. W. Spicer 2584* (DAO), Clear lake, 45 25 N, 77 12 W, *J. H. Soper & C. Despins 13646* (CAN), Edmonton Co., *J. White 8066* (CAN), Elgin Co., *J. E. James 1724* (DAO), Essex Co., 42 16 N, 83 05 W, *D. Langedoen s.n.* (CAN), Frontenac Co., *J. M. Gillett 6559* (DAO), Greenville Co., *Bassett & Mulligan 17* (DAO), Grey Co., 44 25 N, 80 43 W, *V. R. Brownell 83-557* (CAN), Hastings Co., *J. M. Gillett 7081* (DAO), Huron Co., *W. H. Minshall 4674* (DAO), Kent Co., 42 05 N, 82 06 W, *K. M. Lindsay 1752* (CAN), Lambton Co., *Garwood 2029* (CAN), Lanark Co., 44 55 N, 76 29 W, *M. J. Schepanek et al. 8731* (CAN), Leeds Co., 44 21 N, 75 58 W, *W. J. Cody 22829* (DAO), Middlesex Co., *J. M. Gillett 10746* (DAO), Norfolk Co., *W. M. Bowden 262-62* (DAO), Northumberland Co., 44 00 N, 77 42 W, *S. Darbyshire 515* (CAN), Ontario Co., 44 05 N, 78 54 W, *D. Hoy et al. 3376* (DAO), Ottawa Co., 45 27 N, 75 37 W, *S. Darbyshire & H. L. Dickson 812* (CAN), Oxford Co., *J. K. Shields 164* (CAN), Peel Co., 43 40 N, 79 39 W., *J. M. Webber 2956* (CAN), Prescott Co., 45 32 N, 74 31 W, *M. J. Schepanek & A. Dugal 958* (CAN), Renfrew Co., 45 19

N, 77 37 W, *M. J. Schepanek et al.* 7677 (CAN), Russell Co., *H. A. Senn* 1966 (DAO), Sidney Co., 44 13 20 N, 77 35 05 W, *P. M. & V. R. Catling* 9919 (DAO), South Gloucester Co., 45 18 N, 75 33 W, *A. W. Dugal* 655A (CAN), Stewartville Co., 45 24 00 N, 76 30 10 W, *A. Vogt & K. MacDonald* 94 (CAN), Stormont Co., *W. H. Minshall* 2396 (DAO), Waterloo Co., *C. Frankton* 1878 (DAO), Wellington Co., *J. F. Alex* 684 (DAO), Wentworth Co., *A. Tamsalu* 3297 (DAO), York Co., *E. Haber* 100 (CAN). **Ottawa:** Carleton Co., *L. Jenkins* 4712 (DAO), Lanark Co., *H. A. Senn* 66 (DAO), Pontiac Co., *H. A. Senn et al.* 737 (DAO). **Prince Edward Island:** Prince Co., *D. Erskine* 2224 (DAO). **Quebec:** Argenteull Co., *L. Jenkins* 9149 (DAO), Charlevoix Co., *G. Claude* 76315 (DAO), Gatineau Co., 45 26 N, 75 50 W, *A. W. Dugal & M. Camfield* 4314 (CAN), Huntingdon Co., *A. Bouchard* 67092 (CAN), Montmorency Co., *M. Victorin et al.* 45277 (OKL), St. Armand West Co., *I. J. Bassett* 2488 (DAO), St. Genevieve Co., *H. J. Scoggan* 139 (CAN), Vaudreuil Co., 45 28 N, 74 18 W, *E. Parnis* 493 (DAO). **Saskatchewan:** 52 38 40 N, 101 48 45 W, *V. L. Harms et al.* 35125 (CAN). **GUATEMALA.** **Alta Verapaz:** without precise locality, *H. V. Turckheim* 1377 (GH). **Chimaltenango:** Acatengo, *F. W. Hunnewell* 17135 (GH), Tecpan, *A. Molina et al.* 16106 (NY). **El Quiche:** Chichicastenango, *W. M. Antonia et al.* 16318 (TEX), Chunama, *G. R. Proctor* 25010 (TEX), Nebaj, *E. Contreras* 5238 (TEX), Utatlan Ruins, *A. R. Molina* 25071 (NY). **Hehuatenango:** Sierra de los Cuchumatanes, *J. A. Steyermark* 49121 (GH). **MEXICO. Chiapas:** Dan Cristobal, *O. Tellz* 7207(MEXU), La Trinitaria, *D. E. Breedlove* 38748-A (MO), Oxchuc, *F. G. Santiz* 166 (MEXU), Pueblo Nuevo Solistahuacan, *O. F. Clarke* 295 (NY), San Cristobal de Las Casas, *A. Mendez G.* 8461 (MEXU), SanJuan Cancuc, *J. Brett* 105 (TEX), San Juan Chamula, *C. S. Ruiz* 1051 (TEX), Tenejapa, *D. E. Breedlove* 15253 (NY), Yajalon, *A. M. Ton* 6696 (MEXU), Zincantan, *A. M. Ton* 9270 (TEX). **Coahuil:** Ocampo, *D. H. Roskind et al.* 1747 (TEX), Sierra del Carmen, *P. P. Lowry & M. J. Warnock* 3138 (TEX). **UNITED STATES.** **Arizona:** Coconino Co., *M. A. Baker* 8589 (TEX). **California:** Dan Diego Co., *W. P. Armstrong* 1208 (DAO), Lake Co., *A. A. Heller* 5978 (GH), Mendocino Co., *J. McMurphy s.n.* (DAO), San Bernardino Co., *P. A. Munz* 8680 (GH), San Diego Co., *M. F. Spencer* 979 (MU), Sierra Co., *J. G. Lemmon* 1019 (GH). **Colorado:** Boulder Co., *J. Salamus & J. A. Erdman s.n.* (MU). **Connecticut:** Fairfield Co., *D. M. Porter* 793 (GH), Franklin Co., *R. W. Woodward s.n.* (GH), Greenville Co., *G. R. Lumsden* 36678 (CAN), Hartford Co., *C. H. Bissell s.n.* (GH), Litchfield Co., *A. E. Evans s.n.* (GH). **Georgia:** Murray Co., *R. Kral* 50787b (GH). **Illinois:** Cook Co., *T. G. Lammers* 10291 (MU), Peoria Co., *V. H. Chase* 15416 (TEX). **Indiana:** Cass Co., *R. C. Friesner* 9306 (TEX), Pulaski Co., *C. C. Deam* 56575 (GH). **Iowa:** Clayton Co., *B. Shimick s.n.* (TEX), Dickinson Co., *R. F. Thorne* 13214 (DAO). **Kansas:** Lawrence Co., *W. H. Horr* 4606 (GH). **Maine:** Aroostook Co., *M. L. Fernald* 1936 (GH), Blue Earth Co., *J. W. Moore* 26982 (CAN), Camden Co., *G. G. Kennedy s.n.* (GH), Hancock Co., *E. Rand s.n.* (GH), Kennebec Co., *M. L. Fernald & B. Long* 13917 (GH), Knox Co., *R. C. Friesner* 24591 (OKL), Lincoln Co., *E. B. Chamberlain* 363 (GH), Oxford Co., *A. S. Pease* 37192 (GH), Penobscot Co., *M. L. Fernald s.n.* (GH), Waldo Co., *M. L. Fernald & B. Long* 13914 (GH), Woolwich Co., *S. F. Blake s.n.* (TEX), York Co., *E. B. Chamberlain s.n.* (GH). **Massachusetts:** Barnstable Co., *M. L. Fernald & B. Long* 18595 (GH), Dukes Co., *M. L. Fernald & J. M. Fogg, Jr.* 927 (GH), Essex Co., *C. H. Knowlton s.n.* (GH), Franklin Co., *S. K. Harries & R. G. Poland* 25193 (GH), Hampden Co., *H. E. Ahles* 89224 (CAN), Hancock Co., *C. F. Batchelder* 4277 (GH), Lincoln Co., *E. F. Williams s.n.* (GH), Lowell Co., *F. S. Beattie s.n.* (OKL), New Bedford Co., *E. W. Hervey s.n.* (GH), Norfolk Co., *G. G. Kennedy s.n.* (GH), Worcester Co., *N. F. Woodward s.n.* (GH). **Michigan:** Cheboygan Co., *R. G. Webb s.n.* (OKL), Houghton Co., *O. A. Farwell* 9891 (DAO), Mackinac Co., *F. G. Gates* 21688 (TEX), Washtenaw Co., *I. L. Wiggins* 11932 (MU). **Minnesota:** Center city, *B. C. Taylor s.n.* (MU),

Crookston Co., *C. R. Child s.n.* (MU). **Missouri:** Macon Co., *B. F. Bush 7842* (GH). **Nebraska:** Minden Co., *H. Hapeman s.n.* (MU). **New Hampshire:** Carroll Co., *S. K. Harris 32815* (GH), Grafton Co., *M. L. Fernald 11737* (GH), Strafford Co., *A. S. Pease 27883* (GH). **New Jersey:** Hunterdon Co., *B. Long 51427* (GH). **New York:** Albany Co., *G. R. Colley 12103* (MU), Cayuga Co., *T. G. Lammers 10129* (MU), Chemung Co., *T. F. Lucy 2680* (MU), Essex Co., *H. F. Heady 668* (DAO), Lowville Co., *C. L. Booth s.n.* (MU), Orange Co., *H. M. Raup 7415* (GH), St. Lawrence Co., *E. C. Ogden et al., s.n.*(DAO), Suffolk Co., *R. Lathan 5889* (DAO), Washington Co., *H. D. House 28786* (OKL). **North Carolina:** Avery Co., *F. W. Hunnewell 12834* (GH), Transylvania Co., *S. Stewart s.n.* (DAO), Yancey Co., *A. E. Radford 45056* (TEX). **North Dakota:** Dunn Co., *O. A. Stevens 1292* (DAO). **Ohio:** Ashtabula Co., *J. S. McCormac 6891* (MU), Athens Co., *L. D. Cribben 252* (OKL), Butler Co., *C. R. Werth & W. P. Pusateri s.n.* (MU), Champaign Co., *J. S. McCormac 4043* (MU), Erie Co., *J. S. McCormac 1607* (MU), Garrettsville Co., *R. J. Webb s.n.* (GH), Lorain Co., *A. E. Ricksecker s.n.* (MU), Lucas Co., *J. S. Shelly 68* (MU), Mansfield Co., *E. Wilkinson 36674* (CAN), Montgomery Co., *S. J. Stine, Jr. 35* (MU), Portage Co., *R. L. Gardner 1857* (MU), Richland Co., *E. Wilkinson s.n.* (OKL), Summit Co., *B. A. Andreas 4058* (MU). **Oklahoma:** Delaware Co., *U. T. Waterfall 8220* (OKL). **Pennsylvania:** Center Co., *F. W. Hunnewell 10192* (GH), Juniata Co., *H. A. Wahl et al. 13879* (DAO), Monroe Co., *D. E. Dimmick 460* (DAO), Philadelphia Co., *A. MacElwee 911* (DAO), Somerset Co., *D. L. Nickrent 822* (MU). **Rhode Island:** Newport Co., *J. Franklin s.n.* (GH), Providence Co., *A. E. Lownes s.n.* (GH). **Tennessee:** Unicoi Co., *C. Crowder & B. E. Woodford 76* (DAO). **Texas:** Bowie Co., *D. S. Correll 31260* (TEX), Giles Co., *H. Gentry 766* (TEX). **Vermont:** Addison Co., *C. H. Knowlton s.n.* (GH), Bennington Co., *M. A. Day 367* (GH), Chittenden Co., *C. H. Knowlton s.n.* (GH), Grand Isle Co., *C. H. Knowlton s.n.* (GH), Washington Co., *D. Potter s.n.* (GH), Windham Co., *W. W. Merrill 1849* (GH), Windsor Co., *M. A. Loreland s.n.* (GH). **Virginia:** Augusta Co., *F. W. Hunnewell 19716* (GH), Giles Co., *E. L. Core s.n.* (MU). **West Virginia:** Allegheny Co., *E. S. Steele 138* (GH), Tucker Co., *H. A. Allard 9200* (TEX). **Wisconsin:** Brown Co., *M. A. Vincent 10769* (MU), Madison Co., *C. Heimsch 16W* (MU), Outagamie Co., *W. E. Rogers s.n.* (MU), Saul Co., *R. Buston 75* (MU), Trunk B. Co., *T. G. Hartley 1918* (DAO), Vernon Co., *H. P. Hansen 117* (TEX).

APPENDIX 3-7. Representative Specimens Examined of *Agrimonia hirsuta*. **BRAZIL.** **Parana:** Laranjeiras do Sul, Campo Novo, *G. Hatschbach 24146* (VDB). **Santa Catarina:** Cadador, Pinheriral, *L. B. Smith & R. Klein 10893* (NY), R. Xapeco, Abelardo Luz., *A. Castellanos 8279* (NY).

APPENDIX 3-8. Representative Specimens Examined of *Agrimonia incisa*. **UNITED STATES.** **Florida:** Alachua Co., *W. A. Murrill s. n.* (FLAS), Columbia Co., *B. Herring 430* (FLAS), Dixie Co., *L. E. Arnold s.n.* (FLAS), Duval Co., *A. H. Curtiss 5753* (FLAS), Duval co., *A. H. Curtiss 5753* (TEX), Gadsden Co., *L. E. Arnold 4671* (FLAS), Gilchrist Co., *W. A. Murill s.n.* (FLAS), Hillsborough Co., Tampa Bay, 1834, *Dr. Burrows s.n.* (NY), Jackson Co., 30 42 20 N, 84 53 17 W., *S. L. Orzell & E. L. Bridges 18075* (TEX). Jefferson Co., 30 28 40 N, 83 54 36 W., *S. L. Orzell & E. L. Bridges 13175* (TEX), Madison Co., *R. K. Godfrey et al. 53990* (FLAS), Marion Co., *W. A. Murrill s.n.* (FLAS), Suwannee Co., *W. G. Darcy 1412* (FLAS), Washington Co., *L. E. Arnold 2945* (FLAS). **Georgia:** without precise locality, [about 1830], *Leconte s.n.* (NY). **Mississippi:** Harrison Co., *D. Demaree 33010* (TEX), Perry Co., *S. W. Leonard 9543-1* (TEX). **South Carolina:** Charleston Co., *R. D. Porcher 1559* (GH), Colleton Co., *J. B. Nelson 17891* (USCH), Georgetown Co., *J. B. Nelson 22239*

(USCH), Orangeburg Co., *A. B. Pittman 1127301* (USCH). **Texas:** Angelina Co., *J. Singhurst 3471* (TEX), Angelina Co., *B. R. & M. H. MacRoberts 3114* (TEX), Jasper Co., 31 04 00 N, 94 08 23 W, *S. L. Orzell & E. L. Bridges 11054* (TEX), Jasper Co., *J. Singhurst 3473* (TEX), Newton Co., *B. R. & M. H. MacRoberts 3140* (TEX), New Co., *J. Singhurst 3475* (TEX), Sabine Co., *J. Singhurst 3474* (TEX).

APPENDIX 3-9. Representative Specimens Examined of *Agrimonia microcarpa*.

UNITED STATES. Alabama: Henry Co., *K. M. Wiegand & W. E. Manning 1437* (GH). **Connecticut:** Hartford Co., *G. H. Bissell s.n.* (GH). **Florida:** Alachua Co., *D. B. Ward 5437* (FLAS), Columbia Co., *B. Tan 790* (FLAS), Gadsden Co., *G. V. Nash 2395* (GH), Jackson Co., *S. C. Hood 2627* (TEX), Leon Co., *E. West & L. E. Arnold s.n.* (FLAS), Liberty Co., *R. Kral 3092* (GH), Suwannee Co., *L. Martin 953* (FLAS), Union Co., *D. B. Ward 4151* (FLAS). **Georgia:** De Kalb Co., *J. K. Small s.n.* (GH). **Louisiana:** Lincoln Co., *J. A. Moore & D. Spencer 6331* (GH), Rapides parish, *D. S. & H. B. Correll 9882* (GH), Vernon parish, *C. M. Allen et al. PLK0697* (OKL). **Mississippi:** Lincoln Co., *J. D. Ray, Jr. 5419* (GH), Saratoga Co., *S. M. Tracy 8700* (GH). **North Carolina:** Durham Co., *W. V. Brown s.n.* (TEX), Nash Co., *R. K. Godfrey 5142* (GH). **Oklahoma:** McCurtain Co., *M. Hopkins & M. V. Valkenburgh 6151* (OKL). **South Carolina:** Aiken Co., *W. R. Kelley & W. T. Batson s.n.* (USCH), Barnwell Co., *W. R. Kelley & W. T. Batson s.n.* (USCH), Berkeley Co., *J. B. Nelson 5679* (USCH), Calhoun Co., *C. Douglass 626* (USCH), Charleston Co., *C. R. Reeves 31* (USCH), Edgefield Co., *J. B. Nelson 17805* (USCH), Georgetown CO., *J. B. Nelson 22138* (USCH), Greenwood Co., *R. J. Davis s.n.* (TEX), Newberry Co., *J. B. Nelson 3586* (USCH), Richland Co., *A. B. Pittman 09279601* (USCH), Williamsburg Co., *R. K. Godfrey & R. M. Tryon Jr. 1641* (GH). **Tennessee:** Hickman Co., *R. Karl 53704* (TEX), Knox Co., *A. Ruth 324* (GH). **Texas:** Angelina Co., 31 04 27N, 94 14 30 W, *S. L. Orzell & E. L. Bridges 11434* (TEX), Bowie Co., *A. A. & E. G. Heller 4183* (GH), Carthage Co., *B. C. Tharp s.n.* (TEX), Gregg Co., *C. L. York s.n.* (TEX), Hardin Co., *C. L. Lundell 14086* (TEX), Jasper Co., 30 58 22N, 94 04 05W, *W. R. Carr 11396* (TEX), Newton Co., *B. C. Tharp 44354* (TEX), Polk Co., *E. Garvin s.n.* (TEX), Sabine Co., 31 11 22N 93 43 12 W, *S. L. Orzell & E. L. Bridges 5639* (TEX), San Jacinto Co., *V. L. Cory 19649* (GH), Shiner Co., *B. C. Tharp 2376* (TEX), Wood Co., *J. A. Mears 870* (TEX). **Virginia:** Greensville Co., *M. L. Fernald & B. Long 11046* (GH), Isle of Wight Co., *M. L. Fernald et al. 6606* (GH), Northampton Co., *M. L. Fernald et al. 5315* (GH), Southampton Co., *M. L. Fernald & B. Long 10678* (GH), Sussex Co., *M. L. Fernald & B. Long 6225* (GH).

APPENDIX 3-10. Representative Specimens Examined of *Agrimonia nipponica*.

CHINA. Fujian: without precise locality, *F. H. Niao 9628* (GH). **Guangdong:** Zengcheng, *C. Huang 1662248* (MO), Gaoyao, *C. Huang 162248* (MO), Guangzhou, *L. Deng 9802* (MO), Huaiji, *K. L. Shi 15149* (GH), Lian'an, *P. Tan 59329* (MO), Longmen, *Z. Li 2027* (MO), Yangshan (Mt.), *Z. Li 1426* (MO), Zhaoging, *G. Shi 132* (MO). **Guangxi:** Longsheng, *Q. Lui & Y. Wei 20019* (MO), Longzhou, *X. Liang 65960* (MO), Pingnan, *J. Zhong 84786* (MO). **Guizhou:** Jiangkou Xian, *L. T. Ling 1988* (NY), Yinjiang, *Sino-American ex. 1363* (GH). **Hubei:** Tongchenhue, *Y. Cunsu 8283* (MO). **Hunan:** Chongyang, *C. Ye 8709* (MO), Linyang, *B. Xiong 2631* (MO), Yangling, *B. Xiong 4688* (MO). **Jiangsu:** without precise locality, *W. Z. Fang 7914* (MO). **Jiangxi:** Lu Mt., *C. Tan 941021* (MO). **Jianyang:** Fujian, *Y.-T. Zhang 2610* (MO). **Kwangsi:** Hin Yen, *R. C. Ching 7125* (NY). **Schandung:** Tsingtao, *E. Licent 13362* (GH). **Sichuan:** Dujiangyan, *D. E. Boufford & B. Bartholomew 24211* (GH). **Yunnan:** without precise locality, *K. M. Eng 12889* (GH). **Zhenjiang:** Hangzhou, *S. Zhang 6180* (MO).

JAPAN. Chubu: Aichi, *H. Ohba & S. Akiyama 3237* (TI), Gifu, *J. & H. Murata 27067* (TI), Shizuoka, *H. Tahahashi 577* (MO), Toyama, *J. Jutila & H. Fujino 716* (MO). **Chugoku:** Yamaguchi, 34 10N, 132 10E, *J. Ohwi 1845* (MO). **Hondo:** Hida, *R. Tomioku s.n.* (GH), Mino, *T. Kato 9316* (GH), Omi, *K. Shiota 1062* (GH). **Kansai:** Hyogo, *N. Kurosaki 13829* (TI), Kyoto, *D. E. Boufford 19610* (MO). **Kanto:** Chiba, *H. Hara 172* (TI), Ibaraki, *T. Yateishi & K. Sato 9846* (GH), Saitama, *J. Murata et al. 8485* (TI), Tochigi, *J. Murata & H. Chashi s.n.* (TI), Tokyo, *S. Suzuki 312* (GH). **Kyushu:** Kagoshima, *J. Murata et al. 15434* (TI), Miyazaki, *J. Murata 10061-a* (TI), Oita, *J. Murata 10037* (TI). **Shikoku:** Kagawa, *M. Takahashi 1125* (GH), Kochi, 32 51 N, 132 51 E, *H. Sada et al. FOK-059768* (SMU). **Tohoku:** Aomori, *T. Naito s.n.* (MO), Miyagi, 38 14 N, 140 50 E, *Y. Tateishi & T. Kurosawa 14503* (MO). **Toyama:** Nakanikawahun, *J. Jutila & H. Fujino 716* (NY). **Tokohama:** without precise locality, *unknown s.n.* (GH). **SOUTH KOREA. Chungcheongbuk-do:** Mt. Sokri, *T. B. Lee s.n.* (SNUA). **Chungchenonganam-do:** 36 52N, 126 13E, *B. R. Yinger et al. 2626* (SNUA). **Gangwon-do:** Mt. Pukamryong, *J. I. Jeon & U. Kang PA011* (SNUA). **Gyeonggi-do:** Kwanglung, *H. Kim 1003* (SNUA). **Gyeongsangnam-do:** Guje Is. *Oh et al. 7-03-0822-054* (CBU). **Jeju-do:** Mt. Halla, *T. B. Lee 1495* (SNUA). **Jellanam-do:** Mt. Jili, *Chunwangbong, Oh et al. 040917-242* (CBU).

APPENDIX 3-11. Representative Specimens Examined of *Agrimonia parviflora*.

BRAZIL. Parana: Juruqui, *G. Hatschbach 41182* (MU). **CANADA. Ontario:** Elgin Co., *W. Stewart 2862* (CAN), Essex Co., 42 15 05N, 83 02 00W, *M. J. Oldham 2765* (CAN), 42 15 00N, 83 04 00 W, *D. Langendoen s.n.* (CAN), Kent Co., 42 05 00 N, 82 26 00 W, *K. M. Lindsay 1442* (CAN), Middlesex Co., *M. J. Oldham 11698* (CAN). **DOMINICA REPUBLIC. Cienaga da:** Manabao, Jarabacoa, *Bro. Alain H. 12087* (NY). **San Juan:** Sabana Nueva, *R. A. & E. S. Howard 9078* (GH), **Santo Domingo,** 70 56 00N, 18 59 00 E, *T. Zaroni & Garcia 41569* (MO). **MEXICO.** without precise locality, *F. Muller 1855* (NY). **UNITED STATES. Alabama:** Clay Co., *F. S. Earle s.n.* (GH). **Arkansas:** Phillips Co., *D. Demaree 19752* (OKL). **Connecticut:** Fairfield Co., *D. Norris s.n.* (GH), Litchfield Co., *L. W. Scerau s.n.* (GH), New Haven Co., *E. B. Garcer 797* (GH). **Delaware:** Middletown Co., *J. R. Churchill 494* (GH). **Georgia:** Catoosa Co., *A. Cronquist 5603* (GH), Chickamauga Co., *R. M. Harper 374* (GH). **Illinois:** Cook Co., *R. Bebb 1074* (OKL), Illinois Co., *T. G. Lammers 10282* (MU), Marion Co., *G. S. Winterringer 1332* (DAO). **Indiana:** Cass Co., *R. C. Friesner 9397* (TEX), Clay Co., *R. C. Froesner 21831* (MU), Jasper Co., *R. Kral 48464b* (TEX), Monroe Co., *V. Foley s.n.* (TEX), Porter Co., *C. H. Knowlton s.n.* (GH), Ripley Co., *T. G. Lammers 6835* (MU), Sullivan Co., *W. Pichon & H. Parker 666* (DAO). **Iowa:** Mahaska Co., *D. W. Augustine 495a* (OKL). **Kansas:** Riley Co., *J. B. Norton 143* (GH), Shawnee Co., *P. Maus s.n.* (MU), Washington Co., *W. H. Horr 4442* (TEX). **Kentucky:** Clark Co., *M. R. Beckett 770* (GH), Jackson CO., *D. D. Taylor 9804* (MU). **Maryland:** Harford Co., *F. L. Johnson & M. D. Proctor ABR0066* (OKL), Hyattsville Co., *S. F. Blake 7911* (TEX). **Massachusetts:** Berkshire Co., *P. Weatherbee 2844* (GH). **Michigan:** Berrien Co., *W. T. Gillis & M. Kohring 14109* (GH), Saginaw Co., *H. H. Bartlett & C. D. Richards 11* (DAO), St. Clair Co., *C. K. Dodges s.n.* (MU), Washtenaw Co., *G. W. Parmelee 646* (TEX). **Mississippi:** Allenton Co., *G. W. Letterman s.n.* (CAN). **Missouri:** Dade Co., *J. Stone & K. Sikes 1403* (TEX), Jasper Co., *E. J. Palmer 5982* (GH), Reynolds Co., *D. L. Nickrent 1134* (MU). **New Jersey:** Somerset Co., *H. N. Moldenke 8237* (CAN), Union Co., *A. L. Moldenke & H.N. 26089* (TEX). **New York:** Albany Co., *H. D. House 25014* (TEX), Erie Co., *D. D. Taylor 9714* (MU). **North Carolina:** Forsyth Co., *H. E. Ahles & J. G. Haesloop 52683* (GH), Granville Co., *R. K. Godfrey 6112* (GH), Macon Co., *J. R. Bozeman 7587* (TEX), Orange Co., *S. W. Leonard 3436*

(DAO), Scotland Co., *R. K. Godfery 5081* (GH), Wake Co., *R. K. Godfrey 6747* (GH). **Ohio:** Adams Co., *J. S. McCormac 1319* (MU), Belmont Co., *J. S. McCormac 1758* (MU), Butler Co., *D. D. Taylor 8168* (MU), Champaign Co., *K. Neff 088* (MU), Clermont Co., *M. A. Vincent 10412* (MU), Cuyahoga Co., *A. Gerogius 48* (MU), Dayton Co., *A. Lonsing s.n.* (MU), Fairfax Co., *M. A. Vincent 3310* (MU), Gallia Co., *J. C. Bryant 314* (MU), Hamilton Co., *A. M. Swanson 130* (MU), Logan Co., *J. S. McCormac 3176* (MU), Lorain Co., *A. E. Ricksecker s.n.* (MU), Mahoning Co., *T. G. Lammers 6946* (MU), Ottawa Co., *R. M. Lowden 770* (DAO), Oxford Co., *G. M. Kalter s.n.* (MU), Portage Co., *R. J. Webb 344* (GH), Preble Co., *H. Ball s.n.* (MU), Trumbull Co., *T. G. Lammers 6943* (MU). **Oklahoma:** Bryan Co., *J. & C. Taylor 2035* (OKL), Cherokee Co., *C. S. Wallis 196* (OKL), Cleveland Co., *P. Falley 2660* (OKL), Comanche Co., *F. B. McMurry 546* (OKL), Craig Co., *G. W. Stevens 2203* (OKL), Delaware Co., *C. S. Wallis 2560* (OKL), Kiowa Co., *D. R. Segal 5308* (OKL), Mayes Co., *C. S. Wallis 2589* (OKL), McCurtain Co., *M. Hopkins & G. L. Cross 2554* (OKL), Okfuskee Co., *P. Folley 1561* (OKL), Oklahoma Co., *U. T. Waterfall 2539* (OKL), Woods Co., *P. Nighswonger 1738* (OKL), Woodward Co., *J. E. Engelmann s.n.* (OKL). **Pennsylvania:** Delaware Co., *W. M. Thompson 514* (DAO), Lancaster Co., *J. K. Small s.n.* (MU), Westmoreland Co., *P. E. Pierron 36680* (CAN). **South Carolina:** Berkeley Co., *K. W. H. s.n.* (GH), Cherokee Co., *J. B. Nelson 5537* (USCH), Fairfield Co., *J. B. Nelson 4291* (USCH), Laurens Co., *C. N. Horn 5850* (MU), Oconee Co., *J. B. Nelson 6998* (USCH), Pickens Co., *S. R. Hill 18828* (GH), Richland Co., *E. Humphries s.n.* (USCH), York Co., *J. B. Nelson 3197* (USCH). **Tennessee:** Carroll Co., *H. K. Stevenson 358* (GH), Cater Co., *L. F. & F. R. Randolph 1205* (GH), Dickson Co., *M. Bierner 90-187* (TEX), Gatlinburg Co., *D. H. Linder s.n.* (GH), Lewis Co., *C. B. King 226* (GH), Sevier Co., *T. Leroy 040* (MU). **Texas:** Bowie Co., *D. S. Correll 33381* (GH), Hemphill Co., *C. W. Rowell 105959* (OKL), Henderson Co., *J. Singhurst 6996* (TEX), Wood Co., *E. McMullen s.n.* (TEX). **Virginia:** Bedford Co., *A. H. Curtiss 36682* (CAN), Norfolk Co., *M. L. Fernald & B. Long 2968* (GH), Orange Co., *M. Sewell 125* (MU). **West Virginia:** Craig Co., *E. S. Steele 43* (MU), Greenbrier Co., *F. W. Hunnewell 7091* (GH), Harrison Co., *M. A. Vincent 3320* (MU).

APPENDIX 3-12. Representative Specimens Examined of *Agrimonia pilosa*. **CHINA.**
Anhui: Q. X. Liu s.n. (MO). L. L. Zhao 084 (MO). Q. Wu 98-203 (MO). G. Yao & Q. Wang 156 (MO). F. Konta Ch-3256 (GH). K. Yao 11893 (NY). H. L. Yin 6083 (MO). **Beijing:** Z. Guo 70 (MO). I. Guo 48 (MO). **Daganshuan:** Fenyi city, K. Yao 9197 (GH). **Fujian:** H. Guosheng s.n. (MO). G. He 4522 (MO). G. He 4276 (MO). G. He 5322 (MO). **Gansu:** L. Xu & J. Zhang 1723 (MO). Y. Lian s.n. (MO). **Guangxi:** Li s.n. (MO). **Guizhou:** Z. Liu s.n. (MO). **Hebei:** Y. Liu s.n. (MO). **Heilongjiang:** X. Lin 8803 (MO). X. Lin 83143 (MO). X. Lin 82920 (MO). X. Lin 92714 (MO). **Henan:** Zheng s.n. (MO). **Hubei:** K. S. Chow 2368 (NY). K. R. Liu 83 (MO). Z. W. Jiang 88 (MO). C. Ye 9315 (MO). **Hunan:** J. Zuo 0970 (MO). Q. S. Wang 3395 (MO). B. Xiong 3656 (MO). C. Ye 7625 (MO). J. Zuo 0970 (MO). B. Xiong 9709 (MO). **Inner Mongolia:** Yin s.n. (MO). Q. R. Wu s.n. (MO). **Jiangsu:** N. Sheng s.n. (MO). N. Sheng et al. L-028 (MO). W. Wu s.n. (MO). Jurong city, X. Q. Wang et al. 179 (NY). **Jiangxi:** M. Niu 91137 (MO). C. S. Ye 395 (MO). C. S. Ye 4423 (MO). S. S. Lai & H. R. Shan s.n. (MO). S. Lai & H. Shan 1988 (MO). W. Xiong 179 (MO). W. Z. Xiong s.n. (MO). S. Lai & H. Shan 4224 (MO). M. Niu 91384 (MO). K. S. Chow s.n. (MO). **Jilin:** Changbaishan, Wan & Chow 81042 (MO). Changbahan, B. B. Wan & K. S. Chow 81042 (NY). **Kwngsi:** P. P. Wan & K. S. Chow 79115 (NY). Manchuria: F. H. Chen 151 (NY). M. H. Bohnhof 140 (NY). **Mongolia:** Eastern region, Bainoola, A. A. Yunatov 10906 (GH). T. Z. Liu 217 (MO). **Nenjiang:** S. Zhaong 30053 (MO). **Shaanxi:** Y. Lian s.n. (MO). Q. L. Li 96 (MO).

Shangdong: *Q. X. Liu s.n.* (MO). **Shanxi:** *Q. R. Wu s.n.* (MO). *T. Liu & Z. Zeng 2445* (MO). *X. Liu 5422* (MO). **Shennongjia:** Laojunshan Yaowan Canyon, *Sino-American Expedition 451* (NY). **Sichuan:** *Guan et al 1847* (MO). *C. Li s.n.* (MO). *Dujiangyan, D. E. Boufford & B. Bartholomew 23991* (NY). **Yunnan:** *Bao s.n.* (MO). **Zhejiang:** *P. L. Chiu 3776* (MO). *Zhejiang Team 725* (MO). *P. L. Chiu 03306* (MO). **JAPAN. Chubu:** Yamanashi, *F. Konta 12137* (GH). *Ishikawa, H. Takahashi 1686* (NY). **Kansai:** Kyoto, *R. Nishimura 211* (GH). *Kyoto, T. Nagasawa 82* (MO). *S. Takahashi 2062* (MO). **Tohoku:** *L Fukushima, H. Koyama 3067* (NY). **SOUTH KOREA. Chungcheongbukdo:** Mt. Walak, *Oh et al. 050831-171* (CBU). **Gyonggido:** Mt. Chunma, *H. Kim 759* (SNUA). Mt. Dodeok, *H. Kim 976* (SNUA). **Gyengsangnamdo:** Kwangyang-si, *Oh et al. 040827-051* (CBU). **Jeollanamdo:** Mt. Jili, *Oh et al. Gulyegun 041009-072* (CBU). Mt. Jili, *C. S. Chang & S. Park 0534* (SUNA). **TAIWAN. Miaoli:** *Hsuechien, T. C. Huang & S. F. Huang 15425* (NY). *H. Ohahi et al. 20744* (GH). *H. Ohashi et al 20753* (GH). **VIETNAM. Cao Bang:** *Nguyen Binh, L. Averyanov et al. CBL 025* (MO).

APPENDIX 3-13. Representative Specimens Examined of *Agrimonia pringlei*. **MEXICO. Puebla:** without precise locality, *F. Miranda 3658* (MEXU), *Villa Juarez, Rzedowski 17216* (TEX), *Xalacapa, E. Hernandez-Xolocotzi & A. J. Sharp s.n.* (MEXU), *Zochitlan, F. Ventura A. 22350* (XAL). **Tamaulipas:** *Sirerra, 23 56N, 99 15W, M. C. Hohnston 12812* (TEX). **Veracruz:** *Coacoatzintla, F. Venture A. 18946* (MEXU), *Huatusco, 19 09N, 96 58W, J. I. Calzada 7978* (XAL), *Ranco guadalupe, 10 30N, 96 55 W, R. Ortega O. 01369* (XAL), *Tlacolulan, F. Venture A. 9129* (MEXU), *Xalapa, 19 30N, 96 55 W, R. Ortega O. 01369* (TEX).

APPENDIX 3-14. Representative Specimens Examined of *Agrimonia procera*. **AUSTRIA.** Tyrol, *A. Polatschek s.n.* (NY), *Ubergang. North Tyrol, A. Polatschek s.n.* **DENMARK. Zealand:** *Tisvile Hegn. J. Svendsem 490* (NY). **GERMANY. Bayern:** *Oberfalz, Donau, F. Schuhwerk 704013* (NY), *Schwaben, F. Schuhwerk 792713* (NY). *Bergisches, A. Schumacher s.n.* (BRIT), *Rheinland, A. Chumacher s.n.* (NY). **GREECE,** without precise locality, *unknown s.n.* (GH). **HUNGARY.** without precise locality, *unknown, s.n.* (BRIT). **RUSSIA. Kapuga:** *Zhizdra, A. K. Skvortsov s.n.* (GH). **SCANDINAVIA.** without precise locality, *J. Punum s.n.* (NY). **SCOTLAND.** *Argyll, J. McNeill 12245* (DAO). **SWEDEN.** without precise locality, *A. Hulyphers s.n.* (DAO). *Narke, U. Starback s.n.* (BRIT), *Uppland, T. Vestergrew s.n.* (NY). *Vastergotland, T. E. Hasselrot s.n.* (BRIT). **SWITZERLAND.** without precise locality, *unknown s.n.* (NY).

APPENDIX 3-15. Representative Specimens Examined of *Agrimonia pubescens*. **CANADA. Ontario:** *Essex Co., UTM414541, M. J. Oldham 3474* (CAN), *UTM607238, M. J. Oldham 2052* (CAN), *Kingsville, Macoun 34294 9* (CAN), *Lambton Co., 42 37N, 82 30W, D. Langendoen & P. F. Maycock s.n.* (CAN), *Middlesex Co., M. J. Oldham 7244* (DAO), *Northumberland Co., 44 23N, 78 39 W, S. L. Gray & M. N. Campbell 1645* (CAN), *Kent Co., Rondeau park, H. J. Scoggan 14830* (CAN), *Wentworth Co., R. S. Haines 79* (DAO), *York Co., 43 38 45N, 79 27 45 W, K. L. McIntosh s.n.* (DAO). **UNITED STATES. Alabama:** *Jackson Co., L. V. Porter s.n.* (GH). **Arkansas:** *Independence O., E. Sundell 10036* (MU), *Sebastian Co., R. A. Thopson et al. C0954* (OKL). **Connecticut:** *Fairfield Co., E. H. Eames s.n.* (GH), *Hartford Co., C. H. Bissell s.n.* (GH), *New Haven Co., G. H. Barlett s.n.* (GH), *New London Co., R. W. Woodward s.n.* (GH). **Illinois:** *Adams Co., R. Brinker 1303* (TEX), *Carroll Co., R. P. Wunderlin 122* (MU), *Champaign Co., A. S. Pease s.n.* (GH), *Edgar Co., T. C. Hodge 79*

(MU), Peoria Co., *V. H. Chase* 9027 (OKL), Quincy Co., *R. Brinker* 1285 (TEX), Sullivan Co., *P. Parker & McClain* 443 (DAO). **Indiana:** Clay Co., *R. C. Friesner* 21854 (OKL), Hamilton Co., *R. C. Friesner* 17213 (GH), Indianapolis Co., *R. C. Friesner* 10103 (OKL), Knox Co., *J. D. Lipps s.n.* (OKL), Monroe Co., *M. Springer* 302 (TEX), Putnam Clay Co., *R. C. Friesner* 21854 (MU), Wells Co., *C. C. Deam* 58046 (OKL). **Iowa:** Warren Co., *T. V. Bruggen* 3508 (DAO). **Kansas:** Douglas Co., *C. C. freeman* 4438 (OKL), Lyon Co., *K. Schaefer* 1164 (OKL), Pottawatomie Co., *W. T. Barker* 4802 (GH), Woodson Co., *T. Bilderback* 1150 (FLAS). **Kentucky:** Boone Co., *J. W. Thiert & J. R. Baird* 60444 (MU), Bullitt Co., *R. A. Thompson et al.* K0008 (OKL), Clark Co., *M. R. Beckett* 720 (GH), Harlan Co., *H. Kearney, Jr.* 307 (CAN). **Maryland:** Prince Georges Co., *W. D. Lonbottom* 4293 (MU), Washington D. C., *E. S. Steele s.n.* (MU). **Massachusetts:** Hampden Co., *B. A. Sorrie* 2600 (GH), Middlesex Co., *M. L. Fernal s.n.* (GH), Suffolk Co., *B. A. Sorrie* 2292 (GH). **Michigan:** Livingston Co., *F. L. Hermann* 6951 (GH), Washtenaw Co., *G. W. Parmelee* 2583 (TEX). **Minnesota:** Nicollet Co., *C. A. Ballard* 36675 (CAN). **Missouri:** Franklin CO., *J. C. Solomon* 18771 (OKL), Jasper Co., *E. J. Palmer* 5979 (GH), Pulaski Co., *R. T. Overbo et al.* W0556 (OKL), Ralls Co., *B. Hinterthuer* 706 (DAO), Reynolds Co., *D. L. Nickrent & P. Nelson* 1043 (MU). **Nebraska:** without precise locality, *J. M. Winter s.n.* (MU). **New Jersey:** Cold spring Co., *O. H. Brown* 10740 (GH), Sussex Co., *M. A. Chrysler s.n.* (DAO), Warren Co., *L. Grison* 9729 (GH). **New York:** Bronx Co., *S. H. Burnham* 203 (OKL). **North Carolina:** Granville Co., *R. K. Godfrey* 5513 (GH), Cabarrus Co., *H. E. Ahles* 19583 (DAO). **Ohio:** Adams Co., *D. M. Ambrose* 267 (MU), Ashland Co., *G. T. Jones* 69-7-23-811 (MU), Butler Co., *T. J. Cobbe s.n.* (MU), Butler Co., Oxford, *C. Heimsch* 132 (MU), Champaign Co., *K. Neff* 112 (MU), Clermont Co., *M. A. Vincent* 01237 (MU), Darke Co., *M. A. Vincent* 1949 (MU), Dayton Co., *Lonsing s.n.* (MU), Erie Co., *E. L. Modeley s.n.* (GH), Greene Co., *S. M. Sulgrove* 90072633 9 (MU), Medina Co., *G. T. Jone* 70-8-12-511 (MU), Miami Co., *S. M. Sulgrove* 90071248 (MU), Montgomery Co., *M. Hinds* 540 (MU), Portage Co., *R. L. Gardner* 1615 (MU), Preble Co., *H. Ball s.n.* (MU), Sandusky Co., *G. T. Jones* 69-7-27-836 (MU), Warren Co., *M. Hinds* 401 (MU). **Oklahoma:** Adair Co., *C. S. Wallis* 2512 (OKL), Bryan Co., *C. Taylor* 874 (OKL), Cherokee Co., *C. S. Wallis* 835-1 (OKL), Choctaw Co., *E. F. Alder s.n.* (OKL), Mayes Co., *C. S. Wallis* 5750 (OKL), McCurtain Co., *P. Folley & J. Norman* 1528 (OKL), Nowata Co., *N. McCarty & A. Buthod* OOL509 (OKL), Osage Co., *G. W. Steven* 2134 (OKL), Ottawa Co., *C. S. Wallis* 5910 (OKL), Pontotoc Co., *D. McCoy* 1229 (OKL). **Pennsylvania:** Centre Co., *H. A. Wahl* 427 (GH), Lehigh Co., *H. W. Pretz* 4161 (DAO), Luzerne Co., *S. L. Glowenke* 11499 (DAO), York Co., *T. Moul* 2310 (TEX). **Rhode Island:** Providence Co., *E. J. Palmer* 48410 (GH). **South Carolina:** Clarendon Co., *R. K. Godfrey & R. M. Tryon, Jr.* 982 (GH), York Co., *J. B. Nelson* 5006 (USCH). **Tennessee:** Carroll Co., *H. K. Svenson* 380 (GH), Cheatham Co., *R. Kral* 53737 (TEX), Lewis Co., *C. B. King s.n.* (TEX), Montgomery Co., *F. L. Johnson & W. J. Anderson* CAM0323 (OKL). **Vermont:** Chittenden Co., *C. H. Knowlton s.n.* (GH). **Virginia:** Caroline Co., *M. D. Proctor & G. E. Brown* HIL0479 (OKL), Lee Co., *H. L. Chance s.n.* (OKL). **West Virginia:** Cabell Co., *F. A. & C. R. Gilbert* 880 (OKL), Rockingham Co., *A. Heller & G. Halbach s.n.* (DAO). **Wyoming:** Outagamie Co., *G. Hofman s.n.* (MU).

APPENDIX 3-16. Representative Specimens Examined of *Agrimonia repens*.

APPENDIX 3-17. Representative Specimens Examined of *Agrimonia rostellata*.

UNITED STATES. **Alabama:** Coosa Co, *F. S. Earle s.n.* (MU). **Arkansas:** Baxter Co., *P. E. Hyatt* PEN 1987 03 (OKL), Garland Co., *V. H. Chase* 9942 (TEX), Independence Co., *R. D.*

Thomas 20449 (CAN), Marion Co., *B. Shaols 30178* (TEX), Perry Co., *D. Demaree 20179* (OKL), Poinsett Co., *D. Demaree 3668* (TEX), Polk Co., *L. E. Brown 17438* (TEX), Sharp Co., *R. D. Thomas & Ark. Native Plat Soc. group 125596* (MU), Silver Co., *E. Brinkley 317* (TEX). **Connecticut:** Easthampton Co., *E. B. Harger 7505* (GH), New London Co., *R. W. Woodward s.n.* (GH). **Florida:** Jackson Co., *D. B. Ward 9181* (FLAS). **Georgia:** Cherokee Co., *W. H. Duncan 8580* (GH). **Illinois:** Cook co., *T. G. Lammers 10290* (MU), Fayette Co., *M. A. Feist & D. Busemeyer 604* (DAO), Peoria Co., *V. H. Chase 13773* (DAO). **Indiana:** Franklin Co., *S. McCoy 4151* (TEX), Vermillion Co., *R. C. Friesner 19414* (GH). **Kansas:** Cherokee Co., *G. L. Clothier 1002* (GH). **Kentucky:** Fleming Co., *E. L. Braun 3402* (GH), Harlan Co., *H. Kearney, Jr. 168* (CAN), Lawrence Co., *M. A. Vincent 6681* (MU), Madison Co., *J. Marfman s.n.* (TEX), Madison Co., *M. E. Whaerton 10444* (GH). **Louisiana:** Caddo parish Co., *D. S. & H. B. Correll 10137* (GH), Iberia parish Co., *D. S. & H. B. Correll 9522* (GH). **Maryland:** without precise locality, *S. F. Blake 7171* (TEX). **Mississippi:** Forrest Co., *K. E. Rogers 6804* (GH), Oktibbeha Co., *J. D. Ray, Jr. 6714* (GH), Oxford Co., *J. A. & W. W. Barkley 665* (OKL), Tishomingo Co., *J. D. Ray, Jr. 7437* (GH). **Missouri:** Christian Co., *J. W. Blankeinship s.n.* (GH), Douglas Co., *J. A. Steyermark 34630* (DAO), Madison Co., *W. G. D'Arcy 3778* (DAO), Pulaski Co., *R. T. Overebo et al. 0577* (OKL). **New York:** New York, *H. N. Moldenke 70* (CAN), Suffolk Co., *R. Latham 20399* (DAO). **North Carolina:** Ashe Co., *A. E. Radford 41191* (DOA), Biltmore Co., *B. Herbarium 5697* (CAN), Caswell Co., *R. K. Godfrey 5571* (GH), Chatham Co., *E. C. Swab 472* (GH), Jones Co., *A. E. Radford 6660* (GH), Lee Co., *D. F. Houck 584* (GH), Orange Co., *S. W. Leonard 3436* (TEX), Swain Co., *F. W. Hunnewell 10392* (GH), Wake Co., *R. K. Godfrey 5019* (GH). **Ohio:** Adams Co., *J. Howell s.n.* (MU), Butler Co., *A. M. Carlson 48* (MU), Cincinnati Co., *O. G. Lloyd 36.681* (CAN), Crawford Co., *G. T. Jones 70-8-3-454* (MU), Dark Co., *M. A. Vincent 931* (MU), Erie Co., *G. T. Jones 71-8-6-436* (MU), Hamilton Co., *A. M. Swanson 113* (MU), Highland Co., *M. A. Vincent 1029* (MU), Huron Co., *G. T. Jones 67-8-13-803* (MU), Montgomery Co., *D. C. Dister s.n.* (MU), Portage Co., *R. J. Webb 1373* (GH). **Oklahoma:** Bryan Co., *C. Taylor 913* (OKL), Cherokee Co., *R. S. Mitchell 3745* (TEX), Choctaw Co., *B. Hoagland & A. Buthod HUG0668* (OKL), Delaware Co., *U. T. Waterfall 8220* (TEX), Johnston Co., *J. & C. Taylor 13911* (OKL), Le Flore Co., *G. W. Stevens 2772* (OKL), Marshall Co., *D. Alder s.n.* (OKL), McCurtain Co., *P. Folley B011* (OKL), Murray Co., *M. Hopkins 1956* (OKL), Oklahoma Co., *T. R. Stemen & W. S. Myers s.n.* (OKL), Ottawa Co., *Bo Hoagland AB-3895* (OKL), Pawnee Co., *A. Buthod & B. Hoagland AB-5028* (OKL), Pittsburg Co., *T. A. Zaroni 4013* (OKL), Pontotoc Co., *R. T. Robbins 2787* (OKL), Tulsa Co., *M. B. Clark 689* (OKL). **Pennsylvania:** Bedford Co., *F. W. Hunnewell 10181* (GH). **South Carolina:** Abbeville Co., 34 10 92N, 82 36 68W, *J. B. Nelson 20855* (USCH), Beaufort Co., *C. A. Smith 2026* (USCH), Berkeley Co., 33 21 10N, 79 92 66W, *A. B. Pittman 08170011* (USCH), Charleston Co., 32 80 00N, 80 41 42W, *J. B. Nelson 25081* (USCH), Clemson Co., *C. C. Douglass 1859* (USCH), Colleton Co., 32 85 54N, 80 43 75W, *J. B. Nelson 24894* (USCH), Darlington Co., *B. E. Smith 52* (USCH), Dorchester Co., 33 05 85N, 80 40 30W, *A. B. Pittman 08100003* (USCH), Fairfield Co., *D. A. Rayner 1047* (USCH), Florence Co., *A. B. Pittman 08230203* (USCH), Greenville Co., *J. B. Nelson 3707* (USCH), Lexington Co., *J. B. Nelson 14905* (USCH), McCormick Co., *C. N. Horn 9740* (USCH), Orangeburg Co., 33 27 19N, 80 36 80W, *A. B. Pittman 07250109* (USCH), Pickens Co., 87 45 06N, 33 69 53 E, *A. B. Pittman 09200604* (USCH), Richland Co., *Barton & Kelley 399* (GH), Saluda Co., *unknown s.n.* (USCH), York Co., *J. B. Nelson 6801* (USCH). **Tennessee:** Davidson Co., *R. Kral 56537* (GH), Hickman Co., *R. Kral 53704* (TEX), Knoxville Co., *H. M. Jennison s.n.* (GH), Lewis Co., *C. B. King 191* (TEX), Morgan Co., *K. E. Rogers 40578* (GH), Rutherford Co., *H. K. Syenson 9000* (GH). **Texas:** Bowie Co., *B. C.*

Tharp 2375 (TEX), Golden Co., *E. McMullen s.n.* (TEX), Grayson Co., *H. Gentry 51-1335* (TEX), Gregg Co., *C. L. York s.n.* (TEX), Harrison Co., *D. S. Correll 13193* (TEX), Jasper Co., *D. S. & H. B. Correll 38248-B* (TEX), Marion Co., *D. S. Correll 34469* (TEX), Morris Co., *D. S. Correll 26340* (GH), Polk Co., *E. D. Gerin s.n.* (TEX), Red River Co., *D. S. Correll 37884* (TEX), Smith Co., *H. E. Moore, Jr. 860* (GH), Tyler Co., *W. R. Carr 18592* (TEX). **Virginia:** Craig Co., *E. S. Steele 44* (MU), Frederick Co., *F. W. Hunnewell 14452* (GH), Princess Anne Co., *M. L. Fernald & L. Grison 2822* (GH), Rockbridge Co., *J. R. Churchill s.n.* (GH), Southampton Co., *M. L. Fernald & B. Long 10679* (GH). **West Virginia:** Hampshire Co., *F. E. Hunnewell 19344* (GH).

APPENDIX 3-18. Representative Specimens Examined of *Agrimonia striata*. **CANADA.**
Alberta: Bonnyville, 54 23N, 110 39 W, *K. W. Baldwin & J. Macpherson 10632* (CAN), Buck Lake, *M. G. Dumais & G. Swala 4594* (DAO), Cold Lake, *H. Groh 1107* (DAO), Edmonton, *E. H. Moss 2582* (GH), Lac La Biche, 54 54N, 111 54W, *W. K. K. Baldwin 11267* (CAN). **British Columbia:** Burton, *J. A. Calder 10050* (DAO), Creston, *J. W. Eastham s.n.* (DAO), Hazelton, *J. W. Eastham 12018* (CAN). **Manitoba:** Brandon, *B. Boivin 13963* (GH), Lake Winnipeg, *Richardson 8063* (CAN), Riding Mt., 50 42 05N, 99 53 04W, *W. J. Cody & W. A. Woitas 24848* (DAO), Turtle Mt., *G. M. Dawson 7595* (CAN). **New Brunswick:** Albert, *D. R. Lindsay 1807* (DAO), Bass River, *J. Fowler s.n.* (CAN), Blue Bell Mt., *M. O. Malte & W. R. Watson 704* (CAN), Campbellton, *M. O. Malte 759* (CAN), Gloucester, *S. F. Blake 5437* (TEX), Madawaska Co., *I. J. Bassett & G. A. Mulligan 2801* (DAO), Northumberland Co., *P. R. Roberts & D. E. Drury 63-969* (DAO), Victoria Co., 46 50 48 N, 67 41 30 W, *E. Haber et al. 3975* (CAN), York Co., 45 34N, 67 25 W, *M. J. Shepanek & A. W. Dugal 7164* (CAN). **Newfoundland and Labrador:** Lomond river, *A. Bouchard 73-6* (CAN), Gros Morne National Park, *M. B. Duncan 30162* (SMU), St. Barbe South Dist., 49 58N, 57 46 W, *A. Bouchard 72-60* (CAN). **Nova Scotia:** Cumberland Co., *W. B. Schofield & D. H. Webster 5674* (DAO), Digby Co., *M. L. Fernald & B. Long 21651* (GH), Guyborough Co., South river lake, *E. C. Smith et al. 18016* (MU), Inverness Co., *E. C. Smith et al. 2884* (DAO), Kent Ville Co., *J. W. McLellan s.n.* (DAO), Pictou Co., *E. C. Smith et al. 18151* (DAO). **Ontario:** Algoma Dist., 47 00N, 84 45W, *T. M. C. Taylor et al. 2048* (DAO), Carleton Co., *L. Jenkins 3399* (DAO), Clearwater Bay, *W. L. Gordon s.n.* (DAO), Essex Co., 42 15N 83 04W, *D. Langendoen s.n.* (CAN), French River, *J. H. Soper & C. E. Heidenreich 8766* (CAN), Glengarry, *V. R. Brownell & P. M. Catling s.n.* (DAO), Hawkesbury, *D. F. Vick 1433* (DAO), Minnitaki Lake, 50 03N, 91 54 W, *W. K. W. Baldwin 9032* (CAN), Nipigon, 48 45N, 87 15W, *R. S. Hosie 1896* (CAN), Prescott Co., *W. H. Minshall 2559* (DAO), Prince twp., 46 33N, 84 33W, *S. T. B. Losee 2283* (CAN), Renfrew Co., *G. A. Mulligan 600* (DAO), South Gloucester, 45 18N, 75 33W, *A. W. Dugal 1265* (CAN), Thunder Bay dist., 48 21N, 89 27 W, *C. E. Garton 19141* (CAN), Kenora dist., 49 46N, 92 45W, *M. J. Schepanek & A. Dugal 5447* (CAN). **Ottawa:** Gatineau Co., *R. L. Gutteridge 891* (DAO), Papineau Co., *L. Jenkins 6271* (DAO). **Prince Edward Island:** Prince Co., *D. Erskine 2225* (DAO), Queens Co., *I. J. Bassett 1527* (DAO). **Quebec:** Saint Juan Lake, *F. Marie-Victoria & F. Rolland-Germain 43175* (CAN), Bonaventure Co., *I. T. Quern 4* (DAO), Bouaventure Is., *J. M. Gillett 15156* (DAO), Champlain Co., *I. J. Bassett & A. Hamel 2018* (DAO), Charlevoix Co., *G. Claude 76270* (DAO), Gaspé Co., *J. F. Collins et al. s.n.* (GH), Gatineau Co., *L. Jenkins 7342* (DAO), Harvey Co., *S. Brisson 62178* (CAN), Kamouraska Co., *R. Paquin & A. Payette s.n.* (DAO), Lake Monroe, 46 20N, 74 30 W, *Rolland-Germain 520* (CAN), Montebello, *J. E. Charlebois 1202* (DAO), Rouville Co., *O. B. Maryniak 3013* (DAO), Saint-Adolphe, *D. Breton 117* (CAN), St. Genevieve, *H. J. Scoggan 139* (CAN), Timiskaming Co., *W. K. W. Baldwin 4458*

(CAN). **Saskatchewan**: Alternate Dam, *V. L. Harms 38210* (DAO), Meadow Lake, *A. J. Breitung 8247* (DAO), Wallwort, *A. J. Breitung 380* (CAN), Wood Mt., *B. Boivin & J. M. Gillett 8882* (GH). **MEXICO**. **Chihuahua**: Chuhuichupa, *H. LeSueur 665* (TEX), Mohinora, *R. M. Straw 2036* (MEXU), Sierra Madre Mts., *M. E. Jones s.n.* (NY). **Hidalgo**: Tulandingo, *H. E. Moore, Jr. 3513* (GH). **Sinaloa**: Ochrachui, Sierra Surotato, *H. S. Gentry 6166* (GH). **Sonora**: El Tigre, *S. S. White 3351* (MEXU), Rio de Bavispe, El Rancho del Roble, *S. S. White 4354* (GH), Rio de Bavispe, *E. A. Phillips 639* (GH). **UNITED STATES**. **Arizona**: Bisbee Co., *L. N. Goodding 671* (GH), Pima Co., *J. R. Crutchfield 3349* (TEX), Rincon Mts., *J. C. Blumer 2284* (GH), Santa Catalin Mts., *F. Shreve 5405* (GH). **Arkansas**: Gila Co., *W. Hodgson 1633* (OKL). **California**: Humboldt Co., *J. P. Tracy 16571* (DAO). **Colorado**: Boulder Co., *J. Ewan 11462* (GH), El Paso Co., *W. A. Wener & B. Willard 11546* (OKL), San Miguel Co., *E. P. Walker 480* (GH), Windsor Co., *G. E. Osterhout 5506* (OKL). **Connecticut**: Harford Co., *C. H. Bissell s.n.* (GH), New London Co., *R. W. Woodward s.n.* (GH), Windham Co., *C. A. Weatherby 6708* (GH). **Indiana**: Greencastle Co., *W. L. Baxter s.n.* (DAO). **Iowa**: Hardin Co., *M. E. Peck s.n.* (GH). **Maine**: Aroostook Co., *M. L. Fernald 1937* (GH), Clinton Co., *R. C. Bean s.n.* (GH), Franklin Co., *E. B. Chamberlain & C. H. Knowlton s.n.* (GH), Hancock Co., *E. L. Rand s.n.* (GH), Oxford CO., *R. C. Bean s.n.* (GH), Piscataquis Co., *M. L. Fernald & B. Long 13919* (GH), Somerset Co., *J. F. Collins & E. B. Chamberlain s.n.* (GH), Waldo Co., *G. B. Rossbach 492* (GH), Washington Co., *C. H. Knowlton s.n.* (GH), York Co., *K. Furbish s.n.* (GH). **Massachusetts**: Berkshire Co., *J. R. Churchill s.n.* (GH), Bristol Co., *F. W. Hunnewell 13231* (GH), Brookline Co., *F. F. Forbes s.n.* (TEX), Franklin Co., *S. K. Harris s.n.* (MU), Hampden Co., *C. W. Johnson s.n.* (GH), Hampshire Co., *B. L. Robinson 676* (GH), Hardwick Co., *C. F. Batchelder s.n.* (GH), Suffolk Co., *N. T. Kidder s.n.* (GH). **Michigan**: Becker Co., *V. E. McNeilus 97-517* (GH), Houghton Co., *C. D. Richards 963* (CAN), Schoolcraft Co., *H. H. Bartlett & C. D. Richards 591* (DAO). **Minnesota**: Becker Co., *V. E. McNeilus 970517* (TEX), Clearwater Co., *J. B. Moyle 559* (MU), Cook Co., *F. K. Butters et al. 340* (DAO), Minneapolis, *O. Lakela 4686* (DAO), St. Louis Co., *O. Lakela 11965* (DAO), Woods Co., *J. W. & M. F. Moore 11169* (DAO). **Mississippi**: Stoughton Co., *S. F. Blake 100* (TEX). **Missouri**: Greene Co., *J. W. Blankinship s.n.* (MU). **Nebraska**: Valentine Co., *W. L. Tolstead 412* (GH). **New Hampshire**: Coos Co., *A. S. Pease 25982* (NEBC), Grafton Co., *W. V. Brown 1880* (TEX), Hillsborough Co., *C. F. Batchelder s.n.* (GH), Sullivan Co., *M. C. Cowden s.n.* (GH). **New Jersey**: Morris Co., *G. A. Loughridge s.n.* (MU), York Co., *P. R. Roberts & D. E. Drury 63-1452* (DAO). **New Mexico**: Catron Co., *L. M. Shultz 1441* (GH), Colfax Co., *R. J. Hartman s.n.* (TEX), Grant Co., *M. G. Shelton 73* (TEX), Lincoln Co., *B. Hutchins 2450* (TEX), Otero Co., *W. Hess 290* (OKL), San Miguel Co., *M. J. Warnock 1574* (TEX), Sandoval Co., *L. M. Rohrbaugh 594* (OKL). **New York**: Albany Co., *H. D. House 26896* (OKL), Fowler Co., *O. P. Phelps 597* (CAN), Ithaca, *P. Harington 148* (OKL). **North Dakota**: Benson Co., *J. Lunell s.n.* (OKL), Nelson Co., *O. A. Stevens 277* (CAN). **Ohio**: without precise locality, *J. M. Greenman 923* (GH). **Pennsylvania**: Lehigh Co., *H. W. Pretz 721* (DAO), Pocono plateau, *J. W. Harshberger s.n.* (GH). **Rhode Island**: Providence Co., *J. F. Collins & T. Hope s.n.* (GH). **Vermont**: Addison Co., *C. H. Knowlton s.n.* (GH), Caledonia Co., *A. S. Pease 27663* (GH), Chittenden Co., *S. F. Blake 1982* (TEX), Essex Co., *A. S. Pease 30851* (GH), Lamoille Co., *C. H. Knowlton s.n.* (GH), Orange Co., *D. E. Bouffoard 23301* (GH), Orleans Co., *A. S. Pease 28012* (GH), Rutland Co., *C. H. Knowlton s.n.* (GH), Washington Co., *C. H. Knowlton s.n.* (DAO), Windham Co., *R. J. Eaton s.n.* (GH), Windsor Co., *R. C. Bean s.n.* (GH). **Virginia**: Kent Co., *J. Ebinger 3839* (MU). **West Virginia**: Pocahontas Co., *J. M. Greenman 187* (GH). **Wisconsin**: Armstrong Creek, *H. H. Bartlett & C. D. Richards 803* (DAO), La Crosse Co., *T. G. Hartley 2146* (DAO), Langlade Co., *H. H.*

Bartlett & C. D. Richards 848 (DAO), Lincoln Co., *F. C. Seymour 13195* (GH), Woods Co., *V. E. MaNeilus 98-630* (TEX). **Wyoming:** Crook Co., *R. D. Dorn 2179* (CAN).

APPENDIX 3-19. Representative Specimens Examined of *Agrimonia villosa*. **BRAZIL.**
Parana: Guarapuava, *J. Bornuller 95* (GH), Palmeira, *G. Hatschbach 47591* (MO). **Rio Grande do Sul:** Bom Jesus, *B. Rambo 34591* (NY). **Santa Catarina:** Campos Novos, *O. S. Ridas 1240* (NY), R. Xapeco, Abelardo Luz., *A. Castellanos 24655* (NY).