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Reexamination of Sectional Classification in Far Eastern *Euphorbia* Subgenus *Esula* (Euphorbiaceae) Using Morphological and Phenolic Data

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Reexamining the classification proposed by Hurusawa, the phylogeny of Far Eastern *Euphorbia* subgenus *Esula* was analyzed using thirteen morphological and seventeen phenolic compound data. These data were analyzed independently and in combination using PAUP under the assumptions of Fitch parsimony. Ten species, comprised of three sections and five subsections within Far Eastern *Euphorbia* subg. *Esula* and one outgroup from subg. *Chamaesyce*, were used as terminal taxa. The phylogenetic results did not support the sectional classifications within subg. *Esula* proposed by Hurusawa. Section *Decussatae* was nested in the paraphyletic sect. *Esula* in all of the analyses, and the relationship of sect. *Helioscopiae* was equivocal among data sets. The disagreement of data sets over the placement of *Euphorbia ebracteolata* is probably due to a hybrid origin of the species and missing phenolic data for *E. pallasii*. A sister-group relationship of the Korean endemic *E. fauriei* with the widespread *E. pekinensis* was strongly supported by the morphological and phenolic data.

Keywords: *Euphorbia*, morphological data, phenolic data, phylogeny, subg. *Esula*

Euphorbia subgenus *Esula* Pers. consists of about 500 species and is a native Eurasian group recognized by exstipulate leaves, bibractate cyathia, four glands that lack petaloid appendages, and ecarunculate seeds (Hurusawa, 1940; Webster, 1967; Carter and Radcriffe-Smith, 1988; Park, 1998). The primitive woody members of subg. *Esula* occur in Macronesia, Australia, and Melanesia (Kuzmanov, 1964; Molero and Rovira, 1992; Forster, 1994). Because of their large size, close similarity among species, and wide distribution, diverse classifications of species in subg. *Esula* have been proposed by several authors (Prokhanov, 1949; Hurusawa, 1954; Khan, 1964; Ma and Wu, 1992). Elucidation of the phylogentic relationships among the taxa within the subgenus is critical to the reexamination of classification system within the subg. *Esula*.

Although a phylogenetic hypothesis of subg. *Esula* has yet to be presented, recent phylogenetic studies including limited numbers of subg. *Esula* species showed that herbaceous Eurasian subg. *Esula* is more closely related to the Macaronesian or Mediterranean woody *Euphorbia dendroides* L. rather than the Australasian *E. plumerioides* Teijsm. ex Hassk. (Park and Elisens, unpublished). Boissier (1862) and Kuzmanov (1964) also hypothesized that the Eurasian herbaceous subg. *Esula* groups originated from the Mediterranean woody members of *Esula* groups such as sects. *Tulocarpa* (Raf.) Prokh., *Murtekias* Prokh., and *Pachycladae* Boiss.

Intensive monographic studies of Far Eastern *Euphorbia* including Korea, Japan, northeast China, and Taiwan were published by Hurusawa (1940, 1954), who described 27 species, and his treatment of subg. *Esula* included four sections, *Decussatae* (Boiss.) Hurusawa, *Helioscopiae* (Hurusawa) Hurusawa, *Chamaesulae*, and *Esula* (Roeper ex Duby) Hurusawa, with three subsections: *Verticillatae* Hurusawa, *Euesulae* Hurusawa, and *Galarrhoei* (Boiss.) Hurusawa. Except for the first three sections, most of the species in sect. *Esula* are native to the Far East.

Recent palynotaxonomic studies of the Korean Euphorbiaceae did not support the recognition of the subg. *Esula* sect. *Helioscopiae* and suggested a close relationship between subsect. *Verticillatae* and ser. *Laevicarpae* Hurusawa of subsect. *Galarrhoei* in sect. *Esula* (Park and Lee, 1988). Based on chemotaxonomic studies on the Korean Euphorbiaceae, Ahn et al. (1995) suggested that sect. *Deccusatae* is closely related to subsect. *Esulae* of sect. *Esula* due to its absence of hydrolyzable tannins, with all the species having crescent-shaped cyathial glands. However, the recognition of sect. *Helioscopiae* was supported by the presence of

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the unique helioscopins A and B in *E. helioscopia*.

Because the distinctions among sections were mainly based on vegetative character differences, a critical phylogenetic reexamination of Hurusawa's classification using most of the available characters is desirable. Using the morphological and phenolic compound data, we conducted a phylogentic investigation of the Far Eastern *Euphorbia* subg. *Esula* for reexamining the classifications proposed by Hurusawa (1940, 1954).

MATERIALS AND METHODS

Terminal Taxa and Characters

Ten species belonging to the Far Eastern *Euphorbia* subg. *Esula* and *E. maculata* of subg. *Chamaesyce* were selected as terminal taxa in this study (Table 1). *E. maculata*, which was used as the outgroup is thought to be closely related to subg. *Esula* on the basis of its basic chromosome number, laticifer shape, and vegetative morphology. Ten ingroup species represent three sections and five subsections of Hurusawa's (1940) *Euphorbia* subg. *Esula* in Far Eastern Asia (Table 1). Due to difficulty in obtaining materials, sect. *Chamaesulae* of subg. *Esula* was not included in this study.

Thirteen morphological characters were identified based on herbarium specimens and collections made by the authors (Table 2). Pollen sculpturing characters

Table 2. List of the morphological (1-13) and phenolic compound (14-30) characters used in the cladistic analysis of Far Eastern *Euphorbia* subgenus *Esula* species.

1. Root: 0 = not succulent; 1 = semi-succulent; 2 = succulent
2. Stem: 0 = glabrous; 1 = hairy
3. Leaf margin: 0 = denticulate; 1 = entire; 2 = serrulate
4. Leaf apex: 0 = round; 1 = acute
5. Terminal bracts: 0 = round; 1 = triangular
6. Gland shape: 0 = orbiculate; 1 = bicornate
7. Capsule surface: 0 = smooth; 1 = verrucose
8. Capsule pubescence: 0 = ciliate; 1 = glabrous
9. Pollen sculpturing: 0 = foveolate; 1 = fossulate
10. Petaloid appendages: 0 = present; 1 = absent
11. Seed caruncle: 0 = absent; 1 = present
12. Seed wrinkle: 0 = present; 1 = absent
13. Stipule: 0 = present; 1 = absent
14. Gallic acid: 0 = absent; 1 = present
15. Gallic acid 3-O-β-D-(6-O-galloyl)-glucoside: 0 = absent, 1 = present
16. Brevifolin carboxylic acid: 0 = absent; 1 = present
17. Quercitrin: 0 = absent; 1 = present
18. Kaempferol 3-O-rhamnoside: 0 = absent; 1 = present
19. Kaempferol 3-O-rutinoside: 0 = absent; 1 = present
20. 3-O-galloyl quinic acid: 0 = absent; 1 = present
21. 3-O-galloyl shikimic acid: 0 = absent; 1 = present
22. 2,3-di-O-galloyl glucose: 0 = absent; 1 = present
23. 1,3,4,6-tetra-O-galloyl-β-O-glucose: 0 = absent; 1 = present
24. 1,2,3,4,6-penta-O-galloyl-β-D-glucose: 0 = absent; 1 = present
25. Corilagin: 0 = absent; 1 = present
26. Geraniin: 0 = absent; 1 = present
27. Euphorbin A: 0 = absent; 1 = present
28. Euphorbin D: 0 = absent; 1 = present
29. Rugosin E: 0 = absent; 1 = present
30. Excoecarianin: 0 = absent; 1 = present

Table 1. Taxa used in the cladistic analysis based on morphological and phenolic data. Classification in *Euphorbia* based on Hurusawa (1940).

Taxon	Species (acronym) used in the study
Genus *Euphorbia*	
Subgenus *Esula* (ingroup)	
Section *Decussatae*	*E. lathyris* L. (LATH)
Section *Helioscopiae*	*E. helioscopia* L. (HELI)
Section *Chamaesulae*	
Section *Esula*	
Subsection *Verticillatae*	*E. pallasii* Turcz. (PALL)
Subsection *Euesulae*	*E. esula* L. (ESUL)
	E. sieboldiana Morr. et Decne. (SIEB)
Subsection *Galarrhoei*	
Series *Quinadenium*	*E. adenochlora* Morr. & Decne. (ADEN)
Series *Pekinenses*	*E. pekinensis* Rupr. (PEKI), *E. jolkini* Boiss. (JOLK)
	E. fauriei Lev. et Van. (FAUR)
Series *Laevicarpae*	*E. ebracteolata* Hayata (EBRA)
Subgenus *Chamaesyce* (outgroup)	*E. maculata* L. (MACU)
Subgenus *Agaloma*	
Subgenus *Euphorbium*	
Subgenus *Arthrothammus*	
Subgenus *Poinsettia*	

were extracted from the literature by the first author (Park and Lee, 1988). Seventeen phenolic compounds were scored from previous reports by the second authors (Ahn et al., 1995; Table 2). Determination of the presence / absence of the phenolic compounds was based on the spots of the fingerprints, which were identified by comparison with authentic samples that were obtained by the column-chromatography of aqueous acetone extracts of *E. pekinensis* and *E. ebracteolata* (Ahn et al., 1995).

Data Analysis

A maximum parsimony analysis (Swofford, 1991) was performed on PAUP version 3.0s with ingroup and outgroup species simultaneously, and the resulting trees were rooted at the internode between ingroup and outgroup taxa. Two analyses based on 13 morphological and 17 chemical data were performed independently, and a combined data matrix including 30 characters was analyzed. In addition to the three analyses, we calculated the most-parsimonious trees from the combined matrix excluding three missing characters for *E. adenochlora* and *E. pallasii*. The search for the shortest tree was carried out using the branch-and-bound algorithm with the accelerated transformation (ACCTRAN). The strict consensus trees obtained from the morphological data, phenolic compound data, combined data, and combined data excluding missing phenolic characters were generated. Bootstrap support (Felsenstein, 1985) for monophyletic groups was estimated based on 100 bootstrap replications with the tree bisection reconnection (TBR) branch swapping algorithm.

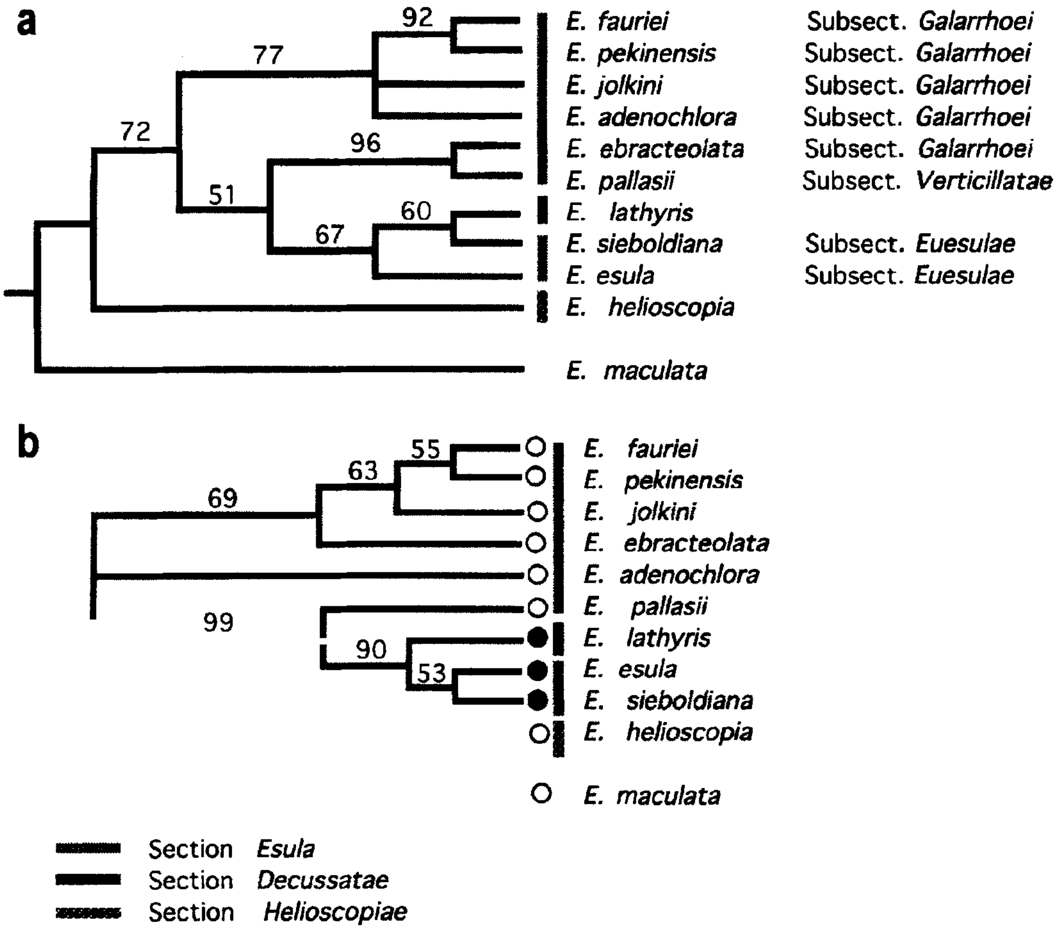

Figure 1. Strict consensus of the equally parsimonious trees of Far Eastern *Euphorbia* subg. *Esula* using morphological and phenolic compound data. Bootstrap support of 100 replicates is shown above the branches. **a.** A single most parsimonious tree obtained from phylogenetic analysis of morphological characters (length = 17; CI = 0.882; RI = 0.913). **b.** Strict consensus of four equally parsimonious trees using phenolic compound data (length = 23; CI = 0.739; RI = 0.865). Solid circle = bicornate glands, open circle = orbiculate glands.

RESULTS

Cladistic analysis of the 13 morphological characters resulted in a single most parsimonious tree of 17 steps with a consistency index of 0.882 and a retention index of 0.913. The values of 100 bootstrap replications were presented (Fig. 1a). This topology did not support the monophyly of subg. *Esula* sect. *Esula*., as the sect. *Decussatae* was nested in sect. *Esula*. In this single most parsimonious tree, *Euphorbia helioscopia*, sect. *Helioscopiae*, was divided at the base and was the sister group of the other species. The resulting tree showed a closer relationship between *E. ebracteolata* of subsect. *Galarrhoei* and *E. pallasii* of subsect. *Verticillatae* than other species of the *Galarrhoei* subsect. Bootstrap analysis indicated that the clade uniting *E. ebracteolata* and *E. pallasii* was strongly supported by the highest bootstrap value (96%) within a study group.

The analysis of phenolic data resulted in four equally parsimonious trees (length = 23; CI = 0.739; RI = 0.865) that were represented by the strict consensus tree shown in Figure 1b. Comparing the morphological analysis, a strict consensus tree of these phenolic data showed no resolution at the base of the tree. However, the strict consensus showed *E. pallasii* as a sister species of *E. lathyris* + *E. esula* + *E. sieboldiana*. The bootstrap analysis indicated good support for the monophyly of the above clade with bootstrap value of 99%. The two data sets differred significantly

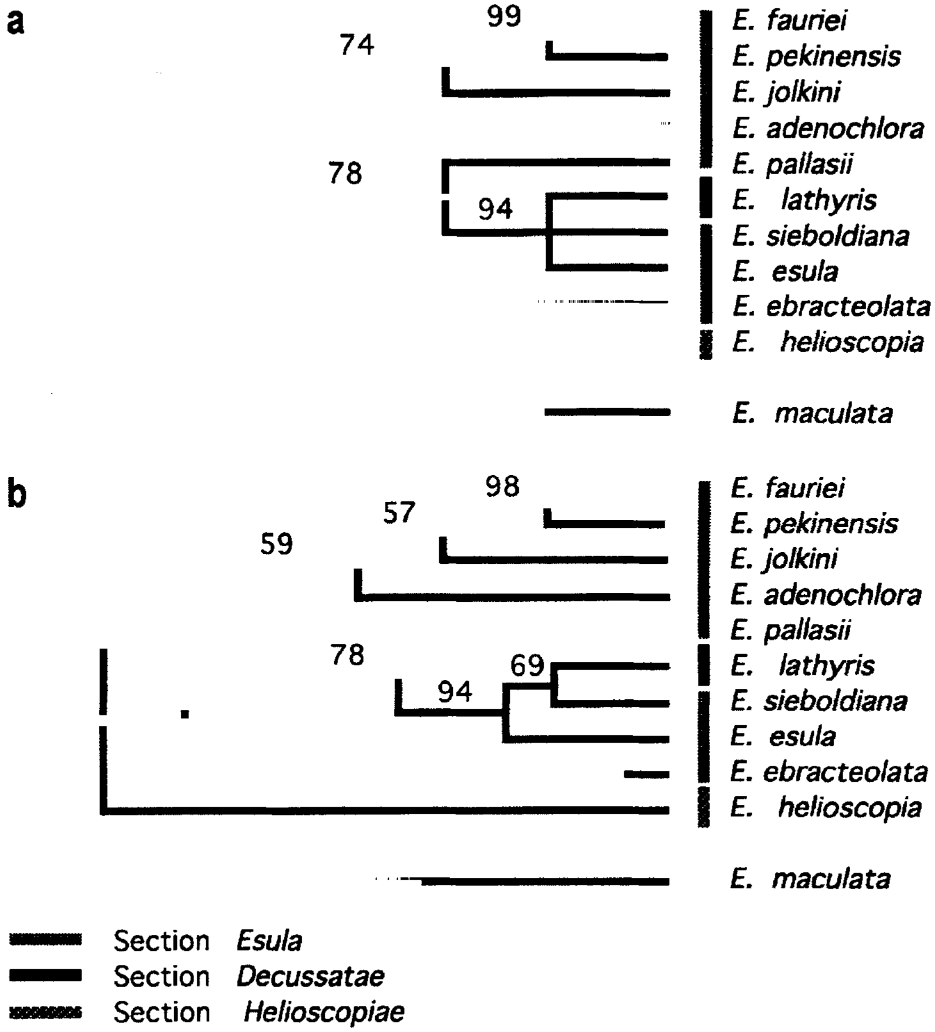

Figure 2. Strict consensus of the equally parsimonious trees of Far Eastern *Euphorbia* subg. *Esula* using combined data from morphology and phenolic compound. Bootstrap support of 100 replicates is shown above branches. **a**. Strict consensus of six equally parsimonious trees for combined morphological and phenolic compound data. **b**. A single most parsimonious tree obtained from combined morphological and phenolic compound data excluding missing characters.

in the placement of *E. ebracteolata*, while the species of sect. *Esula* subsect. *Euesulae* and sect. *Decussatae* formed a clade in both results. The morphology and phenolic compound trees both support the *E. fauriei* + *E. pekinensis* clade.

Phylogenetic analysis of a combined data set consisting of 13 morphological and 17 phenolic characters resulted in six equally parsimonious trees (length = 48), which was represented by the strict consensus shown in Figure 2a. The topology obtained from the combined data was mostly identical to the result from the phenolic characters, differing only by the position of *E. ebracteolata*. In the analysis using combined data without missing characters, a single most parsimonious tree with 41 steps was found (Fig. 2b). The topologies obtained from the analyses before and after excluding missing characters differred on the relationships among basal lineages, the main of which was the recognition of the two major clades within sects. *Esula* + *Decussatae* in the latter.

DISCUSSION

With the intensive revision of Far Eastern *Euphorbia*, the phylogenetic results are not compatible with the sectional or subsectional classifications of Hurusawa (1940, 1954). Although Hurusawa recognized sect. *Decussatae* as distinct from the other three sections based on its unique decussate leaves, all of the data sets strongly suggest the inclusion of sect. *Decussatae* within sect. *Esula*. This interpretation is confirmed by the presence of bicornate glands and the lack of hydrolyzable tannins (Ahn et al., 1995; Fig. 1b).

However, the relationship of sect. *Helioscopiae* is equivocal among data sets. The cladograms obtained by morphological data and combined data without missing characters support the basal dichotomy of ingroup taxa by sect. *Helioscopiae* and the remaining taxa. In the results from phenolic and combined data, *E. helioscopia* was not recognized, and was arranged at the basal polytomy. Palynologically, *E. helioscopia* in sect. *Helioscopiae* is not well separated from the species of sect. *Esula* subsect. *Galarrhoei* (Park and Lee, 1988). However, the recognition of the section is supported by the presence of the unique helioscopins A and B in *E. helioscopia* (Ahn et al., 1995). Therefore, determination of whether it should be included within subsect. *Galarrhoei* or recognized as a distinct sect. *Helioscopiae* depends on a global analysis including other species of *Helioscopiae* and careful evaluation of other character states.

Within sect. *Esula*, our data suggest that subsect. *Galarrhoei* and subsect. *Euesulae* are nonmonophyletic. However, our results strongly support that subsects. *Verticillatae* (*E. pallasii*) and *Euesulae* (*E. esula* and *E. sieboldiana*) form a clade if *E. lathyris* (sect. *Decussatae*) is included in subsect. *Euesulae*. Chemically, the unique absence of two compounds (chars. 26, 27) in these groups strongly supports their monophyly (Ahn et al., 1995). Within subsect. *Galarrhoei*, Hurusawa (1940) recognized three series: *Quinadenium*, *Pekinenses*, and *Laevicarpae* (Table 1). The ser. *Pekinenses* consists of three related species on the basis of morphological and palynological data (Park and Lee, 1988). Our cladistic results mostly support a monophyly of the ser. *Pekinenses* species. In addition, within the series, Korean endemic *E. fauriei* forms a clade with Far Eastern native, *E. pekinensis* in all of the analyses. However, the relationships among three series of subsect. *Galarrhoei* remained unresolved in our analyses.

The most striking point of disagreement in our analyses was the placement of *E. ebracteolata*. According to morpholgy, *E. ebracteolata* is placed in *E. pallasii* clade, and it is strongly supported (bootstrap = 96%). However, the phenolic data places *E. ebracteolata* as a sister group of the ser. *Pekinenses* of subsect. *Galarrhoei* with moderate support (bootstrap = 69%). The combined data produced a third topology, different from morphology and phenolic compound topologies. In case of high support values of conflicting clades each other, some authors recommend that the data should not be combined (de Queiroz, 1993; Kitching et al., 1998).

Recently, the incongruence of different data sets

Table 3. Morphological and phenolic data matrix for the cladistic analysis of Far Eastern *Euphorbia* subgenus *Esula*. "?" designates missing chatacter states. Acronyms are given in table 1.

Taxon	Characters
	000000000111111111122222222223 123456789012345678901234567890
FAUR	112100110111111111111011111000
JOLK	101000110111111101111011111000
PEKI	112100110111111111111011111000
ADEN	1010001101111101? ? ?01011111000
PALL	2010100011111100? ? ?00000100000
EBRA	201010001111111000111111111000
LATH	001111010111100000000000000000
HERI	000000010110110100011100111000
ESUL	001011010111100011000000000000
SIEB	001111010111100010000000000000
MACU	000000000000010100000011111111

has been diversely interpreted as error in phylogenetic inference, and a difference in phylogenetic history between the independent data sets (de Queiroz et al., 1995; Crandall and Fitzpatrick, 1996; Baum et al., 1998; Normark and Lanteri, 1998). The disagreements of data sets over the placement of *E. ebracteolata* is presumably due to a hybrid origin of the species, and significant amounts of missing chemical data for *E. pallasii* (Table 1). In flowering plants, reticulation has been thought to contribute significantly to incongruence between the topologies from different sources of data (Schilling and Panero, 1996; Baum et al., 1998). The hybrid or introgressional origin of *E. ebracteolata* and *E. pallasii* is strongly suggested by the morphological characters. The rounded glands and thick fleshy roots of both are shared with other species of subsect. *Galarrhoei*, while the three angular bracts and glabrous capsules are similar to those found in subsect. *Euesulae* species.

In conclusion, the phylogenetic results using morphology, phenolic, and combined data do not support the sectional classification within subg. *Esula* proposed by Hurusawa (1940, 1954). Thus, based on the global parsimony analysis including most of species in subg. *Esula*, some taxonomic change at the sectional and sub-sectional level should be effected.

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