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R.G. van den Berg, G.W.M. Barendse, G.M. van der Weerden, C. Mariani

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QUANTITATIVE ASSESSMENT OF COROLLA SHAPE VARIATION IN MEXICAN *SOLANUM* SECTION *PETOTA*

D.M. SPOONER[1] AND R.G. VAN DEN BERG[2]

[1] Department of Horticulture, University of Wisconsin, U.S.A.
[2] Plant Taxonomy Group, Wageningen University, Wageningen, The Netherlands

Abstract

Corolla shapes in wild potatoes vary from stellate to rotate, and have been used as major morphological features to distinguish species, series, and superseries. In preparation of a potato flora of North and Central America, we found that corolla shapes, and associated corolla acumen lengths, have been used to distinguish the ser. *Demissa, Longipedicellata,* and *Tuberosa*. However, we found these corolla characters difficult to apply in both herbarium and living material. To better assess corolla sizes and shapes as taxonomic characters in the North and Central American wild potatoes we pressed flowers of germplasm accessions grown in a common field plot. We measured corolla radius, two assessments of shape, and absolute and relative length of acumens. The corolla measurements failed to practically distinguish most these three series. However, there were statistically significant differences of widely overlapping ranges for radius of corolla that distinguished series *Longipedicellata* from ser. *Demissa* (to the exclusion of *S. demissum* that we ally with members of the South American ser. *Acaulia*) and ser. *Tuberosa*. Their wide overlap in ranges of size greatly reduces their utility as practical taxonomic characters.

Introduction

Solanum L. sect. *Petota* Dumort., the potato and its relatives, occurs from the southwestern United States to southern Chile. It consists of seven cultivated and 225 wild species, according to the latest comprehensive taxonomic treatment (Hawkes 1990). Nine of these species are members of separate clades and are alternatively treated in sect. *Etuberosum* (Buk. et Kameraz) A. Child, sect. *Lycopersicum* (Mill.) Wettst., or sect. *Juglandifolium* (Rydb.) A. Child (Child 1990; Spooner et al. 1993).

Most wild potato species grow in the Andes, but 31 species and five hybrid species grow in Mexico and Central America (Hawkes 1990). Hawkes (1990) grouped the species from this region into eight series. In herbarium work for preparation for a potato flora of North and Central America, we found it difficult to identify species of ser. *Demissa*, ser. *Longipedicellata*, and ser. *Tuberosa*. According to Hawkes (1990), ser. *Demissa* contains five species and two hybrids; ser. *Longipedicellata* contains six species, four subspecies, two varieties, and one hybrid; ser. *Tuberosa* contains three species. The North and Central American species in these series range in ploidy level and Endosperm Balance Number (EBN) from 2X (2EBN) to 4X (4EBN) to 6X (4EBN) (Hanneman 1994).

Correll (1962) and Hawkes (1990) had slightly different concepts of inclusion of species in these series (Fig. 1).

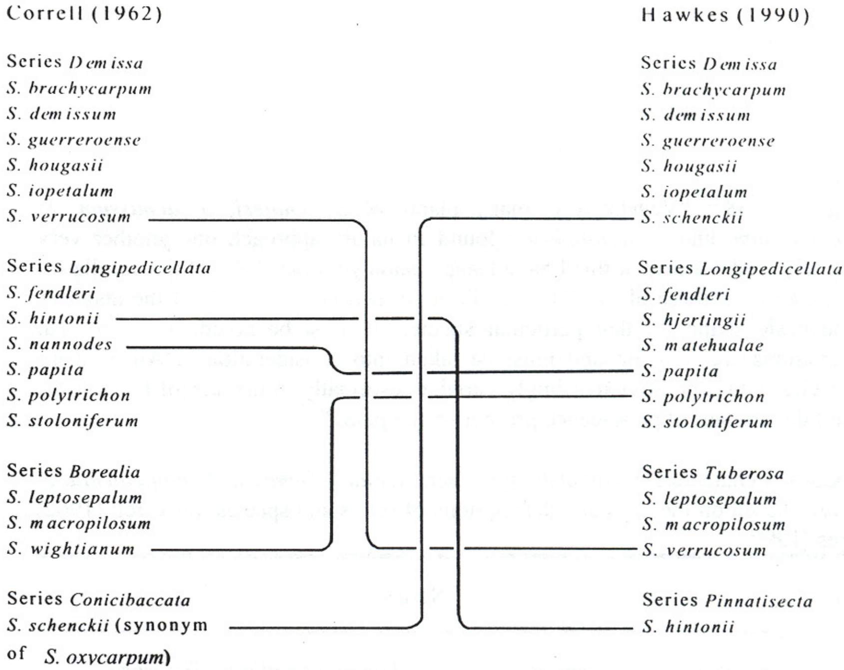


FIGURE 1. Alternative taxonomic concepts of members of *Solanum* ser. *Demissa, Longipedicellata,* and *Tuberosa.*

Correll (1962) and Hawkes (1990) provided similar criteria to circumscribe these series and ser. *Acaulia* (Table 1), but they appear to provide overlapping ranges of character states as is common in sect. *Petota* (Spooner and Van den Berg 1992; Van den Berg et al. 1998).

According to Correll (1962) and Hawkes (1990) corolla diameter appears to be diagnostic only for ser. *Acaulia* in which they place species from South America, but not *S. demissum* as in our interpretation. Correll (1962) noted morphological variation and had difficulty distinguishing *S. verrucosum* (ser. *Tuberosa*), *S. demissum* (ser. *Demissa*), *S. fendleri* (ser. *Longipedicellata*) and *S. stoloniferum* (ser. *Longipedicellata*). Two of his statements reveal his problems clearly:

> pg. 364: "In my opinion, the confusing of some plants of *S. verrucosum* with some plants of *S. demissum* is excusable. In fact, many plants collected in nature cannot be placed with certainty into either category, while specimens of cultivated plants of both *S. verrucosum* and *S. demissum* are exceedingly variable."

pgs. 380-382: "Vegetatively, many plants of *S. fendleri*, *S. demissum*, *S. verrucosum*, and *S. stoloniferum* found in nature approach one another very closely, and because of this I have leaned heavily on the shape of the corolla for separation of these plants. The corolla described here for each of the above is the basic shape for that particular species. It must be noted, however, that variations are frequent and must be taken into consideration"..."All of these species, however, are exceedingly variable, especially in the size of the corolla, and the amount of pubescence present on the plant."

TABLE 1. Corolla characters distinguishing the ser. *Acaulia, Demissa*, *Longipedicellata,* and *Tuberosa,* based on the keys and descriptions of series and species in Correll (1962) and Hawkes (1990).

Character	Series			
	Acaulia[1]	*Demissa*	*Longipedicellata*	*Tuberosa*
Corolla diameter	1 – 2 cm	2 – 4 cm	1 – 3 cm	2.5 – 4 cm
Corolla shape	rotate	rotate-pentagonal	rotate-stellate, rarely rotate-substellate	rotate-pentagonal
Corolla lobes	very short and flat	very short and flat	broadly triangular	broader than long
Acumens	minute	small (1.5 – 2 mm)	prominent, large	triangular, acute

[1]To include *S. demissum* (see text).

The above statements show Correll's reliance on corolla shape to make some final decisions on identifications. As Correll (1962), we are having trouble identifying some species within these three series, and even in distinguishing the series from each other, in our preparation of a monograph of the potatoes of North and Central America. However, we found that less than ten percent of herbarium specimens have corollas pressed adequately flat to clearly determine shapes. As a result, many identifications that ultimately have relied on corolla characters are problematical.

This study tests the taxonomic utility of corolla shapes and sizes to distinguish ser. *Acaulia*, *Demissa*, *Longipedicellata*, and *Tuberosa*. We evaluated *S. demissum* as a member of ser. *Acaulia*. Previous morphological studies, using 37 vegetative and floral characters (Spooner et al. 1995) showed *S. demissum* to cluster with *S. albicans* (ser. *Acaulia*), but not with other members of ser. *Demissa*. Subsequent morphological studies using more accessions of *S. acaule* and *S. albicans* (ser. *Acaulia*) by Kardolus (1999) also supported the relationship of *S. demissum* to members of ser. *Acaulia*. Further, Kardolus and Groendijk-Wilders (1998) showed that *S. demissum* shared a similar inflorescence architecture with ser. *Acaulia*. This relationship also has been supported by molecular data from single- to low-copy nuclear restriction fragment length polymorphisms (RFLPs; Debener et al. 1990), chemical data from steroidal glycoalkaloids (Petersen et al. 1993), and cladistic and one phenetic analysis (but not another phenetic analysis) of Amplified Fragment Length Polymorphisms (AFLPs; Kardolus 1998). Finally, the relationships of ser. *Acaulia* and *Demissa* are fully supported by EBN, as hexaploid *S. albicans* and *S. demissum* are both 6x (4EBN). We interpret these combined morphological, chemical, biological, and two independent DNA data sets as compelling to support the relationships of *S. demissum* to members of ser. *Acaulia*, rather than ser. *Demissa*.

Material and Methods

We examined 45 accessions (Table 2) and from one to four corollas from separate plants per accession (for a total of 112 individuals) of members of ser. *Acaulia* (one accession, three individuals, of *S. albicans*; three accessions, eight individuals, of *S. demissum*), ser. *Demissa* (nine accessions, 16 individuals), ser. *Longipedicellata* (21 accessions, 57 individuals), and ser. *Tuberosa* (nine accessions, 26 individuals). To compare our results to stellate corollas characteristic of Mexican diploid species we included also ser. *Bulbocastana* and *Pinnatisecta* (one accession and one individual per species). These corollas were collected and immediately pressed in the field from plants grown from seeds from the US Potato Genebank (Bamberg et al. 1996), that we grew in a common garden at the University of Wisconsin Hancock Agricultural Research Station.

TABLE 2. Accessions examined for corolla size and shape.

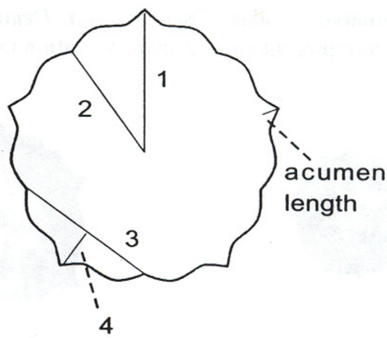
Species	PI[1]	No. of corollas assessed per PI	Series	Location
S. albicans (Ochoa) Ochoa	266381	3	*Acaulia* Juz.	Peru, Cajamarca
S. avilesii Hawkes et Hjert.	498091	4	*Tuberosa* (Rydb.) Hawkes	Bolivia, Santa Cruz
S. brachycarpum (Correll) Correll	558430	2	*Demissa* Buk.	Mexico, Oaxaca
S. bulbocastanum Dunal	255516	1	*Bulbocastana* (Rydb.) Hawkes	Mexico, Jalisco
S. brevicaule Bitter	498111	3	*Tuberosa*	Bolivia, Cochabamba
S. brevicaule	498218	3	*Tuberosa*	Bolivia, La Paz
S. bukasovii Juz.	414155	1	*Tuberosa*	Peru, Apurímac
S. bukasovii	568933	1	*Tuberosa*	Peru, Puno
S. demissum Lindl.	186551	2	*Acaulia*	Mexico, D.F.
S. demissum	205514	3	*Acaulia*	Mexico, Veracruz
S. demissum	275211	3	*Acaulia*	Guatemala, Huehuetenango
S. fendleri A. Gray	275161	4	*Longipedicellata* Buk.	USA, New Mexico
S. fendleri	275162	2	*Longipedicellata*	USA, Arizona

BLE 2. Accessions examined for corolla size and shape (continued).

pecies	PI[1]	No. of corollas assessed per PI	Series	Location
fendleri	458409	3	*Longipedicellata*	USA, New Mexico
fendleri	497995	4	*Longipedicellata*	Mexico, Chihuahua
fendleri	558395	4	*Longipedicellata*	Mexico, Baja California Sur
fendleri	558396	2	*Longipedicellata*	Mexico, Baja California Sur
gourlayi Hawkes	210038	3	*Tuberosa*	Argentina, Jujuy
gourlayi	473019	4	*Tuberosa*	Argentina, Jujuy
gourlayi	558067	4	*Tuberosa*	Argentina, Jujuy
guerreroense Correll	161727	3	*Demissa*	Mexico, Jalisco
guerreroense	161730	2	*Demissa*	Mexico, Guerrero
hjertingii Hawkes	186559	2	*Longipedicellata*	Mexico, Coahuila
hjertingii	498019	2	*Longipedicellata*	Mexico, Coahuila
hougasii Correll	161174	2	*Demissa*	Mexico, Michoacán
hougasii	558402	2	*Demissa*	Mexico, Jalisco
iopetalum (Bitter) Hawkes	275181	1	*Demissa*	Mexico, Puebla
jamesii Torr.	275168	1	*Pinnatisecta* (Rydb.) Hawkes	USA, Colorado
matehualae Hjert. et Tarn	498050	3	*Longipedicellata*	Mexico, San Luis Potosí
polytrichon Bitter	275240	2	*Longipedicellata*	Mexico, Zacatecas
polytrichon	498038	4	*Longipedicellata*	Mexico, Durango
papita Rydb.	251740	1	*Longipedicellata*	Mexico, Zacatecas
papita	251741	3	*Longipedicellata*	Mexico, Durango
papita	275227	4	*Longipedicellata*	Mexico, Durango
papita	275229	4	*Longipedicellata*	Mexico, Zacatecas
schenckii Bitter	275261	1	*Demissa*	Mexico, Oaxaca
schenckii	498041	1	*Demissa*	Mexico, Queretaro
schenckii	498280	2	*Demissa*	Mexico, Oaxaca
stoloniferum Schltdl.	161178	3	*Longipedicellata*	Mexico, Michoacán
stoloniferum	255534	2	*Longipedicellata*	Mexico, Jalisco
stoloniferum	275249	2	*Longipedicellata*	Mexico, Michoacán
stoloniferum	365399	3	*Longipedicellata*	Mexico, Mexico
stoloniferum	558455	2	*Longipedicellata*	Mexico, Queretaro
stoloniferum	558462	1	*Longipedicellata*	Mexico, Mexico
S. *verrucosum* Schltdl.	545747	4	*Tuberosa*	Mexico, Mexico

Jnited States Department of Agriculture Plant Introduction Number.

We pressed the corollas as flat as possible for accurate determinations of corolla size and shape, and scanned them as gray scale images. We measured corollas by the five measures outlined in fig. 2, and assessed corolla shapes by the two ratios outlined in Fig. 2, as used by Spooner and Van den Berg (1992). Our measure of shape 1 (Fig. 2) assesses Correll's (1962) and Hawkes's (1990) assessment of corolla shape (Table 1), while our measure of shape 2 assesses corolla lobes (table 1). We assessed absolute acumen length (measure 4, Fig. 2), and relative acumen length by dividing the radius by the absolute acumen length (measure 1/measure 4, Fig. 2).



$$\text{Shape } 1 = \frac{1}{2}$$

$$\text{Shape } 2 = \frac{3}{4}$$

FIGURE 2. Measures of corolla shape and size.

These accessions have been identified in past years by D. Correll, J.G. Hawkes, and other taxonomists during on-site visits to the US potato genebank in Sturgeon, Bay, Wisconsin (the National Research Support Program-6, NRSP-6; formerly called the Inter-Regional Potato Introduction Project, IR-1) to inspect living representatives in field plots. These plants for identifications frequently were accompanied by prior taxonomic determinations and by chromosome counts performed by the genebank personnel (Spooner and Van den Berg 1992).

We designated accessions to series as delineated by Hawkes (1990; Fig. 2) but we grouped members of ser. *Bulbocastana* and ser. *Pinnatisecta* as "Mexican diploids" to compare them to the rotate-pentagonal to rotate corollas of the other series, and we designated *S. demissum* to ser. *Acaulia*. We determined simple statistics and the presence of statistically significant differences among series using the Tukey-Kramer HSD test (Kramer 1956) in JMP software (SAS Institute Inc. 1994).

Results and Discussion

Figure 3 shows representative corollas of ser. *Acaulia*, *Demissa*, *Longipedicellata*, and *Tuberosa* that we chose to represent corolla shape variation in these series.

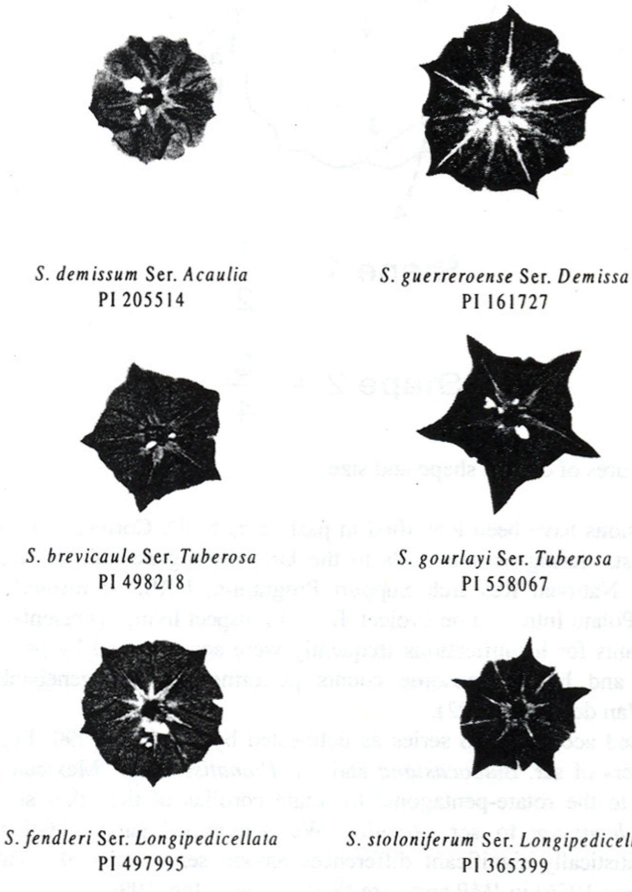


FIGURE 3. Representative corolla shapes in members of *Solanum* ser. *Acaulia*, *Demissa*, *Longipedicellata*, and *Tuberosa*.

We found that the putative measures of corolla shapes outlined by Correll (1962) and Hawkes (1990) fail to distinguish ser. *Demissa* (excluding *S. demissum*), *Longipedicellata*, and *Tuberosa*, but that ser. *Acaulia* differed from these by four of our five measures of corolla shape and size (Fig. 4). There are statistically significant differences (P = 0.05) of widely overlapping ranges for radius of corolla that distinguished ser. *Longipedicellata* from ser. *Demissa* and ser. *Tuberosa*, but the extensive overlapping ranges of this trait make it impractical for distinguishing these series (Fig. 4). Correll's (1962) and Hawkes's (1990) assessment of corolla shape (our shape 1, Fig. 2), and corolla lobes, (our shape 2) and acumen length similarly fail to distinguish the three series. Only ser. *Longipedicellata* and ser. *Tuberosa* show significant differences in widely overlapping ranges of acumen length (Fig. 4). However, the acumens are part of the corolla radius, and when the relative acumen lengths are assessed by dividing the radius by the acumen length (see Material and Methods), there is no significant difference in the lengths.

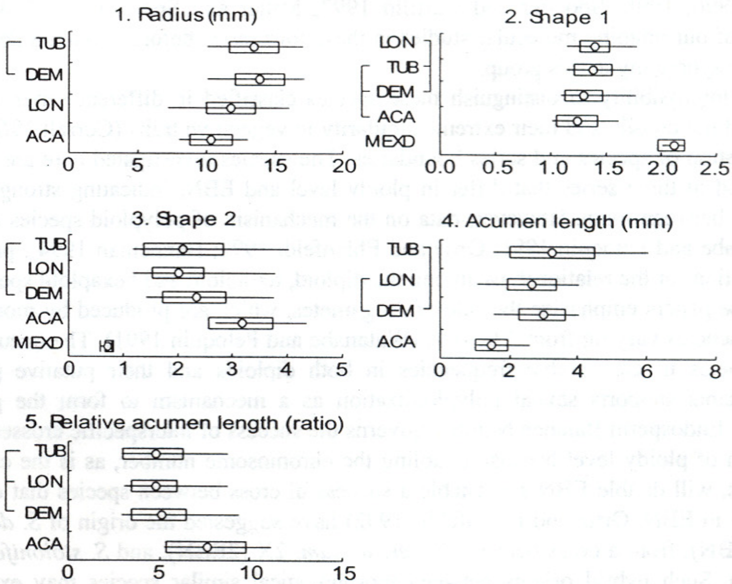


FIGURE 4. Means, ranges, and one standard deviation from the mean for *Solanum* ser. *Aucaulia*, *Demissa*, *Longipedicellata*, and *Tuberosa*, and two Mexican diploid species.

This study was stimulated by our difficulty to identify the North and Central American members of ser. *Acaulia*, ser. *Demissa*, ser. *Longipedicellata*, and ser. *Tuberosa*, and the apparent great reliance given by Correll (1962) and Hawkes (1990) on corolla sizes and shapes to distinguish them. Like Correll (1962), we found particular difficulties to identify many Mexican species in these series. Less than ten percent of the herbarium specimens we examined have corollas present or pressed sufficiently flat to assess these traits. Correll (1962) stated it was necessary to "[lean] heavily on the shape of the corolla" for separation of *S. fendleri*, *S. demissum*, *S. verrucosum*, and *S. stoloniferum*. However, even when it is possible to examine complete herbarium specimens, our data show corolla shape not to distinguish these species from different series, except sometimes for *S. demissum*.

Our herbarium and field experience in Mexico (Spooner et al. 1991, 2000), and our morphological examination of members of ser. *Demissa* (Spooner et al. 1995), and ser. *Longipedicellata* (Spooner et al. 2001) suggest that there may be too many species recognized in sect. *Petota* in North and Central America. We have found this to be true for South American wild potatoes also (Van den Berg and Spooner 1992; Van den Berg et al. 1996, 1998; Spooner and Castillo 1997; Miller and Spooner 1999). We await results of our ongoing molecular studies in these four series before making a decision on possible synonymy in this group.

The impossibility to distinguish these species classified in different series using the shape of the corolla and their extreme similarity in vegetative traits (Correll 1962), raises the question of species and series boundaries. The species investigated here are currently classified in three series that differ in ploidy level and EBN, indicating strong crossing barriers between them. However, data on the mechanism of polyploid species evolution (Watanabe and Peloquin 1991; Ortiz and Ehlenfeldt 1992; Hanneman 1994), provide an explanation for the relationships among the diploid, tetraploid and hexaploid species.

These papers emphasize the role of 2n gametes, which are produced by most species in frequencies varying from 2 to 10% (Watanabe and Peloquin 1991). The occurrence of 2n gametes in comparable frequencies in both diploids and their putative polyploid descendants supports sexual polyploidization as a mechanism to form the polyploid species. Endosperm Balance Number governs the success of interspecific crosses and is a function of ploidy level because doubling the chromosome number, as is the case in 2n gametes, will double EBN and enable a successful cross between species that originally differed in EBN. Ortiz and Ehlenfeldt (1992) have suggested the origin of *S. demissum*, 6X (4EBN), from a cross between *S. verrucosum*, 2X (2EBN), and *S. stoloniferum*, 4X (2EBN). Such hybrid origins between morphological similar species may explain the great taxonomic difficulty in wild potatoes.

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