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Comparative Morphological Studies on Infraspecific Taxa of *Petunia integrifolia* (Hook.) Schinz et Thell. (Solanaceae)

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In order to study the overlap in the characters of infraspecific taxa of *Petunia integrifolia* sensu Wijsman (1982), we compared the morphology of reproductive organs of subsp. *integrifolia* var. *integrifolia*, var. *depauperata*, subsp. *inflata*, and subsp. *occidentalis*, as well as those of *P. littoralis*, for both plants in their native habitats and the cultivated ones. In their native habitat, all subspecies of *P. integrifolia* were separately distributed and were readily distinguished. No location where two or more taxa concomitantly occurred was found, though there were a few places where intermediate forms between var. *integrifolia* and var. *depauperata* occurred. Except for *P. littoralis*, all taxa studied were clearly discriminated by cluster and principal component analyses of 32 characters of reproductive organs, and overlap in the morphology was not detected. The results support the treatments of Fries (1911) rather than those of Wijsman (1982). The morphology of *P. littoralis* resembled that of var. *depauperata*.

Today, the genus *Petunia* sensu Ando and Hashimoto (1993) comprises ten species: *P. axillaris* (Lam.) Britton, Sterns et Poggenb.; *P. integrifolia* (Hook.) Schinz et Thell.; *P. reitzii* L. B. Sm. et Downs; *P. saxicola* L. B. Sm. et Downs; *P. scheideana* L. B. Sm. et Downs; *P. littoralis* L. B. Sm. et Downs; *P. exserta* Stehmann; *P. bonjardinensis* T. Ando et G. Hashim.; *P. altiplana* T. Ando et G. Hashim.; and *P. mantiqueirensis* T. Ando et G. Hashim.. The parental species of garden petunias, *P. axillaris* and *P. integrifolia*, are distributed in the region including Argentina, Bolivia, Brazil, Paraguay, and Uruguay (Wijsman 1982; Sink 1984), whereas the other species are endemic to southern Brazil (Ando et al. 1992; Ando and Hashimoto 1993, 1994).

Petunia integrifolia comprises four infraspecific taxa: subsp. *integrifolia* (Hook.) Wijsman with var. *depauperata* (R. E. Fr.) L. B. Sm. et Downs, subsp. *inflata* (R. E. Fr.) Wijsman, and subsp. *occidentalis* (R. E. Fr.) Wijsman.

Fries (1911) reported that *P. inflata* and *P. occidentalis* could be distinguished from *P. violacea* Lindl. (=*P. integrifolia*) by their inflexed pedicel during fruiting. *Petunia occidentalis* has smaller corollas, narrower corolla-tubes, and larger capsules than does *P. violacea*.

Wijsman (1982) reported that conditions of the pedicel during fruiting in *P. integrifolia* and *P. inflata*

are variable and overlapping, and concluded that separation between the two species is difficult. He also regarded *P. occidentalis* and var. *depauperata* as an extreme form of *P. integrifolia* and a variant growing in poor soil, respectively.

However, we have found that all subspecies of *P. integrifolia* are readily distinguished in the living state, and noted that their floral morphology was uniform without any intermediate form in their localities. We are also aware that the floral morphology of subsp. *occidentalis* seems to be rather unique in this genus though that of *P. littoralis* resembles that of var. *depauperata*.

In order to confirm our expectation, reproductive organs of *P. integrifolia* and *P. littoralis* were morphologically studied.

Materials and methods

Habitat observation The first author, Ando, observed all *Petunia* taxa except *P. exserta* in 685 locations in northern Argentina, southern Brazil, and Uruguay. In *P. integrifolia*, var. *integrifolia* was observed in 141 places; var. *depauperata*, in 21 places; subsp. *inflata*, in 84 places; and subsp. *occidentalis*, in 5 places. *Petunia littoralis* was observed in 6 locations.

Plant materials Thirty-eight specimens (listed in the Appendix) were selected. Among them, specimens from type localities and their adjacent regions were included.

Type specimen of var. *integrifolia* is not available, and the figure of Hooker (1831) was selected as the type by Wijsman (1982). Hooker (1831) had described this type from Rio Negro of Uruguay, from which location three specimens were selected in this study.

For subsp. *inflata*, originally described from Paraguay and Misiones of Argentina (Fries 1911), one specimen from Paraguay and three specimens from Misiones including one from the exact type locality (Ando & Buto A7) were selected.

For subsp. *occidentalis* described from Jujuy Province of Argentina and its adjacent regions, we used five specimens from the type locality.

Var. *depauperata* was described based on the collections from eastern Santa Catarina and Rio Grande do Sul States of Brazil. One specimen from the exact type locality in Santa Catarina (Ando & Buto B222) was used for this study.

In order to study the overlap in the morphological characters, we selected other specimens to cover the total range.

Seeds obtained from the respective specimens were sown in January and the plants were grown in a greenhouse by the standard horticultural procedure for garden petunias. The characters of reproductive organs were measured in May and June.

Type and other specimens used in this study were listed in a previous paper (Ando and Hashimoto 1993).

Characters of reproductive organs As shown in Table 1, thirty-three characters of reproductive organs were measured for five randomly selected flowers on the upper part of the lateral shoots of each plant. The flowers were pollinated by pollen of different plants from the same source, and the angle of pedicel to stem and the size of capsule were measured in the mature capsules.

The mean values of the measurements were used as variables for multivariate analyses conducted by the Computer Center of Chiba University as follows:

Cluster analysis: The cluster analysis was performed by use of BMDP-2M (Biomedical Computer Programs-2M). Euclid distances were calculated from the standardized data of the characters.

Principal component analysis: The principal component analysis was performed by use of BMDP-7M (Biomedical Computer Programs-7M). For derivation of the principal components, a correlation matrix of all characters was used as an information matrix.

Table 1. Morphological characters of reproductive organs of infraspecific taxa of *Petunia integrifolia* and *P. littoralis*, and their abbreviations, examined in this study

Abbreviation	character
X_1	distance between anthers of long and medium stamens
X_2	distance between anthers of medium and short stamens
X_3	distance between two anthers of long stamens
X_4	distance between two anthers of medium stamens
X_5	length of long stamens
X_6	length of basal part of filament affixed to corolla-tube
X_7	proportion of the length of basal part of filament affixed to corolla-tube to whole length of the filament; $(X_6/X_5)\times100$
X_8	length of pistil plus ovary
X_9	length along vertical axis of stigma
X_{10}	length along horizontal axis of stigma
X_{11}	ratio X_9/X_{10}
X_{12}	length of upper calyx-lobe
X_{13}	width of upper calyx-lobe at the central part
X_{14}	length of calyx-tube
X_{15}	ratio X_{12}/X_5
X_{16}	length along long axis of ovary
X_{17}	length along horizontal axis of ovary
X_{18}	length along vertical axis of ovary
X_{19}	ratio X_6/X_{16}
X_{20}	length of pedicel
X_{21}	length of corolla-tube
X_{22}	ratio X_8/X_{21}
X_{23}	ratio X_{12}/X_8
X_{24}	diameter of corolla-tube
X_{25}	length of corolla
X_{26}	width of corolla-limb
X_{27}	ratio X_{25}/X_{26}
X_{28}	number of node bearing the first flower (main stem)
X_{29}	number of node bearing the first flower (side stem)
X_{30}	length of capsule
X_{31}	width of capsule
X_{32}	ratio X_{30}/X_{31}
X_{33}	angle of pedicel to stem during fruiting

To investigate the morphological relationships of reproductive organs, we calculated each component score based on vectors and normalized variables for every character.

Colorimetric determination of corolla lobes Colors of corolla-lobes were measured with a colorimetric meter (Minolta CR-300), and were expressed according to Hunter's L, a, b system (Hunter 1958).

Results

Observations on habitats Var. *integrifolia* was found in central Rio Grande do Sul and northwestern Uruguay. Subsp. *inflata* occurred in rather northern regions such as northern Rio Grande do Sul, western Santa Catarina, Misiones, and Paraguay. We confirmed that subsp. *occidentalis* was endemic to the Andes region and isolated from the other taxa of *P. integrifolia* (Wijsman 1982). We did not find intermediate forms between any two of the taxa mentioned above. There was no location where two or more of such taxa concomitantly occurred.

Var. *depauperata* was evidently concentrated on

the Atlantic sea coast, such as in the Santa Catarina and Rio Grande do Sul States, Brazil. It also occurred in the eastern littoral zones in Uruguay.

In a few cases, it was difficult to determine whether the plant was var. *integrifolia* or var. *depauperata* in the habitat in eastern Rio Grande do Sul State, where the former grows inland and the latter occurs near the sea coast. The few intermediate forms encountered were excluded from the following study.

Subsp. *occidentalis* always possessed inflexed pedicels. In subsp. *inflata*, the pedicels borne on the upper position of the stem were evidently inflexed during fruiting, but those on the lower position of the main stem were often deflexed to some extent. Evidently, var. *integrifolia* and var. *depauperata* had deflexed pedicels during fruiting, but the degree of deflection was variable. In an extreme case, it stood off at an angle of up to 90°.

As described by Smith and Downs (1966), *Petunia littoralis* was found in the bare sand dunes on Santa Catarina Island, and had distinct features in vegetative organs such as glabrous stems and narrow lanceolate, glabrous, and fleshy leaves. However, the floral morphology and corolla color of *P. littoralis* were not different from those of var. *depauperata*. All plants observed had deflexed pedicels when fruiting, though Smith and Downs (1966) stated that the pedicels after flowering were not at all deflexed.

In its native habitat, subsp. *inflata* is easily distinugishable from subsp. *integrifolia* by the differences in corolla color, as mentioned later.

T-test of 33 morphological characters Among the 33 morphological characters measured, no differences between var. *depauperata* and *P. littoralis* were significant by the t-test at the 1% level (Table 2). In contrast, there were a considerable number of characters which were significantly different among the remaining taxa.

Pedicel condition The inflexed or deflexed condition of the pedicel was expressed by the angle of the pedicel to the stem during fruiting (character X_{33}). An angle of less than 90° was regarded as indicating on inflexed condition, and that of more than 90°, as defining the deflexed one.

The angle of the pedicel during fruiting was stable and different in the infraspecific taxa, when they were grown under similar conditions (Table 2). Var. *integrifolia* and var. *depauperata* displayed deflexed pedicels when fruiting while subsp. *inflata* and subsp. *occidentalis* showed inflexed ones at that time.

Cluster analysis As it may be valuable to perform cluster analysis with character X_{33} (angle of pedicel to the stem during fruiting) eliminated to find additional characters distinguishing infraspecific taxa of *P. integrifolia*, we used the remaining 32 characters as variables for cluster analysis, and obtained the dendrogram as shown in Fig. 1. Even though subsp. *integrifolia*, subsp. *inflata* and subsp. *occidentalis* formed respective clusters, var. *depauperata* and *P. littoralis* made another cluster. When character X_{33} was added, the principal structures of the dendrogram were not different from those of Fig. 1.

Principal component analysis Principal component (PC) analysis was also performed on the 32 variables (character X_{33} excluded). Factor loadings and contributions of the first to fourth PCs are shown in Table 3. A scatter diagram of the plants from each location in the first and second PC planes is shown in Fig. 2. Total contribution of the first four PCs came to 77.1% of the whole variation (Table 3). Since the initial first and second PCs explain 55.5% of the whole variation, the scatter diagram for two component scores of plants is convenient to investigate their morphological relationships.

As shown in Fig. 2, var. *integrifolia*, subsp. *inflata*, and subsp. *occidentalis* were separately distributed in the planes. Variety *depauperata* and *P. littoralis*, however, combined again into the same group like in the cluster analyses (Fig. 1) and were not divided into two groups in the scatter diagrams of any combina-

Table 2. Mean values of morphological characters of reproductive organs of infraspecific taxa of *Petunia integrifolia* and *P. littoralis*

Taxa	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
P. integrifolia									
subsp. *integrifolia*	mm	mm	mm	mm	mm	mm	%	mm	mm
var. *integrifolia*	3.94 a	1.85 a	1.22 a	1.40 a	18.5 a	7.25 b	39.3 b	16.0 a	1.43 a
var. *depauperata*	2.95 b	1.49 b	1.00 ab	0.93 bc	17.0 b	6.66 b	39.2 b	15.3 a	1.13 b
subsp. *inflata*	3.41 b	1.78 ab	0.88 b	1.05 bc	15.0 c	4.53 c	30.1 c	13.1 b	0.93 c
subsp. *occidentalis*	1.12 c	1.50 b	1.16 ab	1.17 ab	16.9 b	9.59 a	56.8 a	16.3 a	1.35 a
P. littoralis	3.15 b	1.61 ab	0.91 ab	0.73 c	17.7 ab	6.52 b	37.3 b	16.0 a	1.30 ab

Taxa	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}
P. integrifolia								
subsp. *integrifolia*	mm		mm	mm	mm		mm	mm
var. *integrifolia*	1.55 a	0.91 b	15.5 a	2.03 ab	2.17 b	0.83 a	2.54 b	1.35 b
var. *depauperata*	1.22 b	0.95 b de	10.4 c	1.71 c	2.81 a	0.62 b	2.34 bc	1.31 b
subsp. *inflata*	1.23 b	0.76 c e	13.6 b	1.91 bc	1.27 c	0.91 a	2.25 c	1.12 c
subsp. *occidentalis*	1.08 b	1.26 a	15.0 ab	2.25 a	3.23 a	0.89 a	3.47 a	1.52 a
P. littoralis	0.97 b	1.35 a d	10.7 c	1.74 bc	2.90 a	0.61 b	2.33 bc	1.27 b

Taxa	X_{18}	X_{19}	X_{20}	X_{21}	X_{22}	X_{23}	X_{24}	X_{25}	X_{26}
P. integrifolia									
subsp. *integrifolia*	mm		mm	mm			mm	mm	mm
var. *integrifolia*	1.48 b	2.88 a	22.0 a	23.4 a	0.68 b	0.97 a	10.8 a	31.8 a	38.2 a
var. *depauperata*	1.51 b	2.88 a	19.9 a	19.8 b	0.77 a	0.69 b	10.6 a	29.2 ab	31.0 b
subsp. *inflata*	1.40 b	2.05 b	22.6 a	20.2 b	0.65 b	1.04 a	8.6 b	26.8 bc	34.6 a
subsp. *occidentalis*	1.76 a c	2.78 a	24.3 a	21.7 ab	0.76 a	0.92 a	7.4 c	25.4 c	22.2 c
P. littoralis	1.42 bc	2.82 a	29.5 a	20.3 b	0.79 a	0.67 b	9.8 ab	28.1 abc	32.3 ab

Taxa	X_{27}	X_{28}	X_{29}	X_{30}	X_{31}	X_{32}	X_{33}
P. integrifolia							
subsp. *integrifolia*				mm	mm		°
var. *integrifolia*	0.84 bc	24.5 a	16.5 a	5.52 c	4.05 b	1.36 c	120 b
var. *depauperata*	0.94 b	26.1 a	19.0 a	6.18 b	3.74 bc	1.67 b	144 a
subsp. *inflata*	0.78 c	25.6 a	13.4 b	4.99 d	3.44 c	1.45 c	58 c
subsp. *occidentalis*	1.15 a	23.9 a	15.0 ab	9.70 a	5.02 a	1.94 a	44 d
P. littoralis	0.87 bc	22.8 a	17.2 a	7.01 b	3.93 bc	1.79 ab	142 a

Different letters within columns indicate significant differences by t-test at 1% level.

tions of PC planes (figure not shown).

In relation to the first PC, characters of var. *integrifolia* were regarded as rather similar to those of var. *depauperata*, such that this component was considered as a good discriminator among three groups, that is, subsp. *integrifolia* (including var. *depauperata*), subsp. *inflata*, and subsp. *occidentalis*.

As shown in Table 3, the first PC was highly and positively correlated with the characters like length and proportion of basal part of the filament affixed to the corolla-tube (X_6 and X_7), size and shape of fruit capsule (X_{30}, X_{31} and X_{32}), length of calyx-tube (X_{14}), size of ovary (X_{16}, X_{17} and X_{18}), ratio of length of corolla to width of corolla-limb (X_{27}), and ratio of the length along the vertical axis of the stigma to the length along the horizontal axis of the stigma (X_{11}).

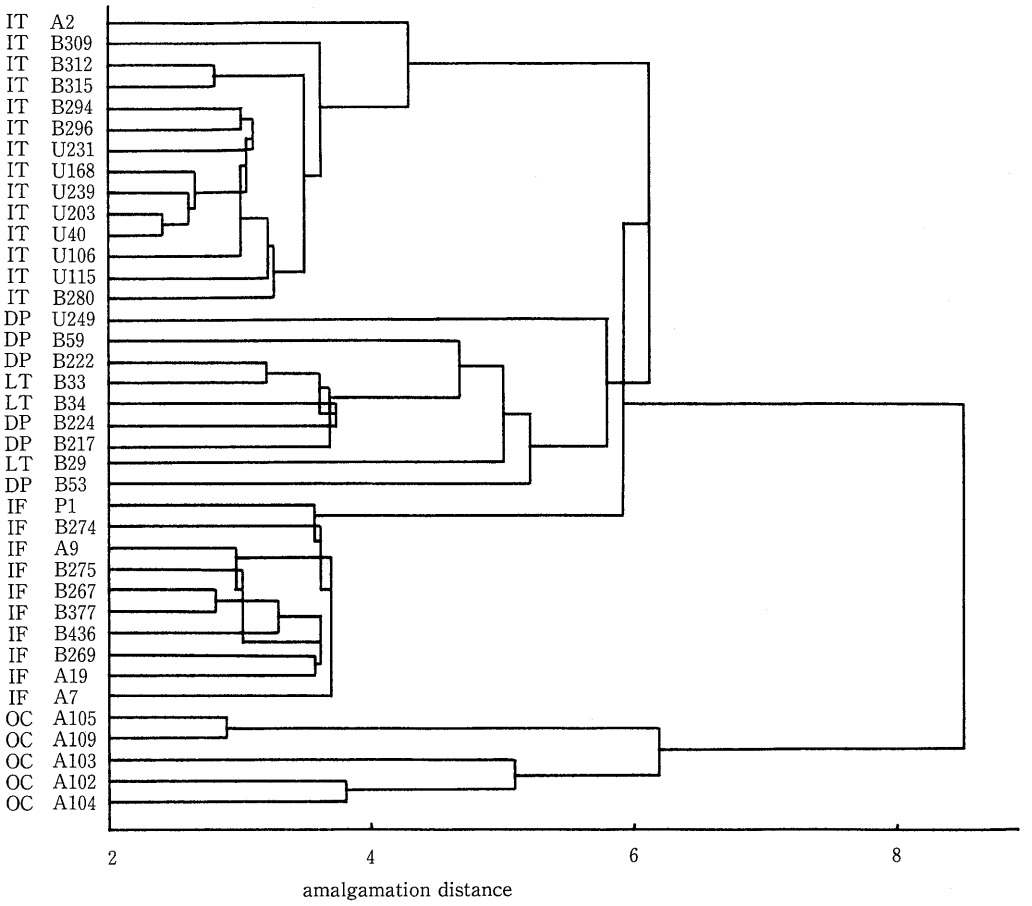

Fig. 1. Cluster analysis using 32 characters (character X_{33} [angle of pedicel to stem during fruiting] eliminated) representing morphology of reproductive organs of infraspecific taxa of *Petunia integrifolia* and *P. littoralis*. IT: subsp. *integrifolia* var. *integrifolia*. DP: subsp. *integrifolia* var. *depauperata*. IF: subsp. *inflata*. OC: subsp. *occidentalis*. LT: *P. littoralis*.

However, this PC was highly but negatively correlated with the distance between anthers of long and medium stamens (X_1).

In relation to the second PC, var. *integrifolia* occupied an isolated position, and this PC was considered as a factor distinguishing var. *integrifolia* from the other taxa.

This component was highly and positively correlated with characters like the length along the horizontal axis of the stigma (X_{10}), length of corolla-tube (X_{21}), length of long stamens (X_5), distance between two anthers of medium stamens (X_4), distance between two anthers of long stamens (X_3), and length of the corolla (X_{25}).

Corolla color As shown in Table 4, the differences in color of corolla lobes among var. *integrifolia*, var. *depauperata*, and *P. littoralis* were not significant by t-test at 1% level.

Significant differences in the 'L' values among remaining taxa indicated that the corolla color of subsp. *inflata* is darker and that of subsp. *occidentalis* is lighter than that of subsp. *integrifolia*. The differences in the 'a' value showed that the color of subsp. *inflata* is more reddish and that of subsp. *occidentalis* is less reddish than that of subsp. *integrifolia*. The difference in the 'b' values indicated that the color of

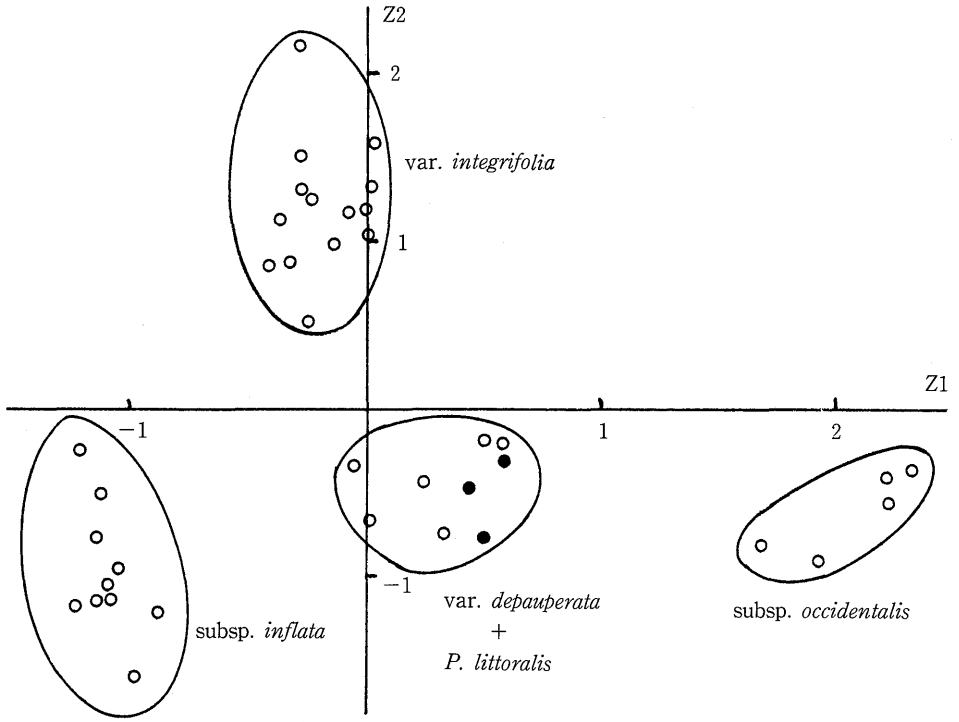

Fig. 2. Scatter diagram of infraspecific taxa of *Petunia integrifolia* and *P. littoralis* from each location in the first (Z1) and second (Z2) principal component planes. Closed circles: *P. littoralis*.

subsp. *inflata* is more bluish than that of subsp. *integrifolia*. The value of $\sqrt{a^2+b^2}$ was also significantly different; that is, subsp. *inflata* was brighter and subsp. *occidentalis* was duller than subsp. *integrifolia*.

Basal part of filament affixed to the corolla-tube

Differences in the length of the basal part of the filament affixed to the corolla-tube (X_6) and its proportion to the whole length of the filament (X_7) were significant among var. *integrifolia*, subsp. *inflata*, and subsp. *occidentalis* (Table 2, Fig. 3). The first PC was most highly correlated with these characters (Table 3). As shown in Table 2, X_6 of subsp. *inflata* was the shortest and only 30% of the whole filament length; whereas that of subsp. *occidentalis* was the longest and nearly 60% of the filament length. The lengths of the affixed basal part of the filament in var. *integrifolia*, var. *depauperata*, and *P. littoralis* were intermediate, and the proportions were about 40%.

The basal part of the corolla-tube where the filament-base was affixed at the inside was narrow in subsp. *occidentalis*. This subspecies was characterized by a corolla-tube with a long, cylindrical basal half (Figs. 3D, 4D), as reported by Fries (1911).

It is also useful to note about the ratio of length of basal part of the filament affixed to the corolla-tube to the length along the long axis of the ovary (X_{19}) (Table 2). In var. *inflata*, the length of the affixed basal part of the filament was about double the ovary length. It was about 2.8 times the ovary length in the other taxa studied.

Conditions of stamens All taxa studied had didynamous stamens. The stigma was always located between the anthers of long and medium stamens (Fig. 3). The degree of the didynamous condition, which can be indicated by the distance between an-

Table 3. Factor loadings and contributions of the first four principal components (PCs)

characters	factor loading			
	1st. PC	2nd. PC	3rd. PC	4th. PC
X_1	–0.726 *	0.513 *	–0.337	–0.066
X_2	–0.251	0.379	0.347	–0.263
X_3	0.236	0.703 *	0.087	–0.188
X_4	–0.021	0.719 *	0.234	–0.069
X_5	0.418	0.722 *	–0.431	–0.042
X_6	0.924 *	0.336	0.052	0.029
X_7	0.942 *	0.113	0.202	0.039
X_8	0.747 *	0.496 *	–0.321	–0.019
X_9	0.512 *	0.611 *	–0.120	–0.151
X_{10}	–0.312	0.827 *	0.017	0.241
X_{11}	0.754 *	–0.196	–0.150	–0.357
X_{12}	0.051	0.624 *	0.659 *	–0.031
X_{13}	0.272	0.396	0.539 *	–0.154
X_{14}	0.874 *	–0.038	–0.305	0.019
X_{15}	–0.280	0.225	0.858 *	–0.029
X_{16}	0.796 *	0.113	0.486 *	0.110
X_{17}	0.831 *	0.351	0.086	0.042
X_{18}	0.717 *	0.070	0.341	0.379
X_{19}	0.637 *	0.382	–0.497 *	–0.099
X_{20}	0.075	–0.157	0.101	0.002
X_{21}	0.183	0.823 *	0.115	0.245
X_{22}	0.693 *	–0.274	–0.509 *	–0.272
X_{23}	–0.458 *	0.292	0.763 *	–0.035
X_{24}	–0.176	0.557 *	–0.657 *	0.067
X_{25}	–0.146	0.705 *	–0.445 *	–0.054
X_{26}	–0.623 *	0.583 *	–0.310	–0.099
X_{27}	0.794 *	–0.217	0.193	0.033
X_{28}	–0.106	0.005	0.029	0.913 *
X_{29}	0.181	0.116	–0.313	0.846 *
X_{30}	0.916 *	–0.225	0.214	0.004
X_{31}	0.764 *	0.215	0.281	–0.099
X_{32}	0.738 *	–0.532 *	–0.016	0.086
Contribution (%)	34.4	21.1	14.6	7.0

Character X_{33} was eliminated from the calculation.
*significant at 1% level.

thers of long and medium stamens (character X_1), was obviously small in subsp. *occidentalis* compared with that of the other taxa (Fig. 3D).

In subsp. *occidentalis*, the value for character X_1 was so small that these anthers often contacted one another; the stigma was sandwiched by and made contact with the anthers (Fig. 3D). The remaining taxa had strongly didynamous stamens, and the anthers of long and medium stamens were clearly separated by considerable distances such that the stigma was hardly in contact with any anthers (Figs. 3A, 3B, 3C).

Stigma Stigmata of subsp. *occidentalis* were unique in being vertically bilobed in contrast to those of the remaining taxa, which displayed rather knuckle-shaped ones. Significant differences in the ratio of the length along the vertical axis to the length along the horizontal axis of the stigma (X_{11}) were also found between subsp. *occidentalis* and the remaining taxa of

Table 4. Color of corolla-lobes in the infraspecific taxa of *Petunia integrifolia* and *P. littoralis* according to Hunter's system

Hunter's system	*Petunia integrifolia*				*Petunia littoralis*
	subsp. *integrifolia*		subsp. *inflata*	subsp. *occidentalis*	
	var. *integrifolia*	var. *depauperata*			
L	45.2	43.7	37.2 *	66.3 *	42.8
a	61.1	63.1	69.3 *	36.5 *	63.6
b	−16.3	−17.9	−24.2 *	−16.4	−18.0
a/b	−3.3	−3.6	−3.0	−2.3	−3.6
$\sqrt{a^2+b^2}$	63.3	65.6	73.5 *	40.1 *	66.2

*significantly different from var. *integrifolia* by t-test at 1% level.

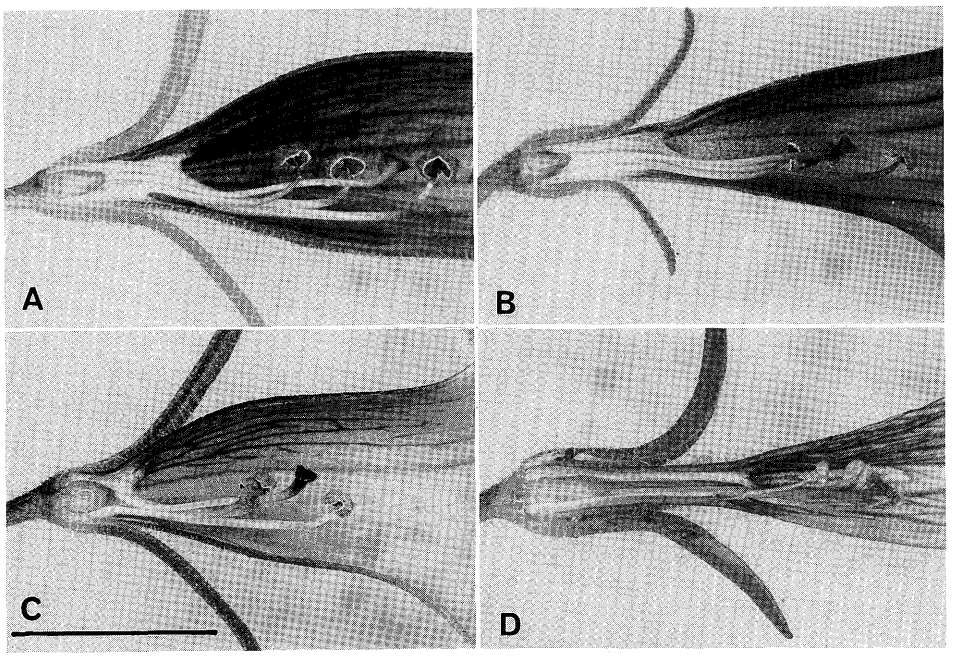

Fig. 3. Side view of inside of corolla in four infraspecific taxa of *Petunia integrifolia*. A. Subsp. *integrifolia* var. *integrifolia*. B. Subsp. *integrifolia* var. *depauperata*. C. Subsp. *inflata*. D. Subsp. *occidentalis*. Two calyx lobes, half of the corolla, and two stamens were removed. Scale bar = 10 mm.

P. integrifolia. The ratio was large in *P. littoralis* because a few of the plants from the same source had extremely narrow stigmata (Table 2).

Ovary and capsule The size of the ovary (X_{16} and X_{17}) and that of the capsule (X_{30} and X_{31}) were significantly different among var. *integrifolia*, subsp. *inflata*, and subsp. *occidentalis*: the smallest was in subsp. *inflata*; and the largest, in subsp. *occidentalis* (Table 2).

The shape of the capsule was also different. In subsp. *occidentalis*, it was a long ovoid; whereas in the others, it was a short ovoid.

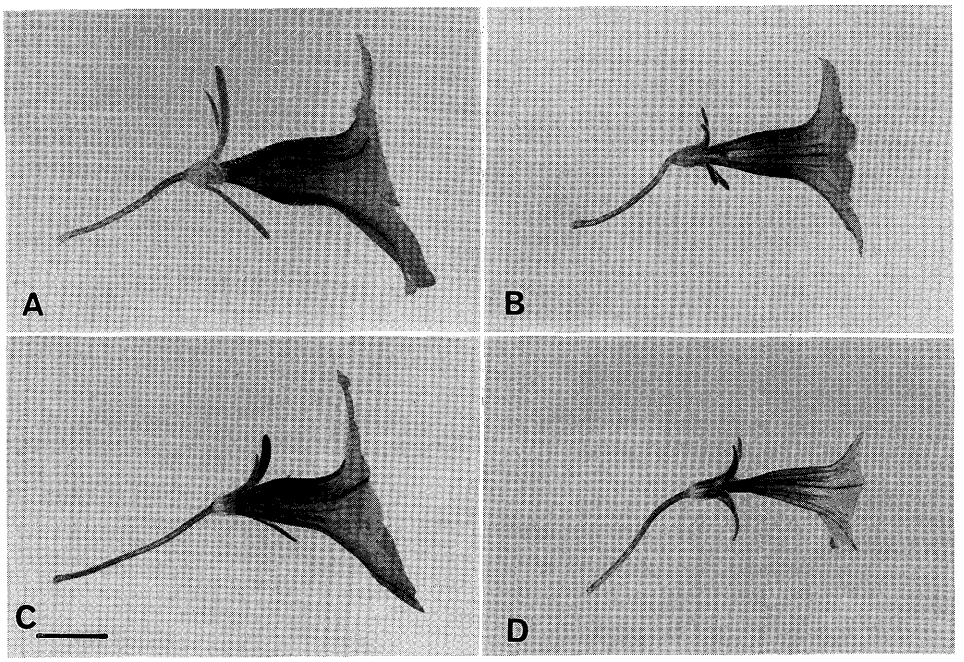

Fig. 4. Side view of corolla in four infraspecific taxa of *Petunia integrifolia*. A. Subsp. *integrifolia* var. *integrifolia*. B. Subsp. *integrifolia* var. *depauperata*. C. Subsp. *inflata*. D. Subsp. *occidentalis*. Scale bar = 10 mm.

Characters of flower Var. *integrifolia* had the largest flower; and subsp. *occidentalis*, the smallest (Figs. 4, 5). As shown in Figs. 4 and 5, the flower shape was different among the taxa. Var. *depauperata* was not different from var. *integrifolia*, though the flowers and calyx-lobes were considerably smaller (Figs. 4A, 4B, 5A, and 5B).

The corolla lobes of var. *integrifolia* and var. *depauperata* were roundish (Fig. 5), whereas those of subsp. *inflata* were triangular, which made the front view somewhat like a regular pentagon. The front view of subsp. *occidentalis* was star-like since its corolla lobes were deeply split.

Flower longevity The flower of subsp. *occidentalis* faded on the next day; that is, it lasted only one day. This feature was unique in this genus, because the flowers of the other species we observed lasted several days after flowering.

Annual versus perennial state Under the cultivated condition, var. *integrifolia*, var. *depauperata* and *P. littoralis* were perennial, whereas subsp. *occidentalis* was evidently annual. Subspecies *inflata* was intermediate; that is, some lived but some died after fruit ripening. These features agree with Fries' (1911), though he supposed subsp. *inflata* to be an annual.

Discussion

Based on his herbarium study, Wijsman (1982) stated, "Obviously, there is (clinal) overlap in the characters of the various taxa (of *P. integrifolia*). Their status as subspecies can be questioned". He regarded subsp. *inflata* and subsp. *occidentalis* as extreme forms of subsp. *integrifolia*, and var. *depauperata* as "a mere variant grown in poor soil". In addition, Smith and Downs (1966) treated subsp. *inflata* as a synonym of subsp. *integrifolia*.

Actually, we encountered a few locations in east-

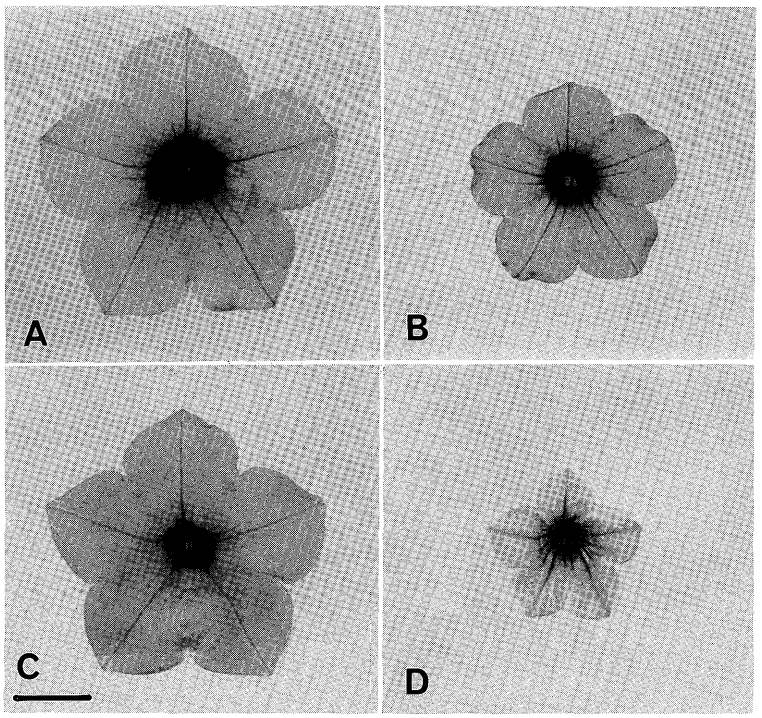

Fig. 5. Front view of corolla in four infraspecific taxa of *Petunia integrifolia*. A. Subsp. *integrifolia* var. *integrifolia*. B. Subsp. *integrifolia* var. *depauperata*. C. Subsp. *inflata*. D. Subsp. *occidentalis*. Scale bar = 10 mm.

ern Rio Grande do Sul State, Brazil, where intermediate forms between var. *integrifolia* and var. *depauperata* were found. Except for these, however, we have never found locations where any intermediate forms between any two of remaining taxa of *P. integrifolia* occurred.

Except in the case mentioned above, it is easy to distinguish all infraspecific taxa of *P. integrifolia* from each other in their native habitat. The distribution areas are well delimited, and we could not detect overlap in the characters between any two taxa of *P. integrifolia* except in the case mentioned above.

Wijsman (1982) concentrated much on pedicel conditions. Actually, this character is fairly variable in the native habitats. When infraspecific taxa of *P. integrifolia* were grown under similar conditions, however, the pedicel condition during fruiting (X_{33}) became stable and was significantly different among the four taxa (Table 2).

We found a considerable number of characters of reproductive organs to be different among the taxa (Table 2). The results of the cluster analysis (Fig. 1) and PC analysis (Table 3 and Fig. 2) well agreed, and all infraspecific taxa of *P. integrifolia* were clearly discriminated even if the character representing the pedicel condition (X_{33}) was eliminated from the calculation. It is thus reasonable to say that the floral structure is uniform in each taxon of *P. integrifolia* and that no overlap in the character at least between any two subspecies of *P. integrifolia* is detectable.

Subsp. *occidentalis* has rather unique characters such as weakly didynamous stamens, a very long basal part of the filament affixed to the corolla-tube (Fig. 3D), a corolla-tube with a long cylindrical base

(Fig. 4D), a large ovary (Fig. 3D), and a large capsule (Table 2). It has the smallest corolla lobe (Fig. 5D) and the shortest period of flower longevity found in this genus.

Variety *depauperata* persisted in displaying a growth habit of prostrated stems with smaller and narrower leaves even when it was cultivated in rich soil. Therefore, such morphology of var. *depauperata* is an innate one, not "a mere variant grown in poor soils" (Wijsman 1982). Even though var. *depauperata* has a small corolla-limb and short calyx-lobes, the overall corolla shapes are not different from those of var. *integrifolia* (Figs. 4, 5). The color of the corolla is not different, either (Table 4).

Our results support those of Fries (1911), who recognized subspp. *inflata* and *occidentalis* as distinct species, and var. *depauperata* as a subspecies of *P. integrifolia*.

Possible overlapping distribution is suggested by the existence of intermediate forms between var. *integrifolia* and var. *depauperata*. *Petunia littoralis* should be united to P. *integrifolia* by resembling floral morphology, especially to var. *depauperata*.

This work could not have been accomplished without the kind assistance of Mr. Masao Udagawa of Montevideo, Uruguay, and Mr. Sebastião T. Nagase, Mr. Nobuyuki Hiranaka, and Mr. Tomio Koshizawa of São Paulo, Brazil, in surveying the habitats of *Petunia*.

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Appendix of Specimens Citations

Seeds were obtained from the following specimens.

Petunia integrifolia (Hook.) Schinz et Thell. subsp. **integrifolia** (Hook.) Wijsman var. **integrifolia.**

ARGENTINA. Entre Rios: Parque San Carlos, Concordia, 11 Dec. 1990, T. Ando & H. Kokubun A2 (SI, Ando). BRAZIL. Rio Grande do Sul: Mun. CACHOEIRA DO SUL: Route BR153, 22.1 Km N. of the junction of route BR290 and BR153 (30°04'37"S 52°52'32"W), 28 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B315 (Ando, Hashimoto). Mun. ROSARIO DO SUL: Route BR290, 27.9 Km N.W. of Rosário do Sul (30°09'13"S 55°11'18"W), 28 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B309 (MVFA, Ando, Hashimoto). Mun. SANTA MARIA: Route BR392, 21.6 Km SSW of the entrance of Santa Maria (29°53'25"S 53°43'53"W), 25 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B280 (Ando, Hashimoto). Mun. SANTANA DO LIVRAMENTO: Route BR293, 15.5 Km W. of the junction of route BR293 and BR158 (30°49'33"S 55°20'15"W), 27 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B294 (MVFA, Ando, Hashimoto); Route BR293, 3.1 Km N.W. of the entrance of Santana do Livramento (30°51'12"S 55°31'50"W), 27 Nov. 1991. G. Hashimoto, T. Ando & S. Iida B296 (Ando, Hashimoto). Mun. SÃO GABRIEL: Route BR290, 13.4 Km E. of São Gabriel (30°21'51"S 54°12'12"W), 28 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B312 (Ando, Hashimoto). URUGUAY. Dep. FLORES: Route 3, 1.9 Km N.W. of Andresito, 13 Nov. 1990, T. Ando & K. Buto U168 (MVFA, SI, Ando, Hashimoto). Dep. RIO NEGRO: Route 2, 1.6 Km N.W. of the bridge over Rio Negro to Fray Bentos (33°13'28"S 58°01'31"W), 28 Nov. 1989, T. Ando & H. Watanabe U106 (MVFA, S, SI, Ando, Hashimoto); Arrayanes (33°14'19"S 58°01'59"W), 21 Nov. 1990 T. Ando & K. Buto U203 (MVFA, S, SI, Ando, Hashimoto); Route 3, 1 Km N.W. of the bridge over the river Negro (33°07'46"S 57°10'19"W), 4 Nov. 1991, T. Ando & S. Iida U239 (MVFA, Ando). Dep. SALTO: Route 3, entrance to Termas del Arapey (30°54'27"S 57°41'37"W), 3 Nov. 1991, T. Ando & S. Iida U231 (MVFA, Ando). Dep. TACUAREMBÓ: Route 59, 4.1 Km S. E. of the junction of route 5 and 59, 23 Nov. 1988, T. Ando & H. Kokubun U40 (MVFA, Ando); Route 26, 2.6 Km W. of the junction of route 5 and route 26, 30 Nov. 1989, T. Ando & H. Watanabe U115b (MVFA, Ando, Hashimoto).

Petunia integrifolia (Hook.) Schinz et Thell. subsp. **integrifolia** (Hook.) Wijsman var. **depauperata** (R. E. Fr.) L. B. Sm. et Downs.

BRAZIL. Rio Grande do Sul: Mun. PALMARES DO SUL: 11 Km S. of Capivari to Palmares do Sul, 7 Nov. 1988, G. Hashimoto, T. Ando & H. Kokubun B53 (Ando, Hashimoto). Mun. SÃO LOURENCO DO SUL: São Lourenco do Sul, 8 Nov. 1988, G. Hashimoto, T. Ando & H. Kokubun B59 (MVFA, SI, Ando, Hashimoto). Santa Catarina: Mun. GAROPABA: 2.5 Km S. of Campo Duna, 5 Nov. 1990, G. Hashimoto, T. Ando & K. Buto B222 (MVFA, SI, Ando, Hashimoto); 7.0 Km S. of Campo Duna, 5 Nov. 1990, G. Hashimoto, T. Ando & K. Buto B224 (MVFA, Ando, Hashimoto). Mun. IMBITUBA: Itapirubá, 5 Nov. 1990, G. Hashimoto, T. Ando & K. Buto B217 (MVFA, Ando, Hashimoto). URUGUAY. Dep. ROCHA: Playa del Barco (33°59'59"S 53°32'12"W), 7 Nov. 1991, T. Ando & S. Iida U249 (MVFA, Ando).

Petunia integrifolia (Hook.) Schinz et Thell. subsp. **inflata** (R. E. Fr.) Wijsman.
ARGENTINA. Misiones: Parque Central, Bonpland, 27 Nov. 1990, T. Ando & K. Buto A7 (SI, Ando, Hashimoto); Route 103, 1.6 Km E. of the junction of route 4 and 103, 28 Nov. 1990, T. Ando & K. Buto A9 (MVFA, SI, Ando, Hashimoto); Posadas Airport, 2 Dec. 1990, T. Ando & K. Buto A19 (Ando). BRAZIL. Rio Grande do Sul: Mun. CARAZINHO: Route BR285, 19.8 Km W. of Carazinho, 350 m, 24 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B267 (Ando, Hashimoto). Mun. CRUZ ALTA: 21 Km S.E. of Ijuí to Cruz Alta (28°32'30"S 53°45'53"W), 25 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B275 (MVFA, Ando, Hashimoto). Mun. PANAMBI: Route BR285, 6.1 Km W. of the junction of route BR285 and BR158, 24 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B269 (MVFA, Ando, Hashimoto). Mun. SANTO ANGELO: São Miguel das Missões (28°32'55"S 54°33'36"W), 24 Nov. 1991, G. Hashimoto, T. Ando & S. Iida B274 (Ando, Hashimoto). Santa Catarina: Mun. ITAPIRANGA: Itapiranga (27°10'52"S 53°43'10"W), 25 Nov. 1992, G. Hashimoto, T. Ando, K. Shibata & M. Kurata B377 (Ando, Hashimoto). Mun. CAMPOS NOVOS: Route BR470, 14 Km E. of Campos Novos (27°22'51"S 51°05'58"W), 29 Nov. 1992, G. Hashimoto, T. Ando, K. Shibata & M. Kurata B436 (MVFA, S, SI, Ando, Hashimoto). PARAGUAY. Route 1, Itá, 28 July 1990, T. Ikemizu & T. Ando P1 (Ando).

Petunia integrifolia (Hook.) Schinz et Thell. subsp. **occidentalis** (R. E. Fr.) Wijsman.
ARGENTINA. Jujuy: Route 19, 8 Km S.W. of the junction of route 34 and 19 to Higueritas (23°56'09"S 64°51'39"W), 14 Nov. 1991, T. Ando & S. Iida A102 (Ando); Route 19, 16 Km S.W. of the junction of route 34 and 19 to Higueritas (23°56'56"S 64°56'11"W), 14 Nov. 1991, T. Ando & S. Iida A103 (SI, Ando); Route 34, entrance of Caimancito (23°43'56"S 64°36'37"W), 14 Nov. 1991, T. Ando & S. Iida A104 (SI, Ando); San Salvador de Jujuy (24°10'10"S 65°22'04"W), 15 Nov. 1991, T. Ando & S. Iida A105 (SI, Ando, Hashimoto); beach of the river Xibi Xibi, San Salvador de Jujuy (24°11'26"S 65°17'58"W) T. Ando & S. Iida A109 (MVFA, S, SI, Ando, Hashimoto).

Petunia littoralis L. B. Sm. et Downs.
BRAZIL. Santa Catarina: Mun. FLORIANOPOLIS: Route SC401, 10 Km E. of the entrance of Jureré, 3 Nov. 1988. G. Hashimoto, T. Ando & H. Kokubun B29 (MVFA, Ando, Hashimoto); Praia Mole, 3 Nov. 1988, G. Hashimoto, T. Ando & H. Kokubun B33 (MVFA, SI, Ando, Hashimoto); Praia do Campeche, 3 Nov. 1988, G. Hashimoto, T. Ando & H. Kokubun B34 (MVFA, S, SI, Ando, Hashimoto).

安藤敏夫，倉田昌泰，佐々木幸子，上田善弘，ハシモト・ゴロウ，エドゥアルド・マルチェシー：

***Petunia integrifolia* の種内分類群の形態比較**

ペチュニア品種の片親である *Petunia integrifolia* の種内分類群（subsp. *integrifolia* var. *integrifolia*, subsp. *integrifolia* var. *depauperata*, subsp. *inflata*, subsp. *occidentalis*）を整理する目的で，自生地での観察に加え，育成個体の32形質を多変量解析（クラスター分析，主成分分析）に供した．var. *depauperata* に近縁と思われた *P. littoralis* も調査に加えた．4分類群は，別々の地域に分布しており，var. *integrifolia*，var. *depauperata* 間を除き，他の分類群間には中間型は認められなかった．多変量解析に於いて，var. *depauperata*, *P. littoralis* は一群を成したが，他の分類群の形質は明確に区別され，Wijsman (1982) の主張する亜種間の形質重複は認められなかった．*P. littoralis* の形質は var. *depauperata* に酷似していた．各種内分類群の分類学的地位が議論された．