●Note

RFLP data of chloroplast DNA in the genus *Petunia sensu* Jussieu (Solanaceae)

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The chloroplast DNA (cpDNA) of 52 taxa of *Petunia sensu* Jussieu and *Nicotiana langsdorffii* was digested by 19 restriction enzymes and hybridized with 12 cloned tobacco cpDNA fragments as probes. A total of 212 restriction fragment length polymorphisms (RFLP) were detected, of which 89 were phylogenetically informative.

Our previous research showed that the genus *Petunia sensu* Jussieu can be divided to *Petunia sensu* Wijsman and *Calibrachoa* using the RFLP of cpDNA. Here we describe detailed nature of the RFLP s and charted on a phylogenetic tree in order to use them as molecular markers to distinguish taxa of *Petunia sensu* Jussieu.

INTRODUCTION

The genus *Petunia* was established by Jussieu in 1803 [1], and Fries wrote the most recent formal generic treatment on *Petunia* using Jussieu's circumscription [2]. Hereafter, we refer to the genus *Petunia* circumscribed by Jussieu as *Petunia sensu* Jussieu. Later, Wijsman and de Jong [3] and Wijsman [4] divided *Petunia sensu* Jussieu into two genera, *Petunia* and *Calibrachoa* La Llave & Lex., based on morphology, chromosome count, and cross compatibility, and transferred 15 species of *Petunia sensu* Jussieu to *Calibrachoa.* We refer to the *Petunia* circumscribed by Wijsman [4] as *Petunia sensu* Wijsman. Subsequently, Stehmann and Semir [5] transferred nine additional species to *Calibrachoa* in a work that described *C. eglandulata* using the circumscription of Wijsman [4].

Recent studies indicated that *Petunia sensu* Wijsman and *Calibrachoa* can be distinguished based on cross-compatibility [6, 7] and seed surface morphology [8]. In another study [9], we studied phylogenetic relationship of taxa in *Petunia sensu* Jussieu based on the restriction fragment length polymorphism (RFLP) of chloroplast DNA (cpDNA). The results clearly indicated *Petunia sensu* Jussieu was divided into *Petunia sensu* Wijsman and *Calibrachoa,* which was the first molecular evidence to the separation of *Petunia sensu* Wijsman and *Calibrachoa.*

The present report is to provide detailed information on the RFLP used in the previous study [9], which was too voluminous to be published within that article, in order to serve as a guide for future molecular studies, such as developing molecular markers, on *Petunia sensu* Jussieu.

MATERIALS AND METHODS

Table 1 shows the taxa used in this study. These 52 taxa covers almost entire genus *Petunia sensu* Jussieu. One *Petunia sensu* Wijsman and some *Calibrachoa* specimens seemed to be undescribed taxa, and designated as unnamed taxon P1, C1, etc. The seeds were collected randomly from respective wild population, and raised in a greenhouse using standard cultivation techniques for garden petunias. Voucher specimens were deposited to appropriate herbaria that were indicated in the table as standard abbreviation [10]. For the detail of collection locality, see Watanabe et al. [6, 7].

Total DNA was extracted from ca. 5 g of fresh leaves by a modified CTAB method [11]. However, unidentified substance, possibly some polysaccharide, prevented the use of this method to many of the *Calibrachoa* species. For those, a method described by Lassner et al. [12] was used.

Ca. 1 μg of the extracted DNA was digested with 19 kinds of 4 to 6 base pair recognition endonuclease (*Alu*I, *Ava*I, *Ava*II, *Bam* HI, *Bcn*I, *Bgl*II, *Cla*I, *Dra*I, *Eco*O109I, *Eco*RI, *Eco*RV, *Eco*T22I, *Hae*III, *Hha*I, *Hin*cII, *Hin*dIII, *Sty*I, *Vsp*I, *Xba*I) under the condition specified by the producer of each enzyme. The recognition sequences of these enzymes were different from each other. DNA fragments were separated by electrophoresis in a 1.5% agarose gel and transferred onto the Hybond N+ nylon membrane (Amersham, Little Chalfont, Buckinghamshire, UK) by capillary blotting with 20xSSC.

Twelve contiguous clones of tobacco cpDNA (pTBa1, pTBa2, pTB7, pTB8, pTB10, pTB15, pTB19, pTB20, pTB22, pTB25, pTB28, pTB29 [13]) that covered 99.4% of total tobacco cpDNA sequence were used as probes to detect the RFLPs.

Table 1. Species and voucher specimens of *Petunia* sensu Wijsman and *Calibrachoa* taxa used in cpDNA RFLP analysis.

Taxa number	Botanical name, collection locality, voucher specimen number and herbaria
Petunia sensu Wijsman (1990), $2n=2x=14$.	
1	*P. altiplana* T. Ando & Hashim. BRAZIL: Rio Grande do Sul, Ando et al. B319 (S[a], US).
2	*P. axillaris* (Lam.) Britton, Sterns & Poggenb. subsp. *axillaris.* URUGUAY: Montevideo, Ando & Kokubun U1 (MVFA, Ando).
3	*P. axillaris* subsp. *parodii* (Steere) Cabrera. URUGUAY: Artigas, Ando & Kokubun U27 (MVFA, Ando)
4	*P. axillaris* subsp. *subandina* T. Ando. ARGENTINA: Salta, Ando & Iida A100 (S, SI, Ando)
5	*P. bajeensis* T. Ando & Hashim. BRAZIL: Rio Grande do Sul, Hashimoto et al. B662 (BM, MBM, MVFA, R, S, SI, SP, U, US, GHSP, Ando).
6	*P. bonjardinensis* T. Ando & Hashim. BRAZIL: Rio Grande do Sul, Ando et al. B174 (S, GHSP, Ando).
7	*P. exserta* Stehmann BRAZIL: Rio Grande do Sul, Hashimoto et al. B931 (GHSP).
8	*P. guarapuavensis* T. Ando & Hashim. BRAZIL: Paraná, Hashimoto et al. B65 (MBM, BM, HBR, S, US, GHSP, Ando).
9	*P. integrifolia* (Hook.) Schinz & Thell. subsp. *integrifolia* var. *integrifolia.* URUGUAY: Rio Negro, Ando & Watanabe U106 (MVFA, S, SI, GHSP, Ando).
10	*P. integrifolia* subsp. *integrifolia* var. *depauperata* (R.E.Fr.) L.B. Sm. & Downs. BRAZIL: Rio Grande do Sul, Hashimoto et al. B59 (MVFA, SI, GHSP, Ando).
11	*P. inflata* R.E.Fr. ARGENTINA: Misiones, Ando & Buto A7 (SI, GHSP, Ando).
12	*P. interior* T. Ando & Hashim. BRAZIL: Santa Catarina, Hashimoto et al. B569 (MBM, BM, R, S, US, GHSP, Ando).
13	*P. littoralis* L.B.Sm. & Downs. BRAZIL: Santa Catarina, Hashimoto et al. B29 (MVFA, GHSP, Ando).
14	*P. mantiqueirensis* T. Ando & Hashim. BRAZIL: Minas Gerais, Hashimoto et al. B357 (S, BM, K, SP, U, US, GHSP, Ando).
15	*P. occidentalis* R.E.Fr. ARGENTINA: Jujuy, Ando & Iida A109 (MVFA, S, SI, GHSP, Ando).
16	*P. reitzii* L.B.Sm. & Downs. BRAZIL: Santa Catarina, Hashimoto et al. B28 (GHSP, Ando).
17	*P. riograndensis* T. Ando & Hashim. BRAZIL: Rio Grande do Sul, Hashimoto et al. B860 (MBM, S, GHSP, Ando).
18	*P. saxicola* L.B.Sm. & Downs. BRAZIL: Santa Catarina, Hashimoto et al. B113 (GHSP, Ando).
19	*P. scheideana* L.B.Sm. & Downs. BRAZIL: Santa Catarina, Hashimoto et al. B109 (BM, MBM, MVFA, S, SI, US, GHSP, Ando).
20	unnamed taxon P1. BRAZIL: Rio Grande do Sul, Hashimoto et al. B990 (GHSP, Ando).
Petunia sensu Jussieu (1803) with $2n=2x=18$ chromosomes sharing similar morphological characters with *Calibrachoa.*	
21	*P. alpicola* L.B.Sm. & Downs. BRAZIL: Santa Catarina, Hashimoto et al. B130 (BM, MBM, MVFA, S, SI, US, GHSP, Ando).
22	*P. helianthemoides* Sendtn. ARGENTINA: Misiones, Ando & Buto A4 (SI, Ando).
23	*P. kleinii* L.B.Sm. & Downs. BRAZIL: Santa Catarina, Hashimoto et al. B105 (MBM, GHSP, Ando).
24	*P. pubescens* (Spreng.) R.E.Fr. URUGUAY: Durazno, Ando & Kokubun U23 (S, MVFA, Ando).
25	*P. variabilis* R.E.Fr. URUGUAY: Tacuarembo, Ando & Kokubun U6 (MVFA, Ando).
Calibrachoa La Llave & Lexarza (1825), $2n=2x=18$.	
26	*C. calycina* (Sendtn.) Wijsman. ARGENTINA: Corrientes, Ando & Buto A15 (MBM, GHSP, Ando).
27	*C. dusenii* (R.E.Fr.) Stehmann & Semir. BRAZIL: Paraná, Hashimoto et al. B484 (MBM, S, GHSP, Ando).
28	*C. eglandulata* Stehmann & Semir. BRAZIL: Santa Catarina, Hashimoto et al. B917 (GHSP, Ando).
29	*C. elegans* (Miers) Stehmann & Semir. BRAZIL: Minas Gerais, Hashimoto et al. B489 (GHSP, Ando).
30	*C. ericaefolia* (R.E.Fr.) Wijsman. BRAZIL: Paraná, Hashimoto et al. B63 (MBM, GHSP, Ando).
31	*C. heterophylla* (Sendtn.) Wijsman. BRAZIL: Rio Grande do Sul, Hashimoto et al. B60 (BM, MBM, GHSP, Ando).
32	*C. linearis* (Hook.) Wijsman. URUGUAY: Río Negro, Ando & Buto U165 (MVFA, Ando).
33	*C. linoides* (Sendtn.) Wijsman. BRAZIL: Rio Grande do Sul, Hashimoto et al. B282 (GHSP, Ando).
34	*C. macrodactylon* (L.B.Sm. & Downs) Wijsman. BRAZIL: Santa Catarina, Hashimoto et al. B138 (Ando).
35	*C. micrantha* (R.E.Fr.) Stehmann & Semir. BRAZIL: Paraná, Hashimoto et al. B486 (BM, MBM, S, US, GHSP, Ando).
36	*C. parviflora* (Juss.) Wijsman. URUGUAY: Tacuarembó, Ando & Kokubun U42 (MVFA, Ando).
37	*C. pygmaea* (R.E.Fr.) Wijsman. URUGUAY: Artigas, Ando & Kokubun U39 (BM, MVFA, S, US, Ando).
38	*C. rupestris* (Dusén) Wijsman. BRAZIL: Paraná, Hashimoto et al. B479 (BM, MBM, GHSP, Ando).
39	*C. selloviana* (Sendtn.) Wijsman. BRAZIL: Rio Grande do Sul, Hashimoto et al. B892 (MBM, S, US, GHSP, Ando).
40	*C. sendtneriana* (R.E.Fr.) Stehmann & Semir. BRAZIL: Santa Catarina, Hashimoto et al. B447 (BM, US, GHSP, Ando).
41	*C. serrulata* (L.B.Sm. & Downs) Stehmann & Semir. BRAZIL: Santa Catarina, Hashimoto et al. B1031 (BM, S, US, U, GHSP, Ando).
42	*C. spathulata* (L.B.Sm. & Downs) Stehmann & Semir. BRAZIL: Santa Catarina, Hashimoto et al. B90 (GHSP, Ando).
43	*C. thymifolia* (A.St.-Hil.) Stehmann & Semir. BRAZIL: Santa Catarina, Hashimoto et al. B36 (BM, MBM, MVFA, S, SI, US, GHSP, Ando).
44	unnamed taxon C1. BRAZIL: Rio Grande do Sul, Hashimoto et al. B7 (GHSP, Ando).
45	unnamed taxon C2. BRAZIL: Rio Grande do Sul, Hashimoto et al. B201 (GHSP, Ando).
46	unnamed taxon C3. BRAZIL: Santa Catarina, Hashimoto et al. B215 (GHSP, Ando).
47	unnamed taxon C4. BRAZIL: Rio Grande do Sul, Hashimoto et al. B755 (GHSP, Ando).
48	unnamed taxon C5. BRAZIL: Rio Grande do Sul, Hashimoto et al. B789 (GHSP, Ando).
49	unnamed taxon C6. BRAZIL: Santa Catarina, Hashimoto et al. B1126 (GHSP, Ando).
50	unnamed taxon C7. BRAZIL: Rio Grande do Sul, Hashimoto et al. B1176 (GHSP, Ando).
51	unnamed taxon C8. BRAZIL: Santa Catarina, Hashimoto et al. B1248 (GHSP, Ando).
52	unnamed taxon C9. URUGUAY: Salto, Ando & Iida U229 (GHSP, Ando).
Nicotiana L. (1735) $2n=18$, 20, 24, 32, 36, 38, 40, 42, 44, 46, 48	
53	*Nicotiana langsdorffii* Weinm. ($2n=2x=18$). BRAZIL: Rio Grande do Sul, Hashimoto et al. HB622 (Ando).

a Abbreviations of herbaria are after Holmgren, Holmgren, and Barnett (1990) [10] except GHSP (Centro de Pesquisas História Natural, São Paulo, Brazil) and Ando (temporary collection of Toshio Ando).

These probes were labeled with ECL direct nucleic acid labeling and detection system (Amersham, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's recommendations and hybridized with *Petunia sensu* Wijsman and *Calibrachoa* cpDNA fragments. The ECL system uses non-RI chemiluminescence detection method.

RESULTS AND DISCUSSION

RFLPs

We detected a total of 212 RFLPs in all 19 restriction enzymes distributed in all 12 tobacco cpDNA clone regions (Table 2). The table lists restriction enzymes used (the column, Enzyme), the tobacco cpDNA clone region in which an RFLP is detected (Probe), loss and gain of fragments (Loss and Gain), and the taxa that showed the particular RFLP (Taxa). Some of the RFLPs were unambiguously identified as gain or loss of a particular restriction site. However, due to the number and size of the fragments produced, we could not identify such restriction sites in some enzymes, especially those with four base recognition sequence such as *Alu*I and *Hae*III. In those cases, the mutation was recorded simply as gain or loss of particular fragments.

Of the 212 mutations, 89 were shared by more than one taxon, which were phylogenetically informative. The rest of mutations occurred in only one taxon, which cannot be used to determine the branching patterns of the parsimonious trees, but can be useful to calculate the distance matrix.

Deletion, insertion and reversion of the cpDNA

Two cases of deletion of DNA fragment were detected in two taxa, *Petunia altiplana* T. Ando & Hashim. and unnamed taxon C1, in the overlapped region of pTB7 and pTB20 of the cpDNA. The length of deletion was estimated as ca. 0.2 kbp for *P. altiplana* and ca. 0.4 kbp for the C1. The deletion in *P. altiplana* was confirmed to be 144 bp by sequencing intergenic region between *trnC* and ORF154 of *P. altiplana* in comparison with a known tobacco sequence (Genbank Z00044, data not shown).

No insertion and reversion of DNA fragment was unambiguously identified.

RFLP mapping on a phylogenetic tree

Figures 1 and 2 show RFLPs as character states on a phylogenetic tree (50% majority rule consensus of MP trees) obtained in a previous study (see Fig. 1 in Ando et al. [9]). Some RFLPs were unique at each branch (indicated by thick lines in the figures), however, many showed homoplasy (indicated by thin lines and italic letters). The numbers of RFLPs that separate *Petunia sensu* Wijsman and *Calibrachoa* assured their separation, but their generic ranks need to be tested using closely related genera such as *Fabiana*.

Phylogeny of Petunia sensu Jussieu

Although an elaborate discussion about the phylogeny of *Petunia sensu* Jussieu was made in the previous paper [9], it may be worth summarizing here.

Petunia sensu Jussieu is divided into two clades, *Petunia sensu* Wijsman and *Calibrachoa* (Fig. 1). *Petunia sensu* Wijsman is characterized by herbaceous stem and chromosome number of $2n=14$; *Calibrachoa* is characterized by woody stem (except *C. parviflora* and *C. pygmaea*, which have herbaceous stem) and chromosome number of $2n=18$. The five species of *Petunia sensu* Jussieu (21 through 25 in Table 1) that have morphological characters of *Calibrachoa* and $2n=18$ chromosomes were included in the *Calibrachoa* clade. In conclusion, the two clades in *Petunia sensu* Jussieu are genetically and morphologically consistent.

Two species of *Petunia sensu* Wijsman, *P. axillaris* and *P. integrifolia* consist of infraspecific taxa. However, neither species formed clades that exclusively consist of their infraspecific taxa. This may not necessarily lead to a conclusion that these species should be revised, because some of the clades within *Petunia* Wijsman were weakly supported by only one RFLP (Fig. 1). Further study is required to clarify the infrageneric relationship in *Petunia sensu* Wijsman and *Calibrachoa*.

The application of the present data

The present data may serve as a guide for further molecular studies on *Petunia sensu* Jussieu, for example genetic diversity within a taxa, molecular markers to identify taxa, and introgression from one taxon to another.

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Table 2. Chloroplast DNA restriction fragment polymorphism in *Petunia* sensu Wijsman and *Calibrachoa*. Taxa numbers correspond with Table 1.

	Enzyme	Probe	Loss	Gain	Taxa
1	*Alu*I	7	2.7	1.6+1.1	53
2	*Alu*I	7	2.3		21–35, 38–52
3	*Alu*I	7–20	2.8	2.6	1
4	*Alu*I	7–20	2.7	2.4	44
5	*Alu*I	15		1.2	53
6	*Alu*I	19	0.6		21–52
7	*Alu*I	19	1.2		1–20
8	*Alu*I	20		2.4	36, 37, 44
9	*Alu*I	22	2.0	1.7	1
10	*Alu*I	25	2.0		3, 4
11	*Alu*I	25	2.0	2.5	21–35, 38–52
12	*Alu*I	28		1.3+1.1	53
13	*Alu*I	29	2.8	1.7+ ?	1
14	*Alu*I	29	0.65	1.1	20, 53
15	*Alu*I	a2	2.7	2.5	21, 23, 25–35, 38–52
16	*Ava*I	7	4.1	3.4+0.7	36
17	*Ava*I	7	1.3	1.0+ ?	53
18	*Ava*I	7–20	3.4	2.4+1.0	1, 16
19	*Ava*I	8	3.2+0.4	3.6	36
20	*Ava*I	19	5.6+1.2	6.8	1–20
21	*Ava*I	20	3.9	3.6+0.6	36
22	*Ava*I	25	6.1+3.4	5.7+3.8	53
23	*Ava*I	29–a1	3.4+4.8	3.9+4.2	53
24	*Ava*I	a1–15	2.3+0.3	2.6	53
25	*Ava*II	7	0.9	1.6	11
26	*Ava*II	7	2.2+0.8	3.0	53
27	*Ava*II	7–20	2.0	2.1	44
28	*Ava*II	8	0.8		21–52
29	*Ava*II	10	1.6	1.3+0.3 ?	53
30	*Ava*II	15		1.6	8
31	*Ava*II	15	2.5	0.8+0.5	53
32	*Ava*II	15–28	1.6	1.4	48
33	*Ava*II	19	4.1+1.9	6.0	53
34	*Ava*II	19	1.6+0.1	1.7	1
35	*Ava*II	19	4.1	3.0+ (1.1)	21–52
36	*Ava*II	19	1.1	0.6+0.5 ?	21–24, 26, 27, 30–35, 38, 39, 41, 43–52
37	*Ava*II	19	1.6	1.5	22, 24–26, 32, 33, 47, 49, 51, 52
38	*Ava*II	19	3.0		25
39	*Ava*II	20		1.5	36, 37, 51
40	*Ava*II	20		2.3+1.2	53
41	*Ava*II	22–29	1.9	1.4+0.5	53
42	*Ava*II	25	2.2		1–20
43	*Ava*II	25	3.0		1–21, 23, 27–31, 34–46, 48, 50
44	*Ava*II	25		0.9	12
45	*Ava*II	28	1.2	1.5	29
46	*Ava*II	28		0.8+0.5	53
47	*Ava*II	a1	3.5	(2.9) +0.5 ?	21–53
48	*Ava*II	a1	(2.9)	1.6+1.3	53
49	*Ava*II	a2	3.9	3.6	22–26, 29–52
50	*Ava*II	a2	5.2	4.6	1–16, 18–20

51	*Ava*II	a2	5.2	5.6	21, 28
52	*Ava*II	a2	5.2	3.4+1.8	27
53	*Bam*HI	7	3.0	1.9+1.1	53
54	*Bam*HI	7-20	3.6	3.4	1
55	*Bam*HI	7-20	3.6	3.0+0.8	11
56	*Bam*HI	7-20	3.6	3.2	44
57	*Bam*HI	10	3.0+1.5+0.6	5.2	53
58	*Bam*HI	19	10	6.0+4.0	1-20
59	*Bam*HI	19	10+2.3	12.3	22-27, 30-35, 38, 39, 43-52
60	*Bam*HI	20	4.5	2.8+1.7	21, 23, 25-31, 33-35, 38-52
61	*Bam*HI	22	4.0	3.3	53
62	*Bam*HI	25	4.0	3.6+0.4	53
63	*Bam*HI	25-28	4.8+2.9	7.7	4, 7, 15
64	*Bam*HI	29	3.4	3.2+0.2 ?	21-52
65	*Bam*HI	29	3.4	2.1+1.3	53
66	*Bam*HI	a1	13.6+6.0	19.6	21-28, 30-53
67	*Bam*HI	a1-22	13.6+3.6	17.2	5
68	*Bam*HI	a2	17	15+2.4	53
69	*Bcn*I	7	5.4		53
70	*Bcn*I	7-20	2.6	2.4	1
71	*Bcn*I	10	2.4+0.3	2.7	21-35, 38-52
72	*Bcn*I	15	2.5	2.7	53
73	*Bcn*I	15	1.3	1.0	22, 24
74	*Bcn*I	15-28		3.5	11
75	*Bcn*I	20	2.6		16
76	*Bcn*I	25	2.4	1.8+0.6	53
77	*Bcn*I	25	2.4+0.9	3.3	21, 28, 29, 40, 41, 51
78	*Bcn*I	25	1.6		22-27, 30-35, 38, 39, 43-50, 52
79	*Bcn*I	25	0.8+0.2	1.0	1-20
80	*Bcn*I	25	2.3	2.2	1-20
81	*Bcn*I	25	1.6	1.7	18
82	*Bcn*I	29	1.8+0.4	2.2	21-35, 38-52
83	*Bcn*I	29	1.8+0.4	1.7+0.5	1-20, 36, 37
84	*Bcn*I	a1	4.6	4.3	53
85	*Bcn*I	a2	10	7	53
86	*Bgl*II	7	4.3	2.9+1.4	10
87	*Bgl*II	7-20	3.5	3.3	1
88	*Bgl*II	7-20	3.5	1.9+1.6	22, 24
89	*Bgl*II	7-20	3.5	3.1	44
90	*Bgl*II	15	2.9+0.2 ?	3.1	27
91	*Bgl*II	19	2.7	2.4+0.3	22-27, 29-35, 38, 39, 41, 43-52
92	*Bgl*II	19	6.4+1.6	8	53
93	*Bgl*II	20	3.0		53
94	*Bgl*II	22	2.7+0.4	3.1	31
95	*Bgl*II	25		0.9	10
96	*Bgl*II	25	4.9+4.2	9	12
97	*Bgl*II	25	4.2	4.5	21-52
98	*Bgl*II	25	4.2		53
99	*Bgl*II	25-28	4.3+3.5	7.8	32, 52
100	*Bgl*II	a1	8	6.4+1.6	24
101	*Bgl*II	a1-15	2.0+1.8	3.8	2, 9, 51
102	*Bgl*II	a2	2.0	1.0+1.0	14, 36, 37
103	*Cla*I	7		3.0	10, 16
104	*Cla*I	15-28	1.0+0.6	1.6	6, 16, 18

105	*Cla*I	19	0.6+0.4	1.0	17
106	*Cla*I	25	2.0+1.0	3.0	10, 14
107	*Cla*I	28	14	6.8+7.2	1–20
108	*Cla*I	a1	3.0+1.5	4.5	4, 7, 15, 53
109	*Dra*I	7		5	6
110	*Dra*I	10		2.4	8
111	*Dra*I	19	5.0	2.0+2.0+1.0	5, 17
112	*Dra*I	19	5.0	4.0+1.0	9
113	*Dra*I	20		4.0	6
114	*Dra*I	20	16.0	13.5+2.5	8
115	*Dra*I	25	2.0	1.7	43, 46
116	*Dra*I	25	2.2	1.2	32
117	*Dra*I	28	1.7	1.9	21–24, 26–35, 38–53
118	*Dra*I	28	1.7		48
119	*Dra*I	28	1.7+1.3	1.9+1.1	25
120	*Dra*I	a2	2.5	2.0+0.5	10, 13, 16
121	*Dra*I	a2	2.2		21–52
122	*Eco*O109I	7–20	3.0	2.8	1
123	*Eco*O109I	7–20	3.0	2.6	44
124	*Eco*O109I	8	1.6+0.7	1.5+0.8	21–43, 45–52
125	*Eco*O109I	8		0.5	44
126	*Eco*O109I	15–28	6.5+4.5	11.0	4, 7, 15, 21, 23, 27–31, 34–46, 48, 50, 53
127	*Eco*O109I	19	2.2	2.3	1
128	*Eco*O109I	19	5.2	5.8	25
129	*Eco*O109I	20	1.6	1.5	53
130	*Eco*O109I	22	1.6	1.4	21–52
131	*Eco*O109I	a1	1.5		21–30, 32–52
132	*Eco*O109I	a1	14	9+5	31
133	*Eco*O109I	a1		2.4	53
134	*Eco*RI	7	2.4	2.2	1
135	*Eco*RI	19	8.0+3.0	11.0	10, 13, 14
136	*Eco*RI	19		0.6	4
137	*Eco*RI	20	2.4+2.1	4.5	10, 13, 14
138	*Eco*RI	20	2.1		1
139	*Eco*RI	25	2.1+0.6	2.5	4, 14
140	*Eco*RI	25	2.5	2.4	9
141	*Eco*RI	28	1.8+1.2	3	2–4, 8, 10, 13, 15, 19, 23, 37, 48
142	*Eco*RI	28	1.8	2.2	14
143	*Eco*RI	a2	2.3+2.0	2.5+1.8	22, 24–26, 31–34, 39, 43, 45, 46, 48, 49, 51, 52
144	*Eco*RI	a2	2.3+2.0	4.3	21, 23, 27–30, 35, 38, 40–42, 50
145	*Eco*RI	a2			44, 47
146	*Eco*RI	a2			53
147	*Eco*RV	7	1.6	1.1+ ?	35, 38
148	*Eco*RV	7	4.5	4.0	44
149	*Eco*RV	7–20	17.8+4.5	22.3	34, 36, 37, 39, 45, 48
150	*Eco*RV	8			13
151	*Eco*RV	8a–a2	13.0+6.0	19.0	53
152	*Eco*RV	15	1.0	0.9	45
153	*Eco*RV	20			1
154	*Eco*RV	20–22		25.2	50
155	*Eco*RV	22–a1	11	8.1+2.9	53
156	*Eco*RV	25	9.4+6.3	11.0+4.7	1, 21–52
157	*Eco*RV	a1	4.1+2.5	6.6	53
158	*Eco*T22I	7–20	2.6	2.7+3.1	1

159	*Eco*T22I	19	1.3+0.2	1.5	1–21, 28, 36, 37, 40, 41
160	*Eco*T22I	19–29	4.5+(1.5)	6.0	13
161	*Eco*T22I	20	3.2	2.8	7
162	*Eco*T22I	20	1.4	1.3	16
163	*Eco*T22I	20	1.4	1.1+0.3 ?	36, 37
164	*Eco*T22I	20	4.0	2.6+1.4	31
165	*Eco*T22I	22–a1	9.1+1.3	10.4	22, 24–26, 31–35, 39, 43, 44, 46–49, 51, 52
166	*Eco*T22I	25	12+6.4	18.4	1–20
167	*Eco*T22I	a1	1.3	1.0+0.3 ?	36, 37
168	*Eco*T22I	a2	5.0	2.5+2.3+0.3	53
169	*Hae*III	8			7
170	*Hae*III	19		1.5	7, 21–39, 41, 43–53
171	*Hae*III	20	1.1	0.9	1
172	*Hae*III	20		1.7	13
173	*Hha*I	7	2.0	2.2	21–35, 38–52
174	*Hha*I	7	2.1	1.9	23, 27, 30, 35, 38, 41, 50
175	*Hha*I	8	1.4+1.9(2.0)	3.5(3.6)	1–20
176	*Hha*I	19	0.6		21–35, 38–52
177	*Hha*I	19	3.0	2.8	22–27, 30–35, 38, 39, 41, 43–48, 50–52
178	*Hha*I	19	1.1	1.0	22, 24–26, 32, 33, 47, 49, 51, 52
179	*Hha*I	19	2.5+1.5	4.0	49, 53
180	*Hha*I	22	8.0	7.5+0.5	53
181	*Hha*I	a1	3.3+0.9	4.2	2
182	*Hha*I	a1	6.9	3.4+3.4	23, 30, 35, 38, 50
183	*Hinc*II	15	1.6	1.2+0.4	21–52
184	*Hinc*II	25		2.2	21–25, 27, 28, 30, 31, 34–46, 48, 50
185	*Hinc*II	25	5.8+2.9	4.8	26, 32, 47, 49, 51, 52
186	*Hinc*II	25	5.8+3.6+2.9+2.2	4.8	33
187	*Hinc*II	25		3.4	36
188	*Hinc*II	a2			12
189	*Hin*dIII	7–20	11.6	7.8+3.8	53
190	*Hin*dIII	8	1.0	0.9	21–52
191	*Hin*dIII	25	3.2	2.1+1.1	53
192	*Hin*dIII	25	(3.2)+2.7	5.9	4
193	*Hin*dIII	a1	7.5	5.3+2.2	1–20
194	*Hin*dIII	a1–22–29	11+7.5	18.5	37
195	*Sty*I	7	1.2	1.6	5, 9, 10, 13, 17
196	*Sty*I	7	7.6	4.0	7
197	*Sty*I	7	1.2	1.4	53
198	*Sty*I	22	7.1	4.3+2.8	1, 14, 20
199	*Sty*I	22	7.1	6.4	53
200	*Sty*I	28		2.8	1, 20
201	*Vsp*I	7	1.3+0.5	1.8	8
202	*Vsp*I	7	2.5+0.5	3.0	13
203	*Vsp*I	7–20	1.3	1.1	1
204	*Vsp*I	15			10
205	*Vsp*I	19	1.1	1.0+0.1	19
206	*Vsp*I	25		1.9	3
207	*Vsp*I	28			10
208	*Vsp*I	a1	8.9	5.9+3.0	1, 8, 14, 19, 20
209	*Xba*I	7–20	6.8	6.6	1
210	*Xba*I	19			9
211	*Xba*I	22	2.0+0.3	2.3	10, 13, 36, 37
212	*Xba*I	22			7

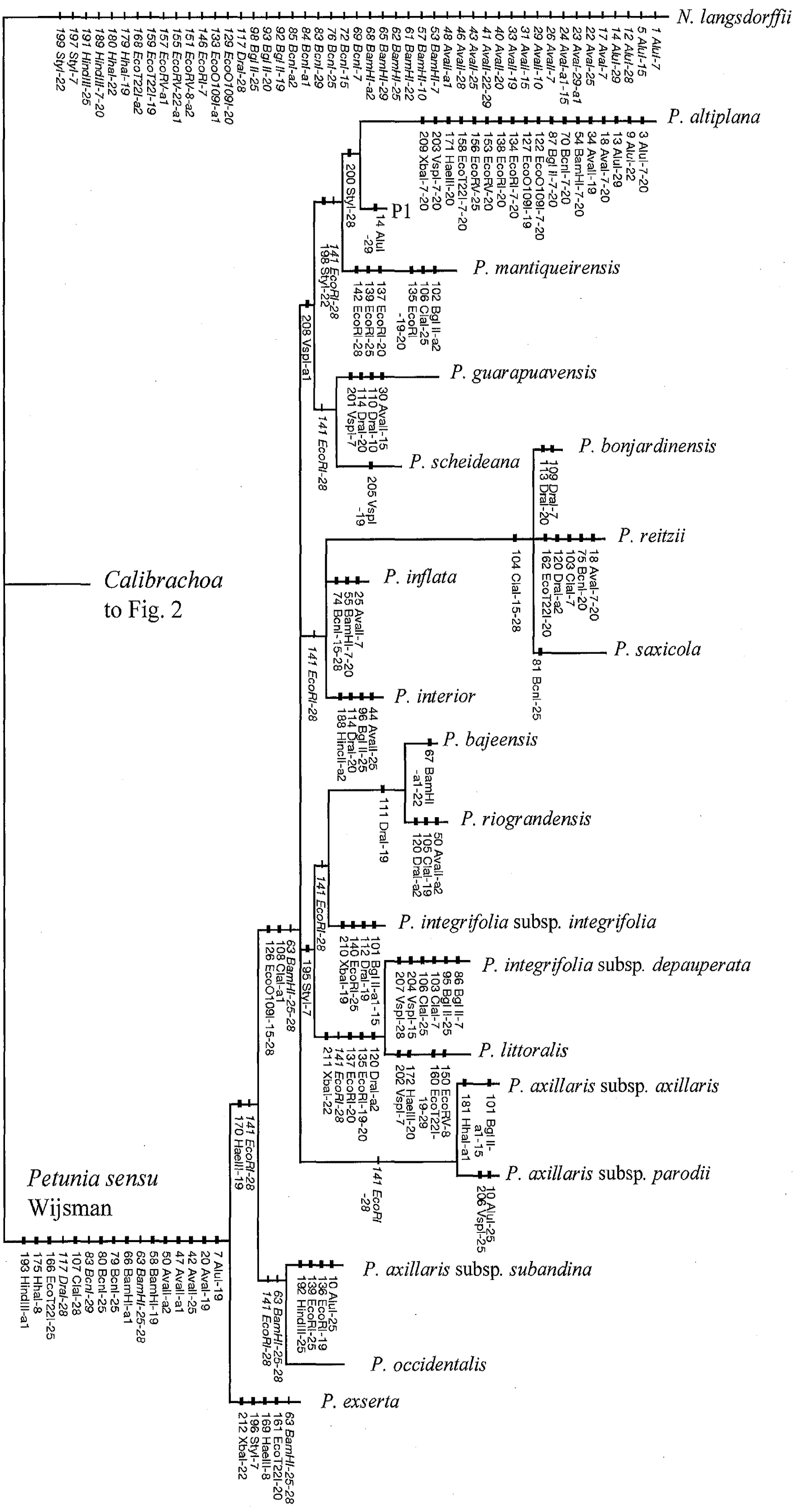

Fig. 1. The 50% majority rule consensus tree of 3740 most parsimonious trees calculated from the present RFLP data in a previous study [9]. The RFLPs that are unique to the branch are indicated by thick bars and roman letters, and what occur on more than one branch (homoplasy) are indicated by thin bars and italic letters.

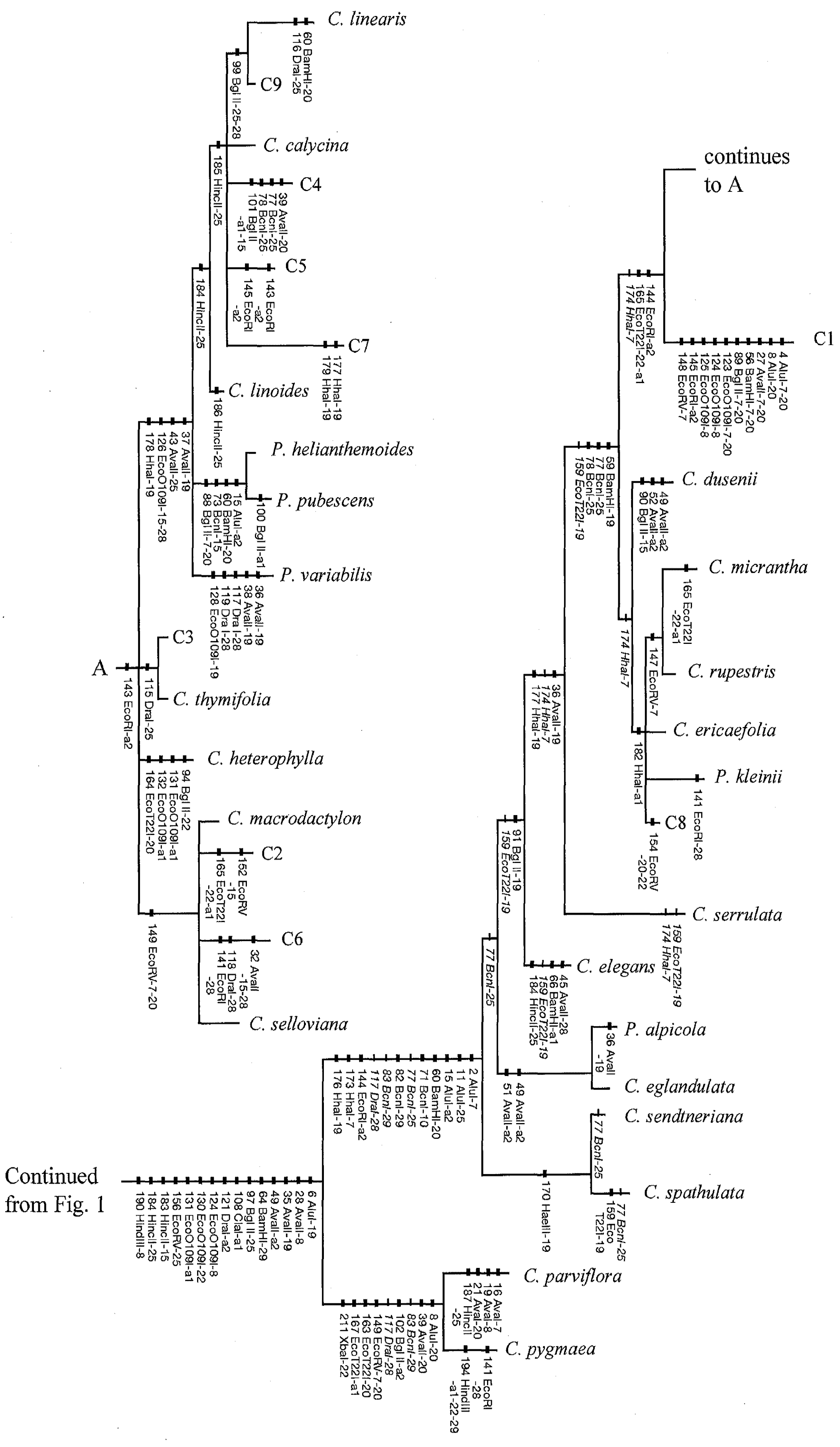

Fig. 2. A part of the phylogenetic tree in Fig. 1 that corresponds with *Calibrachoa*.

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Petunia sensu Jussieu（ナス科）における葉緑体DNAのRFLPの詳細

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摘　要

今後の*Petunia sensu* Jussieuの分子系統学や遺伝子多様性の研究に資するため，野生個体群由来の*Petunia* 52分類群と*Nicotiana langsdorffii*の葉緑体DNAを19種の制限酵素で処理し，12種のタバコ葉緑体DNAプローブによってサザンハイブリダイゼーションを行い，制限断片長多型（RFLP）を調査した。RFLPの詳細をまとめ，前報で報告した系統樹上にマッピングした。