A phylogeny of Japanese dwarf bamboos, the Sasa-group based on RAPD- and morphological data analyses

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Mikio Kobayashi[1] and Ryo Furumoto[2] : **A phylogeny of Japanese dwarf bamboos, the Sasa-group based on RAPD- and morphological data analyses**

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Abstract

A phylogenetic relationship of 30 Japanese bamboo taxa was analyzed including *Phyllostachys bambusoides*, *Shibataea kumasasa*, *Sinobambusa tootsik*, and the species of four most common genera of the Sasa-group ; *Sasa*, *Sasamorpha*, *Pleioblastus* and *Pseudosasa* based on 327 RAPD data by Wagner parsimony rooted at *Streptochaeta spicata*. *Phyllostachys*/*Shibataea* positioned as the earliest divergent clade, *Pseudosasa* was the next most basal, and *Sasamorpha*/*Sasa* sect. *Lasioderma* and *Pleioblastus*/*Sasa* excluding *Lasioderma* formed a sister clade at the terminals. Within genus *Sasa* clade, sect. *Macrochlamys* including *Sasa jotanii* was the most basal, *Monilicladae* was the next most basal, followed by the sister clades of sects. *Sasa* and *Crassinodi* at the terminals. Clusters of a UPGMA dendrogram coincided with the topology of the RAPD tree except for the position of *Pseudosasa*. Differencies between another RAPD tree with representative 20 species and a variety of the Sasa-group and a morphological tree with 14 species and a variety based on 35 characters both rooted at *Phyllostachys bambusoides* were discussed. Section *Lasioderma* was in the *Sasamorpha* clade in the RAPD trees, while the morphological tree showed it as a component of the genus *Sasa*. These results compelled us to exclude the section from the genus *Sasa* and to conserve the genus *Neosasamorpha* Tatew. Character states between culm-sheaths persistent and deciduous, branching pleioclade and monoclade, culm erect and ascending were discussed on the former as the plesiomorphic and the latter as the apomorphic to consider the evolutionary trend among the Japanese bamboos and divergence of genus *Sasa* in accordance with the snowy environment.

Key words : Japanese bamboos, *Neosasamorpha*, phylogeny, *Sasa*, Sasa-group.

Introduction

Muroi (1937) was the first who divided the Japanese bamboos into three main groups ; the "Take", the "Sasa" and the "Bamboo". These are defined by the culm-sheath deciduous, culm-sheath persistent and sympodial rhizome system without considering the culm height, respectively. Recently they have been most acceptably revised by Suzuki (1996), who recognized 6 genera in the Take-group, *Phyllostachys*, *Hibanobambusa*, *Semiarundinaria*, *Sinobambusa*, *Tetragonocalamus* and *Shibataea* ; 8 genera in the Sasa-group, *Sasa*, *Neosasamorpha*, *Sasamorpha*, *Sasaella*, *Pseudosasa*, *Indocalamus*, *Pleioblastus* and *Chimonobambusa* ; and two genera of *Bambusa* and *Dendrocalamus* in the Bamboo-group. The Take-and Sasa-group are distributed naturally in the temperate region, while the Bamboo-group was introduced from tropical Asia (Suzuki 1978) which is laid aside in the present study.

Among the Sasa-group, genera *Sasa* and *Sasamorpha* are representative floristic elements that characterize the plant community in Japan. Genus *Sasa* is almost endemic to the Japan Islands with 32 species, whereas the only two species of *Sasamorpha* are distributed in the Pacific side of the Japan Islands and Korea (Suzuki 1996). Suzuki (1961) established the '*Crassinodi*-line' as a distribution limit which lies longitudinally parallel to the Pacific coast line from north to south of the northeastern district of the Japan Islands (Fig. 1). The line is based on an approximate 50 cm of mean annual maximum snow depth and defines the geographical distribution limits of *Sasa* sect. *Crassinodi* on the Pacific side, while *Sasa* sects. *Sasa* and *Macrochlamys* are distributed in the exceedingly snowy area on Japan Sea side. *Monilicla-*

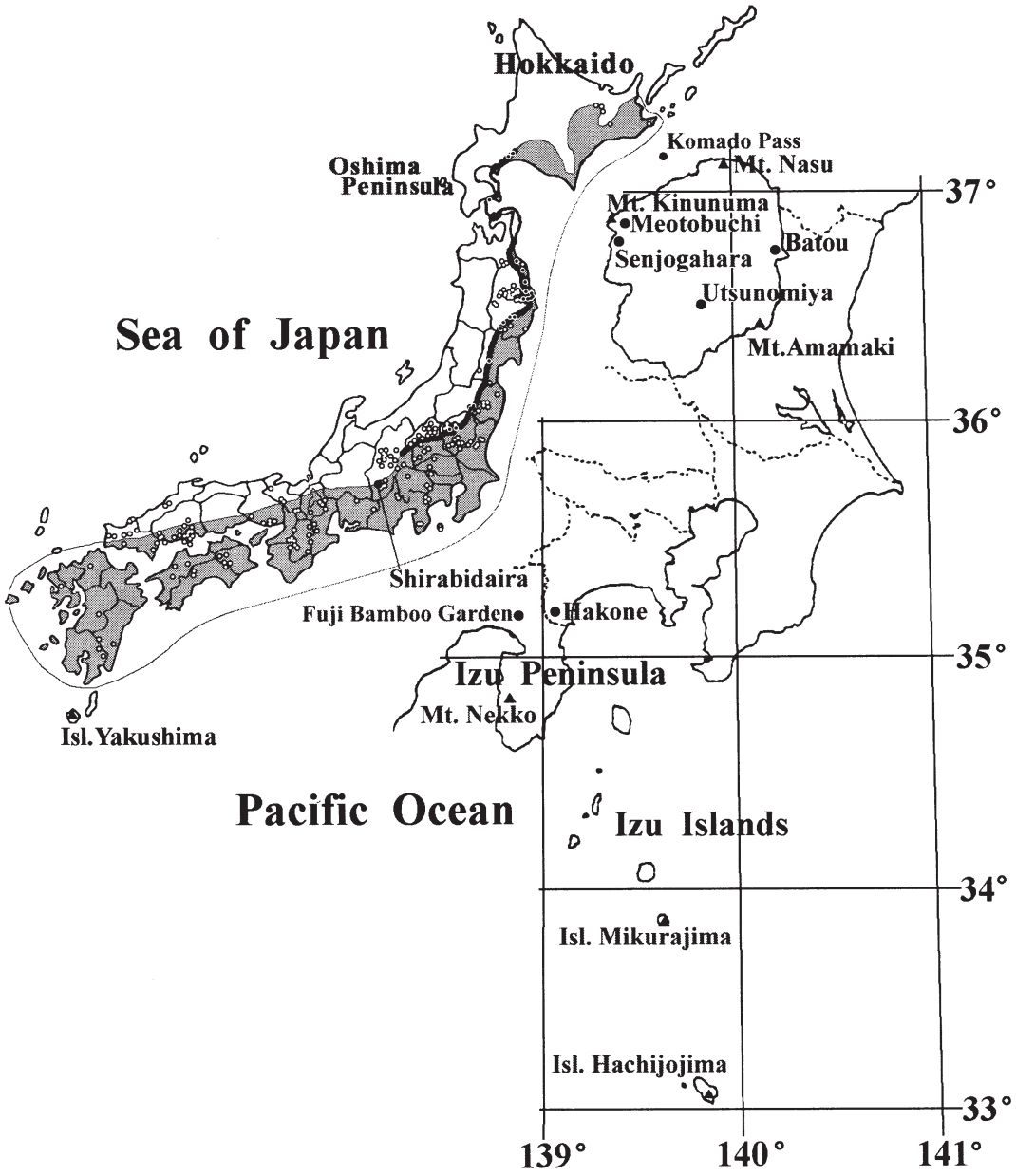


Fig. 1. Map showing the distribution range of genus *Sasa* sect. *Crassinodi* (screened area), the *Crassinodi*-line (bold line), locations of sect. *Lasioderma* plants (open dots), and main locations of sampling site in the Japan Islands.

dae of other section is distributed in a Pacific Ocean environment, whereas sect. *Lasioderma* mostly within the distribution range of the sect. *Crassinodi* (Fig. 1, Suzuki 1978). Key characters of vegetative organs, habitat property and geographical distribution of these dwarf bamboos are schematically summarized in Fig. 2.

Tanimoto (1984) discovered the distribution of a plant like *Sasa kurilensis* of sect. *Macrochlamys* in Isls. Hachijojima and Mikurajima, Izu Islands, even though these islands are located in the Pacific Ocean far south of the Izu Peninsula (Fig. 1). His discovery was so sensational from a phytogeographical viewpoint that controversy

aroused over which taxon the plant should be ascribed to: *Sasa kurilensis* var. *jotanii* (Inoue and Tanimoto 1985), *S. kurilensis* var. *kurilensis* (Kobayashi 1985), or *S. tsuboiana* (Suzuki 1996). Recently, Kobayashi (2000) treated it as a new species, *S. jotanii*, after an intensive study on its flower morphology.

Nakai described *Sasa* sect. *Lasioderma* (Nakai 1931) and sect. *Acrocladula* (Nakai 1934). Tatewaki (1940) recognized these groups as having putative hybrid origin between *Sasa* and *Sasamorpha* and revised them into a new genus *Neosasamorpha*. Koidzumi described two new sections of genus *Sasa*, i.e., *Pseudosasamorpha* (Koidzumi 1939) and *Nanopseudosasamorpha* (Koidzumi 1942), and decided that, contrary to Tatewaki's treatment, the two intercalary sections form continuous intermediaries between *Sasa* and *Sasamorpha*. Suzuki (1978) initially treated those four sections as one, *Sasa* sect. *Lasioderma* Nakai sensu lato. However, Suzuki (1996) later adopted Tatewaki's *Neosasamorpha* while excluding the possibility of hybridity.

Using allozyme evidence on hybridization in the genus *Sasa* and its related genera, Takahashi et al. (1994) decided that these confusing taxonomic treatments were mainly due to intersectional and intergeneric hybridization not only on the F_1 hybrid level but also at the underlying genetic recombination level, in which phylogenetic analysis was laid aside. Thus, distribution patterns and putative hybridity of various Sasa-group require explicit analytical methods to resolve the phylogenetic relationships of each section and position.

In 1990, Williams et al. developed a method of RAPD (random amplified polymorphic DNA) analysis examining the relation between RAPD data with RFLPs. Before that, Nei and Li (1979) proposed the transformation of shared restriction-site data of mitochondorial DNA fragments into genetic distance for RFLPs analysis. Many studies suggest that RAPD banding patterns are good predictors of homology among various subordinate taxa as follows. Dos Santos et al. (1994) compared the modes of genetic similarity among 45 *Brassica oleracea* genotypes between RAPD and RFLPs methods and found that there is high relationship between them; effective classification of Japonica rice cultivars (Mackill 1995); good correlation with RAPD, *rbcL* sequences and RFLPs data for phylogenetic analysis among local populations of *Larix* (Shiraishi et al. 1996); among three species of *Helianthus* as determined by Southern hybridization or correlation with restriction sites of endonucleases, and/or homologous genetic loci (Rieseberg 1996); and high consistency index between most parsimonious trees based on RAPD-dataset and supranuclear dataset of morphological, pollen epidermis texture, chromosomal and chemical variation among sections of genus *Allium* (van Raamsdonk et al. 1997). Thus, applications for various subordinate taxa from cultivars through congeneric species or sections show that RAPD data are effective in analyzing their phylogenetic relationships.

The Japanese dwarf bamboos have a uniformity in genomic size among related genera such that all are tetraploid with 2n=48 (Tateoka 1955). These taxa have self-compatibility and few crossing barriers to forming hybrids even between distantly related genera (Muramatsu 1981) suggesting an underlying homology in chromosomal morphology. These findings suggest that Japanese bamboos are close enough for effective RAPD analysis, considering segregation of a single RAPD marker links well with the configuration of chromosome-specific DNA fragments (Williams et al. 1990). Therefore, we considered that RAPD analysis will be sensitive enough to resolve the phylogenetic relationships among Japanese dwarf bamboos.

As mentioned above, phylogenetic positions of *Sasa jotanii* and *Sasa* sect. *Lasioderma* Nakai sensu lato are ones of the most interesting questions concerning the Japanese dwarf bamboos. In the present study, we at first, tried to analyze a phylogenetic relationship within the dwarf bamboos based on the morphological characters by Wagner parsimony method because no cladistic analysis using the morphological characters of Japanese bamboos has been reported yet since the description of the genus *Sasa* by Makino and Shibata (1901). Next, a UPGMA analysis with RAPD data was carried out to clarify principal clusters among Japanese bamboo taxa. Third, phylogenetic relationship was investigated by

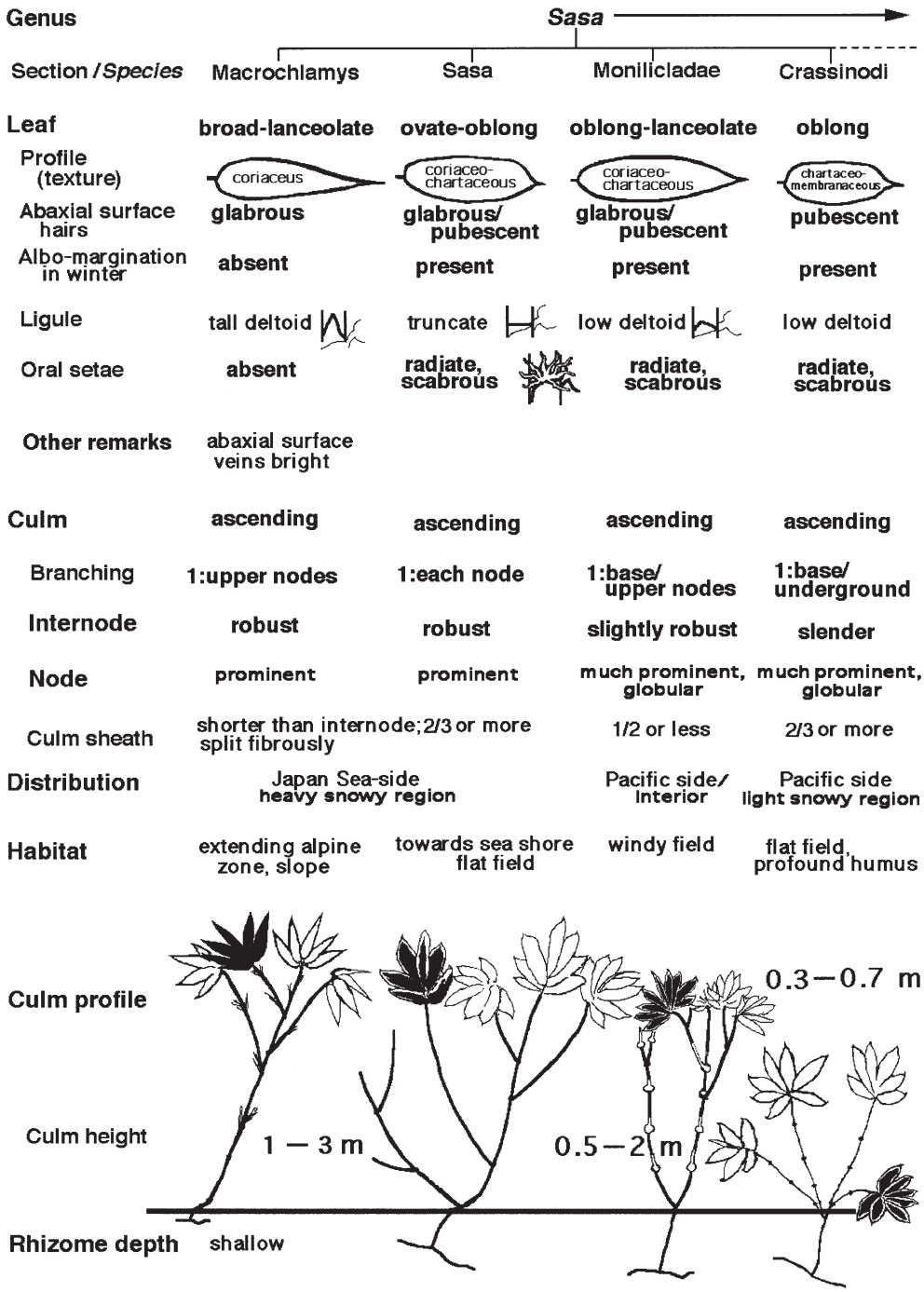
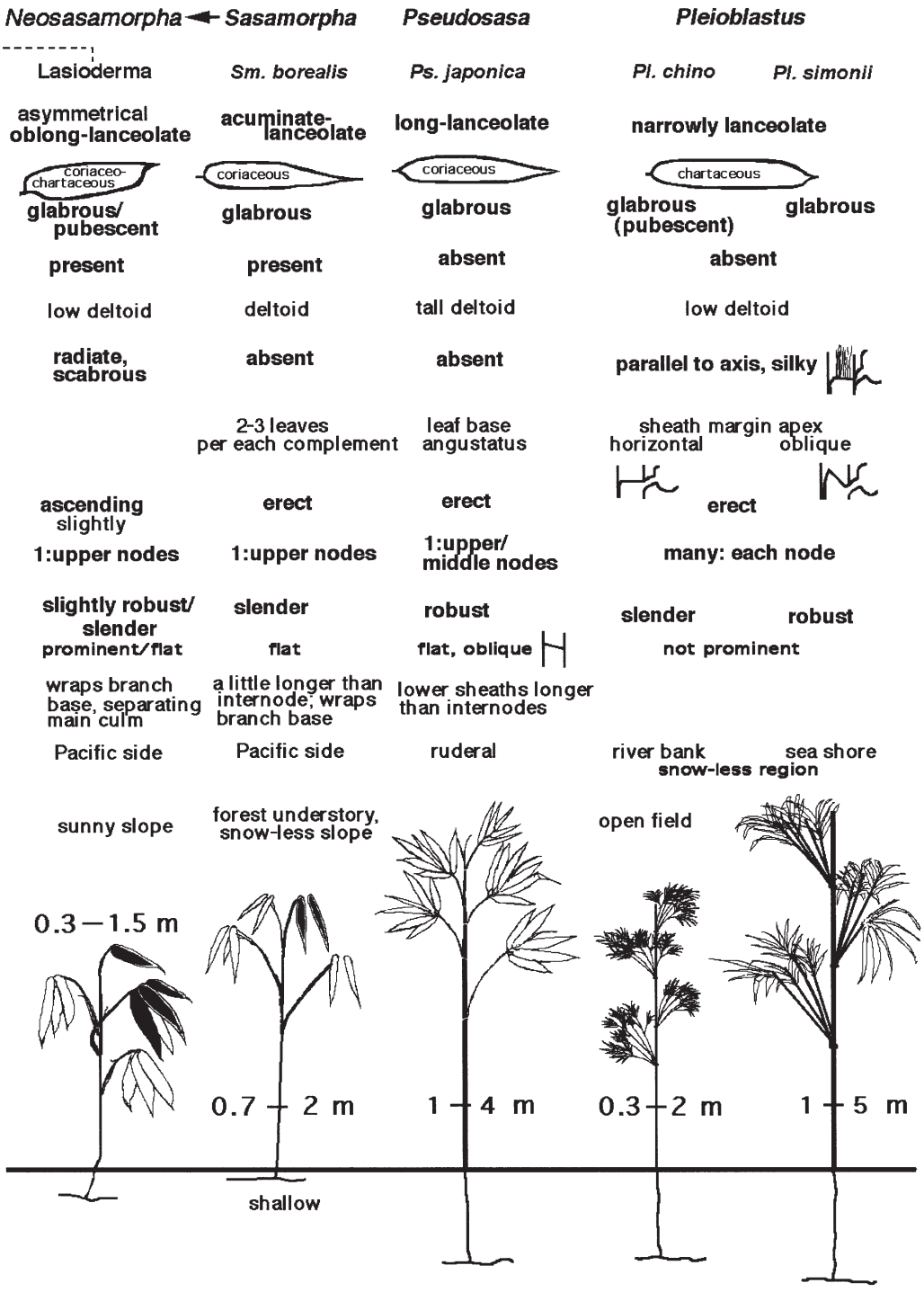


Fig. 2. Index of vegetative organs to Japanese dwarf, culm-sheath persistent bamboos, the Sasa-group



excluding genus *Sasaella*.

means of Wagner parsimony with RAPD data. Lastly, a combined data set with the morphological and RAPD was analyzed, since the method does not require any assumption of evolutionary change or the kind of input data (Nei 1990).

Materials and methods

Plant materials

Table 1 shows 40 samples of bamboo specimens used for UPGMA and parsimony analyses with RAPD data. The taxa included 7 genera and 24 species of Japanese bamboos. One of the most primitive herbaceous bambusoid grasses, *Streptochaeta spicata* was selected for comparison of the genetic distances in UPGMA and as an outgroup for a parsimony analysis to role out the most basal clade among the Japanese bamboos. *Streptochaeta* and *Anomochloa* are the most ancestral clade of the Poaceae, as indicated by *ndhF* sequence data (Clark et al. 1995) and combined sequence data of *ndhF*, *rbcL*, and *PHYB* (Clark et al. 2000). The taxonomic treatment of the bamboos was principally based on the system of Suzuki (1978). Among the collected specimens, two undescribed taxa were included as an intermediate plant between *Sasa kurilensis* and *Sasamorpha borealis*, and as related allies of *Sasamorpha borealis* having an extremely fragile culm, narrowly divaricate branching, and concaved leaves.

Another common genus *Sasaella* was laid aside for the further study for its intergeneric hybridity between *Sasa* and *Pleioblastus* (Takahashi et al. 1994). The first objective of the present study was high-lighted on the position of *Sasa* section *Lasioderma*, a putative hybrid taxon between genera *Sasa* and *Sasamorpha*, avoiding more complicated analytical conditions.

Majority of the specimens were collected from native stands. Some were obtained from the culture collections of the Fuji Bamboo Garden and Utsunomiya University (Fig. 1, Table 1). Voucher specimens were maintained in the herbarium of Department of Forest Science, Faculty of Agriculture, Utsunomiya University.

Morphological data collection

Four most common genera of Sasa-group; *Sasa*, *Sasamorpha*, *Pseudosasa* and *Pleioblastus* were listed up their key characters of vegetative organs of leaf, branch, culm, culm profile, even more the distribution pattern and habitat characteristics (Fig. 2).

Due to limited records on bamboo flowers it was possible to collect the overall complete data of only 16 taxa, as shown in Table 2, which were based on the literatures of Takagi (1963), Suzuki (1978), Kobayashi (2000) and a direct measurement on herbarium specimens as follows; the first flower and/or caryopses records of *Sasa pubiculmis* subsp. *sugimotoi* (RF 573, MK 1668, Aug. 3, 1998) from Mt. Amamaki, Tochigi Prefecture and *Sasamorpha borealis* var. *viridescens* (MK 131, May 6, 1985, Mt. Miharayama, Isl. Hachijojima; MK 1669, Aug. 10, 2001, Ebine Park, Isl. Mikurajima). Additional specimens examined for flower measurements were as follows: *Sasa tsukubensis* (MK 149, Jun. 3, 1985, Senjogahara), *Sasamorpha borealis* (MK 198, May 20, 1986, Senjogahara), *Sasa shimidzuana* (MK 191, May 10, 1986, Mt. Nekko), *Pseudosasa japonica* (MK 121, Feb 10, 1985, Hachioji, Tokyo), *Pleioblastus simonii* (MK 132, May 6, 1985, Isl. Hachijojima). Each value listed on Table 2 shows a mean value of three to 35 measurements of floral organs.

Among these 16 taxa, a total of 35 characters were scored as seen in Table 3 and being composed of the 19 characters of vegetative organs from the index shown in Fig. 2 and the 16 characters of floral organs registered in Table 2. Coding method was the reductive coding (Forey and Kitching 2000) such as all characters were scored as bistate with 1/0 whether it occurs and/or presence or does not occur and/or absence. We took both characters of non-overlapping variations and continuous quantitative variations, though the latter is found to contain less phylogenetic signal (Stevens 2000). When a variation was considered as overlapping one by visual inspection, we used the character only when discrete ranges are recognized among the values. For example, in Table 2, caryopsis length ranged from 5.6 to 18.5 mm, in which two discrete ranges are recognized, i.e., one is 5.8 to 8.0 and another is 11.8 to 18.5. Then we scored them as " caryopsis less than 10 mm" in Table 3.

Phyllostachys bambusoides, one of the Take-

Table 1. Taxa used for RAPD and/or morphological analyses

No.*	Genus	Section	Species**	Abbreviation for figures+	Source/Voucher++
1	*Phyllostachys* Siebold et Zucc.		*Ph. bambusoides* Siebold et Zucc.	*Ph. bambusoides*	Utsunomiya/RF745
2	*Sinobambusa* Makino ex Nakai		*Si. tootsik* (Siebold ex Makino) Makino	*Si. tootsik*	Cult.FBG/RF741
3	*Shibataea* Makino		*Sh. kumasasa* (Zoll.) Makino	*Sh. kumasasa*	Cult.UU/RF749
4	*Pseudosasa* Makino ex Nakai		*Ps. japonica* (Steud.) Makino ex Nakai	*Ps. japonica*	Utsunomiya/RF749
5			*Ps. owatarii* (Makino) Makino	*Ps. owatarii*	Isl. Yaku/KN, Jul. 4,1997
6	*Pleioblastus* Nakai	*Caespitosae* Koidz.	*Pl. linearis* (Hack.) Nakai	*Pl. linearis*	Cult.FBG/RF742
7		*Medakea* Koidz.	*Pl. simonii* (Carrière) Nakai	*Pl. simonii*	Cult.FBG/RF743
8		*Nezasa* Koidz.	*Pl. chino* (Franch. et Sav.) Makino var. *viridis* (Makino) Sad.Suzuki	*Pl. c. viridis*	Cult.FBG/RF744
9			*Pl. chino* (Franch. et Sav.) Makino	*Pl. chino*	Utsunomiya/RF745
10			var. *vaginatus* (Hack.) Sad.Suzuki	*Pl. c. vaginatus*	Hakone/MK, Mar. 1,1997
11	*Sasa* Makino et Shibata	*Monilicladae* Nakai	*S. tsuboiana* Makino	*S. tsuboiana*	Cult.FBG/RF746
12			*S. hayatae* Makino	*S. hayatae*	Isl. Mikurajima/MK1584
13			*S. tokugawana* Makino	*S. tokugawana*, Hakone	Hakone/MK, Mar. 17,1997
14				*S. tokugawana*, Batou	Batou/Cult.UU
15		*Macrochlamys* Nakai	*S. kurilensis* (Rupr.) Makino et Shibata	*S. kurilensis*, Nasu	Mt. Nasu/RF747
16				*S. kurilensis*, Meoto.	Meotobuchi/RF704
17			*S. jotanii* (Ke.Inoue et Tanim.) M.Kobay.	*S. jotanii*, Mikura.	Isl. Mikurajima/MK1585
18				*S. jotanii*, Hachijo.	Isl. Hachijojima/Cult.UU
20			*S. cernua* Makino	*S. cernua*	Senjogahara/RF658
19		*Sasa* (Makino)	*S. palmata* (Lat-Marl. ex N.E.Br.) Nakai	*S. palmata*	Mt. Kinunuma/RF713
21			*S. senanensis* (Franch. et Sav.) Rehder	*S. senanensis*	Meotobuchi/RF684
22			*S. yahikoensis* Makino	*S. yahikoensis*	Komado Pass/Cult.UU
23		*Crassinodi* Nakai	*S. nipponica* (Makino) Makino et Shibata	*S. nipponica*, Senjo.	Senjogahara/MK1583
24				*S. nipponica*, Ama.	Mt. Amamaki/RF596-1
25			*S. chartacea* Makino et Shibata var. *nana* (Makino) Sad.Suzuki	*S. c. nana*, Senjo.	Senjogahara/RF662-1
26				*S. c. nana*, Ama.	Mt. Amamaki/RF596-2
27		*Lasioderma* Nakai s.l.	*S. tsukubensis* Nakai	*S. tsukubensis*	Senjogahara/RF659
28			*S. pubiculmis* Makino ssp. *sugimotoi* (Nakai) Sad.Suzuki	*S. p. sugimotoi*	Mt.Amamaki/RF573
29			*S. shimidzuana* Makino	*S. shimidzuana*	Hakone/MK, Mar.17,1997
30			*S. tsukubensis* Nakai var. *melinacra* (Koidz.) Sad.Suzuki	*S. t. melinacra*	Meotobuchi/RF674
31			aff. *S. kurilensis* x *Sm. borealis*	*S. kurilensis* x *Sm. borealis*	Meotobuchi/RF696
32	*Sasamorpha* Nakai		*Sm. borealis* (Hack.) Nakai	*Sm. borealis*, Senjo.	Senjogahara/MK1289
33				*Sm. borealis*, Ama.	Mt.Amamaki/RF578
34				*Sm. borealis*, Hakone	Hakone/MK, Mar.17,1997
35				*Sm. borealis*, Meoto.	Meotobuchi/RF670
36			aff. *Sm. borealis* (Hack.) Nakai	aff. *Sm. borealis*	Meotobuchi/RF673
37			*Sm. borealis* (Hack.) Nakai var. *viridescens* (Nakai) Sad.Suzuki	*Sm. b. viridescens*, Mikura.	IsI.Mikurajima./MK1586
38				*Sm. b. viridescens*, Hachijo.	IsI.Hachijojima/RF748
39			*Sm. mollis* Nakai	*Sm. mollis*	Shirabi-daira/Cult.UU
40	*Streptochaeta* Schreb.		*St. spicata* Schreb. ex T.Nees	*St. spicata*	Colombia/Cult.UU

* Sample number related to the lane number of agarose gel shown in Fig.4. ** Autonyms are not shown. Citation for author names reffered to Ohrnberger (1999).
+ Abbreviations used for sample names in Figs. 3, 5–8 and Appendix. ++ Note: Cult.:Cultivated plant, where FBG, Fuji Bamboo Garden; UU, Utsunomiya University. Collector prefixes are as follows: MK, M. Kobayashi; RF, R. Furumoto; KN, K. Nakamura.

Table 2. Character states of reproductive organs of Japanese dwarf bamboos, the Sasa-group

Floral part ＼ Species	Sku	Sjo	Spa	Sse	Sts	Sni	Ssu	Stu	Ssh	Smb	Smv	Psi	Pch	Pli	Pse	Phy
Caryopsis length (mm)	7.9*0.8	18.5*1.6	5.9	5.8	8	6.1	5.6	6.6	6.3	5.8	5.9	11.8	12	unknown	7	12
Stigma no.	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Node at style	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present	present	absent	absent	absent	present
Stamen no.	6	6	6	6	6	6	1～6	6	6	6	6	3	3	3	3～4	3
Lodicules (mm)	2.4	3.8*0.4	2.0	2.2	2.1	1.9	2*0.1	1.7*0.08	1.9*0.3	2.2	2.8*0.3	5.0	4.7	3.7	3.0	4.6
Lodicule no.	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Palea,interkeels nerve no.	3	5	3	2	4	2	4	4	4	2	4	4	2	2	5	3
Palea (mm)	9.5	17*0.2	8	8	8	9	9.2	7	8.2	8.2	9.5	12.5	12	12	11	25
Lemma nerve no.	7	13	7	7	7	7	9	7	9～12	11～16	6～12	13	9	9	17	16
Lemma (mm)	7.9	21.8*1.5	7.2	8.6	8.4	6.8	10.7*1.1	7.9*0.7	8.3*1.2	9.1*0.6	11.4*0.6	13.2	15.6	9.1	15	26.5
Floret no.	6～9	4	5～10	4～7	4～6	5～8	2～8	4～9	3～8	5～11	5～12	5～13	8～12	4～5	5～7	1～3
Glume II (mm)	4.2	16.5*4.7	2.1	2.9	3.6	1.2	3.8*0.4	6.8*0.8	5.6*1.3	6.6*0.9	8.6*1.2	12.1	13.1	14.3	12.5	22
Glume I (mm)	1.6	8.7*2.8	0.8	2.2	0.7	0.6	2.2*0.7	3.1	2.4*0.6	3.4*0.7	4.7*0.8	9.2	19.4	9.6	10.5	0
Glume no.	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0～2
Spikelet (mm)	25～35	35.2*3.1	25～40	20～25	25～30	20～30	15～40	14～28	13～31	15～32	22～43	30～110	60～110	40～50	30～50	20～25
Spikelet: F,fusiform; L,linear	F	F	L	L	L	L	F	F	F	F	F	L	L	L	L	F

Sku, *Sasa kurilensis*; Sjo, *S. jotanii*; Spa, *S. palmata*; Sse, *S. senanensis*; Sts, *S. tsuboiana*; Sni, *S. nipponica*; Ssu, *S. pubiculmis* ssp. *sugimotoi*; Stu, *S. tsukubensis*; Ssh, *S. shimidzuana*; Smb, *Sasamorpha borealis*; Smv, *Sm. borealis* var. *viridescens*; Psi, *Pleioblastus simonii*; Pch, *P. chino*; Pli, *P. linearis*; Pse, *Pseudosasa japonica*; Phy, *Phyllostachys bambusoides.* Majority of the data referred to Takagi (1963), Suzuki (1978) and Kobayashi (2000). *Presented arithmetic mean with only known standard deviation.

group, was selected as an outgroup for parsimony analysis of 35 morphological characters in 15 ingroup taxa. This choice seemed appropriate since RFLP analysis had suggested that *Phyllostachys bambusoides* was positioned more basal in the East Asiatic bamboo clade than the Sasa-group (Kobayashi 1997).

RAPD analysis

Samples were taken as rolled or immediately after unrolled leaves to avoid any epiphytic fungal contaminations (Zhang et al. 1997). Total DNA was extracted from fresh or silica gel-dried (Chase and Hills 1991) leaves using a modified CTAB method (Hasebe and Iwatsuki 1990).

Sample DNA in a TE buffer was used as template DNA for RAPD analysis after Williams et al. (1990). Forty primers of the Operon's 10–mer kits (Kit-A and Kit-C) were used as the random primers for amplification. Amplification reactions were performed in volumes of 10 μl containing 15 to 60 ng template DNA, 0.25 U *Taq* DNA polymerase (Gene *Taq*, Nippon Gene), 0.3 mM primer, 0.25 mM of each dNTP, 1.65 mM $MgCl_2$, 11 mM Tris-HCl (pH 8.8), 0.1 mM EDTA, 55 mM KCl, 0.11% Triton X 100, 0.25% glycerol, and 5 mM DTT. Polymerase chain reaction (PCR) was performed in a Perkin Elmer Gene Amp PCR System 2400 programmed for an initial denaturation of 45 sec at 94℃ followed by 43 cycles of denaturation for 30 sec at 92℃, annealing for 1 min at 48℃, extension for 2 min at 72℃, and post-elongation for 5 min at 72℃. Amplification products were analyzed by electrophoresis in 2% agarose gels in 0.5×TBE buffer (45 mM Tris-base, 45 mM borate, 1.0 mM EDTA, pH 8.3) by pulse-field electrophoresis (CHEF DRII ; BioRad) and detected by staining with ethidium bromide. To confirm that the bands were stable and amplified genomic DNA, any sample DNA was omitted from the control lane for each primer and confirmed to be insensitive to DNA template concentrations varying from 10 to 100 ng/ml. Segregating polymorphic banding patterns were scored for presence/absence as 1/0 data matrices only when reproducible markers between 2 or 3 replicate PCRs for each random primers were detected.

Genetic distance between each samples was calculated according to a formula introduced by Mackill (1995) based on Nei and Li's (1979) estimator F in which the shared DNA fragment F transforms restriction-site data into genetic distances.

$$GD = 1 - F = 1 - \{2N / (N_i + N_j)\}$$

In which N_i and N_j are the number of bands for samples i and j, respectively. N is the number of shared bands between the two samples. A program software written in N 88 BASIC produced by ourselves for calculating these genetic distances from PHYLIP data file as in the Appendix is available for corresponding to the author. The genetic distance data were used to construct a cluster dendrogram by UPGMA using NEIGHBOR in PHYLIP (Felsenstein, J. 1995. PHYLIP (Phylogeny Inference Package) version 3.57 c. Seattle : University of Washington, URL : http : evolution.genetics.washington.edu/phylip/). An optional mode of Neighbor-Joining method in the NEIGHBOR program gave no systematically meaningful result such that majority of each clusters were composed of different generic taxa.

Phylogenetic analysis was carried out by Wagner parsimony with a 1,000–replicate bootstrap confidence using SEQBOOT, MIX and CONSENSE in the program package PHYLIP version 3.57 provided by Felsenstein (1995). In using each program, data input was made with randomizing input order by three times of jumbling, without any character weighting. Calculating ensemble consistency and retention indices, CI and RI was referred to Maddison and Maddison (1992) and ambiguous states at all nodes were omitted to avoid overestimation of those tree statistics.

Results

Morphological phylogeny

Wagner parsimony analysis of 35 morphological characters produced a single tree, with a length of 82 steps, a CI of 0.72, and an RI of 0.47 (Fig. 3). *Genus Pleioblastus* was the most basal clade, the next most basal was *Pseudosasa japonica* with 68% bootstrap confidence. *Sasa jotanii* positioned outside the clade of *Sasamorpha* and *Sasa* including sect. *Lasioderma*. *Sasa* sect. *Lasioderma* was monophyletic with *Sasa tsuboiana* — *S. nipponica* group with 45% boot-

Table 3. Morphological data matrix of Japanese dwarf bamboos, the Sasa-group. Abbreviations for species names are the same as in Table 2

Characters ＼ Species	Sku	Sjo	Spa	Sse	Sts	Sni	Ssu	Stu	Ssh	Smb	Smv	Psi	Pch	Pli	Pse	Phy
1 caryopsis < 10 mm	1	0	1	1	1	1	1	1	1	1	1	0	0	?	1	0
2 style-node present	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1
3 stamen no. 6	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0
4 lodicules ≦ 3 mm	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	0
5 palea, interkeels nerve no. ≦ 3	1	0	1	1	0	1	0	0	0	1	0	0	1	1	0	1
6 palea, interkeels nerve no. 5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
7 palea < 13 mm	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
8 lemma nerve no. 7	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0
9 lemma nerve no. 9	0	0	0	0	0	0	1	0	1	0	1	0	1	1	0	0
10 lemma nerve no.≧10	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1	1
11 lemma length < 20 mm	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
12 floret no. ≦ 4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13 glume Ⅱ < 10 mm	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0
14 glume Ⅰ < 5 mm	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	1
15 spikelet length ≦ 40 mm	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1
16 spikelet shape fusiform	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	1
17 leaf albo-margin in winter	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0
18 leaf abaxial pubescent	0	0	0	1	0	1	1	0	1	0	0	0	1	0	0	0
19 leaf coreaceous	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	0
20 leaf apex rostratus	1	1	0	0	0	0	1	0	0	1	1	0	0	1	1	0
21 leaf blade broad	1	1	1	1	1	1	0	1	1	0	1	0	0	0	0	0
22 leaf no. per complement ≦ 3	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
23 sheath-margin fimbriae present	0	0	1	1	1	0	1	1	0	1	1	1	1	0	0	1
24 oral setae radiate	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	1
25 oral setae silky	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
26 oral setae present	0	0	1	1	1	1	1	1	1	0	0	1	1	1	0	1
27 culm sheaths slitted fibrously	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 culm sheaths embrace new branchbase	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
29 culm sheaths cover internode	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0
30 culm sheaths persistent	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
31 one branch at each node	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0
32 branching upper node	1	1	0	0	1	0	1	1	1	1	1	0	0	0	0	0
33 node prominent	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
34 culm erect	0	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1
35 rhizome shallow	1	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0

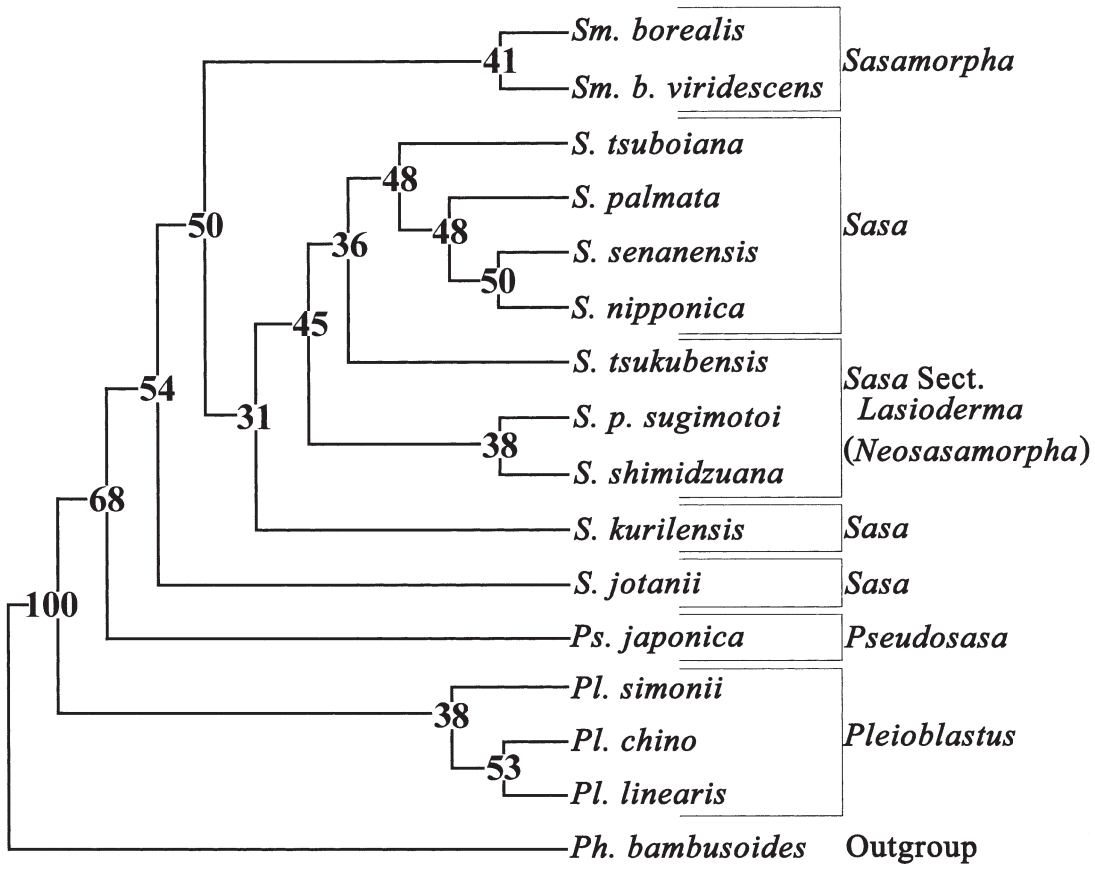


Fig. 3. A single most parsimonious tree of 35 morphological characters in 16 taxa rooted at *Phyllostachys bambusoides* with CI, RI, and tree length of 0.72, 0.47, and 82 steps, respectively. The numbers on each node indicate a 1,000 replicate-bootstrap confidence (%).

strap confidence.

RAPD analysis

Nine out of 40 primers were selected to give stable electrophoretic banding patterns (Table 4). A total of 327 polymorphic bands were detected and scored as 1/0 data matrices as shown in Appendix. Identification of RAPD bands was made by visual inspection of the original pictures of the gels (Fig. 4). In evaluating each banding patterns, the criteria for informative band was referred to the bands within the size range of the DNA Molecular Weight Marker VI between 2,176 bp and 154 bp. Among them, the 11 th band of 220 bp was the finest, to which band with the same or stronger intensity were scored as significant. In the Appendix, data matrix of the whole RAPD bands was shown in order as scored for 1/0 from top (well side) to bottom (front side) for each primer in each gel of A through I in Fig. 4.

The average band number for each Japanese bamboo specimen was 62; while the band of *Streptochaeta spicata* was 11, which fewer band number probably dues to ploidy level that the taxon is diploid, while the Japanese bamboos are tetraploid (Tateoka 1955; Soderstrom 1981).

UPGMA

A UPGMA dendrogram (Fig. 5) of genetic distances between 40 specimens showed four principal clusters within the Japanese bamboos; (1) *Phyllostachys* and *Shibataea*; (2) *Pseudosasa japonica*, *Sinobambusa* and *Pleioblastus*; (3) *Sasa* excluding sect. *Lasioderma*; and (4) *Pseudosasa owatarii* and the *Sasamorpha-Lasioderma* group.

Table 4. Random primers used and numbers of bands produced for 40 samples listed on Table 1

Primer	Nucleotide sequence	No. of bands
OPA-01	5'-CAGGCCCTTC-3'	32
OPA-06	5'-GGTCCCTGAC-3'	42
OPA-08	5'-GTGACGTAGG-3'	48
OPA-17	5'-GACCGCTTGT-3'	36
OPC-03	5'-GGGGGTCTTT-3'	28
OPC-06	5'-GAACGGACTC-3'	35
OPC-14	5'-TGCGTGCTTG-3'	37
OPC-16	5'-CACACTCCAG-3'	38
OPC-18	5'-TGAGTGGGTG-3'	31
Total		327

The third cluster consisted of two main groups, *Sasa* sect. *Macrochlamys* and three sections of *Monilicladae*, *Sasa* and *Crassinodi*. Two specimens of *S. tokugawana*; one from Batou and the other from Hakone were placed in different groups.

Phylogenetic analysis based on RAPD data

Wagner parsimony analysis of 327 RAPD bands from 40 samples yielded 8 equally parsimonious trees in which the number of steps, CI, and RI of each tree were 1200, 0.86, and 0.44, respectively. In a majority rule consensus tree (Fig. 6), four major clades were resolved. The basal clade consisted of *Phyllostachys bambusoides* and *Shibataea kumasasa*. The next most basal clade contained both species of *Pseudosasa*, showing the most distinct point with the topology of the UPGMA dendrogram (Fig. 5). The remainder of the Sasa-group was strongly supported in 90% of the bootstrap confidence. *Sasa* sect. *Lasioderma* was placed in the same lineage as the genus *Sasamorpha*, though this was supported weakly in not more than 7% of the bootstrap confidence. Another group was subdivided into two principal clades, one that included the genera *Pleioblastus* and *Sinobambusa*, and another that was formed by the genus *Sasa* excluding sect. *Lasioderma* (hereafter called as the *Sasa* clade). Within the *Sasa* clade, the species of sect. *Macrochlamys* together with two other taxa formed the most basal group, followed by those of sect. *Monilicladae* at the next most basal position. The species of *Sasa* sect. *Sasa* was sister to sect. *Crassinodi* at the terminal.

In general, hybrid taxa render a phylogenetic tree analysis ambiguous (McDade 1995; Riesebeg and Morefield 1995). Ambiguous polymorphisms in RAPD analysis may result from poor discrimination by a primer against alternative priming sites of slightly different nucleotide sequences (Williams et al. 1990). Hybrid taxa probably caused many ambiguous banding patterns in RAPD-PCR products. Thus we removed suspected hybrid taxa from the 40 specimens with respect to the system of Suzuki (1978). This procedure led to a reduced set of 22 taxa, including two to three representative species of each genus or section of the Sasa-group and an outgroup.

A Wagner parsimony analysis of 289 RAPD data rooted at *Phyllostachys bambusoides* produced a single most parsimonious tree (Fig. 7; tree length=723 steps, CI=0.87, RI=0.57). In this tree, two main clades were resolved. One comprised the genera *Pseudosasa*, *Sasamorpha* and *Sasa* sect *Lasioderma*, the other consisted of the genus *Pleioblastus* which was sister to the *Sasa* clade. Within the *Sasa* clade, sect. *Macrochlamys* was the most basal, followed by the *Monilicladae* and the sects. *Sasa* and *Crassinodi* as sister clades at the terminal.

RAPD and morphological data set

Analysis of combined RAPD and morphological data sets of 298 characters in 16 taxa rooted at *Phyllostachys bambusoides* produced five equally parsimonious trees (length 639 steps, CI=0.83, RI=0.59). The majority rule consensus tree (Fig. 8) resolved three major clades. *Sasa* clade and *Sasamorpha*/sect. *Lasioderma* may appear as sister to each other with that clade sister to the *Pseudosasa*/*Pleioblastus* clade. In each clade, sister relationship of *Pseudosasa japonica* to genus *Pleioblastus*, genus *Sasamorpha* to *Sasa* sect. *Lasioderma*, and sect. *Macrochlamys* to other sections of *Sasa* clade is supported with 62%, 54% and 25% of bootstrap confidence, respectively.

Discussion

Evolutionary trends in Japanese bamboos

Naturally distributed Japanese bamboos are

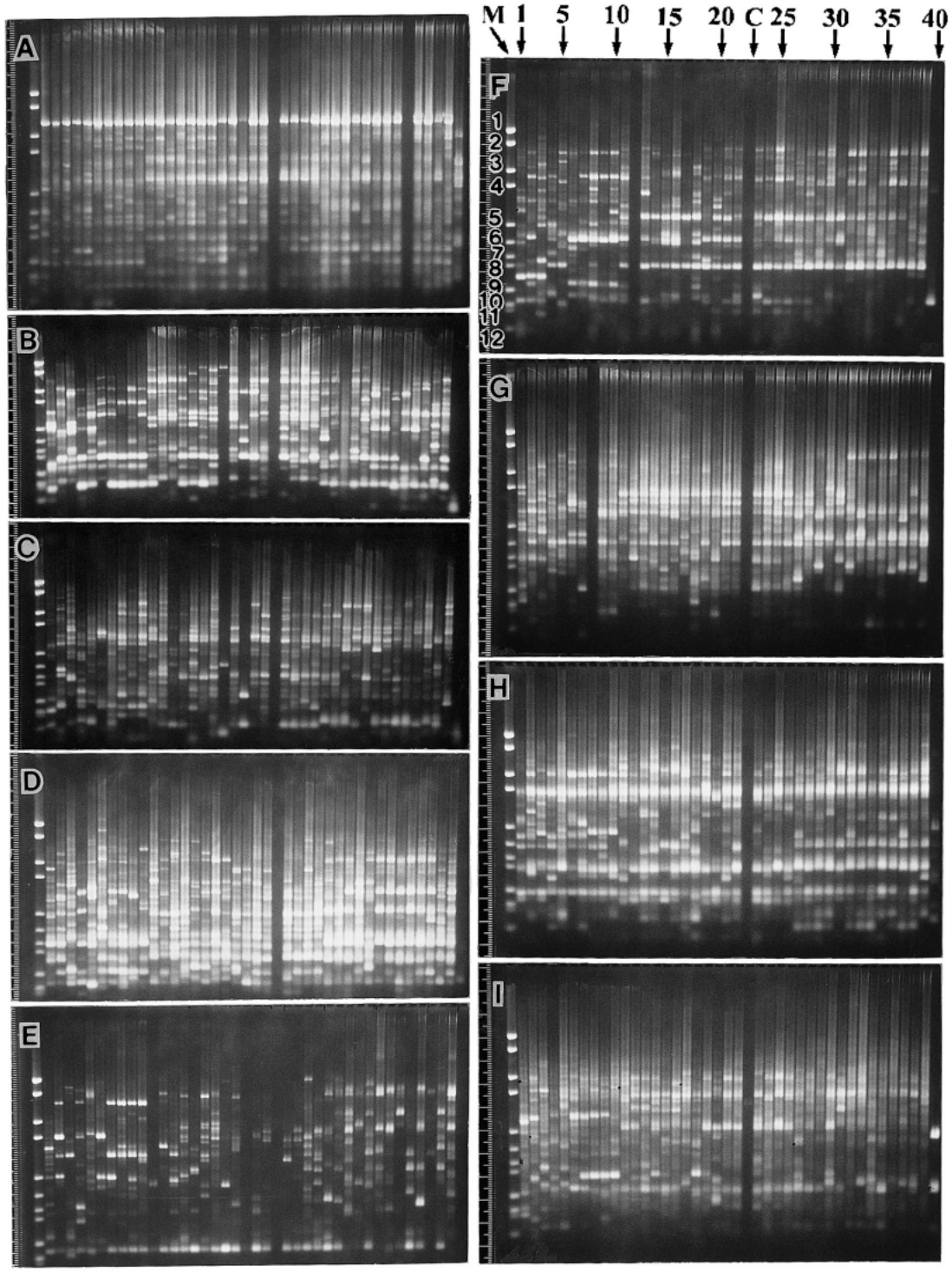


Fig. 4. RAPD banding patterns for primers of A, OPA–01 ; B, OPA–06 ; C, OPA–08 ; D, OPA–17 ; E, OPC–03 ; F, OPC–06 ; G, OPC–14 ; H, OPC–16 ; I, OPC–18 in the Japanese bamboos. The numbers indicated in the row of photograph F refer to the sample number as shown in Table 1. Two lanes containing control mixture (C),and a DNA size marker, Boehringer Mannheim's DNA Molecular Weight Marker VI(M),in which numbers 1 through 12 in the column designate 2176, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234, 220 and 154 bp, respectively.

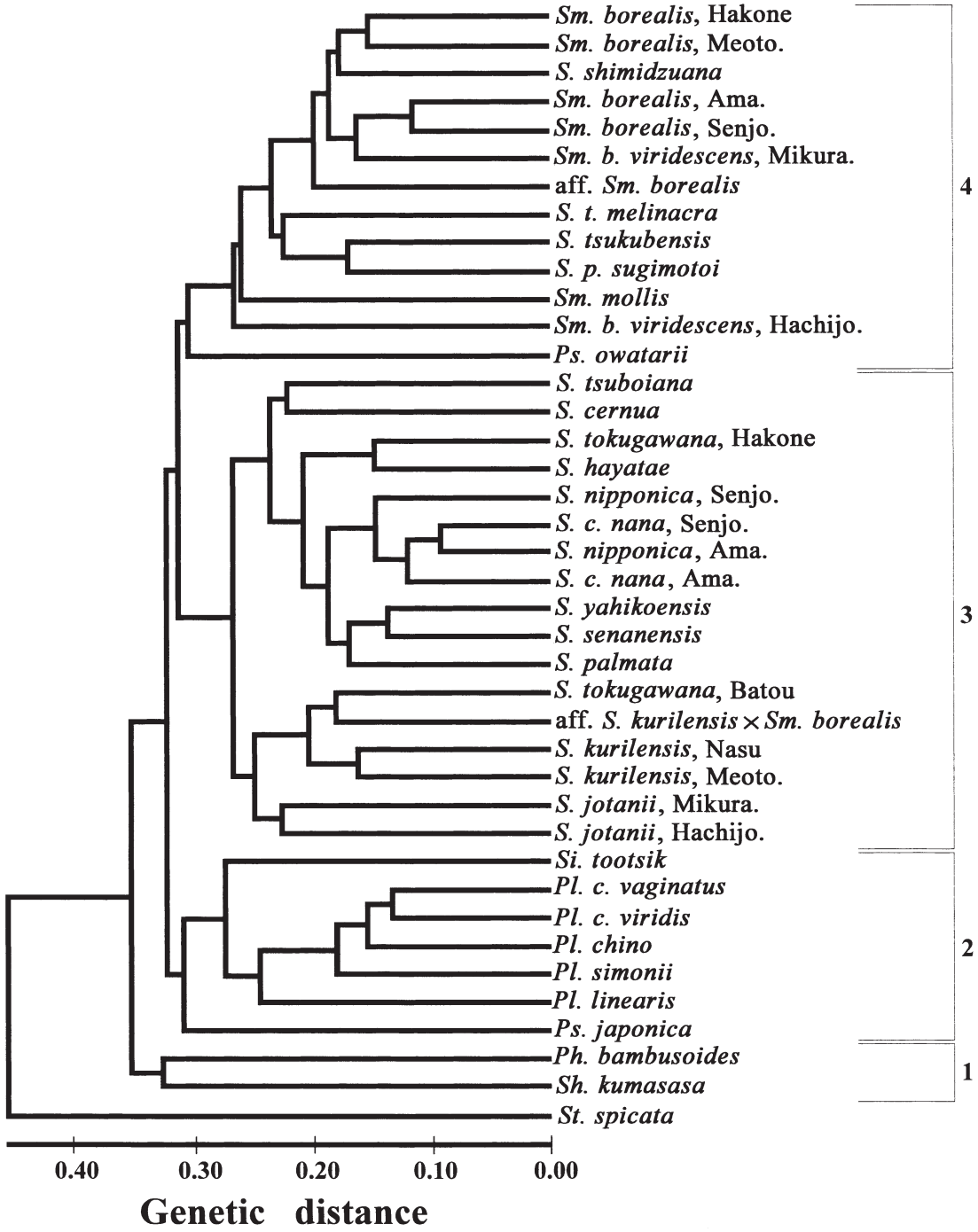


Fig. 5. UPGMA dendrogram of 40 samples based on Nei and Li's genetic distances with RAPD data listed in Appendix.

all temperate species with the somatic chromosome number of 2n=4x=48 (Tateoka 1955; Soderstrom 1981) which are resolved as monophyletic with a chloroplast DNA analysis (Kobayashi 1997). Diagnostic characters of Japanese bamboos are mainly based on the external mor-

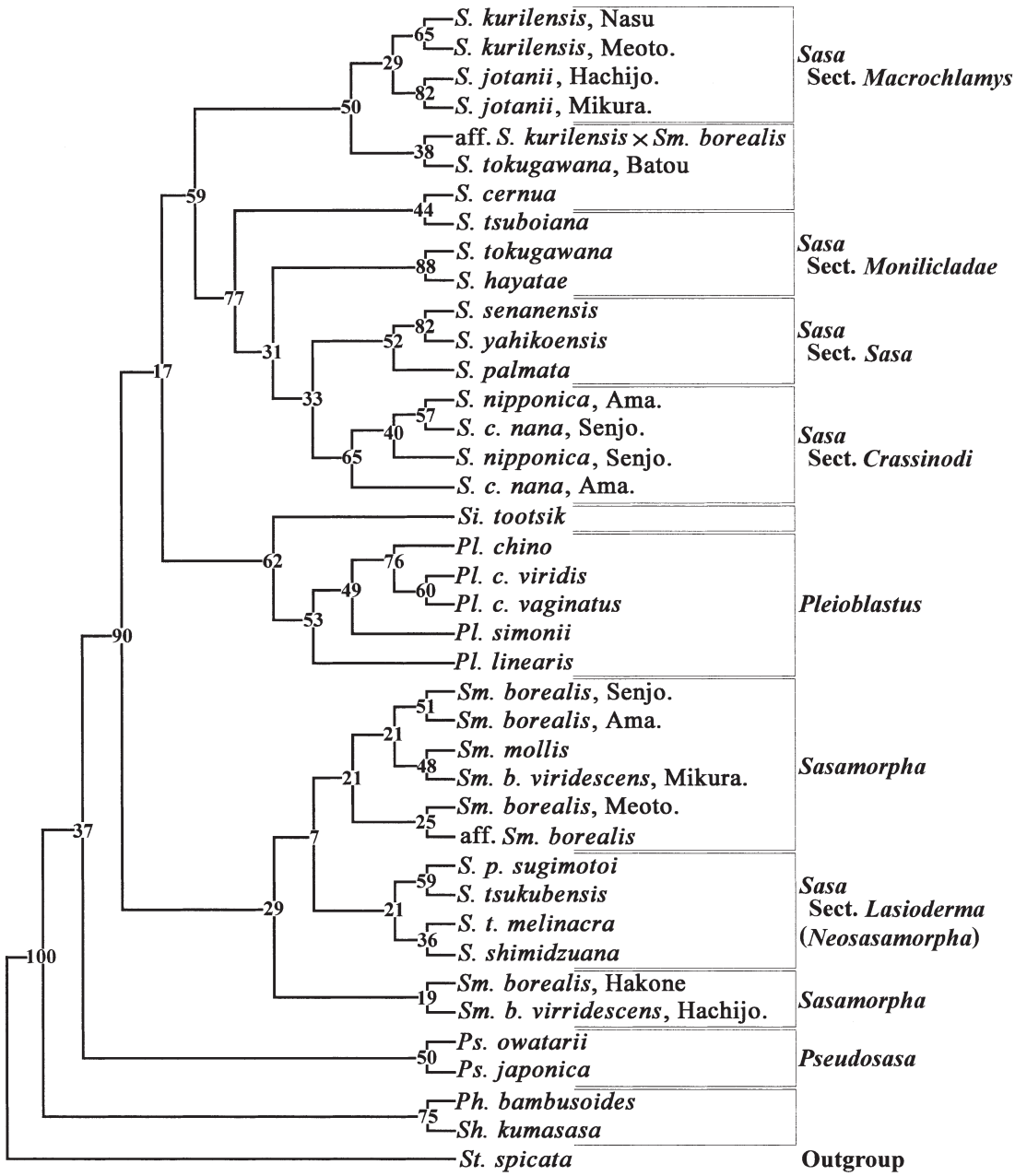


Fig. 6. Majority rule consensus tree of eight equally parsimonious trees based on 327 RAPD data in 39 Japanese bamboo samples and an outgroup rooted at *Streptochaeta spicata* with CI, RI, and tree length of 0.86, 0.44, and 1,200 steps, respecttively. Numbers on each node show a 1,000 replicate-bootstrap confidence (%).

phology of vegetative organs due to the rarity of flowering, e.g., the culm-sheath deciduous or persistence and other vegetative organs as shown in Fig. 2. Both a RAPD based tree (Fig. 6) and the chloroplast DNA-tree (Kobayashi 1997) show *Phyllostachys* and *Shibataea*, the Take-group are more basal than the Sasa-group suggests the deciduous culm-sheath is a plesiomophic character, while persistent is an apomorphic one.

Pleioblastus-Sinobambusa clade is relatively basal in tree topology than the *Sasa*-clade (Fig.

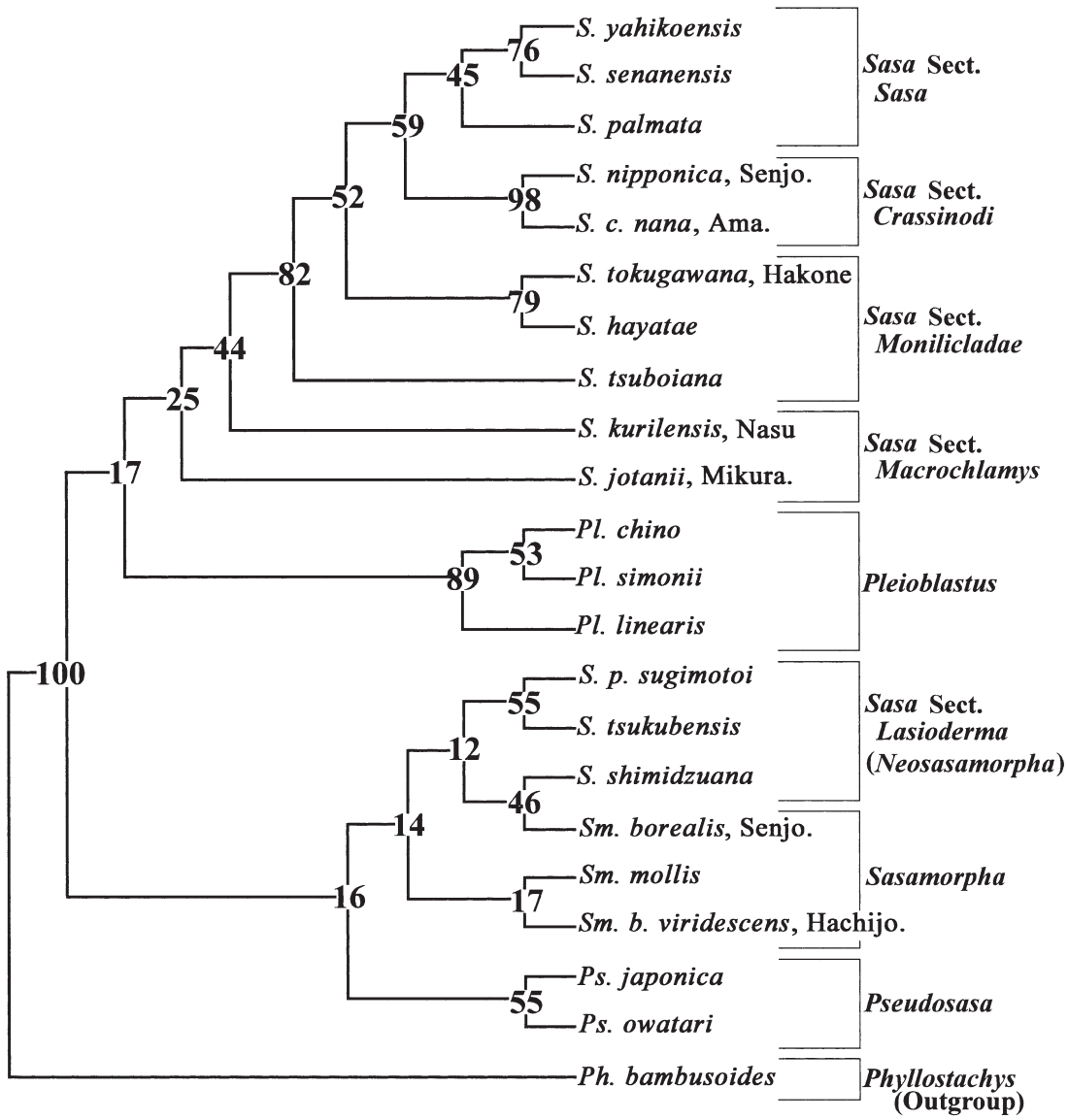


Fig. 7. A single most parsimonious tree of 289 RAPD data in 21 taxa of Sasa-group and an outgroup of *Phyllostachys bambusoides* with CI, RI, and tree length of 0.87, 0.57, and 723 steps, respectively. Numbers on each node show a 1,000 replicate-bootstrap confidence (%).

6). Genus *Pleioblastus* is more basal than the other Sasa-group in the morphological and RAPD-morphological combined trees (Figs. 3 and 8). The species of the Take-group; *Phyllostachys bambusoides*, *Shibataea kumasasa* and *Sinobambusa tootsik* bear two, five and more than three branches per node, respectively, whereas genus *Pleioblastus* bears more than three. While genus *Sasa* clade as well as *Pseudosasa* and *Sasamorpha* are monoclade (Fig. 2). Thus, those species which are relatively basal than the *Sasa* clade all have the multiple branching habit suggesting that the character state of multiple branching per node or pleioclade is plesiomorphic, while one branch per node or monoclade is apomorphic.

The branch number per each node is originated from the primordial axis number per each mid-culm bud. In this respect, Usui (1957) reported four types of mid-culm branch buds and

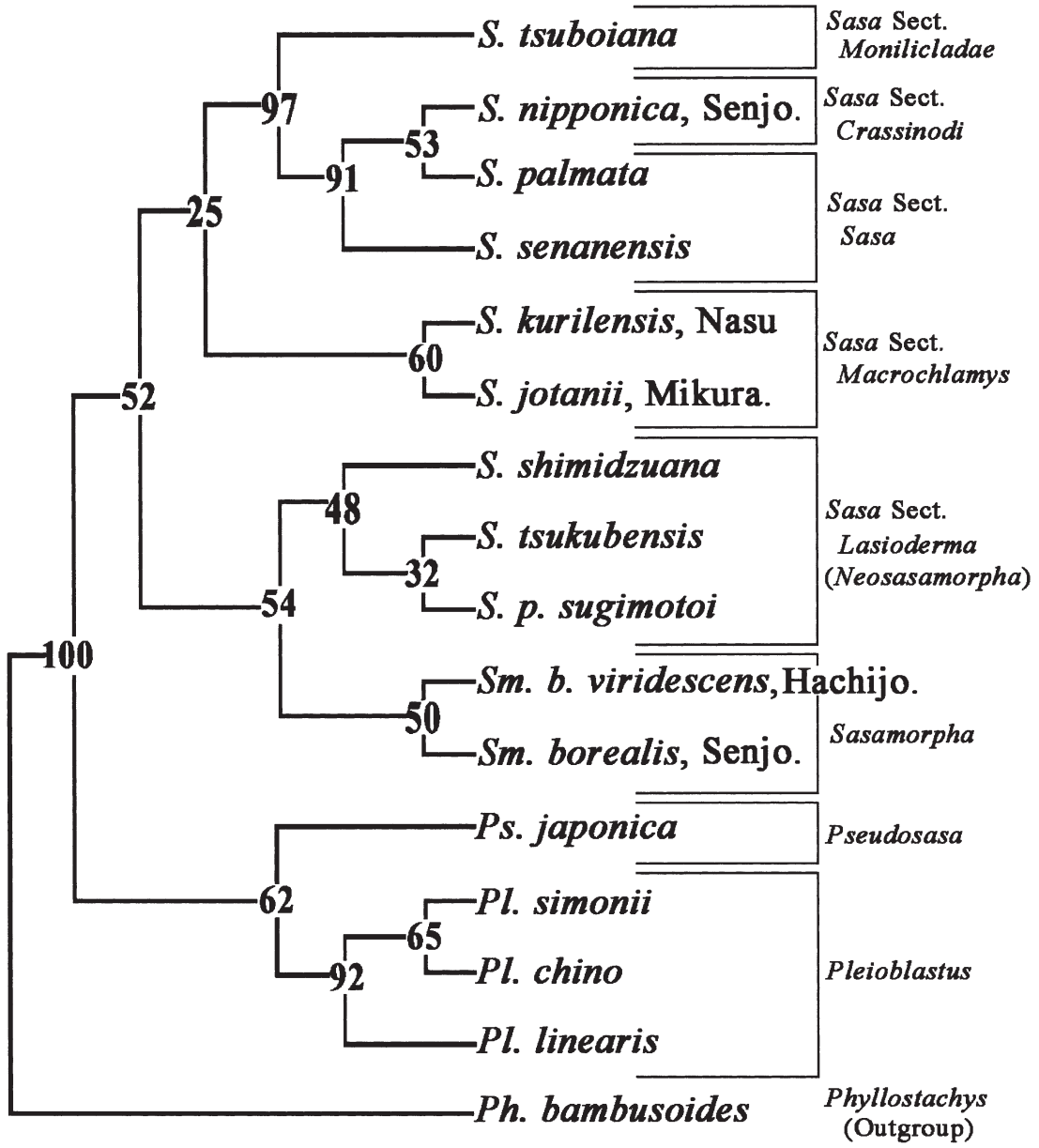


Fig. 8. Majority rule consensus tree produced from five equally parsimonious trees of 298 combined RAPD/morphological data in which CI, RI, and tree length are 0.83, 0.59, and 639 steps, respectively. Numbers on each node show a 1,000 replicate-bootstrap confidence (%).

corresponding branch complements among the Japanese bamboos as genus *Sasa*-, *Pleioblastus*-, *Shibataea*- and *Phyllostachys*-types. Each type is characterized by one- or two-keeled prophyllum that enclosed a primordial branch axis and subsidiary buds developed before and/or after a rupture of the prophyllum in the bud stage. Phylogenic transformation was supposed to have taken place with a fusion of a one-keeled prophyllum and a foliage leaf primordium to produce a two-keeled prophyllum, accompanying with a reduction of the branch numbers from many to one. Considering on the evolutionary trend, Usui speculated that the earliest diversified was the *Shibataea*-type, next was the *Phyllostachys*-type, followed by the *Pleioblastus*-

type, and the most advanced was the *Sasa*-type. In the present phylogenetic analyses, it was shown that the Take-group was the most ancestral, *Pleioblastus* followed them, and genus *Sasa* was the most advanced. These results suggest that the Usui's scheme is adequate.

Phylogenetic position of *Sasa jotanii*

Suzuki (1996) persisted that the *Sasa kurilensis* -like plant distributed in the Southern Izu Islands should be referred to *Sasa tsuboiana* Makino of the sect. *Monilicladae* and even a variety of *Sasa kurilensis* was not believed to distribute in the Izu Islands far from the heavy snowy region of the Japan Sea side. On the contrary, Maekawa (1971) pointed out that *Sasa jotanii* is a remarkable example of a vicarious species with *Sasa kurilensis* which shows a disjunctive distribution over the Pacific side of the Japan Islands, discussing the phytogeographical differentiation in *Platanthera ophrydioides* F.Schmidt. He gave some other examples of vicarious taxa such as *Carex doenitzii* Boeck. var. *okuboi* (Franch.) Kük., *Patrinia triloba* (Miq.) Miq. var. *kozushimensis* Honda, and other Orchidaceous plants to show the Izu Islands is a general vicarious area with the Japan Sea side. Kobayashi (2000) placed *S. jotanii* within the genus *Sasa*, and considered it most closely related to *S. kurilensis* of the sect. *Macrochlamys*, based on a morphological comparison of *S. jotanii*, *S. kurilensis* and *S. tsuboiana*. RAPD-based and RAPD-morphological combined trees (Figs. 6 and 8) included *S. jotanii* in the sect. *Macrochlamys* which supported Maekawa's phytogeographical viewpoint and suggested that the taxonomic treatment of *S. jotanii* as a distinct species of sect. *Macrochlamys* (Kobayashi 2000) is adequate.

However, the morphological tree (Fig. 3) separated *S. jotanii* from the *Sasa* clade. *Sasa jotanii* has unicellular long hairs at the margin of prophyllum that embraces a bud borne on the node near the apex of monopodial rhizome (Kobayashi and Yachimori 2000). Takenouchi (1932) clarified that the rhizomatous prophyllum hairs in the Take-group, *Phyllostachys*, *Tetragonocalamus* and *Chimonobambusa* which is occasionally included in the Sasa-group though, have unicellular long ones, while in the genera of Sasa-group, they have a mixed type of unicellular and multicellular hairs. Thus, *S. jotanii* shares a common character with Take-group in other morphological traits than the analyzed characters as in Table 3. Those characteristics imply that *S. jotanii* has a possibility in positioning the more basal clade as in the morphological tree than in the RAPD trees. The decision is laid aside for the further phylogenetic study with other molecular markers, because the bootstrap confidences of many clades of RAPD trees in the present study are generally low.

Divergence of genus *Sasa*

Not concerning with the branch numbers, the Take-group, *Pleioblastus*, and even *Pseudosasa* are relatively basal in tree topology than genus *Sasa* (Figs. 3, 6, 7 and 8). This signifies that erect culm is the more ancestral state than an ascending one. Ascending culms as well as culm height in the genus *Sasa* plants have been considered as an adaptation to the snow depth environment (Suzuki 1961; Kobayashi 1985). Figure 2 shows the morphological aspect of vegetative organs, habitat, and the distribution patterns on the horizontal view of the Japan Islands from the Japan Sea side through the Pacific side. Genera *Pseudosasa* and *Pleioblastus* are situated on the snow-less area on the right end, which are distributed both sides of the Japan Islands other than Hokkaido, though only two species of *Pleioblastus* are sporadically distributed around the Oshima Peninsula in Hokkaido (Suzuki 1978).

Suzuki (1961) considered the mean annual snow depth of snow-cover is one of the most important limiting factors for the distribution of genus *Sasa* plants. In the regions where the snow depth is more than 75 cm, the sect. *Sasa* habit is predominant. Species of sect. *Macrochlamys* are always distributed sympatrically with sect. *Sasa*, but occur generally more inland and in higher elevation. While in the regions of less than 50 cm snow depth, species of sect. *Crassinodi* are dominant. In the regions of least snow, e.g. in the southern parts of the Kanto district, they become rare and usually genus *Pleioblastus* appears in dense thickets.

RAPD trees showed the intergeneric relation-

ships as the genus *Sasa* excluding sect. *Lasioderma* was more advanced than the genera *Sasamorpha* and *Pleioblastus*, while as for the intrageneric relationships of genus *Sasa*, sect. *Macrochlamys* was the most basal, *Monilicladae* was the next most basal, and sects. *Sasa* and *Crassinodi* were the most advanced (Figs. 6 and 7). Coincidence of these phylogenetic relationships with the habitat segregation and the distribution patterns as shown in Fig. 2 supports the Suzuki's consideration above cited. The distribution patterns suggest that the Japanese dwarf bamboos extended their distribution ranges northwards, diversifying genus *Sasa* plants from warm region towards the snowy area around the Japan Sea region.

At the first time of diversification, the species of the most basal clade in genus *Sasa*, the sect. *Macrochlamys* show wide range of distribution, thus *S. jotanii* inhabits in a mild climate of Southern Izu Islands on one hand, while *S. kurilensis* in heavy snowy areas surrounding the Sea of Japan on the other hand, though *S. jotanii* is considered as the vicarious species as discussed earlier.

In all trees, the sects. *Sasa* and *Crassinodi* or parts of them are sister to each other at terminal nodes, suggesting that the two taxa are the closest and most advanced lineages in the genus *Sasa*. The sister relationship with a small genetic distance between them corresponds to the geographical distribution pattern in the Japan Islands such that occurred side by side around the *Crassinodi*-line. In addition, Suzuki (1961) reported an occurrence of intermediate plants between both sections alongside the *Crassinodi*-line in Northern Japan. Niimiya and Ito (1983) also reported the occurrence of the intermediate plants in Hokkaido and studied their morphological characteristics in special reference to the portions of winter buds. Kobayashi and Hamamichi (2001) studied the intermediate plants distributed around the Senjogahara as the clonal complex of *Sasa nipponica* — *S. palmata* which was selectively predated by Sika-deer, *Cervus nippon* Temminck. Moreover, the present findings confirm the results of the allozyme polymorphism analysis by Takahashi et al. (1994). They showed that genetic differences of allozyme phenotypes on 10 enzyme systems between the two sections were small and the sections formed a "composite type". These findings suggest that sects. *Sasa* and *Crassinodi* differentiated most lately in accordance with the habitat segregation appeared contrastively side by side the *Crassinodi*-line located on the Pacific side interior.

Taxonomic position of sect. *Lasioderma*

In all trees (Figs. 3, 6, 7 and 8), the two genera *Sasa* excluding sect. *Lasioderma* and *Sasamorpha* were located in distinct clades, suggesting that the two taxa have a distinct generic limit each other and the taxonomic treatment of *Sasa* sect. *Sasamorpha* (Nakai) Muroi annotated by Ohrnberger (1999) was inaccurate.

On the contrary, a discrepancy between morphological and RAPD trees; morphological tree placed the sect. *Lasioderma* into the same lineage as the genus *Sasa* (Fig. 3), while in RAPD trees it fell into the *Sasamorpha* clade (Figs. 6, 7 and 8), gives us insight into the generic limit problem among *Sasa*, *Sasamorpha* and sect. *Lasioderma*.

Diagnostic morphological characters of sect. *Lasioderma* are: (1) several foliage leaves per branch complement, (2) developing radiate-scabrous oral setae, (3) leaf blades usually asymmetrically oblong-lanceolate, (4) slightly ascending culm, (5) culm-sheath slightly shorter than internode, and (6) culm-sheath wraps branch base with main culm separately (Fig. 2; Suzuki 1978). Among them, the former two, (1) and (2) are limited to genus *Sasa*, while the last one, (6) is to *Sasamorpha*, whereas other three, (3)～(5) are the intermediate characters characteristically seen in sect. *Lasioderma*.

Tatewaki (1940) was the first to recognize that *Sasa* sects. *Lasioderma* and *Acrocladula* had putative hybrid origins as *Sasamorpha*×*Sasa* and revised them into a new genus *Neosasamorpha*. Takahashi et al. (1994) pointed out that *Sasa* sect. *Lasioderma* Nakai s.l. had distinctive genetic characters caused by successive recombination which were not present in the parental taxa.

For a long time, sect. *Lasioderma* has been treated as a component of genus *Sasa* (Suzuki 1978) which was supported by our morphological

tree. However, RAPD trees separated it from the genus and showed the more closed allies of the genus *Sasamorpha* on the genetic basis which makes us exclude it from the genus *Sasa*. In addition, the taxon has some distinctive genetic characteristics and intermediate morphology. According to them, we at present conserve the genus *Neosasamorpha* Tatew. instead of sect. *Lasioderma*.

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小林幹夫[1]・古本　良[2]：日本産ササ類における RAPD および形態形質データに基づく最節約法による系統類縁関係の解析

マダケ，オカメザサ，トウチク，ならびにアズマザサ属を除く日本産ササ類を含む 30 分類群について，327 個の RAPD データに基づき *Streptochaeta spicata* を外群としたワグナーの最節約法によって系統類縁関係の解析を行った。マダケ・オカメザサ群が最も祖先的な位置を占め，ヤダケ属がそれに続き，スズダケ属・ササ属ナンブスズ節群とメダケ属・トウチク・ナンブスズ節を除くササ属群の 2 大系統群が末端に位置した。ナンブスズ節を除くササ属内部では，ミクラザサを含むチシマザサ節が最も早い分岐群となり，次いでアマギザサ節，末端にチマキザサ節とミヤコザサ節が姉妹分岐群を形成して位置した。UPGMA 樹状図もヤダケ属を除いて同様なクラスター配置を示した。次に，マダケを外群とし，主なササ類の 20 種・1 変種について RAPD の，また，14 種・1 変種について 35 個の形態形質データに基づき解析した。ナンブスズ節は RAPD 系統樹ではスズダケ属と同一の分岐群を形成するのに対して，形態系統樹ではササ属と同一の系統に属した。

本研究では，ナンブスズ節に対して，以上の検討結果と中間的形質ならびに遺伝的形質の存在を考慮し，ササ属，スズダケ属のいずれからも独立したスズザサ属として扱う見解を採った。また，日本産タケ類の系統分岐に関する，稈鞘の脱落性と宿存性，1 節多分枝と単一分枝，稈の直立性と斜上性について前者を原始的，後者を派生的とみなす形質の論証を行い，積雪深に依存したササ属の系統分岐について考察した。

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Appendix. Data matrix obtained from 327 RAPD bands as 0/1 for absence/presence.

Sample No.	Species*	Random primers** OPA-01	OPA-06	OPA-08	OPA-17
1	*Ph. bambusoides*	0010100000010010001100100100000	0001000000000100010000000000111011000000	0000000100000000011000000000100100001010000000	0001001010100010011101100100000100000
2	*Si. tootsik*	0010000000000100010000100011000	0000010000110100000110000101011000000000	0000001000000101000000000000011000000001000001	0011000010110100010010010100000000000
3	*Sh. kumasasa*	1010100000010010100001000001000	0000000100000001101000010101000000000	0110010010110010100100001001000000100001100000	0000000100000000000000010100000000
4	*Ps. japonica*	0010000000000000000000000100001	0000010000000100001000000010001000000000	0000000000000000000000010000001010010000000010	0000000001000100000100101100000000
5	*Ps. owatarii*	0010000001001010000111000110000	0000010100000101001100100011010100000000	0000000100001010010101010100001000001000000010000	1000000000100000100100000001000010000
6	*Pl. linearis*	0010101001000010000000000000010110	0000010000010010000000010001011001010000	0000000000001000000010000000011000000000000010	0101000000000110011000101010110000000
7	*Pl. simonii*	0010100001000010000100100101010110	0000000000010010000000010001000100000000	0000000010000110010100000000000100000010000000	0001000001010100001100101010000000000
8	*Pl. c. viridis*	0011000101000010001000100011010 0	0000000000010000000000000001010100000000	0001001100000100101000000000100101000000000000	1001000001000000101100101001000000000
9	*Pl. chino*	0010100001000011000101111011010 0	0000001000010000000101001000101010000000	0000001000000010000000010000000101000000000001	0001100000100100001100101001000001000
10	*Pl. c. vaginatus*	0010100100000010000001110011010 0	0000001000010010000000000000101010000000	0010001000000100010000000001001000000000000000	1101010001011000001100101000000000000
11	*S. tsuboiana*	0010010011000010000100100100110 0	0011000100000010110000000001001100000000	0000001000110010000001010000001100000000000000	0011100100000000001011100100000000000
12	*S. hayatae*	0010100011000010000100100100110 0	0001000100000101100001000011001000000000	0000001000000010001000101000001000001000000000	0001000000000101000101001100000000010
13	*S. tokugawana*, Hak.	0010000011000010001001000100110 0	0001000100000101100000000011001000000000	0000000000000010000000010000000000010000000000	1000000100000101011011100100001000000
14	*S. tokugawana*, Bat.	0011000011000010000101100110010 0	1011000100100000100000000001000100000000	0000000000001100000000010000000000010000000000	0001000010000101001100111000000000000
15	*S. kurilensis*, Nasu	0010010100100011000000000111110 0	1000000100000010000000001001000100100000	0001000000110110010000100100100100000000000000	0001000010100000001100110100000000000
16	*S. kurilensis*, Meo.	0010100100100011000001100110100 0	0000000100100010100000000001001100000000	0000000001000110000000000100001000000000000000	0011000011100000001100110000000000000
17	*S. jotanii*, Mik.	0010100001000011000000000111110 0	1101000010001010100000000001011100000000	0001010001000100010100100100000000100000000000	0001000011100100001100110000000001 000010
18	*S. jotanii*, Hac.	0100000001000011000010100111110 0	0001100100010010101000000000101000000000	0001010000000001000100100100001000000000000000	0001000010100100000100110000000100 000000
19	*S. palmata*	0010100010000010000100100110010 0	0101000100000011101000000011101010000000	0000000000100001001000001000001000000000000010	0000000010100101001101010100000000000 001000
20	*S. cernua*	0100100010000010000100100100100 0	0000100100001010001000000001000100000000	0000000001100100000100101000110000100000000000	0000100000100100000100100100000000000 000000
21	*S. senanensis*	0010101010000010000100100110010 0	0001100101100011000000000001001010000000	0010000000100110001100001010010000000000000000	0000000000100001011100110100000000000 000000
22	*S. yahikoensis*	0010101010000010000101100100010 0	0001000101001010000000000011100000001100	0000010000100110001000001011100110000000000000	0000010000100101001100110000000000000 000000
23	*S. nipponica*, Sen.	0010000010000010000110000110110 0	0111000001000011100010100011000100000000	0001010000100010000000001000000100001000000000	0000000000000101010100100000000010 000000
24	*S. nipponica*, Ama.	0010000010000010000100100010000 0	0111000000000111001000000011010000000000	0000010000100010001000001110000000001000000000	0000000000000101011100110000000000000 000000
25	*S. c. nana*, Sen.	0010000010000010000000100110000 0	0111000000000011100100000010101000000000	0000010000000010010000001010010000001000000000	1000000000000101011100110100000000000 000000
26	*S. c. nana*, Ama.	0010000010000010000100100100000 0	0011000000000011110100000001110100000000	0000010000000010000000001001000100000000000000	0000001010010101011100110100000000000 000000
27	*S. tsukubensis*	0010000010000010000000000111000 0	0001000000000011100100100000010100000000	0000010001000010100000001100100000010000000000	1001000100101000000100100100000000000 000000
28	*S. p. sugimotoi*	0010100010000010000010000110000 0	0011000000000011000100000010100000000000	0000010000000000000000000100010110001000000000	0001000000101000000100100100000000000 000000
29	*S. shimidzuana*	0011000000000010000010000110000 0	0000001100000010000100000001010100000000	0010000000100010001000000001010010100000000000	0001000001001000000100100100000000 000011
30	*S. t. melinacra*	0011000010000010000010000100000 0	0101100000000010000000100011000100000000	0010000000100110001000001000000000100000000000	0000000011001000000100100000000000 010001
31	*S. kur.* x *Sm. bor.*	0010000011000010000000100111000 0	1001000100100010100000000101000100000000	0010000001010101000000001001001000000000001000	0001000010000000011010001000000001 010000
32	*Sm. borealis*, Sen.	0011000000000010000010000110000 0	0000000110000010001001000001010100000000	0000000000110010011000000100100000010000000000	0001000001001000001100000100000000 010001
33	*Sm. borealis*, Ama.	0011000000000010000010000110000 0	0000010000000010000101000001010100000000	0000000000110010010000010010010000010000000100	0001000001001000000100100000000000 010001
34	*Sm. borealis*,Hak.	0010100000000010000010000111010 0	0000000000100010000100000010101000000000	0000100000000100000000010000000011000000000000	0000000010010000001001000000000000 000010
35	*Sm. borealis*,Meo.	0011000000000010000010000110000 0	0000000100000010000000000000010100000000	0010000000000010100000000101100000110000000000	0001000001001000000100100100000000000 000000
36	aff. *Sm. borealis*	0011010000000010000011100011000 0	0001010000000010000101000000101000000000	1000000101010000000000001001000001000000000000	0001010001001000000100100100000000 100001
37	*Sm. b. virid.*, Mik.	0011101001000010000010000110000 0	0001010000000100000000000001010100000000	0010001000110010100100000100100001100000000000	0000010001001010001100110100000000 010000
38	*Sm. b. virid.*, Hac.	0100000000000010000010001000010 0	0100000000000010000000010000101000000100	0000010000000010000010000000000001000000000000	0010000000001000000100100100000000000 000010
39	*Sm. mollis*	0011101000000000000000100011000 0	0000001000100010001000000001010100000110	0010100000010001100101000000100000000000000000	0000000001101000010010011010000000000 010000
40	*St. spicata*	0001000000000010001000000011000 0	0000000000000000000000000000000000000001	0000000000000000000000000000000000000000000000	0000000000000000000000000000000000000000 000000

Appendix. (continued)

Sample No.	Species *	Random primers** OPC-03	OPC-06	OPC-14	OPC-16	OPC-18
1	*Ph. bambusoiddes*	0101000000000010000000	0000001001001000010101000101000000	0000000000000100000101100110100000000	0000001010100001000000000000100000001	0100010011000000000100000111000
2	*Si. tootsik*	0100100010000000000000	1101001100100100010101001000000000	0100000001000100000000100100000001	0000100000100000101010001000001000	0110000001100001000000001000000
3	*Sh. kumasasa*	0000100000000011000000	0010000101001000100010100010100000	0000001001010100100000100100000010000	1000110001010010110001000000001000100	0010000010101100010000000000000
4	*Ps. japonica*	0000000000000010110000	0001001100000000100001010100010100	0000000001000100101011001000000001000	0010000001100100000110001000000000000	0000000000010001001000100000000
5	*Ps. owatarii*	1000000101000101000000	0001001100000101000010010001000000	0000110000100000100010000001100000	0100000100101000001101010000000000000	0111100000010100100001000000000
6	*Pl. linearis*	0100100010000000000000	0001001100100100100101000100000000	0000100000000100001000011000000000000	0010100001100000101001000000000000000	0100001000000000010000010000001
7	*Pl. simonii*	0000100010000001010000	0001001100000100100101100100001000	0000100001000101001000011000000000000	0000000000111000010100000000000000001	0010001000001010010000010000000
8	*Pl. c. viridis*	0010100000000001000000	0001001100000001000101100100000000	0000100001010100101000010100000000000	0001000011111000000110000100000000100	0110000000001010010000010000000
9	*Pl. chino*	0010100010100001000000	0001001100000001000101000100000000	1000100001000101001000010100000000000	0001000000111001000110000100000000100	0110000000001010001000010000000
10	*Pl. c. vaginatus*	0000100010000001001000	0001001100001001000001100100000000	0000100001010101001000010100000000000	0001001000111000000110010000000000100	0010000000001010010000000000000
11	*S. tsuboiana*	0010000000000001000100	0101001100000000001000010100000000	0001000001000011001001010100000000000	0001000101110000010100000000000000000	0001100100000000010000000000000
12	*S. hayatae*	0010000000000001000000	0001001100000000000001000101000000	0000000010000100010010000000000000	0000010010110011000110000001000000000	0101001100000100010010000000000
13	*S. tokugawana*,Hak.	0010100011000101000000	0001001100000000000001101010101000010	0000000001001010001001000100000000000	0011000010110011000110000001000000000	1001000000000100010010000000000
14	*S. tokugawana*.,Bat.	0000101000000011000000	0100001100000010000011000100010000	0000000000000001000110100000000000000	0000000010110000000101000000000000000	0101001100000010010001000000000
15	*S. kurilensis*, Nasu	0000010000000001000000	0010001010000000000101000101000000	0000000000000001000100100110000000000	0000000010111000100100000011100000000	0001001000100000010001000000000
16	*S. kurilensis*, Meo.	1000100000000001000000	0010001100000000000101010101000100	0000000010000010001101001000000000000	0011000010111000000110000000100000000	0001001000100000010001000000100
17	*S.jotanii*, Mik.	0100100000000001000000	0010001100000000000101010101000100	0000000010000010001101001000000000000	0011000010111000000110000000100000000	0001001000100000010001000000100
18	*S.jotanii*, Hac.	0100000000000001000000	1010001100000001000001000010000000	0010000000000100101010110100000000000	0000000010110000001010000010010011000	0000000000010010010100000000000
19	*S. palmata*	0010000000100001000000	0110001100010000000000100010100000000	0001000001000010001001001000000000000	0001100010110000000100000011000000000	1001000100000010010000000000000
20	*S. cernua*	0000000000001001000000	0010001100000001000001000010000000	0000000000000001000100100000000010	0001000010111000001010001001000010010	1001000100000001010000000000
21	*S. senanensis*	0010000100000001000000	0110001010000001000001010100010001	0001000001001010001001000100000000000	0000000010111000100101000011000000000	1001000100000000010000000000000
22	*S. yahikoensis*	0100000000000001000000	0110001010000000000101000101010000	0001000010000100010010011000000000	0000000010110000100110000001000000000	0101000100000000010000000000000
23	*S. nipponica*, Sen.	0010000000000001000000	0000001100000010000011000100000000	0001000001000010001101001100000000000	0000000010110001000101000001000000000	1001000100000000010000000000000
24	*S. nipponica*, Ama.	0010000000000001000000	0000001100010000000011000010000000	0001000001000010001001000100000000000	0000000010110011000101000001000000000	1101000100000100010000000000000
25	*S. c. nana*, Sen.	0010000000000001000000	0001001101000000000011000001000000	0001001001000100011010001000000000000	0000000010010010100101000001000000000	1001000100000000010000000000000
26	*S. c. nana*, Ama.	0100001000000001000000	0001001101000000000011000001000000	0001001001000100011010001000000000000	0000000010010010100101000001000000000	1001000100000000010000000000000
27	*S. tsukubensis*	0000000000010001000000	0000010100000000010001001100001000	0000000000000010001011000000000000000	0000000000111000001100000011000000000	0101000000100001010000000000000
28	*S. p. sugimotoi*	0000001000000001000000	0000010100000000000101000100000000	0000100000101110001001000000000000000	0000000010110000001100000000000000000	0011000100000000010000000000000
29	*S. shimidzuana*	0000000000001000000000	0000010100001000000101000100010000	0000110000100110001011000000000000000	0000000000110000011001000000000000000	0001000001000001000000000000000
30	*S. t. melinacra*	0000000000000100000000	0000010100010000000101000100000000	0000100000100010001001000000000000000	0000000011010000001100000001000000000	0001000100000010100000000000000
31	*S. kur. x Sm. bor.*	0001010000000001000001	0001001100000000000101000100000000	0000000010000010001101001000000000000	0000000010110000000101000001000000000	0101010000000000010001000000000
32	*Sm. borealis*, Sen.	0000000100000000000000	0001001100000000010101000100010000	0000100000100010000011100000000000000	0100000000010100001100100000000000000	0101010000000000000000000000000
33	*Sm. borealis*, Ama.	0000000100000000000000	0001001100001000000101000000101000	0000100100100111000001100000000000000	0100000000000100001110000000000000000	0101010000000001000000000000000
34	*Sm. borealis*, Hak.	0000000000000001000000	0000001100000000000101000000101000	0000100000000010000011000010000000000	0000000000010100000110000000000000000	0001010000100000010000000000000
35	*Sm. borealis*, Meo.	0000001000000001000000	0001001100001000000101000100010000	0000100000000011000001100000000000000	0100010000010100001110000000100000000	0001010100000000000000000000000
36	aff. *Sm. borealis*	0000001000001001000000	0001101100001000000101000100100000	0000110100100010000001000000000000000	0100011000010000001100001000000000000	0001000000000001000000000000000
37	*Sm. b. virid.*, Mik.	0001000000000000000000	0001101100000000001101000000010000	0000100000100010000010000000000000100	0100000000101000011000100000000000000	0001010000100000100000000000000
38	*Sm. b. virid.*, Hac.	0000001000000001000000	0000001100000000001010000100010000	0000100000100000101001100100000000000	0100000010010000011001000000000000000	1001000001000001100000000000000
39	*Sm. mollis*	0001000000000000000010	0001101100000000000101001010100000	0000100100100100000111100000010000000	0100000000100000011000001000000000000	0001110000010000000000000000000
40	*St. spicata*	0000000000000000000000	0000000000000000100000010000000000	0000000000000000000000000001000000000	0000000000000000000000000000000000000	0000000001000000000000000000010

* Abbreviation for sample names is shown in Table 1. Locality note: Ama., Mt. Amamaki; Bat., Batou; Hac., Isl. Hachijojima; Hak., Hakone; Meo., Meotobuchi; Mik., Isl. Mikurajima; Nasu, Mt. Nasu; Sen., Senjogahara. ** Data of each primer are shown from left through right for top to bottom of each gel as shown in Fig. 4.