

Short communication

Two New Putative Natural Hybrids in Japanese *Arisaema* (Araceae)

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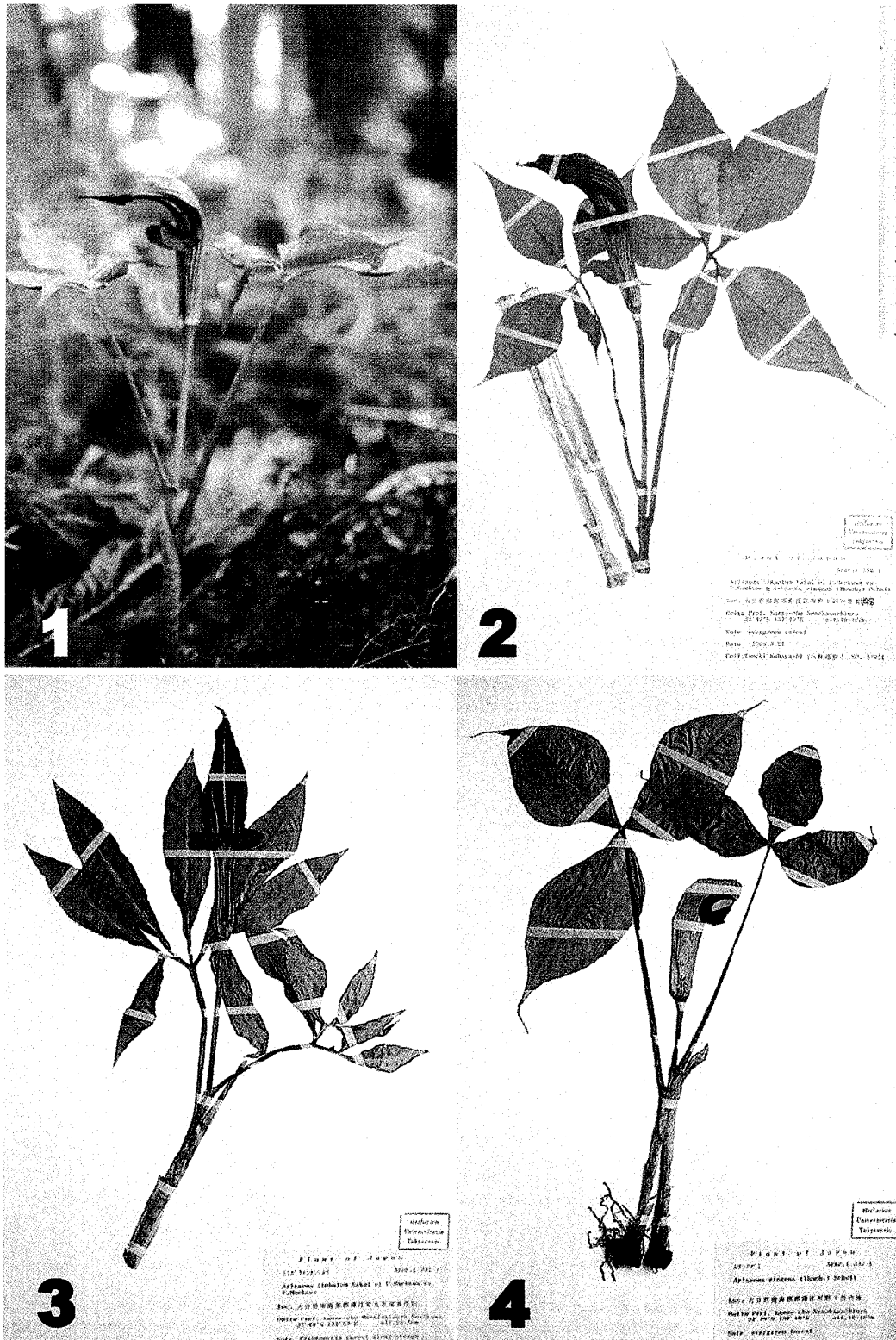
Two new putative natural hybrids, *Arisaema limbatum* Nakai \times *A. ringens* (Thunb.) Schott and *A. ovale* Nakai var. *sadoense* (Nakai) J. Murata \times *A. monophyllum* Nakai have been reported. The former hybrid was male and the latter female. Both hybrids had the combined and/or intermediate gross morphology between their putative parental species with distinct external morphology, and were easily recognized from their parental species in the field. Both putative hybrids were produced by parents with different chromosome numbers of $2n = 26$ and $2n = 28$ in the former combination, and with $2n = 52$ and $2n = 28$ in the latter, respectively. And the pollen stain-ability in the former hybrid was the lowest (32.6%) among the hybrids previously reported.

Key words: Araceae, *Arisaema*, *A. limbatum*, *A. monophyllum*, *A. ovale* var. *sadoense*, *A. ringens*, inter-specific hybrid

Japan is a center of diversity for *Arisaema* sect. *Pedatisecta*. It consists of 31 species in Japan (Murata 1990). Most of them are diploids with $2n = 28$ and are inter-fertile to produce F_1 -hybrids. Thirteen putative inter-specific hybrids between the diploid parents with the same chromosome number $2n = 28$, recognized by their intermediacy in morphology, have been reported (Murata 1962, Serizawa 1975, Ohno & Tsukada 1986, Murata & Ohno 1989). All of putative hybrids reported previously, except for *A. ehimense* J. Murata et Ohno, however, do not give rise to populations and soon disappear. Even in *A. ehimense* suspected as a hybrid origin species between *A. serratum* ($n = 14$) \times *A. tosaense* Makino ($n = 14$) (Murata & Ohno 1989, Murata 1990), no alleles showing additivity of parental allozyme markers were found (Maki & Murata 2001). Although the artificial hybrids such as *A.*

limbatum Nakai ($n = 13$) \times *A. kishidae* (Thunb.) Schott ($n = 14$) and *A. kishidae* \times *A. limbatum* were produced (Murata & Ohno unpublished), there has been no report on the natural hybrids between parents with the different chromosome numbers. Recently, we found two new putative hybrids between the parental species with different chromosome numbers and distinct external morphology, in the course of extensive survey of distribution of Japanese *Arisaema*.

Field observation and collection of one of putative hybrid plants and its parental species *Arisaema limbatum* and *A. ringens* were made around and in the evergreen forest of the shrine, Kamae-cho, Ohita Prefecture. A small number of *A. limbatum* and a large number of *A. ringens* occurred sympatrically in this shrine forest. Around this shrine forest, however, a large number of *A. lim-*



FIGS. 1-4. Photographs of hybrid and its putative parental *Arisaema* species. 1. Putative hybrid between *A. limbatum* and *A. ringens* at Kamae-cho, Ooita Prefecture. Note to the helmet-like spathe (characterize to *A. ringens*) with broad mouth (characterize to *A. limbatum*) and five leaflets (intermediate) with long-tailed tip (characterize to *A. ringens*). 2. A voucher specimen of putative hybrid between *A. limbatum* and *A. ringens*. 3. A voucher specimen of *A. limbatum*. 4. A voucher specimen of *A. ringens*.

batum occurred but *A. ringens* did not occur at all. Figures 1 - 4 show the putative hybrid at Kamae-cho on 21 March 2003 and the voucher specimens of putative hybrid, its parental species, *A. limbatum* Nakai and *A. ringens*, respectively. Table 1 shows the results of 18 morphological traits measured. The gross morphology of a male putative hybrid appears the combined and/or intermediate features between *A. limbatum* and *A. ringens* in its character association. Namely some of morphological traits of putative hybrid such as the lengths of leaflet, peduncle and pseudo-stem, the auricle size at the mouth of spathe, the outer color of spathe and the tip of spathe blade rather resemble to those of *A. limbatum* (Fig. 3). On the other hand, other morphological traits of putative hybrid such as the length of petiole, the number of leaflets, the width of leaflets, the feature of the tip of leaflets, the shape of spathe blade, the number of vertical strips in spathe, the appendage of inflorescence rather resemble to those of *A. ringens* (Fig. 4). The remaining morphological traits such as the state of rachis, the width/length ratio of leaflet, the length ratio of peduncle/petiole, and the position of spathe against leaves show intermediate states between *A. limbatum* and *A. ringens*. The chromosome number of *A. limbatum* from Kamae-cho was $2n = 26$ (Watanabe *et al.* 1998 as *A. limbatum* var. *conspicuum*) and that of *A. ringens* from Kamae-cho was $2n = 28$. Unfortunately this hybrid transplanted and cultivated at Akashi City, Hyogo Prefecture died before the count of chromosome number. Its pollen stain-ability by the aceto-carmin solution for the voucher specimen of putative hybrid was estimated to be 32.6 %, in contrast to the high percentage of stained pollen grains for the putative parental species, *A. limbatum* (91.8%) and *A. ringens* (88.8%). According to the combination and/or intermediacy of morphological traits (Table 1) and the low pollen stain-ability, this plant was strongly suggested to be the hybrid between *A. limbatum* and *A. ringens*. This putative hybrid, however, seemed to scarcely produce F_2

hybrid in the field because of its low pollen stain-ability. *Arisaema ringens* had been temporarily classified in the section *Pistillata* by Hara (1971) and Ohashi & Murata (1980) based on its two trifoliolate leaves and its helmet-like spathe-limb. *Arisaema limbatum* is certainly distinct from *A. ringens* morphologically and their hybrid has been able to recognize easily from both species in the field. In addition, *A. limbatum* is a member of *A. undulatifolium* group characterized by the relatively early flowering season within the Japanese *Arisaema* (Serizawa 1980, Kobayashi *et al.* 2003). Thus these two species have the different flowering times in the field and are isolated reproductively from each other. Nevertheless we found a putative natural hybrid between *A. limbatum* and *A. ringens*. The reasons of the rarity and production of hybrid between these two parental species are considered to be as follows: 1) *A. limbatum* and *A. ringens* occurred sympatrically are rather restricted to the narrow regions, *i. e.*, south-western Ehime Prefecture and south-eastern Oita Prefecture, 2) The flowering time of *A. ringens* delays two weeks than that of *A. limbatum* under the cultivated condition at Akashi City (Kobayashi 1995) and do not overlap. The flowering season of these two species, however, were overlapped spontaneously (or occasionally) at early to middle March in southeastern Ohita Prefecture. Murata (1995) included *A. ringens* into the section *Pedatisecta*. Gusman & Gusman (2002) classified both *A. limbatum* and *A. ringens* in the subsection *Pistillata* of section *Pedatisecta* according to the shape of the first seedling leaf (simple eophyll). The low pollen stain-ability of putative hybrid might be due to the meiotic irregularities by the combination of the different of chromosome numbers of parental species, or *A. limbatum* might be more distantly related with *A. ringens* as suspected by Hara (1971) and Ohashi & Murata (1980).

Field observation and collection of another putative hybrid plant and its parental species *Arisaema ovale* Nakai var. *sadoense* and *A. mono-*

TABLE 1. Comparison of morphological traits among *Arisaema limbatum*, *A. ringens* and its putative hybrid

Morphological traits	<i>A. limbatum</i>	Putative hybrid	<i>A. ringens</i>
Number of samples examined	28	1	3
Length of petiole (cm)	9.1 ± 1.9 (4.5 ~ 13.6)	18.5	15.7 ± 1.6 (13.5 ~ 19.0)
Rachis	developed	slightly developed	not developed
Number of leaflets	8.2 ± 1.2 (6 ~ 11)	5	
Length of leaflet (cm)	13.0 ± 2.9 (7.4 ~ 20.0)	15.5	8.9 ± 1.4 (7.0 ~ 10.0)
Width of leaflet (cm)	3.5 ± 1.1 (1.9 ~ 7.0)	7.0	5.4 ± 0.6 (5.0 ~ 6.2)
Width/length ratio of leaflet	0.27 ± 0.05 (0.19 ~ 0.36)	0.45	0.61 ± 0.08 (0.51 ~ 0.71)
Tip of leaflet (cm)	not elongated caudately	elongated caudately (1 ~ 2)	elongated caudately (1 ~ 2)
Length of peduncle (cm)	9.8 ± 2.5 (4.2 ~ 14.2)	11.7	5.0 ± 2.2 (3.0 ~ 8.0)
Length ratio of peduncle/petiole	1.08 ± 0.24 (0.46 ~ 1.50)	0.63	0.31 ± 0.10 (0.22 ~ 0.46)
Position of spathe against leaves	above leaves	same position	beneath leaves
Color of the outside of spathe	dark purple	dark purple	white-green
Shape of spathe blade	not swelling	swelling	swelling
Tip of spathe blade	sharp and elongated	sharp and elongated	collapsed and not elongated
Edge of spathe blade	not auriculated	auriculated	auriculated
Number of vertical stripes in spathe	about 10	more than 20	more than 20
Auricle size at the mouth of spathe (mm)	15.2 ± 3.0 (7 ~ 21)	17.0	9.3 ± 0.9 (8 ~ 10)
Appendage of inflorescence	slightly protruded out of the spathe tube	largely protruded out of the spathe tube	largely protruded out of the spathe tube
Length of pseudo-stem (cm)	15.1 ± 5.2 (3.5 ~ 26.7)	24.0	10.2 ± 3.4 (7.0 ~ 15.0)

Numerals indicate mean, standard deviation and range (minimum-maximum), respectively.

phyllum were made in the deciduous forest of Nikko City, Tochigi Prefecture. Figures 5 - 8 show the voucher specimens of the putative hybrid and its parental species, *A. ovale* var. *sadoense* and *A. monophyllum*. The chromosome numbers of *A. ovale* var. *sadoense* and *A. monophyllum* from Nikko City were $2n = 52$ and $2n = 28$ (Watanabe *et al.* 1998), respectively. The growth morphology of a female putative hybrid appears the combined and/or intermediate states between *A. ovale* var. *sadoense* and *A. monophyllum* in its character association (Table 2). Namely some morphological traits such as the number of leaflets, the short peduncle, the strongly raised white veins on the outside of the spathe tube (Fig. 6), the presence of 1-2 accessory buds at the axile of major leaf and wide leaflets rather resemble to *A. ovale* var. *sadoense* (Fig. 7). On the other hands, other morphological traits such as the leaves with a long rachis and the spathe limb with purple band transversed inside (Fig. 6) rather resemble to *A. monophyllum* (Fig. 8). The remaining morphological traits such as the lengths of petiole and peduncle and the width/length ratio of leaflets show intermediate states between *A. ovale* var. *sadoense* and *A. monophyllum*. According to the combination and/or intermediacy of morpho-

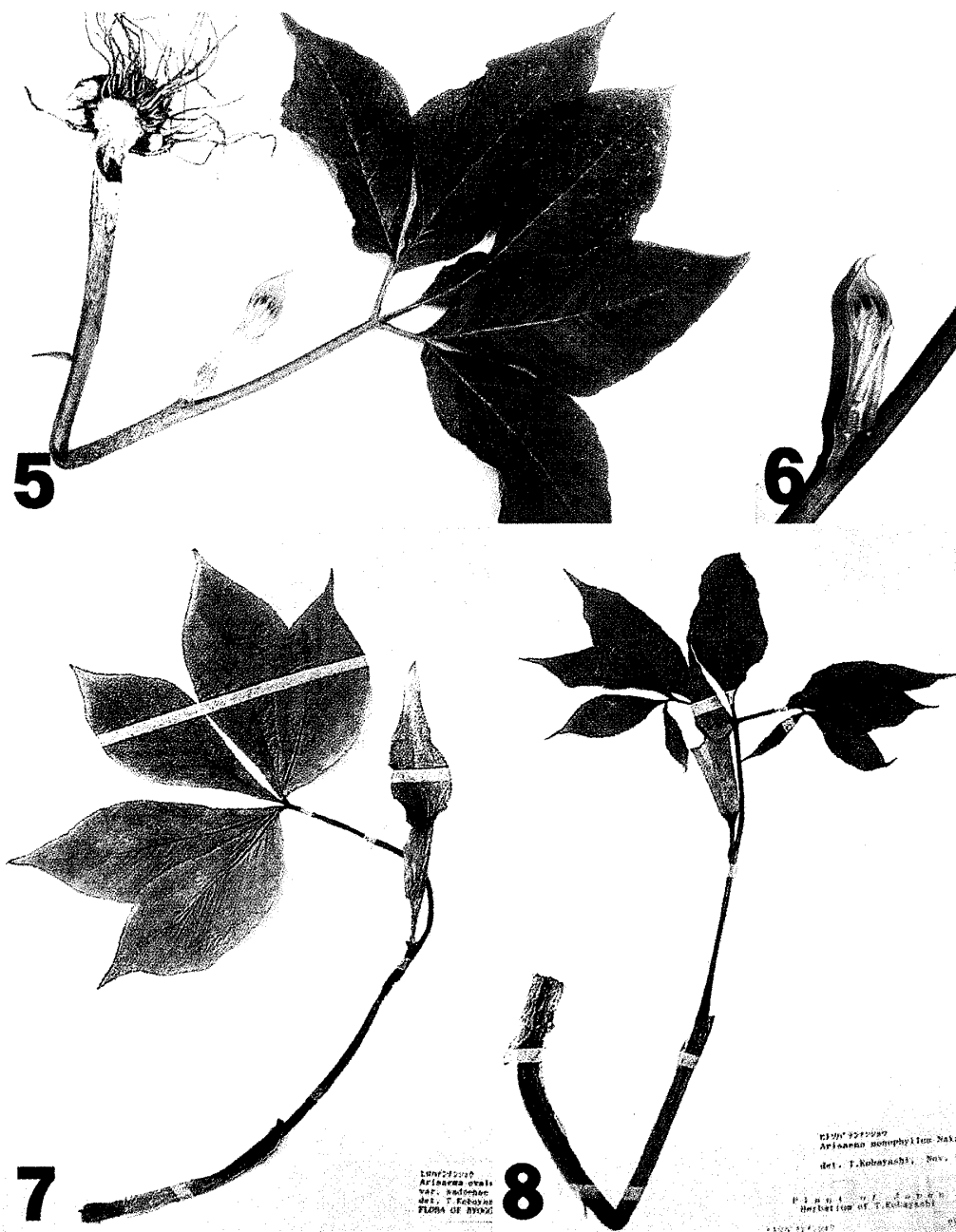
logical traits, this plant was strongly suggested to be the putative hybrid between *A. ovale* var. *sadoense* and *A. monophyllum*. Since several other individuals of putative hybrid have been found in the restricted areas of Nikko, these putative hybrids seem to have originated from many seeds or to reproduce vegetatively. Unfortunately this putative hybrid transplanted and cultivated at Akashi City, Hyogo Prefecture also died before the count of chromosome number. And its pollen stain-ability could not be counted because of the pistillate plant (Fig. 6).

Voucher specimens for two hybrids examined are as follows.

***Arisaema limbatum* Nakai × *A. ringens* (Thunb.) Schott** (Mimigatatennansho-Musashiabumi): Ooita Prefecture. Kanae-cho, Nonokawachiura (*T. Kobayashi* 37951 TI, Figs. 1 and 2)

***Arisaema ovale* Nakai var. *sadoense* (Nakai) J. Murata × *A. monophyllum* Nakai** (Hirohatennansho-Hitotsubatennansho): Tochigi Prefecture. Nikko City, Jakko Waterfall (collected in 1995 and cultivated in Tokyo Metropolitan University until 20 th May 1996. *J. Murata & T. Saito* 960520 TI, Figs. 5 and 6)

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FIGS. 5-8. Photographs of hybrid and its putative parental *Arisaema* species. 5. A voucher specimen of putative hybrid between *A. ovale* var. *sadoense* and *A. monophyllum*. 6. The close-up of spathe in Fig. 5. Notes to the strongly raised vein outside spathe tube (characterize to *A. ovale* var. *sadoense*) and the purple band transversed inside of spathe limb (characterize to *A. monophyllum*). 7. A voucher specimen of *A. ovale* var. *sadoense*. 8. A voucher specimen of *A. monophyllum*.

TABLE 2. Comparison of morphological traits among *Arisaema ovale* var. *sadoense*, *A. monophyllum* and its putative hybrid

Morphological traits	<i>A. ovale</i> var. <i>sadoense</i>	Putative hybrid	<i>A. monophyllum</i>
Number of samples examined	18	1	11
Length of rachis (cm)	0.4±0.2(0.2~0.9)	2.0	2.5±1.2 (0.8~4.3)
Number of leaflets	5	5	(7±11)
Length of peduncle (cm)	3.0±1.4 (0.7~5.3)	1.0	5.3±3.4 (1.1~12.5)
Purple band transversed inside of spathe limb	absent	present	present
Vein on the outside of spathe tube	strongly raised	strongly raised	not raised
Accessory buds at the axile of major leaf	(1~4)	(1~2)	absent
Width of leaflet (cm)	4.7±1.4 (2.5~7.5)	7.2	3.8±2.0 (1.6~7.6)

Numerals indicate mean, standard deviation and range (minimum-maximum), respectively.

References

- Gusman, G. & L. Gusman. 2002. The genus *Arisaema* - a monograph for botanists and nature lovers. Koeltz Scientific Books, Koenigstein.
- Hara, H. 1971. A revision of the eastern Himalayan species of the genus *Arisaema* (Araceae). In :Hara, H. (ed.) Flora of eastern Himalaya. Univ. Mus. Univ. Tokyo, Bull. (2): 321-354.
- Kobayashi, T. 1995. The growth characters of the genus *Arisaema* distributed in Hyogo Prefecture. Plants of Hyogo (5): 19-26. (in Japanese)
- , J. Murata, T. Suzuki & K. Watanabe. 2003. Taxonomic revision of the *Arisaema undulatifolium* group (Araceae). Acta Phytotax. Geobot. 54: 1-17.
- Maki, M. & J. Murata. 2001. Allozyme analysis of the hybrid origin of *Arisaema ehimense* (Araceae). Heredity 86:87-93.
- Murata, G. 1962. Taxonomic notes 7. Acta Phytotax. Geobot. 19: 67-72. (in Japanese)
- Murata, J. 1990. Introduction to the plants of *Arisaema* recently recognized from Japan. Aroideana 13: 34-43.
- . 1995. Diversity in the *Arisaema serrata* group. Acta Phytotax. Geobot. 46: 185-208. (in Japanese)
- & J. Ohno. 1989. *Arisaema ehimense* J. Murata et Ohno (Araceae), a new species from Shikoku, Japan, of putative hybrid origin. Journ. Jpn. Bot. 64:341-351.
- Ohashi, H. & J. Murata. 1980. Taxonomy of the Japanese *Arisaema*. J. Fac. Sci. Univ. Tokyo III. 12: 281-336.
- Ohno, J. & M. Tsukada. 1986. *Arisaema yamatense* × *sikokianum*, a new natural hybrid of *Arisaema* (Araceae) in Japan. Journ. Jpn. Bot. 61: 89-90. (in Japanese)
- Serizawa, S. 1975. *Arisaema* of Tokyo-to. Tokyo-to no shizen (3): 1-7. (in Japanese)
- . 1980. Studies on the genus *Arisaema* in Japan. (1) Group of *Arisaema undulatifolium* (Araceae). Journ. Jpn. Bot. 55: 148-156. (in Japanese)
- Watanabe, K., T. Kobayashi & J. Murata. 1998. Cytology and systematics of Japanese *Arisaema* (Araceae). J. Plant Res. 111: 509-521.

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