Analyses of reticulate evolution in the apogamous species of the *Dryopteris varia* complex (Dryopteridaceae) using five nuclear genetic markers

複数の核遺伝マーカーを用いたイタチシダ類（オシダ科）の無配生殖種における網状進化の解明（英文）

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Summary

Several lineages of ferns perform apogamous reproduction, which is considered as a type of asexual reproduction. However, many apogamous species show large morphological and genetic variation. Therefore, the "hybridization cycle hypothesis" was proposed, supposing that triploid apogamous species sometimes produce diploid sperms through unequal meiosis and hybridize with haploid ovum originating from other diploid sexual species (Lin *et al.*, 1995; Yamamoto, 2012). Moreover, apogamous species may hybridize with distantly related diploid sexual species because apogamous species sometimes have even greater genetic variation than their relatives with sexual reproduction.

In Chapter 1, I revealed reticulate evolution in the apogamous *Dryopteris varia* complex using the biparentally inherited nuclear *PgiC* gene as well as the maternally inherited plastid *rbcL* gene as genetic markers. The obtained data suggested that the apogamous species hybridized with distantly related species because some species of the complex had the same nuclear *PgiC* gene as *D. caudipinna* and *D. chinensis*, which had not been included in the *D. varia* complex. These two diploid sexual species were also shown to be distantly related to the complex by phylogenetic analysis using *rbcL* (Ebihara, 2011). When the *PgiC* sequences of the diploid sexual species *D. varia*, *D. saxifraga, D. protobissetiana*, *D. caudipinna*, and *D. chinensis* are represented by A, B, C, D, and E, respectively, the genetic constitution of the apogamous species of the *D. varia* complex was suggested to be as follows: *D. bissetiana* (B + C), *D. pacifica* (α, A + C; β, A + B + C; γ, A + C + D), *D. sacrosancta* (A +C + E), and *D. kobayashii* (B + C + E).

However, the use of a single nuclear marker may not be sufficient to understand the behaviors of the genome of the parental species in apogamous species. If many crosses with sexual relatives occur, the genome constitution of several nuclear genes can differ from each other. To solve this problem, in Chapter 2, I used five single-copy nuclear markers encoding enzymes that had often been used for allozyme analyses. For each marker, five types of alleles, namely A, B, C, D, and E, were recognized in the sexual types of *Dryopteris varia*, *D. saxifraga*, *D. protobissetiana*, *D. caudipinna*, and *D. chinensis*, respectively. The genetic constitution of the apogamous species was consistent with those of Chapter 1, which used only one nuclear marker, the *PgiC* gene. Therefore, the results presented in Chapter 2 strongly suggest that apogamous species of the *D. varia* complex can cross only a few times or they maintain their genome constitutions even if they cross several times with diploid sexual species.

The results of Chapter 2 clearly show that the former classification of the apogamous species of the *Dryopteris varia* complex (Iwatsuki 1992) had taxonomical problems, particularly for *D. pacifica*, because three genotypes (α, β, and γ types) were recognized in this species. The α type comprises the genomes of *D. varia* (A) + *D. protobissetiana* (C), the β type comprises *D. varia* (A) + *D. saxifraga* (B) + *D. protobissetiana* (C), and the γ type comprises *D. varia* (A) + *D. protobissetiana* (C) + *D. caudipinna* (D). Therefore, in Chapter 3, the species classification of the *D. varia* complex was revised on the basis of the genome constitution of each species as elucidated in Chapter 2. Firstly, diploid sexual taxa with distinct chloroplast and nuclear genomes are treated as independent species. Then, apogamous species are recognized on the basis of differences in the combination of genomes. In other words, apogamous cytotypes with different nuclear genome constitutions are classified as independent

species. I summarized the taxonomical treatment of 11 species of the *D. varia* complex [*D. bissetiana*, *D. chichisimensis*, *D. erythrovaria* (=*D. pacifica* γ), *D. hikonensis* (=*D. pacifica* α), *D. insularis*, *D. kobayashii*, *D. protobissetiana*, *D. sacrosancta*, *D. saxifraga*, *D. subhikonensis* (=*D. pacifica* β), and *D. varia*]

It is still debatable whether fern taxonomists should use several nuclear markers because the allele constitutions of five nuclear loci were concordant in the *Dryopteris varia* complex. If apogamous ferns do not cause chromosome recombinations through unequal meiosis, there will be no problem in estimating genome constitutions and discussing the evolution of the apogamous fern complex using only one biparental nuclear marker. However, if some apogamous ferns cause chromosome recombinations through unequal meiosis, pteridologists must use several nuclear DNA markers to understand the origin and reticulate evolution of the apogamous fern complex. To resolve this issue, similar studies to this study but using several unlinked nuclear genetic markers of several phylogenetically distant apogamous complexes of ferns should be conducted.

General Introduction

The life cycle of ferns is characterized by sporogenesis and the existence of a free-living gametophyte (prothallium), which produces eggs and sperm. In most diploid ferns, motile sperm from the prothallium must swim through water to reach the eggs in the mature archegonia of a different prothallium that is growing nearby. Fertilization usually cannot occur within a gametophyte (intra-gametophytic selfing), possibly due to the existence of recessive deleterious genes (Soltis and Soltis 1990; Watano and Iwatsuki 1988). Therefore, fertilization is a highly risky process in ferns, especially under dry conditions.

However, this only applies to sexual reproduction. Apogamy or agamospory in ferns is a type of asexual reproduction. In most sexual ferns, the archesporial cell undergoes four mitotic divisions to yield 16 spore mother cells (SMCs) in each sporangium, which subsequently undergo meiosis to form 64 spores. By contrast, in most apogamous ferns, sporogenesis follows the Döpp–Manton scheme (Manton 1950), where the fourth mitosis is terminated in the early anaphase and a restitution nucleus is formed. The resultant eight SMCs possess doubled sets of chromosomes. Subsequently, the SMCs undergo meiosis (two sequential cell divisions) and produce 32 unreduced spores. Next, the gametophytes formed from the spores produce the sporophytes for the next generation without fertilization (Manton 1950). Therefore, apogamous ferns do not need water for their fertilization.

Apogamous reproduction is common in ferns. About 10% of all fern species (Lovis 1977) and approximately 13% of Japanese fern taxa are reported to exhibit apogamous reproduction (Takamiya 1996). Unlike other asexual reproductive modes,

such as vegetative reproduction via gemma or adventitious buds, apogamous reproduction involves the production of spores that can tolerate dry conditions. Therefore, apogamous reproduction is advantageous, especially for long distance dispersal and for the formation of new populations from a small number of spores, which might explain why apogamous ferns are common.

However, apogamous reproduction has a severe drawback in terms of the production of genetic variation within populations or even within species because the normal processes of meiosis (recombination) and fertilization, which contribute to the maintenance of intra-specific genetic variation in sexual reproduction, are lacking. Genetic variation is evolutionarily important. In the case of apogamous reproduction, only genetic clones of the parental individual are produced, and thus genetic variation will eventually disappear within the species. In this situation, the extinction risk is high in an apogamous species if extreme environmental changes or an infectious disease epidemic occurs.

Apogamous fern species are expected to exhibit low levels of genetic variation. Indeed, Darnaedi *et al.* (1990) reported that *Dryopteris yakusilvicola* Kurata, a triploid apogamous species of recent hybrid origin that is endemic to Yakushima Island, did not exhibit any allozyme variation in five enzymes within 56 individuals examined in this species. Comparisons of the enzyme banding patterns suggested that the genome of *D. yakusilvicola* was derived through hybridization between *D. sabaei* and either a sexual tetraploid or an agamosporous triploid of *D. sparsa*. Cytological evidence (Darnaedi *et al.* 1989) supports the idea that the sexual tetraploid cytotype of *D. sparsa* is a parent because the hybrid between diploid sexual and triploid apogamous cytotypes is expected to be tetraploid rather than triploid. This monomorphic pattern implies that *D.*

yakusilvicola originated from a single hybrid between the parental species, and that it is a neo-endemic of Yakushima Island.

However, this situation is not common. Despite the clonal nature of apogamous reproduction, many apogamous fern species exhibit high morphological variation and at least some genetic variation (Watano and Iwatsuki 1988; Suzuki and Iwatsuki 1990; Lin *et al.* 1995; Takamiya *et al.* 2001). Previous analyses of genetic variation in apogamous fern species using enzyme electrophoresis found 45, four, and 14 different clones from *D. nipponensis* Koidz. (Ishikawa *et al.* 2003a), *Hymenasplenium hondoense* N. Murak. et Hatanaka (Watano and Iwatsuki 1988), and *D. bissetiana* (Baker) C. Chr. (Lin *et al.* 1995), respectively. Furthermore, genetic and cytological variations have been reported in apogamous ferns. Six different triploid and five diploid clones have been reported in *Pteris cretica* L. (Suzuki and Iwatsuki 1990), and one tetraploid and four triploid clones have been reported in *Diplazium doederleinii* (Luerss.) Makino (Takamiya *et al.* 2001). Thus, despite the clonal nature of apogamous reproduction, many apogamous fern species exhibit high genetic variation.

Several hypotheses have been proposed to explain the increased genetic variation within apogamous fern species: (1) the recurrent origin of apogamous races from sexual species (Gastony and Gottlieb 1985); (2) genetic segregation by homoeologous chromosome pairing (Klekowski 1973; Ishikawa *et al.* 2003 a, b); and (3) hybridization with closely related sexual species (Walker 1962; Suzuki and Iwatsuki 1990). The specific details of these hypotheses are as follow.

(1) Gastony and Gottlieb (1985) proposed a hypothesis that assumes the recurrent origin of apogamous species from related sexual species, thereby leading to genetic variation within an apogamous species. Apogamous species would acquire

genetic variation from their sexual relatives if the former originated repeatedly from the latter. However, the process of sporogenesis that produces unreduced spores in apogamous ferns is extremely complicated and precise, as noted earlier. It might be unlikely that mutations generated such a complicated sporogenesis process independently several times in several apogamous species belonging to different fern families. Therefore, this hypothesis cannot explain the major factors that produced genetic variation in apogamous fern species.

(2) Klekowski (1973) first proposed that the pairing of homoeologous chromosomes could be a mechanism that generates genetic variation in apogamous ferns, although no positive evidence was provided in his study. For example, we can say that assuming that the genotype of the parental triploid apogamous sporophyte is *abc*, the genotype of its SMCs should be *aabbcc*. In the usual process of apogamous reproduction, the genotype of all offspring from such an aporophyte would also be *abc* because only sister chromatid pairing (a-a, b-b, c-c) occurs in the SMCs. By contrast, if homoeologous chromosome pairing (*a*-*b*, *a*-*b*, *c*-*c*) occurs, then gametophytic progeny with *aac* and *bbc* genotypes will be produced in addition to those with the *abc* genotype. In the same manner, if homoeologous chromosome pairing (*a*-*c*, *a*-*c*, *b*-*b*) occurs, then progeny with *aab* and *bcc* genotypes will also be produced. Thus, genetic segregation via homoeologous chromosome pairing can generate genetic variation among clones within an apogamous fern species.

Ishikawa *et al*. (2003a) first reported positive evidence to support genetic segregation via homoeologous chromosome pairing in apogamous ferns using *Dryopteris nipponensis* Koidz. as their test material. They compared the genotypes of the parent sporophyte and its progeny using the allozyme method. Among 250 progeny

gametophytes that developed from the spores of a parental sporophyte of *D. nipponensis* with the *PgiC* genotype *abc*, four had genotypes that differed from that of the parent (three *aac* and one *bbc* gametophytes). Similarly, among 34 sporophyte offspring of the parent sporophyte with the *abc* genotype, one had the *bcc* genotype, whereas the other 33 had *abc*. Thus, Ishikawa *et al*. (2003a) clearly demonstrated the existence of occasional genetic segregation, possibly via homoeologous chromosome pairing in apogamous ferns.

Subsequently, Otsuki *et al.* (2012) obtained more robust data to support genetic segregation in apogamous ferns via homoeologous chromosome pairing using *Cyrtomium fortunei* J. Sm. as plant materials and nucleotide sequence information from *pgiC* as genetic markers. They examined a total of 732 progeny (250 gametophytes and 482 sporophytes) obtained from a parental sporophyte, where the *pgiC* genotype was estimated as *aab*. Their results indicated that 11 (4.4%) gametophytes and eight (1.7%) sporophytes had a genotype (*aaa*) that differed from that of the parent sporophyte. They analyzed a large number of progeny and detected sufficient numbers of segregated offspring, so they were able to demonstrate that genetic segregation occurs in apogamous *C. fortunei* at a relatively high frequency. Moreover, they showed that the segregation frequency in gametophytes is significantly higher than that in sporophytes in the next generation ($\chi^2 = 4.90$, $P = 0.027$). They hypothesized that these results might suggest that apogamous fern species harbor deleterious genes and that these genes might be expressed in greater numbers in sporophytes, with a more complex morphology than that in gametophytes.

It is now evident that genetic segregation via homoeologous chromosome pairing is an important mechanism for generating genetic variation in apogamous ferns,

but this process is not sufficient to maintain the levels of genetic diversity that have been observed in most apogamous species. If genetic segregation occurs at a high frequency in apogamous ferns, heterozygosity will be lost rapidly within individuals and eventually within species. However, a high heterozygosity was observed in them (Watano and Iwatsuki 1988; Darnaedi *et al.* 1989; Suzuki and Iwatsuki 1990; Lin *et al.* 1995; Takamiya *et al.* 2001; Yamamoto 2013); therefore, apogamous species may possess mechanisms that increase heterozygosity.

(3) Hybridization with related sexual species can explain the observed levels of heterozygosity in a species and/or even within individuals of an apogamous fern species. The ability of apogamous ferns to cross with closely related sexual species was first demonstrated by Walker (1962) in artificial crossing experiments between apogamous and sexual races of *Pteris* species. He reported that the reduced egg of the sexual diploids is fertilized by the unreduced sperm of the apogamous triploid to produce tetraploid hybrids, which can reproduce apogamously. Suzuki and Iwatsuki (1992) supported this hypothesis by showing that the wild populations of apogamous *P. cretica* L. in Japan were derived via hybridization between the apogamous parents of the species and the closely related sexual species *P. kidoi* Kurata.

However, crossing between sexual and apogamous species is still not sufficient to explain the existence of genetic variation in apogamous fern species. If an apogamous species crosses with a sexual species in the manner suggested by Walker (1962), the resultant apogamous species would become higher polyploid (tetraploid, pentaploid, hexaploid, *etc*.) because the genome of the sexual species is added to that of the apogamous species in every cross. However, most apogamous fern species are actually triploid and tetraploids or higher polyploids are extremely rare in apogamous ferns.

Thus, apogamous species should also possess mechanisms that decrease the ploidy levels (Takamiya 1996).

The unequal meiosis reported by Lin *et al.* (1992) can cause ploidy reduction in apogamous ferns. In order to elucidate the origin of its intra-specific cytological variation, they performed cytological and genetic studies using *Dryopteris pacifica* (Nakai) Tagawa, in which both diploid and triploid apogamous cytotypes have been recorded. In each sporangium, they found that triploid apogamous *D. pacifica* produced 16 SMCs, some with n = 41II + 41I chromosomes, in addition to eight SMCs with n = 123II. In the former case, the 16 SMCs usually underwent abnormal meiosis to yield about 50 spores, some of which were regular in shape where the eight SMCs multiplied into 32 spores by normal meiosis. Furthermore, they reported that two (1.3%) of the 150 gametophytes that they examined cytologically were diploid and one (0.9%) of the 110 sporophyte offspring was diploid. They also cytologically observed unequal meiosis in the triploid apogamous sporophyte to produce diploid spores. Thus, they showed that diploid spores and diploid gametophytes could arise from the spores, while diploid apogamous sporophyte offspring are also formed occasionally from parental triploid apogamous sporophytes. However, they could not clarify whether this process truly contributes to the genetic variation observed in wild populations of apogamous ferns.

Lin *et al.* (1992, 1995) proposed the "hybridization cycle hypothesis," which assumes that a recurrent cycle with ploidy reduction (oligoploidization) from triploid to diploid apogamous plants, and polyploidization from diploid to triploid by crossing with related sexual diploid species (from triploid apogamous to diploid apogamous, and from diploid apogamous back to triploid apogamous) might explain the existence of genetic variation within a triploid apogamous fern species (Figure GI-1). According to their

hypothesis, triploid apogamous fern species can incorporate genetic variation from related diploid sexual species without polyploidization. Some individuals (gametophytes and/or sporophytes) of the apogamous species decrease their ploidy by unequal meiosis in the first step. In the second step, the apogamous individuals with decreased ploidy hybridize with sexual species that can normally perform meiosis and produce haploid eggs.

The hybridization cycle hypothesis has not yet been verified because each step in the hypothesis has been documented separately in different groups of apogamous ferns under experimental conditions. This hypothesis can be justified only when all the steps occur together in nature. Yamamoto (2013) selected a triploid apogamous species, *Dryopteris erythrosora* (Eaton) O. Kuntze., and its closely related diploid sexual species, *D. caudipinna* Nakai, as the plant materials to determine whether all the steps assumed by the hybridization cycle hypothesis occur in anapogamous species. Yamamoto (2013) performed artificial crossing 596 times between apogamous *D. erythrosora* and sexual *D. caudipinna*, and obtained 31 hybrids that shared nuclear genetic markers of the two parental species. Among the 31 hybrids, 22 (71%) were tetraploids and the remaining nine (29%) were triploids. Thus, triploid hybrids between the triploid apogamous and diploid sexual species were clearly produced under the experimental condition.

However, Yamamoto (2013) did not discuss how many times hybridization occurred between sexual and apogamous species in nature. If apogamous fern species cross repeatedly with related sexual species, then their chromosomes would be replaced mostly by those of the sexual species. To test this possibility, the genome constitutions of apogamous species must be clarified by using several nuclear genetic markers.

This study used the *Dryopteris varia* complex (subg. *Erythrovariae*, sect. *Variae*

Fraser-Jenk., Dryopteridaceae) as plant materials. This fern group is characterized by the lowest basiscopic pinnules on the lowest pinna being markedly elongated with densely scaled petioles. Six species and one variety (*D. bissetiana* (Baker) C. Chr., *D. insularis* var. *insularis* Kodama, *D. insularis* var. *chichisimensis* (Nakai ex H. Ito) H. Ito, *D. pacifica* (Nakai) Tagawa, *D. sacrosancta* Koidz., and *D. saxifraga* H. Ito, *D. varia* (L.) Kuntze) were listed by Iwatsuki (1995). Recently, the classification of the *D. varia* complex has been subjected to revision. Serizawa (2009) recently separated *D. kobayashii* Kitag. from *D. sacrosancta sensu lato* on the basis of observations that the former has thinner fronds and curved sickle-shaped pinna. In addition, Hori *et al*. (2015) discovered *D. protobissetiana* Hori et N. Murakami, which is a new species of the *D. varia* complex from Yakushima Island, Japan. This species is similar to *D. bissetiana* due to its slightly bullate scales and a dark green lamina surface, but it differs in terms of its flat, serrate margins at the apex of the upper pinnae. At present, eight species and one variety are recognized as Japanese members of the *D. varia* complex.

Most species of the *Dryopteris varia* complex are triploid apogamous, such as *D. bissetiana* (Hirabayashi 1967; Nakato *et al.* 1995; Lin *et al.* 2003; Lee *et al.* 2006), *D. insularis* var. *chichisimensis* (Lin *et al*. 2003), *D. pacifica* (Hirabayashi 1970; Lin *et al.* 1992; Nakato *et al.* 1995; Lin *et al.* 2003; Lee *et al.* 2006), *D. sacrosancta* (Mitui 1965; 1968; Hirabayashi 1969; Nakato *et al*. 1995; Lin *et al.* 2003; Lee *et al.* 2006), and *D. varia* (Mitui 1966;1968; Hirabayashi 1970; Nakato *et al.* 1995; Lin *et al.* 2003; Lee *et al.* 2006; Ebihara *et al.* 2014). Only two species are known to be diploid sexual, *i.e*., *D. saxifraga* (Hirabayashi 1967; Mitui 1975; Lee *et al.* 2006) and *D. protobissetiana* (Hori *et al*. 2014). Ebihara *et al*. (2014) recently reported that *D. varia* also has a diploid sexual cytotype. In addition, a few species have been reported as having diploid

apogamous cytotypes in addition to triploid apogamous cytotypes, such as *D. bissetiana* (Hirabayashi 1966; Lee *et al.* 2006), *D. pacifica* (Lin *et al.* 1992; Nakato *et al.* 1995), and *D. varia* (Hirabayashi 1966, 1967, 1974). Therefore, the *D. varia* complex is useful to clarify reticulate evolution between sexual species and apogamous species via the hybridization cycle.

Large morphological variations are observed in each species of the *Dryopteris varia* complex, which cause taxonomic problems, especially for *D. pacifica*. This apogamous species exhibits large variations in the texture of the lamina (coriaceous or papyraceous), margin of the pinnae (deeply or shallowly serrated), and indusia (red or translucent) (Figure GI-2). The taxonomic problems also affect *D. sacrosancta*. Serizawa (2009) recently separated *D. kobayashii* from *D. sacrosancta sensu lato*, as mentioned above. Thus, each species of the *D. varia* complex exhibits large and continuous morphological variation, which makes it difficult to classify the members of this complex based only on their morphological characteristics. In order to clarify the classification of this complex, it is important to elucidate the genetic background and variations in each species of the complex, especially those in the apogamous species.

In this study, I carefully investigated numerous individuals of the *Dryopteris varia* complex from many localities throughout Japan and Taiwan in order to clarify the genetic constitution of each species. To clarify reticulate evolution between diploid sexual and triploid apogamous species, I analyzed the *D. varia* complex using one nuclear marker, *PgiC*, as described in Chapter 1. Subsequently, I could postulate a schematic summary of evolution in the apogamous *D. varia* complex. In Chapter 2, I present an analysis of whether the *D. varia* complex underwent recurrent reticulation or not on the basis of the genome constitutions of the members of the complex determined

using five nuclear markers. Finally, I revised the species classification for the *D. varia* complex on the basis of the genome constitution, as described in Chapter 3.

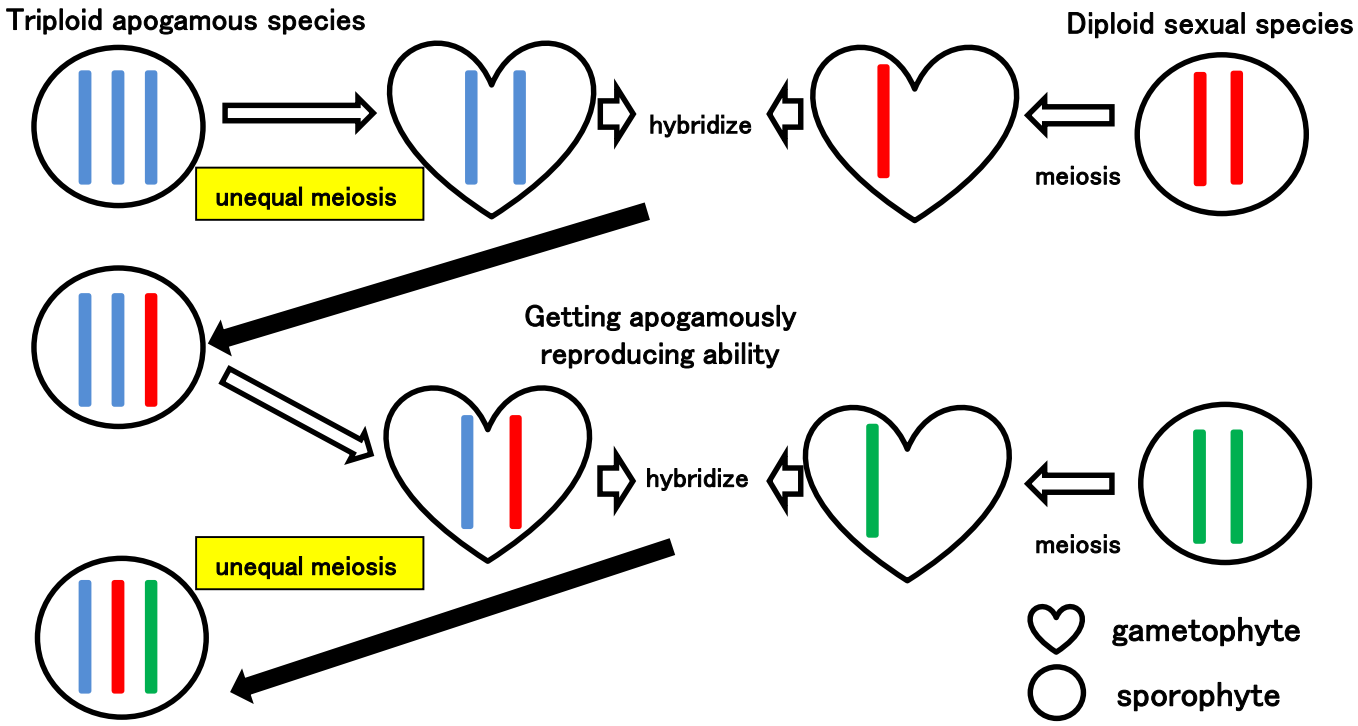

Figure GI-1. Hybridization cycle hypothesis (Lin *et al*. 1992, 1995).
Hybridization occurs between triploid apogamous species and diploid sexual species without polyploidization. The 'hybridization cycle hypothesis' assumes a recurrent cycle with ploidy reduction (oligoploidization) from triploid to diploid apogamous plants and polyploidization from diploid to triploid by crossing with related sexual diploid species. Circles indicate sporophytes and hearts indicate gametophytes. Bars indicate genomes. Detailed explanation is in the text (p. 10).

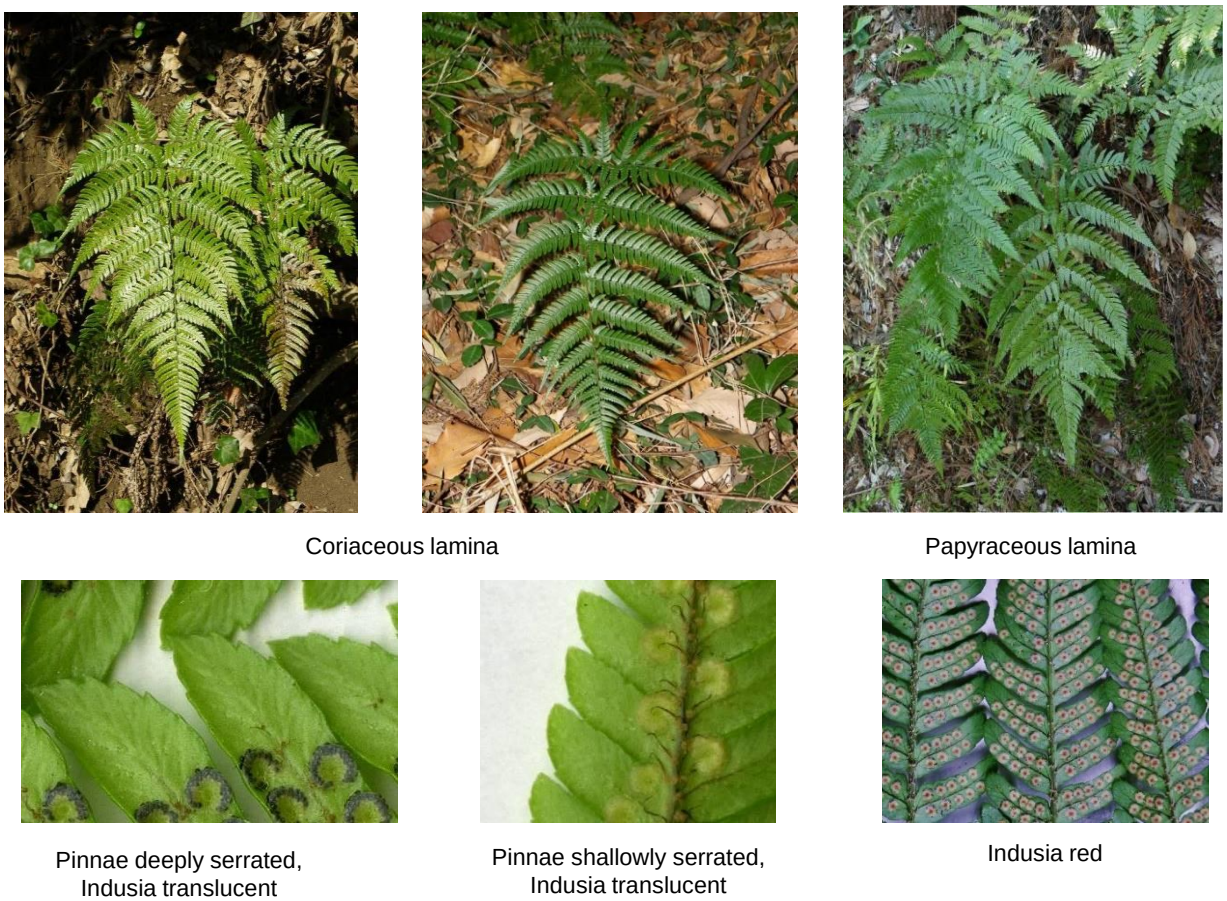

Figure GI-2. Morphological variations of apogamous *Dryopteris pacifica*.

Chapter 1. Reticulate evolution in the apogamous *Dryopteris varia* complex inferred from their nuclear *PgiC* allele constitutions

1.1 Introduction

Apogamy in ferns is a type of asexual reproduction where unreduced spores are formed and the resultant gametophytes produce the sporophytes for the next generation without fertilization (Manton 1950). In apogamous ferns, all of the offspring from a sporophyte are genetically the same unless mutations occur during reproduction. Therefore, the amount of genetic variation within an apogamous species is expected to be very low unless the apogamous species has an old or recurring origin.

However, if the apogamous species has undergone reticulate evolution via hybridization between apogamous species and sexual species, then the apogamous species can acquire genetic variation from the sexual species. Yamamoto (2013) showed that apogamous *Dryopteris erythrosora* has a relatively high ability to hybridize with its closely related sexual species, *D. caudipinna*. In addition, Yamamoto (2013) showed that the two species share genetic variation, where the amount of genetic variation in apogamous *D. erythrosora* was higher when they grew together in Izu-Oshima Island. Thus, it was suggested that reticulate evolution via hybridization with sexual species can occur in apogamous fern species in nature.

The *Dryopteris varia* complex (subg. *Erythrovariae*, sect. *Varia*e Fraser-Jenk.) contains many apogamous species and a few sexual species. The apogamous species in this complex exhibit large and continuous variation, and it is difficult to distinguish the members of the *D. varia* complex. In Japan, sexual cytotypes of the *D. varia* complex

are known only in *D. saxifraga* and all the other species reportedly have apogamous cytotypes (Figure 1-1). Recently, Ebihara *et al.* (2014) discovered the diploid sexual *D. varia* in Taiwan, and more recently, Hori *et al.* (2015) discovered a new diploid sexual species, *D. protobissetiana* on Yakushima Island. Therefore, this complex may be a useful material for elucidating reticulate evolution in apogamous ferns because several sexual relatives are known.

However, how these three diploid sexual species (or cytotypes) participate in forming apogamous species in the complex has not been well resolved. Genetic analyses using both biparentally inherited nuclear and maternally inherited plastid markers are necessary to elucidate the occurrence of reticulate evolution in this complex. Lee *et al.* (2013) recently attempted to elucidate reticulate evolution in species from the *Dryopteris varia* complex in Korea: *D. varia*, *D. pacifica*, *D. sacrosancta sensu lato*, *D. bissetiana*, and *D. saxifraga*, where they analyzed the nucleotide sequences of the nuclear *PgiC* gene as well as those of plastid *rbcL*, *trnL–trnF* intergenic spacer (IGS), and *atpF–atpH* IGS regions. They recognized 14 *PgiC* genotypes and five cpDNA haplotypes, and they concluded that gene flow has occurred between the apogamous *D. bissetiana* and sexual *D. saxifraga* in Korea because these two species shared the same genetic variation in the nuclear *PgiC*. In addition, their results strongly suggested that the apogamous triploid *D. varia* is probably of autopolyploid origin because nuclear *PgiC* haplotypes from the species formed a monophyletic group in the molecular phylogenetic tree of the complex. Their results also suggested cytoplasmic gene flow from *D. sacrosancta* to *D. chinensis* (Baker) Koidz. *Dryopteris chinensis* belongs to another subgenus of *Dryopteris* (subg. *Dryopteris*, sect. *Aemulae*), and its morphological characteristics are clearly different

from those of the *D. varia* complex. They reached this anomalous conclusion because the same plastid DNA sequences were shared by the two species, but the nuclear sequences were not shared. However, they did not clarify how the plastid DNA flowed between these two distantly related species.

The results obtained by Lee *et al.* (2013) suggest that wider taxon sampling is necessary to understand reticulate evolution in the *Dryopteris varia* complex. According to the molecular phylogenetic tree obtained for most Japanese fern species based on the *rbcL* sequences (Ebihara 2011), members of the *D. varia* complex are as closely related to diploid sexual species, *i.e.*, *D. caudipinna* (sect. *Erythorovariae*), *D. gymnophylla* (sect. *Aemulae*), *D. koidzumiana* (sect. *Erythrovariae*), *D. hasseltii* (sect. *Nephrocystis*), *D. polita* (sect. *Politae*), and *D. sordidipes* (sect. *Variae*), as they are to *D. chinensis* (sect. *Aemulae*). With the exception of *D. gymnophylla*, Lee *et al*. (2013) did not include these species in their study. Therefore, even wider taxon sampling than that performed by Lee *et al.* (2013), *i.e.*, not restricted to the *D. varia* complex, is needed to clarify the origin and reticulate evolution in the *D. varia* complex.

The method employed by Lee *et al.* (2013) also has some problems because they used a cloning method to separate multiple nuclear DNA sequences present within a fern sample. However, the cloning involves the risk of missing some alleles or selecting false alleles due to PCR errors. Thus, I consider that PCR-single strand conformation polymorphism (SSCP) analysis is more effective for separating nuclear DNA with different sequences in each sample. This method can separate DNA fragments with different nucleotide sequences on polyacrylamide gel according to differences in their three-dimensional folding conformation, where each fragment appears as a separate band on the gel (Ebihara *et al.* 2005; Adjie *et al.* 2007; Jaruwattanaphan *et al.* 2013).

After extracting each DNA band from the gel, the DNA obtained can then be re-amplified and subjected to direct sequencing. Even if false alleles due to PCR errors are present in the same bands, their number should be sufficiently low to not influence the results obtained by direct sequencing, thereby avoiding the problems in the cloning method.

In Chapter 1, the following two questions were addressed by using *PgiC* as the nuclear genetic marker and the plastid *rbcL* as a maternally inherited marker for indicating the maternal parent of the hybrids. (1) How did reticulate evolution occur in the *Dryopteris varia* complex? (2) Did other *Dryopteris* species that were not previously considered as members of the *D. varia* complex, such as *D. chinensis*, participate in reticulate evolution in the *D. varia* complex?

1.2. Materials and methods

Plant materials

Leaf samples were collected from 274 individuals belonging to the *Dryopteris varia* complex and its closely related species, mainly in my original field surveys throughout 19 prefectures in Japan. The reproductive mode of most individuals among the samples was estimated by counting the number of spores per sporangium (32, apogamous; 64, sexual). Four leaf samples were collected in Taiwan. Moreover, 44 living stocks of the *D. varia* complex were collected in 20 localities in Japan. In addition, a single leaf sample of the triploid apogamous *D. pacifica* (2*n* = 123), called "K111," which has been reported to produce diploid apogamous progeny (Lin *et al*. 1992), was collected from cultivated stocks in the Koishikawa Botanical Garden of the University of Tokyo. Material from *D. shibipedis* was not available, so this species was excluded from my study. Species identification was performed on the basis of gross morphology using the keys provided in previous studies (Iwatsuki *et al*. 1995; Serizawa 2009). *Dryopteris expansa* and *D. sieboldii*, which are classified in different subgenera of the genus *Dryopteris* (Fraser-Jenkins 1986), were used as outgroups. All of the voucher specimens have been deposited in Makino Herbarium of Tokyo Metropolitan University (MAK) and/or National Museum of Nature and Science Herbarium (TNS).

Ploidy analyses

To examine the ploidy level in the plant materials, the DNA content (2C-value) of each nucleus extracted from 31 fresh pinnae samples was measured by flow cytometry using CyFlow Ploidy Analyzer PA-II (Partec, Munster, Germany) and a CyStain UV

Precise P Kit (Partec). A segment of pinnae (approximately 100 mm^2) was torn into several pieces, chopped finely with a razor blade, and placed in 0.25 mL of nuclei extraction buffer from the kit. Next, 0.8 mL of staining solution from the kit was added to the chopped tissues. The crushed tissue and buffers were filtered through a 30-μm nylon mesh (Partec). The filtered samples were measured using the Ploidy Analyzer as soon as possible. Approximately 1,000–2,000 nuclei were measured for each sample. For most of the samples, the measurement was performed only once because high reproducibility was confirmed with five samples. Fresh leaf tissues from *Nicotiana tabacum* L., which has a genome size of 11.71 pg per nucleus (Narayan 1987), (approximately 25 mm^2) were used as the internal standard.

Molecular analyses

For the molecular analyses, a small amount of leaf sample was dried in a small plastic bag using silica gel. Subsequently, the total DNA was extracted by using CTAB solution, according to the method described by Doyle and Doyle (1987).

In this study, *rbcL* was used as the cpDNA marker. PCR amplification of a *rbcL* fragment was performed by using the primers aF and cR, as described by Hasebe *et al.* (1994), with an annealing temperature at 50 °C. The nucleotide sequences of *rbcL* were determined by direct sequencing. Two primers, aR (Hasebe *et al*. 1994) and D. paci-bf (5′-TATCCTTTGGATCTATTCGAAGAAGGTTC-3′), which were developed in this study, were only used to sequence the *rbcL* fragment. PCR amplification was performed by using PrimeSTAR Max DNA Polymerase (Takara, Kyoto, Japan) with a Model 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

A *PgiC* fragment including exons 14–16 and introns 14–15 was used as the nuclear marker. The *PgiC* fragment was amplified by using the primers 14F and 16R, as described by Ishikawa *et al.* (2002), with an annealing temperature at 58 °C.

PCR-SSCP analysis

The PCR-SSCP analysis was used to determine allelic variation at the nuclear *PgiC* locus for each individual according to the method described by Jaruwattanaphan *et al.* (2013). A portion of the PCR sample (3.5 μL) was mixed with 6.5 μL of formamide dye solution (90% formamide, 0.005% bromophenol blue, and 8% glycerol) and then denatured for 3 min at 95 °C. The denatured samples were cooled on ice and 5 μL of the sample was loaded onto a 0.5× MDE gel (180 mm × 180 mm × 1 mm; Takara Bio., Shiga, Japan). The electrophoresis was performed by using gels containing 2% glycerol at 18 °C for 16 h and 350 V in 50% TBE Buffer (50 mM Tris, 41.5 mM boric acid, and 0.5 mM EDTA-Na_2), followed by silver staining. For silver staining, the gels were shaken in 250 mL of 10% acetic acid for 20 min. The gels were then shaken three times in 250 mL of distilled water for 2 min, before shaking in 250 mL of 0.1% $AgNO_3$ water solution (containing 250 μL of 37% formaldehyde). Next, the gels were shaken in 250 mL of distilled water for 30 s, followed by 250 mL of developing solution (250 mL distilled water, 6.25 g Na_2CO_3, 250 μL of 37% formaldehyde, and 250 μL of 2% $Na_2S_2O_3$) for 2–10 min until the bands are visualized, before shaking in 250 mL of stop developing solution (250 mL of distilled water, 3.65 g EDTA 2Na) for 5 min. Finally, the gels were shaken in 250 mL of medium (75 mL ethanol, 11.5 mL of 50% glycerol, and 163.5 mL of distilled water) for 30 min and then stored at 4 °C overnight.

To sequence the bands separated on the SSCP gels, the polyacrylamide gel was

dried after silver staining by sandwiching the gel between Kent paper and a cellophane sheet on an acrylic back plate at 55 °C for 3 h. To extract the DNA, a piece of the DNA band was peeled from the dried gel by using a cutter knife and triturated in 100 μL of distilled water. After removing the distilled water, the small piece of gel was incubated in 50 μL of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) at 65 °C for 1 h. The supernatant solution was used as a template for further PCR amplification with the same primer set employed for the original PCR amplification.

The PCR products were purified by using ExoSAP-IT (USB, Ohio, USA) or Illustra ExoStar 1-Step (GE Healthcare, Wisconsin, USA) and used as templates for direct sequencing. Reaction mixtures for sequencing were prepared by using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Byosystems). The reaction mixtures were analyzed by using an ABI 3130 Genetic Analyzer (Applied Biosystems).

All of the plant samples were classified based on their PCR-SSCP banding patterns and the genomic constitution of each band pattern was identified by determining the nucleotide sequence of each DNA band separated on the SSCP gel.

Phylogenetic analyses

In the phylogenetic analyses, only one sequence representing each haplotype for cpDNA (*rbcL*) and each allele for the nuclear DNA (*PgiC*) were used in our data sets. The cpDNA and nuclear DNA data sets were analyzed separately by maximum parsimony (MP) analysis with MEGA version 5 (Tamura *et al*. 2011) and Bayesian Markov chain Monte Carlo (B/MCMC) analysis with MrBayes 3.2 (Ronquist *et al*. 2012). The MP tree was obtained using the subtree-pruning-regrafting algorithm (Swafford et al. 1996) at search level 1, where the initial trees were obtained by the

random addition of sequences (10 replicates). The bootstrap method with 10,000 replicates was employed to estimate the confidence levels of monophyletic groups. In the B/MCMC analysis, the best fitting sequence evolution model for each DNA region was selected by jModelTest 2 (Posada 2008). The *PgiC* tree was constructed with the HKY+I model and the *rbcL* tree with the SYM+I model. Indels were treated as missing characters for the *PgiC* data sets, as recommended by the MEGA and MrBayes documentations. No indels were found in the *rbcL* fragment. Four rounds of MCMC were run simultaneously and sampled every 100 generations for a total of 1 million generations. Tracer v1.5 (Rambaut and Drummond 2007) was used to examine the posterior distribution of all the parameters and their associated statistics, including the estimated sample sizes. The first 2,500 of the sample trees from each run were discarded as a burn-in period.

1.3. Results

Ploidy analyses

The DNA content of the K111 individual (triploid apogamous *Dryopteris pacifica*) was 1.87 times that of the internal standard (*Nicotiana tabacum*) according to our ploidy analyses. Given that the 2C-value of *N. tabacum* is approximately 11.71 pg per nucleus (Narayan, 1987), the DNA content of this triploid material was estimated as approximately 21.89 pg. The DNA contents of the other 22 samples (including *D. bissetiana*, *D. kobayashii*, *D. pacifica*, *D. sacrosancta*, and *D. varia*) were similar to that of K111 (20.95–27.16 pg). The DNA contents of the other eight samples (including *D. pacifica* and *D. protobissetiana*) were 14.63–17.08 pg. The DNA contents of these eight samples were approximately two-thirds of those of the 22 samples and K111. Therefore, the ploidy levels of these eight samples were estimated to be diploid and those of the other 23 samples were estimated to be triploid.

Plastid rbcL sequence variation and phylogenetic analysis results

Seven types of *rbcL* sequences (Types A–E) were found in the *Dryopteris varia* complex and its closely related species (Figure 1-2). Among the 1,205 sites, 84 (7%) were polymorphic and 35 (3%) were parsimony informative. MP and B/MCMC analyses showed that the phylogenetic trees basically had the same topology. The 50% majority-rule consensus tree obtained from the Bayesian trees with bootstrap percentages (BP) based on the MP analysis and Bayesian posterior probabilities (PP) is shown in Figure 1-2.

Nuclear PgiC sequence variation and phylogenetic trees based on them

Several different sequences of nuclear *PgiC* were detected by the SSCP analyses in most of the samples. However, the number of different sequences was never more than the assumed ploidy level of the sample. According to nucleotide sequences from DNA sequencer, no double peaks were observed for each band separated by the SSCP analyses. Thus, all the distinct sequences in the *PgiC* fragment among the samples were separated on our SSCP gel. In total, 31 different sequences were identified in the samples from the *Dryopteris varia* complex and the length of the sequences varied between 629–684 bp. The data matrix for phylogenetic analyses included 691 characters after editing, where 133 (19%) were polymorphic and 63 (9%) were parsimony informative. The 50% majority-rule consensus tree obtained from the Bayesian trees with BP based on MP analysis and Bayesian PP is shown in Figure 1-3. Five monophyletic groups (groups A, B, C, D, and E in Figure 1-3) were recognized, each of which was supported by the high BP and PP values according to Bayesian inference (Figure 1-3).

The genotype estimated for each sample is summarized in Appendix 2-1 together with according to the data obtained in Chapter 2. It should be noted that the current method cannot distinguish differences in gene dosage in polyploids; therefore, unidentified genomes are denoted by asterisks (*e.g.*, A_1C_1* indicates a genotype of either $A_1A_1C_1$, A_1C_1, or $A_1C_1C_1$, whereas A_1A_1* indicates a genotype of either $A_1A_1A_1$ or A_1A_1).

1.4. Discussion

Reticulogram of the Dryopteris varia *complex based on the plastid* rbcL *and nuclear* PgiC *genes*

The *PgiC* tree (Figure 1-3) shows that the *PgiC* sequences found in the *Dryopteris varia* complex can be grouped into five clades (A–E). Except for *D. varia*, all of the apogamous individuals in the *D. varia* complex had several different *PgiC* sequences from different clades, thereby supporting their hybrid origin. Each clade contained sequence(s) from at least one sexual diploid species (or cytotype), and thus hypothetically, the genome of its sexual progenitor had been transferred to apogamous taxa. Moreover, the information based on plastid *rbcL* (Figure 1-2) could be used to trace the maternal line of each taxon with a hybrid origin. According to the constitution of the nuclear *PgiC* and the maternally inherited plastid *rbcL* identified for each plant sample, I considered the evolutionary diversification processes in the *D. varia* complex by hybridization between the progenitor diploid sexual species and the apogamous species derived from them. Thus, the hypothetical complex reticulate relationships among the species in the *D. varia* complex are summarized in Figure 1-4 as a reticulogram. The reticulogram excludes some of the results obtained in this study, which were not directly related to the formation of the species in the *D. varia* complex.

Sexual diploid progenitors involved in reticulate evolution in the Dryopteris varia *complex*

To understand complex reticulate evolution, it is important to identify the diploid sexual species (or diploid sexual cytotypes when intra-specific cytotypic variation is observed) involved in the *Dryopteris varia* complex. This is because diploid sexual species or cytotypes should be progenitors of apogamous species and they can also be the sources of genetic variation in apogamous species via hybridization. In this study, I first attempted to identify the progenitor diploid sexual species of apogamous taxa in the *D. varia* complex.

Four diploid sexual species (*Dryopteris caudipinna*, *D. koidzumiana*, *D. protobissetiana*, and *D. saxifraga*) as well as the diplod sexual cytotypes of *D. chinensis* and *D. varia* were identified as the progenitor species that probably provided genomes for the *D. varia* complex. These sexual species and the sexual cytotypes of the two species only had one type of *rbcL* and nuclear *PgiC*, excluding *D. varia*. Among these six species or cytotypes, *D. caudipinna*, *D. chinensis*, and *D. koidzumiana* are not included in the *D. varia* complex. According to the classification system proposed for the genus *Dryopteris* by Fraser-Jenkins (1986), *D. caudipinna* and *D. koidzumiana* belong to sect. *Erythrovariae* of subg. *Erythrovariae*, and *D. chinensis* belongs to sect. *Aemulae* of subg. *Dryopteris*, whereas the members of the *D. varia* complex were classified in sect. *Variae* of subg. *Erythrovariae*. Furthermore, the *rbcL* sequences of these species were found to be phylogenetically distant from those of the species in the *D. varia* complex (Ebihara 2011). This demonstrates that the genomes of relatively distant species were also involved in reticulate evolution in the apogamic *D. varia* complex.

Dryopteris caudipinna and *D. koidzumiana* had similar nucleotide sequences for *rbcL* (Type D) and *PgiC* (the sequences in Clade D). Thus, these two species may

well be the same biological species. In this study, the name *D. caudipinna* is used to indicate the genome of *D. caudipinna* or *D. koidzumiana* because the former species is much more widely distributed and more common than the latter. Therefore, it is assumed that five diploid sexual species or cytotypes have participated in the evolution of the *D. varia* complex.

A diploid sexual cytotype of *Dryopteris varia* was found recently in Taiwan (Ebihara *et al.* 2014), in addition to the diploid and triploid apogamous cytotypes (Figure 1-1). A tetraploid sexual cytotype was also reported for this species by Tsai and Shieh (1975, 1985), but this report was not sufficiently reliable. Lee *et al.* (2013) suggested that the triploid apogamous *D. varia* is of autopolyploid origin because the sequences of *D. varia* formed a monophyletic group in both the cpDNA and *PgiC* trees. The data obtained in the present study based on wider sampling surveys in Japan and Taiwan also supported their conclusion because *D. varia* only had one type of nuclear *PgiC* sequence (Clade A). The three *rbcL* sequences (Types A) in *D. varia* were not monophyletic, but these sequences were not shared by other diploid sexual species or cytotypes. Therefore, the results of the present study also demonstrate that the apogamous triploids of *D. varia* are autopolyploid.

Dryopteris protobissetiana is a new diploid sexual species that I recently described from Yakushima Island, Kagoshima Prefecture, Japan (Hori *et al.* 2015). This species had nuclear *PgiC* sequences belonging to Clade C (Figure 1-3). The plastid *rbcL* sequence of *D. protobissetiana* was Type C, and it was shared by *D. bissetiana* and *D. pacifica*. The other three diploid sexual species, *i.e.*, *D. saxifraga*, *D. caudipinna*, and *D. chinensis*, had nuclear *PgiC* sequences in clades B, D, and E, respectively. Therefore, these five clades were resolved in the *PgiC* tree (Figure 1-3)

containing the sequence(s) of their respective sexual species or cytotypes.

All of the genotypes observed in the apogamous species of the *Dryopteris varia* complex, which contained several different nuclear *PgiC* sequences, can be interpreted as combinations of the genomes of the five diploid sexual species or cytotypes represented by clades A–E in the *PgiC* tree (Figure 1-3). Their genomes are denoted by the names of the clades determined in the present study. The genome constitution (*PgiC* constitution) of *D. bissetiana* was B + C (*i.e.*, *D. saxifraga* + *D. protobissetiana*). The genome constitutions of *D. sacrosancta sensu stricto* and *D. kobayashii*, which were separated from *D. sacrosancta sensu lato* by Serizawa (2009), were A + C + E (where E is from *D. chinensis*) and B + C + E, respectively. Thus, the *PgiC* constitutions differed between the two species. These results support the taxonomical treatment of Serizawa (2009) who separated the two species. For *D. pacifica*, three types of genome constitutions were observed in this species: A + C, A + B + C, and A + C + D (where D is from *D. caudipinna*), which are designated as the α type (*PgiC* constitution: A + C), β type (A + B + C), and γ type (A + C + D), respectively. It is difficult to distinguish these types based on their morphology, but the γ type is more readily distinguishable from the others due to its larger fronds (which often reach up to 1 m in length) and papery lamina. Among the apogamous species or apogamous cytotypes in the *D. varia* complex, those containing genomes derived from progenitors outside the *D. varia* complex are as follows: *D. sacrosancta sensu stricto*, *D. kobayashii*, and *D. pacifica* (γ type). *Dryopteris sacrosancta sensu stricto* and *D. kobayashii* contained the genome of *D. chinensis*. The γ type of *D. pacifica* had the genome of *D. caudipinna*.

Possible cytoplasmic gene flow from Dryopteris sacrosancta *to* D. chinensis

Lee *et al.* (2013) suggested that cytoplasmic gene flow might have occurred from *Dryopteris sacrosancta sensu lato* to *D. chinensis* based on their demonstration that they shared a plastid *rbcL* sequence and that *D. sacrosancta sensu lato* did not possess the nuclear *PgiC* sequence of *D. chinensis*. However, in the present study, the nuclear *PgiC* (E) of *D. chinensis* was detected in *D. kobayashii* and *D. sacrosancta sensu stricto*, which were separated from *D. sacrosancta sensu lato* by Serizawa (2009). For *D. chinensis*, only triploid and tetraploid apogamous types have been reported from Japan and China (Figure 1-1). However, I discovered a new diploid sexual cytotype of *D. chinensis* in Japan (Hori *et al.* 2015). Therefore, it is reasonable to consider that these two species originated via hybridization between *D. chinensis* and members of the *D. varia* complex. The contradictory results obtained by Lee *et al.* (2013) and in the present study might be explained by the different methods employed for selecting nuclear sequences, where they used a cloning method to separate and select nuclear *PgiC* sequences from each sample, whereas the SSCP method was used in the present study. The latter method allowed the selection of all the nuclear DNA sequences in each sample more reliable than the cloning method.

Origins of apogamous species in the Dryopteris varia *complex*

Excluding *Dryopteris varia*, all the apogamous species in the complex examined in this study had hybrid origins because their nuclear *PgiC* sequences belonged to two or three clades. Some apogamous species possess sequences from three clades of *PgiC*, which were derived from three different diploid sexual species, so hybridization between two species with different genomes have likely occurred at least

twice during the genetic diversification of apogamous members of the *D. varia* complex.

(1) Apogamous species originated by hybridization between two species

Apogamous species with two *PgiC* types belonging to two different clades, *Dryopteris bissetiana* and *D. pacifica* (α type), can be considered to have originated by hybridization between two species. *Dryopteris bissetiana* (*PgiC* constitution: B + C) is a hybrid of *D. saxifraga* (B) and *D. protobissetiana* (C).

The α type of *Dryopteris pacifica* (A + C) originated by hybridization between *D. varia* (A) and *D. protobissetiana* (C). This species had two plastid *rbcL* sequences: one shared with *D. varia* and another shared with *D. protobissetiana*. Therefore, the maternal parent of *D. pacifica* (α) must be *D. varia* or *D. protobissetiana*. At least two hybridization events have likely occurred during their development.

(2) Apogamous species originated from hybrids between diploid apogamous species and diploid sexual species

In addition, the apogamous species with three *PgiC* types belonging to three different clades (*Dryopteris kobayashii*, *D. pacifica* (β, γ), and *D. sacrosancta*) can be considered to have originated by hybridization between one diploid apogamous species with a previous hybrid origin and one diploid sexual species. These apogamous species might also have developed from at least two hybridization events.

Dryopteris kobayashii (B + C + E) originated by hybridization between the diploid apogamous *D. bissetiana* (B + C) and the diploid sexual *D. chinensis* (E). This species shared one plastid sequence with *D. chinensis*. Therefore, the maternal parent of *D. kobayashii* must be *D. chinensis*. In this study, a diploid apogamous sporophyte of *D.*

bissetiana was not found. However, Lee *et al.* (2006) reported a diploid apogamous *D. bissetiana* from Korea. Even if diploid apogamous sporophytes are not found, the existence of diploid apogamous gametophytes is expected because Lin *et al.* (1992) reported that diploid apogamous spores are produced from triploid sporophytes of members of the *D. varia* complex. The diploid sexual cytotype of *D. chinensis* has not been identified in Japan and China (Kurita 1961; Hirabayashi 1966, 1974; Mitui 1968; Nakato *et al.* 1995), but I found it in Japan, as mentioned above.

Dryopteris sacrosancta sensu tricto (A + C + E) had two plastid *rbcL* sequences, which were shared with *D. varia* (A) and *D. chinensis* (E), respectively. *Dryopteris sacrosancta* individuals with the same plastid *rbcL* sequence as *D. chinensis* might have originated by hybridization between the diploid apogamous *D. pacifica* (α) (A + C) and the diploid sexual *D. chinensis* (E). In this case, *D. chinensis* must be the maternal parent. *Dryopteris sacrosancta* individuals that shared *rbcL* sequences with *D. varia* may have originated by hybridization between sexual *D. varia* (A) and one diploid apogamous strain from the *D. varia* complex (C + E). However, an apogamous strain with the *PgiC* constitution has not been discovered in the *D. varia* complex. Therefore, the latter case may suggest that the diploid apogamous *D. pacifica* (α) (A + C) could act as the maternal parent during the hybridization with *D. chinensis*.

The γ type of *Dryopteris pacifica* (A + C + D) shared two plastid *rbcL* sequences (Type A) with *D. varia.* Therefore, *D. pacifica* (γ) could have originated by hybridization between sexual *D. varia* (A) and one diploid apogamous strain from the *D. varia* complex (C + D), but again an apogamous strain with the *PgiC* constitution has not been discovered in the *D. varia* complex. Therefore, the diploid apogamous *D. pacifica* (α) (A + C), which has the *rbcL* Type A sequence, might be the maternal parent

and the diploid sexual *D. caudipinna* might be the paternal parent. This situation is similar to that in *D. sacrosancta sensu stricto*.

The β type of *Dryopteris pacifica* (A + B + C) had *rbcL* Type A and B sequences, which were shared with *D. varia* (A) and *D. saxifraga* (B), respectively. *Dryopteris pacifica* (β) individuals with the plastid *rbcL* sequence shared with *D. varia* might have originated by hybridization between the diploid apogamous *D. bissetiana* (B + C) and sexual *D. varia* (A). *Dryopteris pacifica* (β) individuals with the plastid *rbcL* sequence shared with *D. saxifraga* might have originated by hybridization between the diploid apogamous *D. pacifica* (α) (A + C) and sexual *D. saxifraga* (B).

The results of the present study suggest that diploid apogamous cytotypes can hybridize with diploid sexual cytotypes because many triploid apogamous species with three types of nuclear *PgiC* belonged to three different clades. These results may support the "hybridization cycle hypothesis" suggested by Lin *et al.* (1992, 1995), although I did not confirm whether apogamous triploid species can produce unequally reduced diploid spores. According to the results of this study, diploid apogamous individuals might be able to act as the maternal species during hybridization with sexual individuals because unknown apogamous strains are required if apogamous individuals are assumed to be only the paternal parent. However, previous studies suggested that apogamous species act only as paternal parents and not as maternal parents (Gastony and Yatskievych 1992; Grusz *et al.* 2009; Jaruwattanaphan *et al.* 2013; Suzuki and Iwatsuki 1990; Walker 1962). To address these problems, I plan to perform artificial crossing between diploid apogamous species, particularly *Dryopteris pacifica* (α), and diploid sexual species, *D. caudipinna* or *D. chinensis*.

The present study provides the most comprehensive information for reticulate

evolution in the *Dryopteteris varia* complex (Figure 1-4). The results demonstrate that any apogamous cytotypes in the complex can be explained by a combination of two or three nuclear genomes from five species (*D. caudipinna*, *D. chinensis*, *D. protobissetiana*, *D. saxifraga*, and *D. varia*) with diploid sexual cytotypes. This study also showed that the genomes of relatively distant diploid sexual species (*D. caudipinna* and *D. chinensis*) were involved in reticulate evolution in the apogamic *D. varia* complex (subg. *Erythrovariae*, sect. *Variae*).

Taxon	Reproductive mode	Ploidy level	Chromosome number	Locality	Literature cited
D. bissetiana	apogamous	2*x*	'*n*' = 82	Japan	Hirabayashi (1966)
		3*x*	'*n*' = 123	Japan	Hirabayashi (1967), Lin et al. (2003)
			2*n* = 123	China	Nakato et al. (1995)
		2*x*, 3*x*	2*n* = 82, 123	Korea	Lee et al. (2006)
	sexual	4*x*	*n* = 82	China	Weng (1989)
D. insularis	apogamous	2*x*	2*n* = 82	Japan	Lin et al. (2003)
D. insularis (var. chichimiensis)	apogamous	3*x*	2*n* = 123	Japan	Lin et al. (2003)
D. pacifica	apogamous	2*x*	'*n*' = 82, 2*n* = 82	Japan	Lin et al. (1992)
			2*n* = 82	China	Nakato et al. (1995)
		3*x*	'*n*' = 123	Japan	Hirabayashi (1970)
			'*n*' = 123, 2*n* = 123	Japan	Lin et al. (1992)
			2*n* = 123	China	Nakato et al. (1995)
			2*n* = 123	Japan	Lin et al. (2003)
			2*n* = 123	Korea	Lee et al. (2006)
D. protobissetiana	sexual	2*x*	2*n* = 82	Japan	Hori et al. (2015)
D. sacrosancta	apogamous	3*x*	'*n*' = 123	Japan	Mitui (1965, 1968), Hirabayashi (1969)
			2*n* = 123	China	Nakato et al. (1995)
			2*n* = 123	Japan	Lin et al. (2003)
			2*n* = 123	Korea	Lee et al. (2006)
D. saxifraga	sexual	2*x*	*n* = 41	Japan	Hirabayashi (1967), Mitui (1975)
			2*n* = 82	Korea	Lee et al. (2006)
	apogamous	3*x*	2*n* = 123	Korea	Lee et al. (2006)
D. varia	sexual	2*x*	2*n* = 82	Tawian	Ebihara et al. (2014)
		4*x*	*n* = 82	Tawian	Tsai & Shieh (1975, 1985)
	apogamous	2*x*	'*n*' = 82	Japan	Hirabayashi (1966, 1967, 1974)
		3*x*	'*n*' = 123	Japan	Mitui (1966, 1968), Hirabayashi (1970)
			2*n* = 123	China	Nakato et al. (1995)
			2*n* = 123	Japan	Lin et al. (2003)
			2*n* = 123	Tawian	Ebihara et al. (2014)
			2*n* = 123	Korea	Lee et al. (2006)

Figure 1-1. The reproductive mode, ploidy level, and chromosome numbers of the *Dryopteris varia* complex.

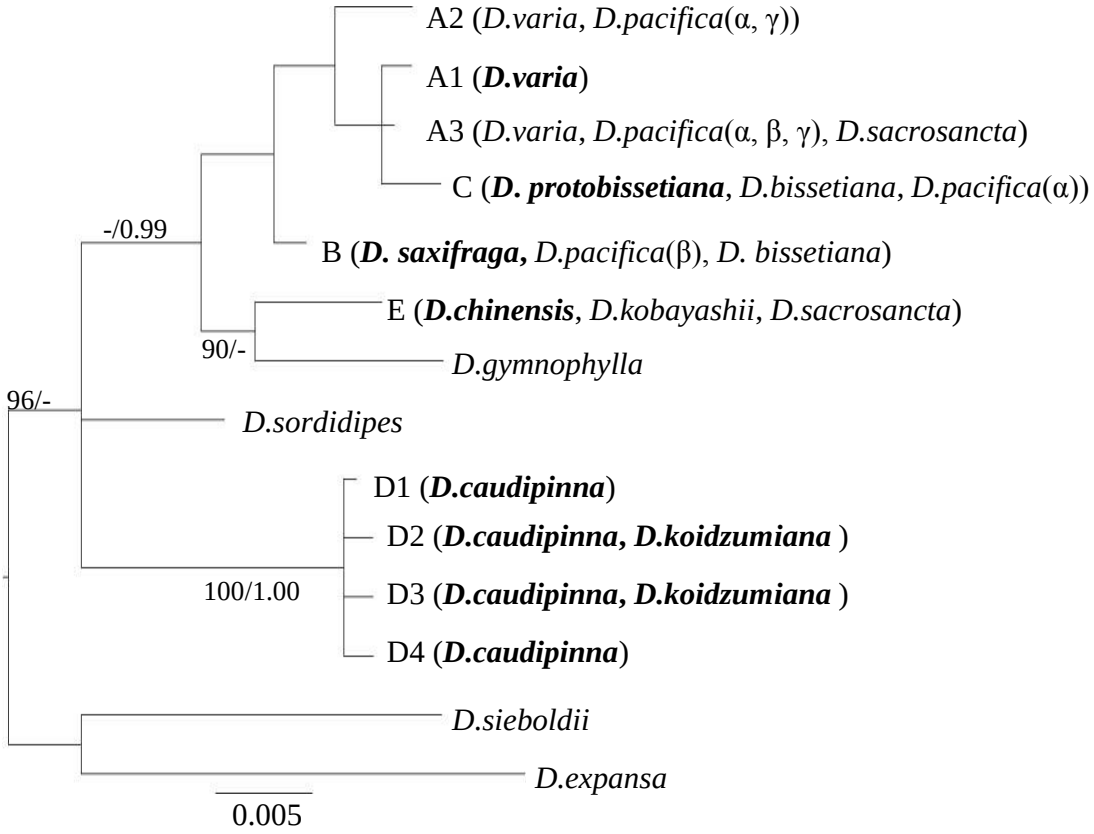

Figure 1-2. A 50% majority-rule consensus tree resulting from Bayesian Markov chain Monte Carlo (B/MCMC) analysis of chloroplast *rbcL* sequences of the *Dryopteris varia* complex.
Numbers at the branches show bootstrap percentages (BP) of maximum parsimony analysis and the posterior probability (PP) of B/MCMC analysis for strong supporting clades (BP ≥80, PP ≥0.95). The scale bar indicates a branch length corresponding to 0.005 substitutions per site. The sexually reproducing species are indicated in bold.

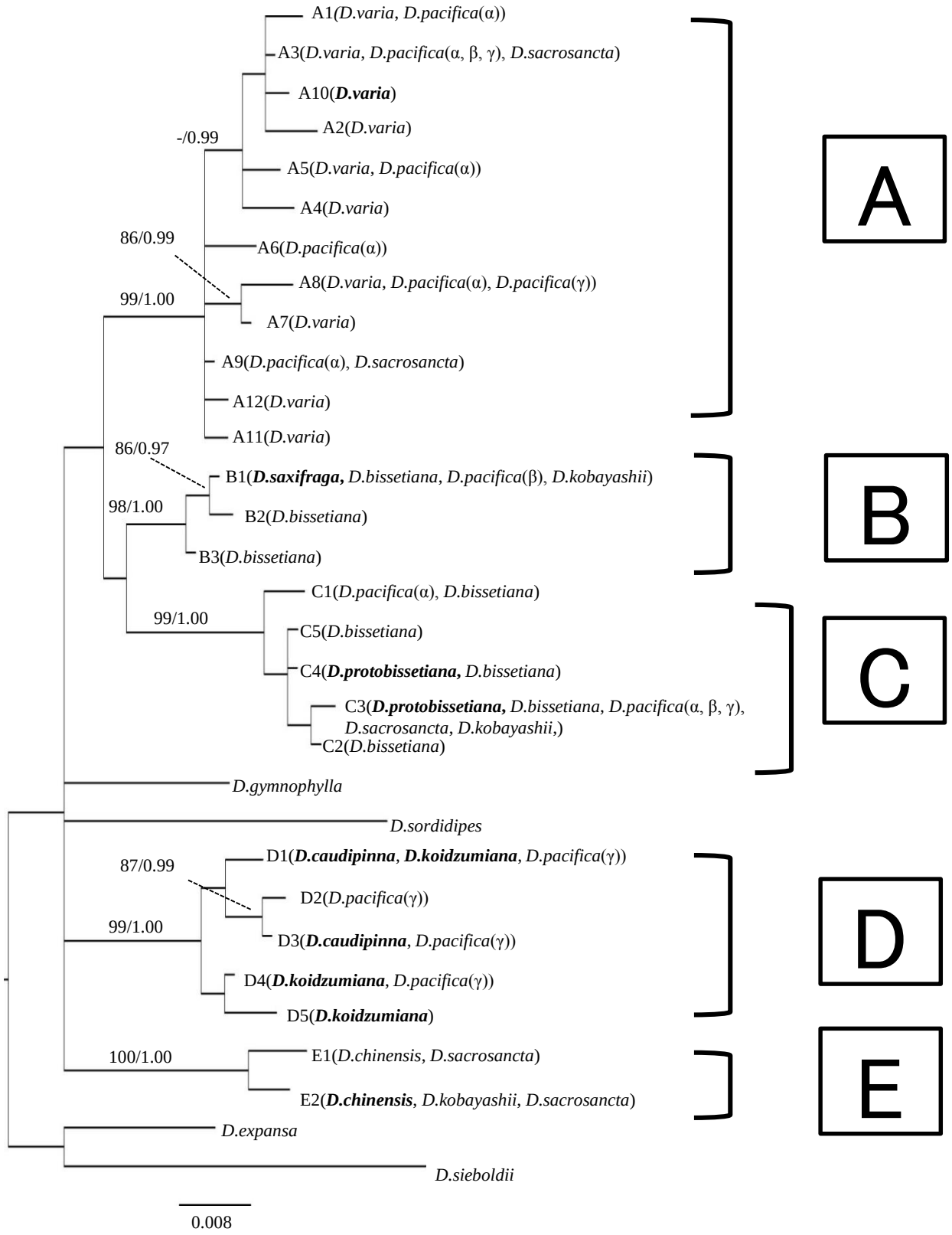

Figure 1-3. 50% majority-rule consensus tree resulting from Bayesian Markov chain Monte Carlo (B/MCMC) analysis of nuclear *PgiC* sequences of the *Dryopteris varia* complex. Numbers at the branches show bootstrap percentages (BP) of maximum parsimony analysis and the posterior probability (PP) of Bayesian analysis for strong supporting clades (BP ≥80, PP ≥0.95). The scale bar indicates a branch length corresponding to 0.008 substitutions per site. The sexually reproducing species are indicated in bold.

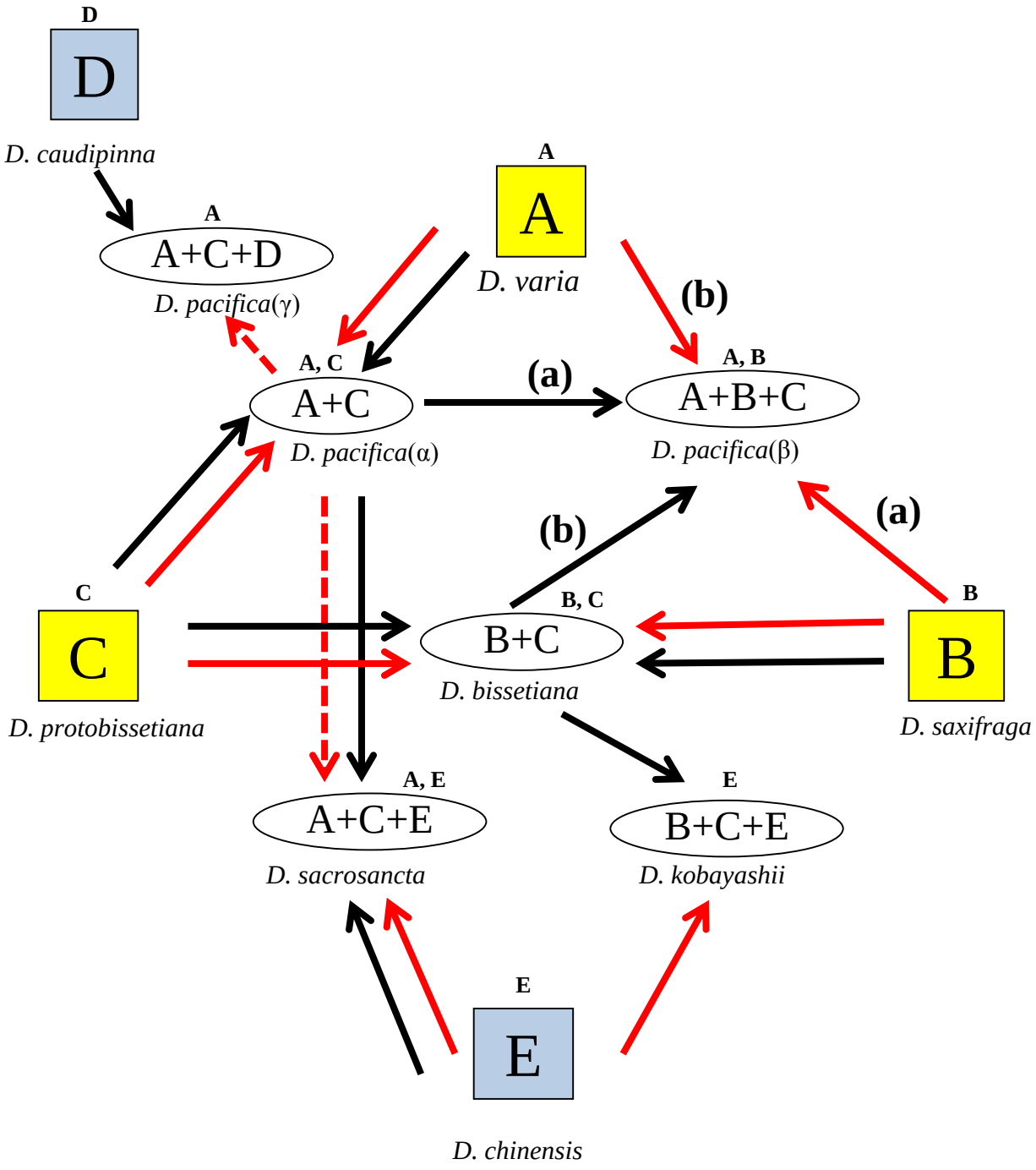

Figure 1-4. The reticulogram of the *Dryopteris varia* complex. The yellow square and the blue square indicate diploid progenitors of the *D. varia* complex and related diploid species other than the complex, respectively. The ellipses indicates apogamous species. The alphabets in squares and ellipses indicate their *PgiC* constitutions. Even in the case that their *PgiC* constituiton is A+A+C or A+C+C, it is simply shown as A+C. The alphabets above the squares or ellipses indicate cpDNA genotypes. Red and black arrows indicate maternal and paternal species, respectively. Red-dashed arrows indicate apogamous maternal species. Two courses of hybridization can be supposed for related to the formation of *D. pacifica* (β) and show the courses as (a) and (b).

Chapter 2

Analyses of reticulate evolution in the apogamous species of the *Dryopteris varia* complex using five nuclear genetic markers

2.1. Introduction

In Chapter 1, the nucleotide sequences of *PgiC* from five diploid sexual species of the *Dryopteris varia* complex and the related species were identified as the following five monophyletic groups of sequences (A-E): A, *D. varia*; B, *D. saxifraga*; C, *D. protobissetiana*; D, *D. caudipinna*; and E, *D. chinensis*. It was also shown that each triploid apogamous species of the *D. varia* complex contained two or three *PgiC* sequences originated from two or three of the above diploid sexual species: *D. bissetiana*, B + C; *D. pacifica* (α), A + C; *D. pacifica* (β), A + B + C; *D. pacifica* (γ), A + C + D; *D. kobayashii*, B + C + E; *D. sacrosancta*, A + C + E. Therefore, these apogamous species may have undergone a complicated reticulate evolution among the diploid sexual species or have undergone recurrent reticulation through unequal meiosis and hybridization with sexual species (Figure 1-3).

If recurrent reticulation occurred in the apogamous species of the *Dryopteris varia* complex, they should have experienced unequal meiosis many times. Meiosis causes genetic recombination by segregation of homologous (or homoeologous, in the case of apogamous species) chromosomes and chromosomal crossing over (Muller 1932), producing offspring with various combinations of both parental and chimeric

alleles. Therefore, allele constitutions can be different among several nuclear loci located on homoeologous chromosomes that originated from different diploid sexual species and are now a single apogamous species. The base chromosome number of *Dryopteris* is $x = 41$. This means that 41 kinds of chromosomes exist and are able to behave independently.

Figure 2-1 explains how chromosome segregation and recombination occur during repeated reticulation in triploid apogamous species, although it assumes the case of $x = 3$ (three non-homologous chromosomes: square, circle, and diamond), instead of $x = 41$. Assume that the first hybridization occurs between a triploid apogamous species (Species A) with only blue chromosomes and a diploid sexual species (Species B) with only red chromosomes. Species A produces diploid apogamous gametophytes with two sets of the three blue chromosomes; whereas, Species B produces haploid sexual gametophytes with a set of the three red chromosomes. If these gametophytes succeed in fertilization, a new hybrid with two sets of blue and a set of red chromosomes is produced. This new hybrid triploid apogamous species (Species C) can produce fertile spores because the apogamous gene is dominant to the sexual gene in ferns, and a hybrid between an apogamous and a sexual species can often reproduce apogamously (Walker 1962). The allele constitution encoded on a locus in one chromosome type (either square, circle or diamond) from Species C should also correspond to the constitution of the three chromosomes; Blue–Blue–Red.

Then, a second hybridization occurs between the triploid apogamous species C and another diploid sexual species (Species D) with only green chromosomes. When Species C produces diploid apogamous gametophytes, their constitution might be

different among the three kinds of chromosomes (i.e., Square chromosome, Blue–Blue; Circle chromosome, Blue–Blue; Diamond chromosome, Blue–Red). On the other hand, Species D produces haploid sexual gametophytes with a set of green chromosomes. If these gametophytes succeed in fertilization, a new triploid apogamous species (Species E) will be produced. The constitution of Species E should be partially different among the three kinds of chromosomes: Square chromosome, Blue–Blue–Green; Circle chromosome, Blue–Blue–Green; Diamond chromosome, Blue–Red–Green.

A third hybridization occurs between the triploid apogamous species E and another diploid sexual species (Species F) that has only orange chromosomes. Species E produces diploid apogamous gametophytes. The constitution of Species E might be again different among the three kinds of chromosomes (i.e., Square chromosome, Blue–Blue; Circle chromosome, Blue–Green; Diamond chromosome, Blue–Red), whereas Species F produces haploid sexual gametophytes with a set of orange chromosomes. If these gametophytes succeed in fertilization, a new triploid apogamous species (Species G) can be produced. The constitution of Species G should be different among the three kind of chromosomes (i.e., Square chromosome, Blue–Blue–Orange; Circle chromosome, Blue–Green–Orange; Diamond chromosome, Blue–Red–Orange.

If such hybridization cycles repeat, the resultant apogamous species can display huge amounts of interclonal genetic variation. In the case of $x = 41$, chromosome constitutions can display a maximum of $3^{41} = 3.6472996e+19$ patterns in a triploid apogamous species. If this is the case, classification of apogamous species according to their genomic constitution must be hopeless. In Chapter 1, only one nuclear genetic marker, *PgiC*, was used; therefore, inconsistencies in allele constitutions among loci on

different kinds of chromosomes were not examined. Other than *PgiC* (as used in Chapter 1), the *GapCp* gene also has been used frequently as a nuclear marker for fern genetic studies. However, if the loci of the two nuclear markers (*PgiC* and *GapCp*) are linked, it is impossible to determine whether recombination of the chromosomes has occurred through the hybridization of these markers. Further evaluation of unlinked nuclear markers is necessary to solve this problem.

In Chapter 2, additional nuclear markers were developed to explore this question by analyzing a total of five nuclear genes: *PgiC*, *GapCp*, *AK1*, *Esterase*, and *G6pdh*. These loci code for enzymes that often have been used for electrophoretic analyses to estimate genetic diversity within a population or among populations of particular plant species (Schall 1980; Levin 1981; Hamrick 1982; Loveless and Hamrick 1984; Gastony and Gottlieb 1982, 1985; Haufler and Soltis 1984; Haufler 1985a-b, 1987; McCauley *et al*. 1985; Holzinger 1987; Smyth and Hamrick 1987; Soltis and Soltis 1987 a-d, 1988; Shinohara *et al.* 2010). Therefore, these nuclear genetic markers are expected to be useful for a wide-range of taxonomic and population genetic studies, including those on the reticulate evolution of apogamous ferns. Additional taxon sampling was conducted in Chapter 2 to include *Dryopteris insularis* var. *insularis* and *D. insularis* var. *chichisimensis*, which are also the members of the *D. varia* complex (Lin *et al*. 1995), because sufficient outgroup materials were not included in the Chapter 1 research.

In Chapter 2, I examine whether or not genome constitutions of the apogamous species in the *Dryopteris varia* complex are different across several nuclear gene loci, at least some of which are unlinked and coded on non-homologous chromosomes. Furthermore, several samples collected from other localities are added to cover genetic

variation within each species of the *D. varia* complex.

2.2. Materials and Methods

Plant materials

The numbers of leaf samples used in this study are as follow for the members of the *Dryopteris varia* complex: *D. varia*, 24; *D. saxifraga*, 18; *D. protobissetiana*, 10; *D. pacifica* (α), 94; *D. pacifica* (β), 13; *D. pacifica* (γ), 40; *D. sacrosancta*, 47; *D. kobayashii*, 14; and *D. bissetiana*, 56; *D. insularis* var. *insularis*, two; *D. insularis* var. *chichisimensis*, two. The *Dryopteris* species not attributed to the *D. varia* complex, but related to it include: *D. chinensis*, 10; *D. caudipinna*, five; *D. koidzumiana*, four. In addition, six samples of *D. sordidipes*, and one sample each of *D. sabaei*, *D. handeliana*, *D. hasseltii*, *D. polita*, *D. monticola*, *D. expansa*, *D. gymnophylla*, *Polystichum lepidocaulon*, *P. retroso-paleaceum*, and *Arachnioides exillis* were used as outgroups. Of these newly collected specimens, 22 samples (*D. varia*, seven; *D. saxifraga*, one; *D. protobissetiana*, one; *D. pacifica* (α), five; *D. pacifica* (γ), one; *D. sacrosancta*, one; *D. kobayashii*, one; *D. bissetiana*, two; *D. insularis* var. *insularis*, one; *D. insularis* var. *chichisimensis*, one) were also collected as living stocks. Voucher information for these samples is listed in the Appendix 2-1. All the voucher specimens have been deposited in MAK and/or TNS.

Cytological observation and estimation of reproductive mode

To observe mitotic chromosomes, root tips of the living stocks were pretreated

with 0.004-M 8-hydroxyquinoline for 7 h at approximately 15°C–18°C. After fixation overnight in ethanol and acetic acid (3:1), the root tips were hydrolyzed in 1-N HCl and 45% acetic acid (1:1) at 60°C for 10 min before being mashed in a 2% aceto–orcein solution. The chromosomes were observed under a microscope (Leica DM2500) and then photographed by using a digital camera (Leica Application Suite LAS ver. 4.4).

To estimate the reproductive mode of each sample or herbarium specimen, the spore numbers in each sporangium were counted. The sample was estimated to be sexually reproduced if the number was 64, whereas it was estimated to be apogamously reproduced if the number was 32 (Manton 1950).

Ploidy analysis

The method for ploidy analysis is described in Chapter 1.

Molecular analysis of plastid and nuclear markers

For molecular analyses, small amounts of leaf samples were dried in small plastic bags of size 20 cm × 10 cm with silica gel. Subsequently, total DNA was extracted from the dried leaves by using cetyltrimethylammonium bromide solution, according to the method of Doyle & Doyle (1987).

Plastid gene *rbcL* was used in this study as the cpDNA marker. Polymerase chain reaction (PCR) amplification of a *rbcL* fragment was performed by using the primers

aF3 (5′-ATGTCACCACAAACGGAGACTAAAGC-3′) and cR3 (5′-GCGGCAGCCAATTCCGGACTCCA-3′), which were newly designed in this study. The nucleotide sequences of *rbcL* were determined by direct sequencing. For sequencing *rbcL*, aF3, aR-D (5′-CGATCTCTCCAACGCATGAATGGCTG-3′), which was also newly designed in this study, D. paci-bf (Hori *et al.* 2014, See also Chapter 1.) and cR3 primers were used.

To analyze nuclear genes, the *PgiC* fragment was amplified by using the primers 14F (5′-GTGCTTCTGGGTCTTTTGAGTG-3′) and 16R (5′-GTTGTCCATTAGTTCCAGGTTCCCC-3′) of Ishikawa *et al.* (2002). The *GapCp* fragment was amplified by using the primers 132F (5′-GTGCTTCCGGAGTTAAATGG-3′) and 488R (5′-CAACATCATCTTCGGTGTATCC-3′) of Hori *et al.* (2016).

For developing new nuclear genetic markers, total RNA was extracted from fresh living individuals of *Dryopteris saxifraga* (diploid sexual species) by using the Spectrum Total Plant RNA Kit (Sigma-Aldrich, St. Louis, Missouri, U.S.A.). cDNA was obtained by the cDNA Synthesis Kit (Roche, Basel) and amplified by PCR. Sequencing was performed on Roche's 454 GS Junior system (Roche, Basel) and approximately 122,963 reads were obtained. The reads were assembled by using SOAPdenovo software (http://soap.genomics.org.cn/soapdenovo.html) and 3,925 contigs (contiguous overlapping sequences) were obtained. Homologs of the genes, which had been often used for the allozyme method, were searched for in databases of the *Arabidopsis thaliana* project (http://pgsb.helmholtz-muenchen.de/plant/athal/) and the 1,000 Plants project (https://www.bioinfodata.org/Blast4OneKP/).

Finally, PCR primers for *Adenylate kinase 1* gene (*AK1*), *Esterase/Lipase/Thioesterase family protein* gene (*Esterase*) and *Glucose-6-phosphate dehydrogenase* (*G6pdh*) gene were designed (Figure 2-2). These newly designed pairs of PCR primers were as follows:

AK4F (5′- GATGAAGCCATCAAGAAACCA-3′) and AKR2 (5′-ATGGATCCAGCGACCAGTAA-3′) for *AK1* (*Adenylate kinase* 1) gene;

EST-F (5′- GGCTGGAGCAGTCTCTCTGT-3′) and EST-R (5′-GCACTAGCAGCTTTCGGAAT-3′) for *Esterase* gene;

G6F (5′-TTTGGTGGCTATGGAGAAGC-3′) and

G6R (5′-CGAATGTTGGGGTATTGGAG-3′) for *G6pdh* gene.

PCR-single-strand conformation polymorphism (SSCP) analysis

PCR-SSCP analysis was performed to examine allelic variation at each nuclear marker, following the method described in Chapter 1.2. Electrophoresis was performed using MDE gel solution (Lonza) under the following conditions: 2% glycerol at 18°C for 16 h at 350 V for *AK1* and *PgiC*; 2% glycerol at 15°C for 14 h at 300 V for *G6pdh* (Figure 2-3); 2% glycerol at 15°C for 9.5 h at 300 V for *GapCp* (Figure 2-4); 5% glycerol at 15°C for 15 h at 300 V for *Esterase*.

Phylogenetic analyses

For phylogenetic analyses, only one sequence representing each allele for the nuclear gene loci (*AK1*, *Esterase*, *GapCp*, *G6pdh*, and *PgiC*) and each haplotype for cpDNA (*rbcL*) was used in the datasets. The chloroplast and nuclear DNA sequences were aligned using MUSCLE (Edgar 2004) and analyzed separately by neighbor-joining (NJ), maximum parsimony (MP), or maximum likelihood (ML) analyses by using MEGA version 6 (Tamura et al. 2013). The NJ tree was obtained with the p-distance method (Nei & Kumar 2000), and the data are expressed as the number of base differences per site. All sites with ambiguous bases were removed from each sequence pair before analysis. The MP tree was obtained by using the subtree-pruning-regrafting algorithm (Swafford et al. 1996) at search level 1, in which the initial trees were obtained by the random addition of sequences (10 replicates). In ML analysis, the best-fitting model of nucleotide substitution for each DNA region was selected by using MEGA version 6 (Tamura et al. 2013). The *AK1* tree was constructed with the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) +I, the *Esterase* tree with the Tamura 3-parameter model (Tamura 1992) + G, the *GapCp* tree with the Tamura 3-parameter model, the *G6pdh* tree with the Tamura 3-parameter model, the *PgiC* tree with the Hasegawa-Kishino-Yano + G model, and the *rbcL* tree using the Kimura 2-parameter model (Kimura 1980) + G. The percentages of trees in which the associated taxa clustered together are shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the NJ method to a matrix of pairwise distances estimated by the Maximum Composite Likelihood approach. The indels were treated as missing characters in MP and ML analyses. The bootstrap method with 1,000

replications was employed to estimate the confidence levels of monophyletic groups.

Genetic linkage of the nuclear markers

AK1 and *Esterase* loci were checked to determine any genetic linkages between the two. Gametophytes were grown for 1 month on agar plates (Yamada *et al.*, 2016) from spores collected from a single individual of the diploid sexual *Dryopteris protobissetiana* (*Hori* 917). Then, total DNAs were extracted from 46 gametophytes that had been silica gel-dried by the same method as mentioned previously for DNA extraction from the sporophyte samples. Genotypes of the *AK1* and *Esterase* genes were estimated by SSCP analysis. Finally, linkage equilibrium between these two nuclear loci was statiscally examined by the chi-square test.

2.3. Results

Ploidies and reproductive modes

Diploid (2*n* = 82) sexual cytotypes were observed in *Dryopteris varia*, *D. saxifraga*; diploid apogamous cytotypes in *D. pacifica* (α), *D. insularis*, and triploid (2*n* = 123) apogamous cytotypes in *D. pacifica* (α), *D. pacifica* (γ), *D. bissetiana*, *D. sacrosancta*, *D. kobayashii*, and *D. insularis* var. *chichisimensis*.

The DNA contents of each species of the complex were estimated as follows: *Dryopteris saxifraga*, 21.12 ± 0.26 pg (*N* = 7); *D. pacifica* (α, diploid apogamous cytotype), 16.42 ± 0.14 pg (*N* = 8); *D. pacifica* (α, triploid apogamous cytotype), 23.60 ± 0.52 pg (*N* = 8); *D. pacifica* (β, triploid apogamous cytotype), 23.76 ± 1.07 pg (*N* = 3); *D. pacifica* (γ, triploid apogamous cytotype), 26.02 ± 0.60 pg (*N* = 4); *D. bissetiana*, 26.23 ± 0.36 pg (*N* = 25); *D. kobayashii* (triploid apogamous cytotype), 23.42 pg (*N* = 1); *D. sacrosancta* (triploid apogamous cytotype), 21.93 ± 0.31 pg (*N* = 7). DNA content data for diploid sexual type of *D. varia*, *D. insularis* and *D. insularis var. chichisimensis* were not available by ploidy analysis.

Molecular phylogenetic trees according to nucleotide sequences of the five nuclear markers

In most of the samples, several alleles were detected by SSCP analyses. In total, 28,

36, 28, 27, and 31 distinct sequences were identified in *AK1*, *Esterase*, *GapCp*, *G6pdh*, and *PgiC* loci, respectively. The length of the sequences varied from 451 to 655 bp, 357 to 594 bp, 301 to 353 bp, 276 to 322 bp, and 617 to 687 bp, respectively. The data matrix for phylogenetic analyses included 767, 674, 374, 344, and 698 characters, respectively, after editing, of which 164 (21%), 182 (27%), 123 (32%), 124 (36%), 230 (33%) were polymorphic and 117 (15%), 110 (16%), 72 (19%), 68 (19%), and 133 (19%) were parsimoniously informative, respectively. The ML trees (highest log likelihood = −2422.5814, −2552.5997, −1659.5745, −1515.4124, and −2850.2708, respectively) according to the sequences of *AK1*, *Esterase*, *GapCp*, *G6pdh*, and *PgiC* with bootstrap percentages (BPs) of NJ/MP/ML analyses are shown in Figure 2-5, 2-6, 2-7, 2-8, and 2-9, respectively.

In each of the molecular trees of the nuclear markers, the sequences from the five diploid sexual species were distinguished (A = *Dryopteris varia*, B = *D. saxifraga*, C = *D. protobissetiana*, D = *D. caudipinna* and *D. koidzumiana*, E = *D. chinensis*) as monophyletic groups except in the *Esterase* tree. As for *Esterase*, diploid sexual *D. varia* had two types of sequences (A and A′), which made different clades (Figure 2-6). However, some individuals of diploid sexual *D. varia* had both A and A′ sequences (in other words, heterozygous of the two types of alleles). Therefore, A and A′ sequences of *Esterase* are likely allelic.

The genotypes (combination of alleles) of each sample of the apogamous species of the complex were the same among the five nuclear loci (Table 2-2): apogamous type

of *Dryopteris varia*, A (AA or AAA); diploid apogamous *D. pacifica* (α), AC; triploid apogamous *D. pacifica* (α), AAC, ACC, or A/C (meaning either AAC or ACC); *D. pacifica* (β), ABC; *D. pacifica* (γ), ACD; *D. bissetiana*, BCC or B/C; *D. sacrosancta*, ACE; *D. kobayashii*, BCE; *D. insularis* var. *insularis* (diploid apogamous), M; *D. insularis* var. *chichisimensis*, ACM. The alleles observed in the samples of *D. insularis* and *D. saxifraga* belong to the same clades in the *GapCp* tree (Figure 2-7), but they were still able to be distinguished by the positions of indels in their *GapCp* sequences. Genotypes of triploid apogamous *D. pacifica* (α) were either AAC or ACC. However, the genotypes of each individual of *D. pacifica* (α) coincided among the five loci. In other words, when the genotype in *AK1* locus was AAC, those in the other four loci were also AAC or A/C, never ACC.

Molecular phylogenetic tree according to rbcL sequences

In the *Dryopteris varia* complex and its diploid sexual relatives, eight types of *rbcL* sequences were recognized. Among the 1,205 sites, 156 (12%) were polymorphic and 88 (7%) were parsimoniously informative. NJ, MP, and ML analyses resulted in phylogenetic trees with similar topology. The ML tree (highest log likelihood = −3118.1608) with BPs of NJ/MP/ML analyses is shown in Figure 2-10.

The haplotype of *rbcL* observed in each species or type of the complex is as follows: *Dryopteris varia* (contains diploid sexual type), A1 or A3; *D. protobissetiana* (diploid sexual species), C; *D. saxifraga* (diploid sexual species), B; *D. insularis* var.

insularis, M; *D. pacifica* α, A1 or A3; *D. pacifica* β, A3 or B; *D. pacifica* γ, A2 or A3; *D. bissetiana*, B or C; *D. insularis* var. *chichisimensis*, A1; *D. kobayashii*, E; *D. sacrosancta*, A3 or E. Each diploid sexual species of the complex had a different type of *rbcL*, except for *D. varia*; however, even *D. varia* did not share haplotypes with the other diploid sexual species. Triploid apogamous species always shared their *rbcL* haplotypes with one of the diploid sexual species of the complex or relatives.

Linkage of the nuclear markers

The sporophyte of *Dryopteris protobissetiana* (*Hori* 917, no. 7) had two alleles in each of the *AK1* (C1 and C6 alleles) and *Esterase* (C4 and C6 alleles) loci. In 46 gametophytes derived from this sporophyte, the p-value of the chi-square test of these two nuclear loci did not indicate marginally significant conflict (P = 0.23). Therefore, *AK1* and *Esterase* are independently inherited nuclear markers. The other three nuclear loci (*GapCp*, *G6pdh*, and *PgiC*) showed too little intraspecific variation within any of the diploid sexual species to test for their independence from other loci.

2.4. Discussion

Recurrent reticulations accompanying chromosomal recombination seemed to occur only a few times within the *Dryopteris varia* complex because the genotypes (allele combinations) were the same among the five nuclear loci used as genetic markers in this study. The genotypes (combination of alleles) of each sample of the apogamous species of the complex were the same among the five nuclear loci (*AK1*, *Esterase*, *GapCp*, *G6pdh*, *PgiC*): apogamous cytotype of *D. varia*, A; diploid apogamous *D. pacifica* (α), AC; triploid apogamous *D. pacifica* (α), AAC, ACC, or A/C (A/C means either AAC or ACC); *D. pacifica* (β), ABC; *D. pacifica* (γ), ACD; *D. bissetiana*, BCC or B/C; *D. sacrosancta*, ACE; *D. kobayashii*, BCE; *D. insularis* var. *insularis* (diploid apogamous), M (MM); *D. insularis* var. *chichisimensis*, ACM. Furthermore, SSCP analyses of the 46 gametophytes derived from the sporophyte of *D. protobissetiana* (*Hori* 917, no. 7), which had two alleles in each of *AK1* (C1 and C6 alleles) and *Esterase* (C4 and C6 alleles) loci, clearly indicated that these two loci are not linked. Therefore, the behavior of chromosomes within reticulate evolution in the *D. varia* complex described in Figure 2-1 is likely inaccurate, and occurs rather as described in Figure 2-12.

In Figure 2-12, the first hybridization occurs between the triploid apogamous Species A with three sets of three blue chromosomes, and the diploid sexual Species B with two sets of red chromosomes, producing a new triploid apogamous species, Species C. The allele constitution of Species C should be Blue–Blue–Red, and this is the same as that in Figure 2-1; however, the behavior of chromosomes in the second hybridization is different from that shown in Figure 2-1. Hybridization occurs between

Species C and another diploid sexual species, Species D, with only green chromosomes. Species C produces diploid apogamous gametophytes, but the allele constitution of the gametophytes is the same among the three kinds of chromosomes: Square, Blue–Red; Circle, Blue–Red; Diamond, Blue–Red. In other words, a set of three kinds of chromosomes from a particular diploid sexual species (i.e., Blue square, Circle, and Diamond chromosomes) always behaves together. If these gametophytes succeed in fertilization with those of species D that has only green chromosomes, a new triploid apogamous species (Species E) will result with three sets of three kinds of chromosomes (Blue, Red, and Green) At this point, the hybridization cycle cannot continue successfully. If it occurs at all, all offspring with chromosome recombinations will likely die and be removed from the gene pool.

In the previous studies, it has been considered that apogamous species can act only as the paternal, and not as the maternal parent (Walker 1962, Suzuki & Iwatsuki 1990, Gastony & Yatskievych 1992, Grusz *et al.* 2009, Jaruwattanaphan *et al.* 2013). However, as discussed in Chapter 1, this study suggests that diploid apogamous individuals might often act as the maternal parent in the hybridization with sexual individuals within the *D. varia* complex. This is because many unknown apogamous strains must be assumed if apogamous individuals can act only as the paternal parent. For example, some individuals of triploid apogamous *D. pacifica* (γ) (genome constitution: A + C + D) and *D. sacrosancta* (genome constitution: A + C + E) shared their *rbcL* haplotype with the diploid sexual *D. varia* (A). If the diploid sexual *D. varia* (A) is the maternal parent, the genome constitution of the diploid apogamous paternal parents should be C + D and C + E, respectively. However, individuals of apogamous

species with such genotypes have yet to be found in the field despite the fact that as many as 338 individuals of this complex have been genetically analyzed. Thus, this study's results suggest that diploid apogamous *D. pacifica* (α) (A + C) can be the maternal parent of *D. pacifica* (γ) and *D. sacrosancta* because plastid genes in ferns are well known as being inherited only from the maternal parent (Gastony & Yatskievych 1992). Therefore, apogamous species can also be maternal parents and involve reticulate evolution in the apogamous fern complex.

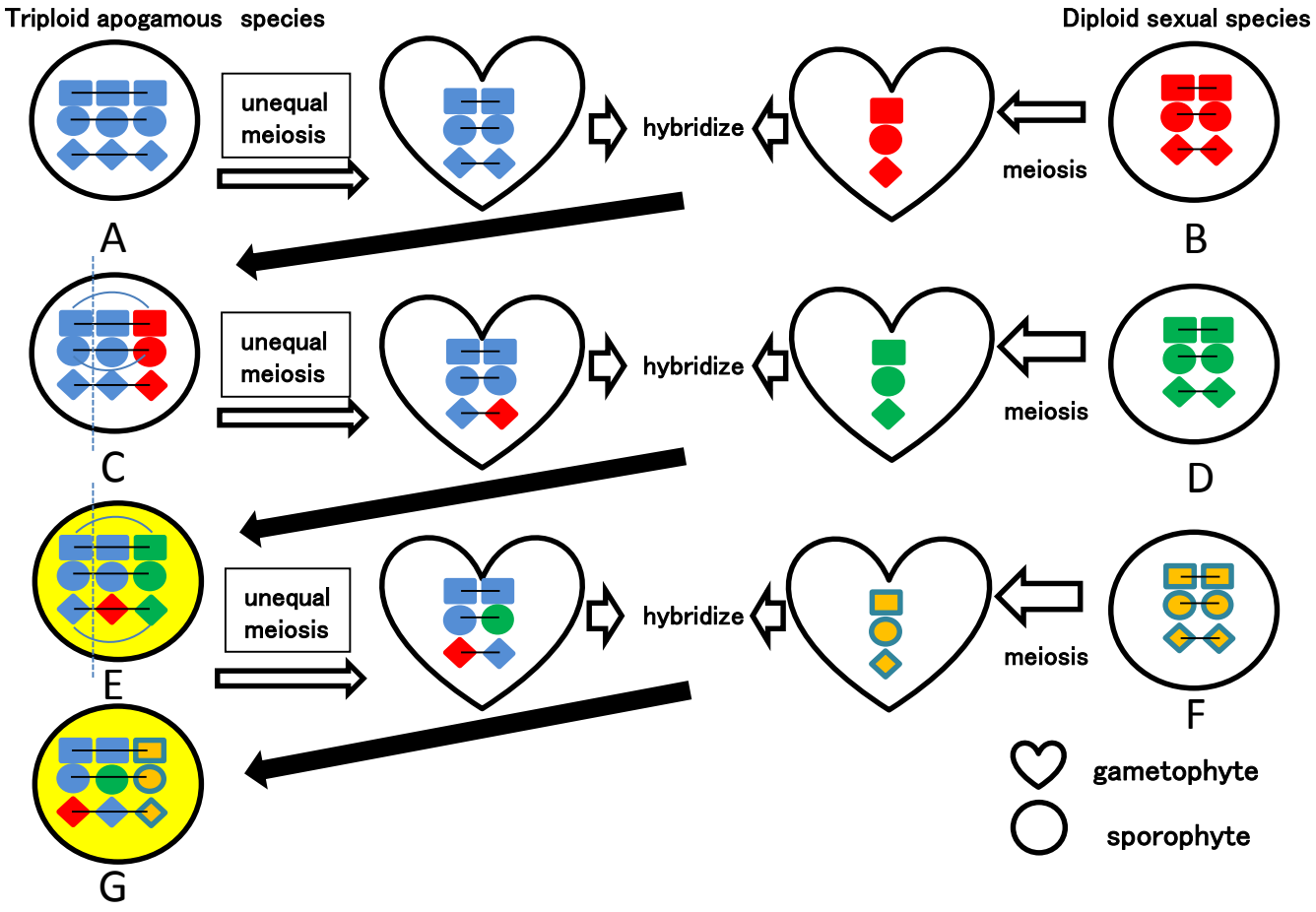

Figure 2-1. The genome constitutions of three kind of chromosomes during repeated hybridization cycles of apogamous species. Each genome consists of three kind of chromosomes (square, circle and diamond). See details in text (p. 40-42.)

Gene	PCR primers (5' - 3')		Homologs and their source plant species		
	Forward	Reverse	*Arabidopsis thaliana*	*Dryopteris saxifraga*	*Polystichun acrostichoides*
AK1 (Adenylate kinase 1) gene	AK4F (GATGAAGCCATCAAGAAACCA)	AKR2 (ATGGATCCAGCGACCAGTAA)	AT5G63400.2	-:-	scaffold-FQGQ-2073286
Esterase (Esterase/Lipase/Thioesterase family protein) gene	EST-F (GGCTGGAGCAGTCTCTCTGT)	EST-R (GCACTAGCAGCTTTCGGAAT)	AT3G50790.1	This study	scaffold-FQGQ-2010471
GapCp - short (glyceraldehyde-3-phosphate dehydrogenase) gene	132F (GTGCTTCCGGAGTTAAATGG)	488R (CAACATCATCTTCGGTGTATCC)	-:-	-:-	-:-
G6pdh (Glucose-6-phosphate dehydrogenase) gene	G6F (TTTGGTGGCTATGGAGAAGC)	G6R (CGAATGTTGGGGTATTGGAG)	AT5G40760.1	This study	scaffold-FQGQ-2071416

Figure 2-2. The list of nuclear markers newly developed in this study and homologs used to design these new PCR primers.

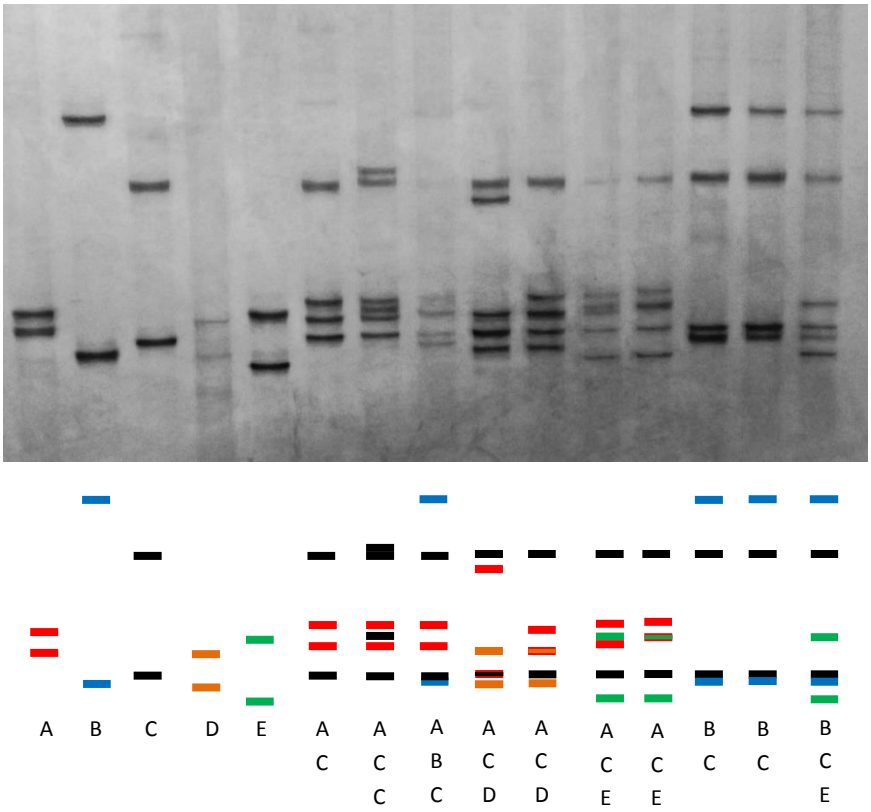

Figure 2-3. The SSCP band patterns of *G6pdh* gene. Electrophoretic band patterns on MDE gel under 2% glycerol at 15° C are shown in the above. The colored bands in below indicate the allele of red (A, *D. varia*), blue (B, *D. saxifraga*), black (C, *D. protobissetiana*), brown (D, *D. caudipinna*) and green (E, *D. chinensis*), each from the diploid sexual species of the *Dryopteris varia* complex.

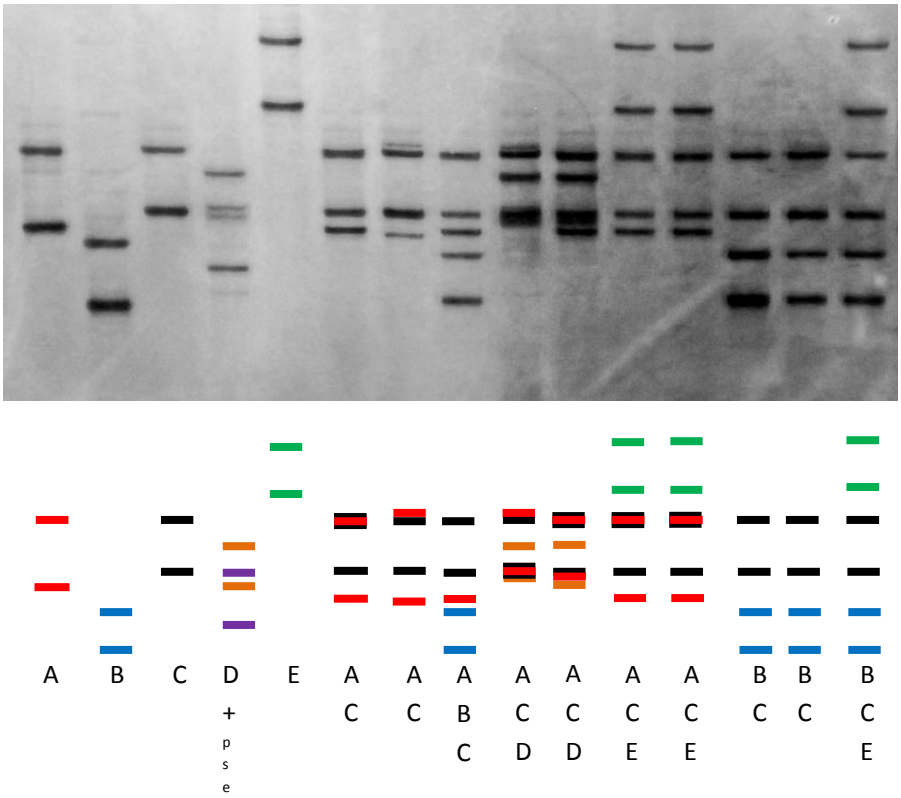

Figure 2-4. The SSCP band patterns of *GapCp* gene. Electrophoretic patterns on MDE gel solution under 2% glycerol at 15˚ C. The colored bands indicate the allele of red (A, *D. varia*), blue (B, *D. saxifraga*), black (C, *D. protobissetiana*), brown (D, *D. caudipinna*), green (E, *D. chinensis*) and purple (pseudo allele amplyfied by PCR), each from the diploid sexual species except purple ones .

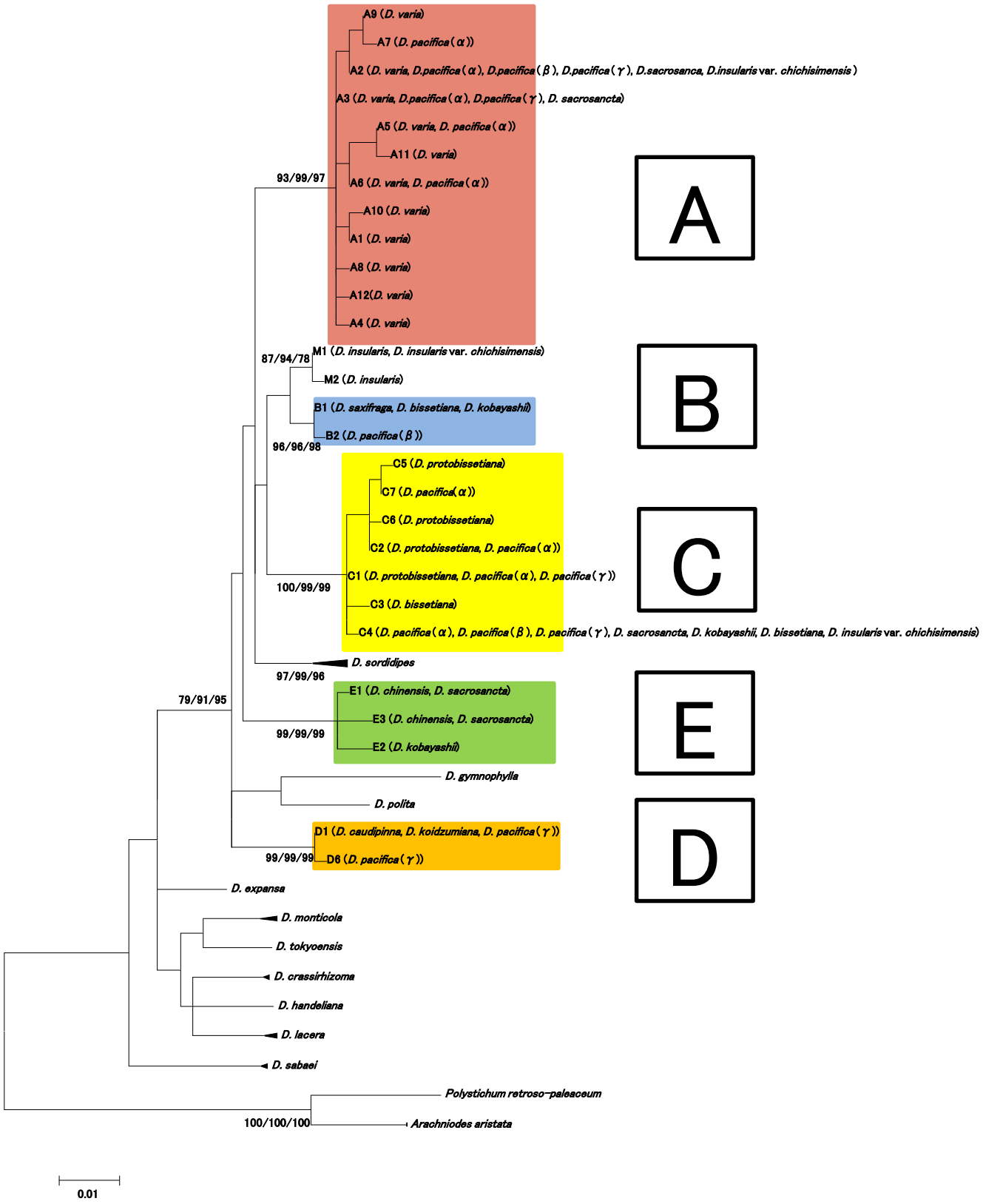

Figure 2-5. The ML tree (highest log likelihood = −2422.5814) based on the sequence variation of the nuclear gene *AK1* with BPs (>70) of NJ/MP/ML analyses on each branch. Square A, B, C, D and E indicate the clades of *Dryopteris varia*, *D. saxifraga*, *D. protobissetiana*, *D. caudipinna* and *D. chinensis*, respectively.

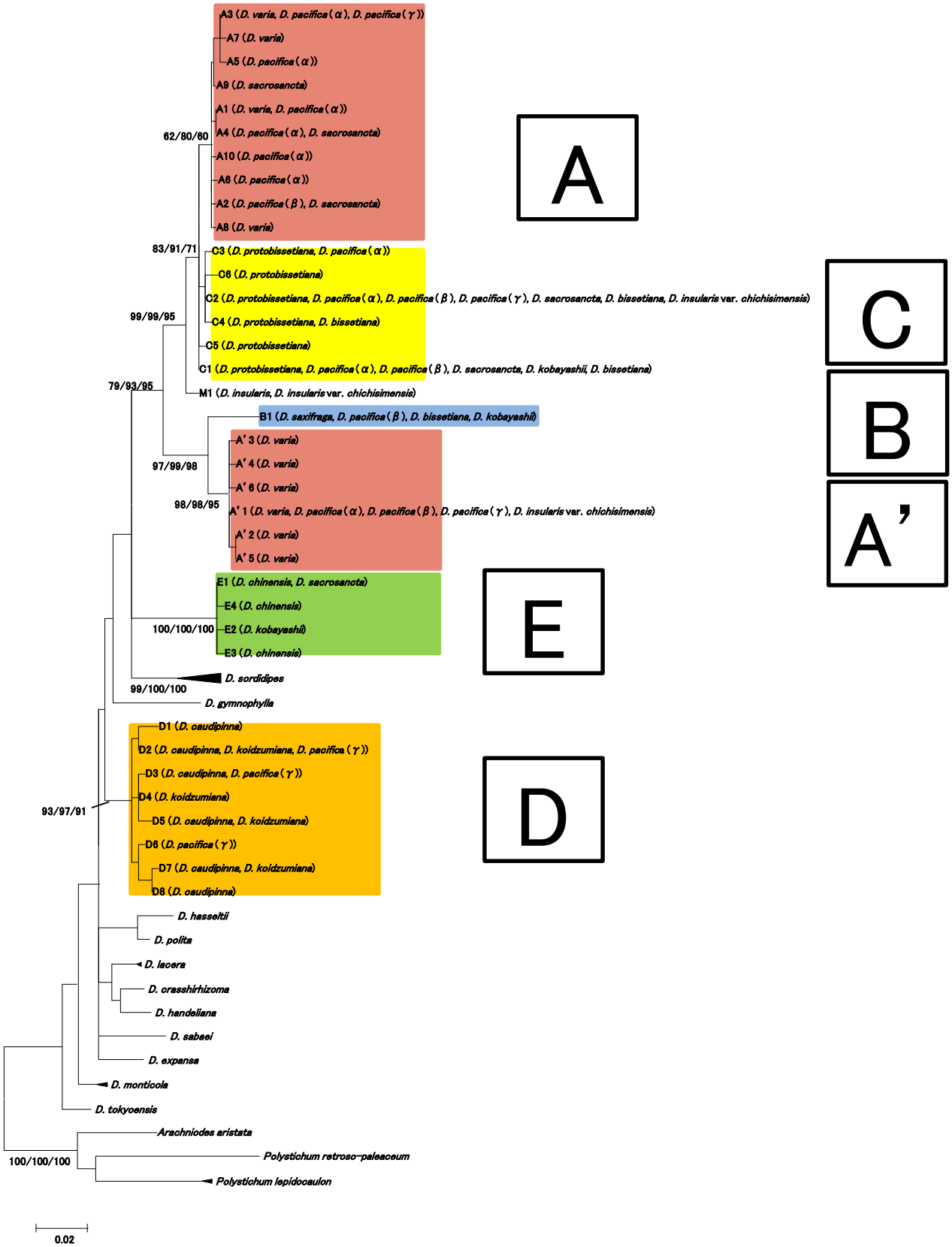

Figure 2-6. The ML tree (highest log likelihood = −2552.5997) based on the sequence variation of the nuclear gene *Esterase* with BPs (>70) of NJ/MP/ML analyses on each branch. Square A, A', B, C, D and E indicate the clades of *Dryopteris varia*, *D. saxifraga*, *D. protobissetiana*, *D. caudipinna* and *D. chinensis*, respectively.

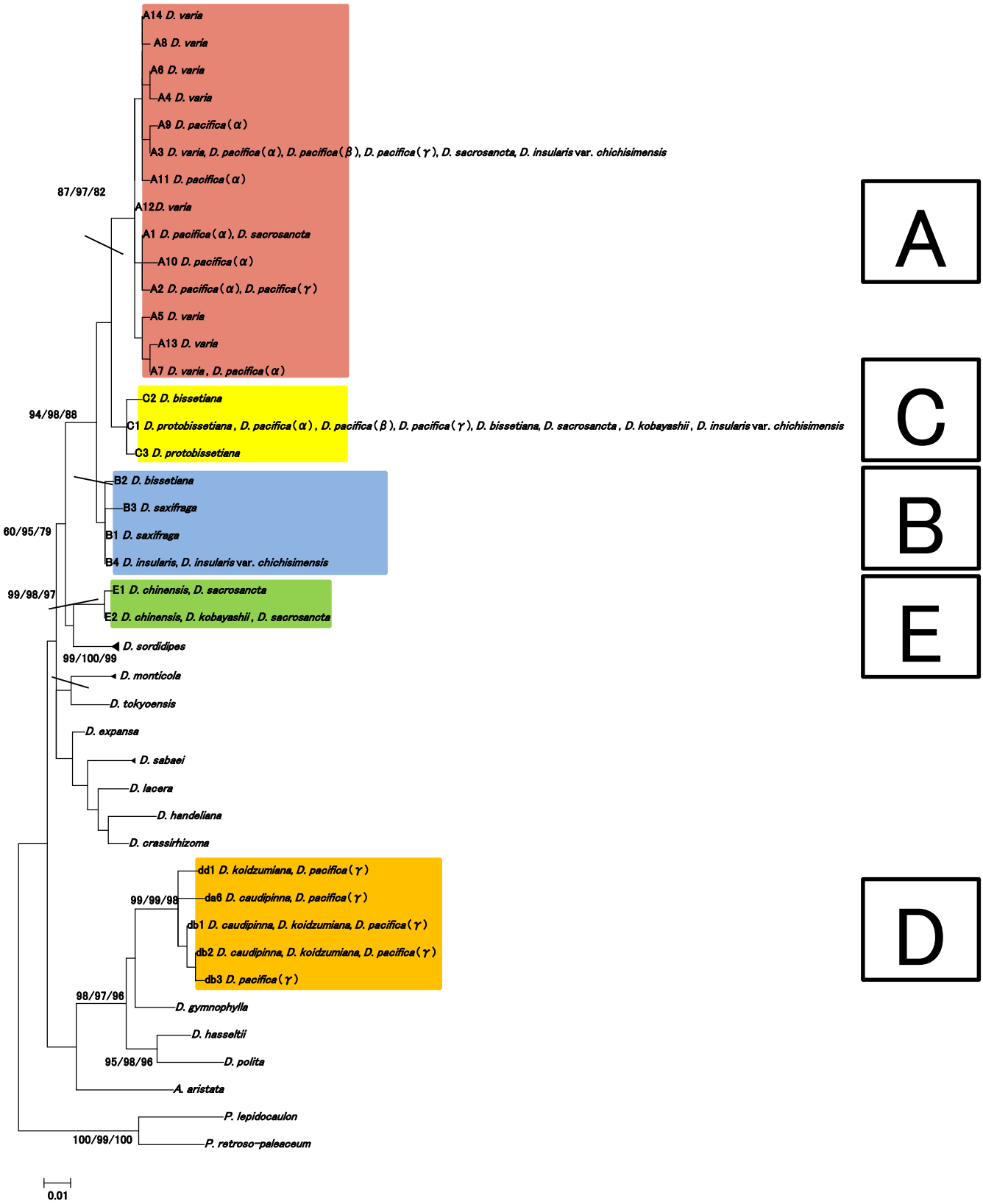

Figure 2-7. The ML tree (highest log likelihood = −1659.5745) based on the sequence variation of the nuclear gene *GapCp* with BPs (>70) of NJ/MP/ML analyses on each branch. Square A, B, C, D and E indicate the clades of *Dryopteris varia*, *D. saxifraga*, *D. protobissetiana*, *D. caudipinna* and *D. chinensis*, respectively.

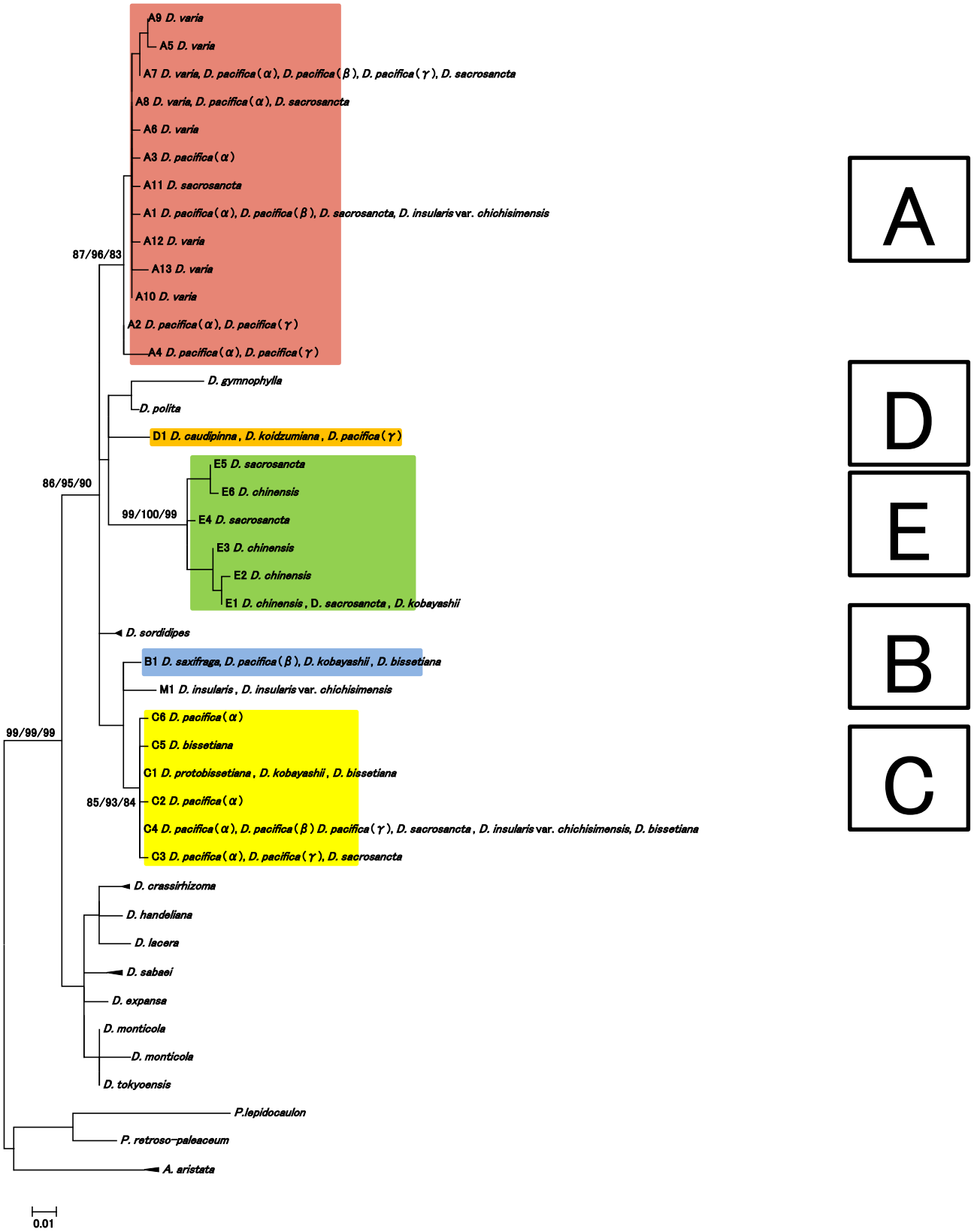

Figure 2-8. The ML tree (highest log likelihood = −1515.4124) based on the sequence variation of the nuclear gene *G6pdh* with BPs (>70) of NJ/MP/ML analyses on each branch. Square A, B, C, D and E indicate the clades of *Dryopteris varia*, *D. saxifraga*, *D. protobissetiana*, *D. caudipinna* and *D. chinensis*, respectively.

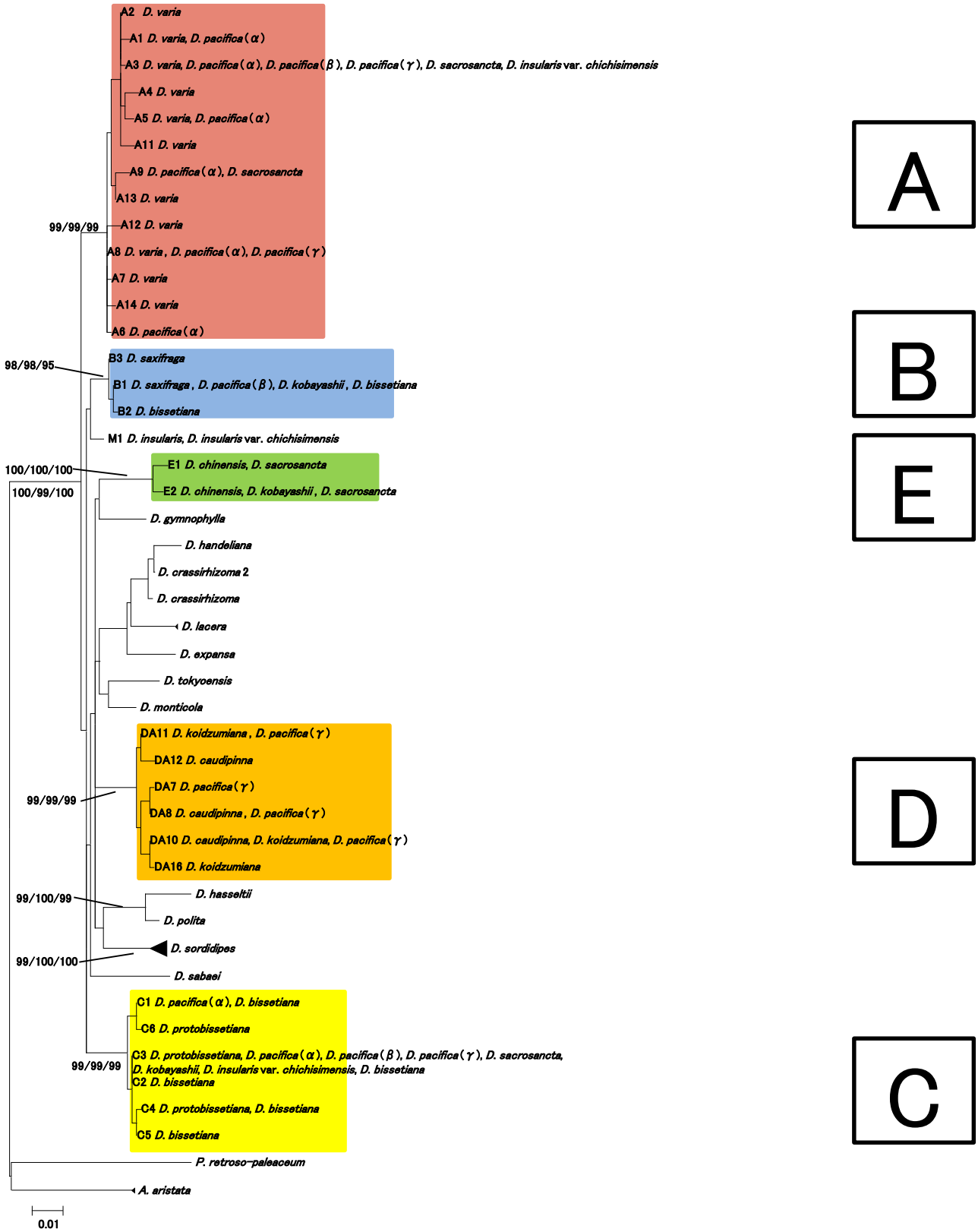

Figure 2-9. The ML tree (highest log likelihood = −2850.2708) based on the sequence variation of the nuclear gene *PgiC* with BPs (>70) of NJ/MP/ML analyses on each branch. Square A, B, C, D and E indicate the clades of *Dryopteris varia*, *D. saxifraga*, *D. protobissetiana*, *D. caudipinna* and *D. chinensis*, respectively.

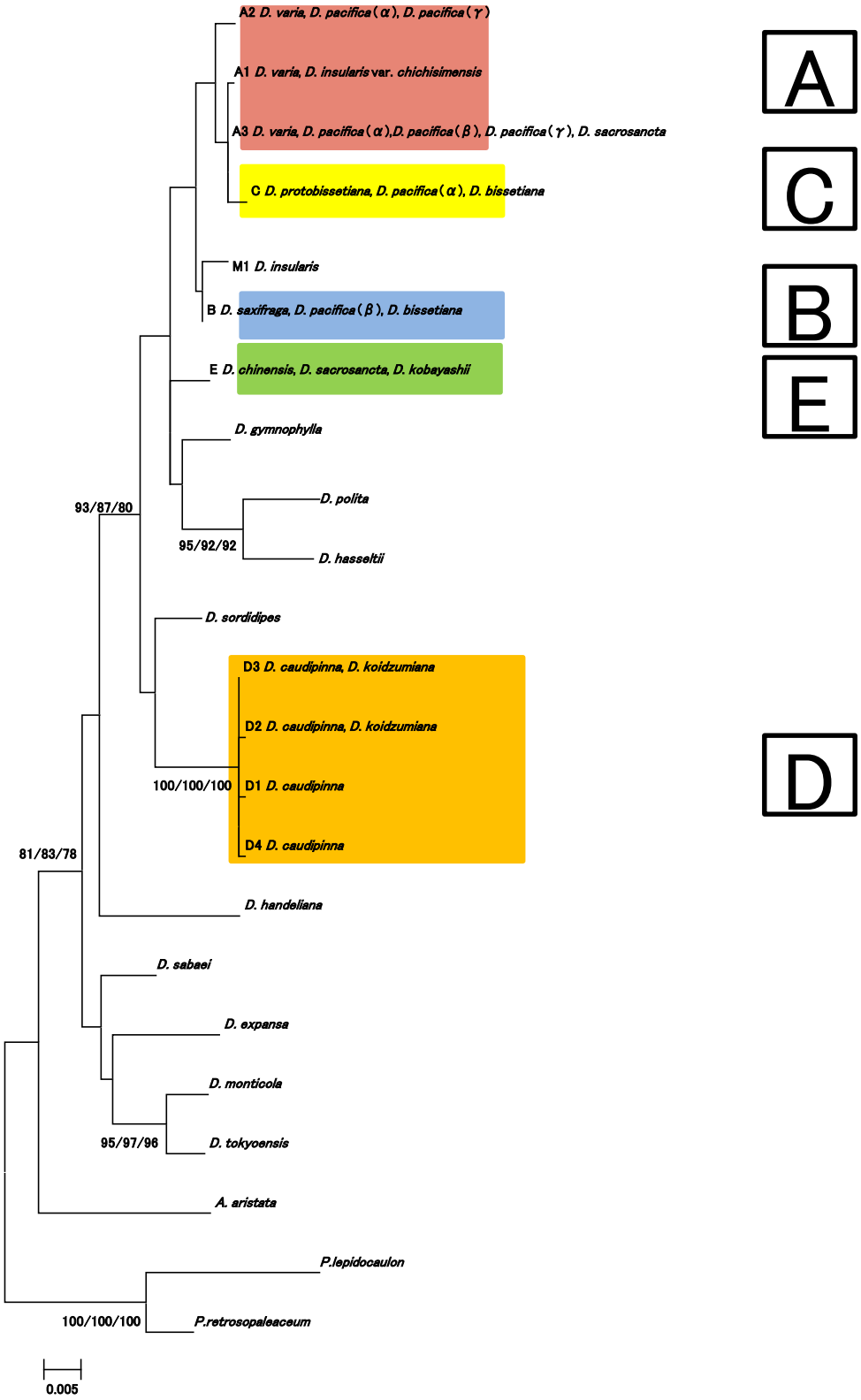

Figure 2-10. The ML tree (highest log likelihood = −3118.1608) based on the sequence variation of the nuclear gene *rbcL* with BPs (>70) of NJ/MP/ML analyses on each branch. Square A, B, C, D and E indicate the clades of *Dryopteris varia*, *D. saxifraga*, *D. protobissetiana*, *D. caudipinna* and *D. chinensis*, respectively.

	AK1	*EST*	*GapCp*	*G6pdh*	*PgiC*	*N*
D. varia	A	A	A	A	A	24
D. saxifraga	B	B	B	B	B	18
D. protobissetiana	C	C	C	C	C	10
D. caudipinna	D	D	D	D	D	9
D. chinensis	E	E	E	E	E	10
D. bissetiana	B C C B / C	B C C B / C	B / C	B C C B / C	B C C B / C	56
D. pacifica (α)	A A C A C C A C A / C	A A C A C C A C A / C	A A C A C	A A C A C C A C A / C	A A C A C C A C A / C	94
D. pacifica (β)	A B C	A B C	A B C	A B C	A B C	12
D. pacifica (γ)	A C D	A C D	A C D	A C D	A C D	38
D. sacrosancta	A C E	A C E	A C E	A C E	A C E	47
D. kobayashii	B C E	B C E	B C E	B C E	B C E	14

Figure 2-11. The geome constituiton of each species of the *Dryopteris varia* complex estimated Genome constitutions "AAC" or "ACC," of apogamous triploids are shown as A/C to simplify. A, B, C, D, E indicate the allele of each diploid sexual species.

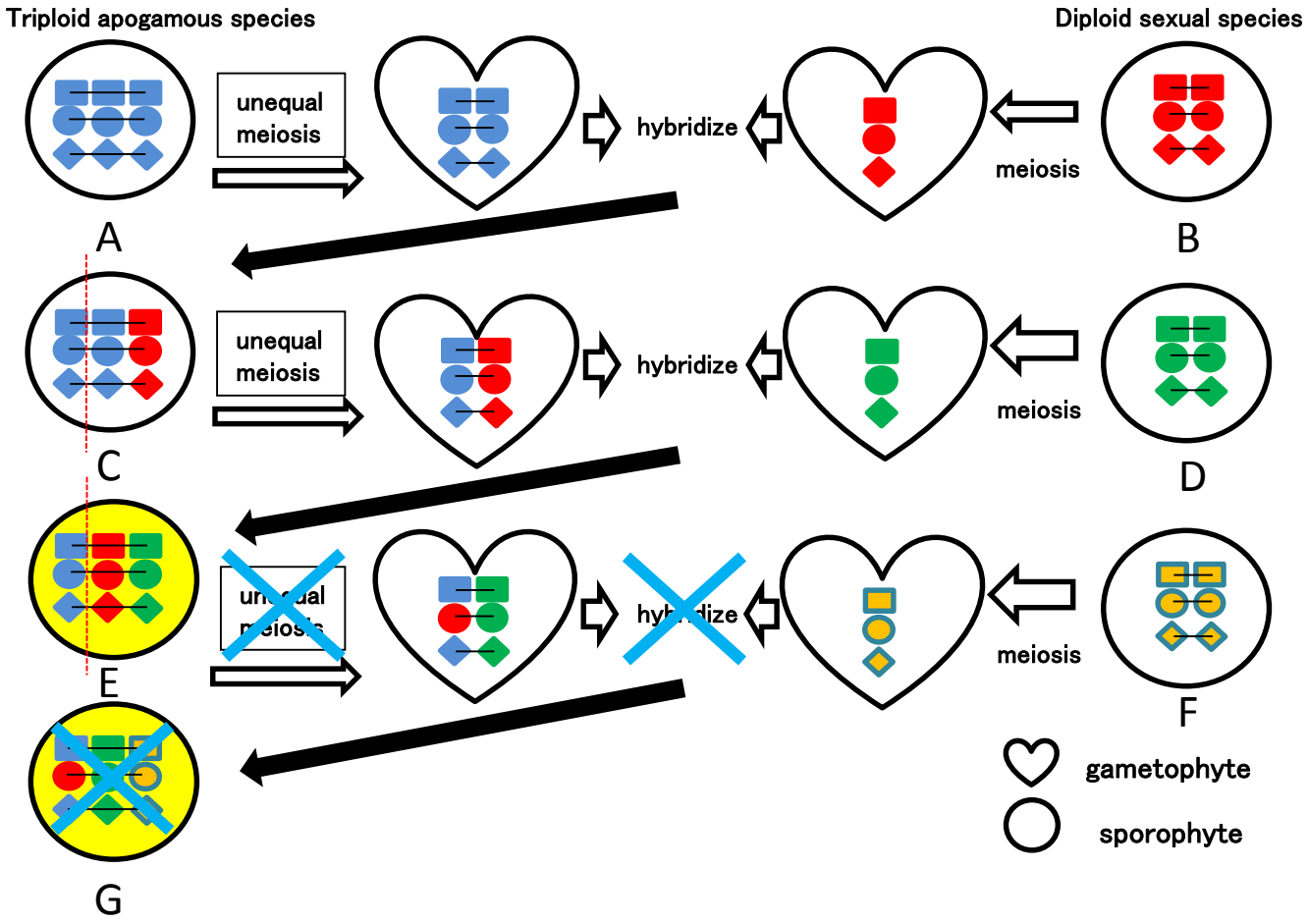

Figure 2-12. The genome constitutions of three kind of chromosomes during hybridization cycles in the case that chromosome recombinations do not occur. This figure shows the case of *x*= 3. Each genome consists of three kind of chromosomes (square, circle and diamond). See details in text (p. 52-53.)

Appendix2-1
Voucher specimens examined in this study. Any genotypes that were identified by sequencing are in boldface. Otherwise, they the genotypes were deduced from comparisons of band positions in SSCP gels. When ploidy is unknown, the genotype is in brackets. For samples with unknown genome dosage, the unidentified genomes are marked by asterisks.

Coll.	MAK	TNS	TAIF	Locality	no.	reproductive mode	chromosome numbers	DNA amount (p.g.)	ploidy	*rbcL*	*AK1*	*EST*	*GapCp*	*G6PDH*	*PgiC*
Hori	405228			Japan, Shizuoka pref., Kamo county	*D. varia* 1					A3	**A3A6A8**	**A1A'1A'2**	**A4***	**A6/A7**	A7*
Hori	405233			Japan, Shizuoka pref., Kamo county	*D. varia* 2					A3	A3A6A8	A1A'1A'2	A4*	**A6/A7**	**A7***
Hori	405245			Japan, Wakayama pref.,Higashimuro county, Taiji town	*D. varia* 3					A3	**A5***	**A1/A'1**	**A5A6A8**	**A6***	**A2A4A7**
Hori	405281			Japan, Mie pref., Minamimuro county, Kihou town, Ida	*D. varia* 4					A3	**A10***	**A7/A'2**	A4*	**A7***	A7*
Hori	405316			Japan, Kouchi pref., Tosashimizu city	*D. varia* 5					A3	**A11***	**A8A'3A'4**	A4*	**A5***	**A2***
Hori	406850			Japan, Hiroshima pref., Inno Is.	*D. varia* 6					A3	**A3/A6**	A1A'1A'2	**A4***	**A6/A7**	**A2/A5**
Hori	406848			Japan, Hiroshima pref., Inno Is.	*D. varia* 7					A3	A3/A6	**A1A'1A'2**	A4*	**A6/A7**	A2/A5
Hori	409211			Japan, Saga pref., Kashima city	*D. varia* 8	apo	2*n* =123	24.88	3*x*	A2	**A3***	**A3/A9**	A4*	**A2/A7**	**A3***
Hori	409195			Japan, Kagawa pref., Takamatsu city, Kinashi town	*D. varia* 9					A3	A3/A6	A1A'1A'2	A4*	**A2/A7**	**A2/A8**
Chun-Ming Chen		1181088		Taiwan, Taipei city	*D. varia* 10					A3	**A5/A9**	**A1/A'2**	A7A12	**A7***	**A3***
Chun-Ming Chen		1181085		Taiwan, Taipei city	*D. varia* 11	sex (Ebihara *et al.* 2014)	2*n* =82		2*x*	A1	**A1A1**	**A1A'1**	A4A4	**A7A7**	**A11A11**
Chun-Ming Chen		1181086		Taiwan, Taipei city	*D. varia* 12	apo (Ebihara *et al.* 2014)	2*n* =123		3*x*	A3	**A5/A9**	A1/A'2	**A7***	**A7A7A7**	A3*
Yih-Hann Chang		1181087		Taiwan, Taipei city	*D. varia* 13					A3	**A4/A10**	**A1/A'1**	A4*	**A7/A10**	**A1/A12**
Hori	409067			Japan, Kumamoto pref., Uki city	*D. varia* 14		2*n* =123		3*x*	A3	**A4/A10**	**A7/A'2**	A4*	A7/A8	**A13***
Hori	423474			Taiwan, Taipei city, Urai	*D. varia* 15	sex				A3	**A2/A3**	A'1A'1	A4A4	A8A8	A14A14
Hori	423476			Taiwan, Taipei city, Urai	*D. varia* 16	sex				A3	**A3/A12**	**A1A'1**	A4A4	A8A8	A14A14
Hori	423480			Taiwan, Taipei city, Urai	*D. varia* 17	sex				A3	**A6/A10**	**A'1A'1**	A4A4	A8A8	A14A14
Hori	423481			Taiwan, Taipei city, Urai	*D. varia* 18	sex				A3	A6/A10	**A'1A'2**	**A3A13**	A8A8	A14A14
Hori	423489			Taiwan, Taipei city, Urai	*D. varia* 19	sex				A3	A3A3	A'1A'1	**A4A4**	**A8A8**	A14A14
Hori	423492			Taiwan, Taipei city, Urai	*D. varia* 20	sex	2*n* =82		2*x*	A3	A3A3	**A1A'1**	A4A4	**A12A13**	**A14A14**
Hori	423494			Taiwan, Taipei city, Urai	*D. varia* 21	sex	2*n* =82		2*x*	A3	A3A3	**A'5A'6**	A4A4	A8A8	**A1A1**
Hori	423497			Taiwan, Taipei city, Shuide Industry Rd, Pinglin District	*D. varia* 22	sex	2*n* =82		2*x*	A3	**A3/A12**	A'1A'2	A4A4	**A9A9**	A14A14
Hori	423498			Taiwan, Taipei city, Shuide Industry Rd, Pinglin District	*D. varia* 23	sex	2*n* =82		2*x*	A1	A3A3	**A'1A'2**	A4A4	A8A8	A14A14
Hori	423499			Taiwan, Taipei city, Shuide Industry Rd, Pinglin District	*D. varia* 24	sex	2*n* =82		2*x*	A3	A3A3	A'1A'1	**A12A14**	A8A8	A14A14
Hori	405345			Japan, Tokyo-to, Nishitama county	*D . saxifraga* 1	sex				B	B1B1	B1B1	**B1B1**	**B1B1**	**B1B1**
Hori	405346			Japan, Tokyo-to, Nishitama county	*D . saxifraga* 2	sex				B	B1B1	B1B1	B1B1	**B1B1**	**B1B1**
Hori	405347			Japan, Tokyo-to, Nishitama county	*D . saxifraga* 3	sex				B	B1B1	B1B1	**B3B3**	**B1B1**	B1B1
Hori	405348			Japan, Tokyo-to, Nishitama county	*D . saxifraga* 4	sex				B	B1B1	B1B1	B1B1	**B1B1**	B1B1
Hori	405349			Japan, Tokyo-to, Nishitama county	*D . saxifraga* 5	sex				B	B1B1	B1B1	B1B1	**B1B1**	B1B1
Hori	411382			Japan, Tokyo-to, Akiruno city	*D . saxifraga* 6	sex				B	**B1B1**	**B1B1**	B3B3	**B1B1**	B1B1
Hori	411383			Japan, Tokyo-to, Akiruno city	*D . saxifraga* 7	sex				B	B1B1	B1B1	B3B3	**B1B1**	B1B1
Hori	411385			Japan, Tokyo-to, Akiruno city	*D . saxifraga* 8	sex				B	B1B1	B1B1	B1B1	**B1B1**	B1B1
Hori	411386			Japan, Tokyo-to, Akiruno city	*D . saxifraga* 9	sex				B	B1B1	B1B1	B1B1	**B1B1**	B1B1
Hori	411387			Japan, Tokyo-to, Akiruno city	*D . saxifraga* 10	sex				B	B1B1	B1B1	B1B1	**B1B1**	B1B1
Hori	411406			Japan, Aichi pref., Kitashitara county	*D . saxifraga* 11	sex				B	B1B1	B1B1	B1B1	**B1B1**	B1B1
Hori	423578			Japan, Akita pref., Sennboku city, Dakigaeri velley	*D . saxifraga* 12	sex		20.07	2*x*	B	B1B1	B1B1	B1B1	B1B1	B1B1
Hori	423579			Japan, Akita pref., Sennboku city, Dakigaeri velley	*D . saxifraga* 13	sex		20.49	2*x*	B	B1B1	B1B1	B1B1	B1B1	B1B1
Hori	423584			Japan, Akita pref., Sennboku city, Dakigaeri velley	*D . saxifraga* 14	sex		21.35	2*x*	B	B1B1	B1B1	B3B3	B1B1	B1B1
Hori	423586			Japan, Akita pref., Sennboku city, Dakigaeri velley	*D . saxifraga* 15	sex	2*n* =82	22.04	2*x*	B	B1B1	B1B1	B1B1	B1B1	B1B1
Hori	423587			Japan, Akita pref., Sennboku city, Dakigaeri velley	*D . saxifraga* 16	sex		21.75	2*x*	B	B1B1	B1B1	B1B1	B1B1	B1B1
Hori	423588			Japan, Akita pref., Sennboku city, Dakigaeri velley	*D . saxifraga* 17	sex		21.29	2*x*	B	B1B1	B1B1	B1B1	B1B1	B1B1
Hori	423590			Japan, Akita pref., Sennboku city, Dakigaeri velley	*D . saxifraga* 18	sex		20.91	2*x*	B	B1B1	B1B1	B1B1	B1B1	B1B1
Hori	410906			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 1	sex	2*n* =82	14.63	2*x*	C	C1C1	**C3C3**	**C1C3**	**C1C1**	**C4C4**
Hori	410907			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 2	sex		15.61	2*x*	C	**C1C1**	**C2C2**	C1C1	**C1C1**	**C3C3**
Hori	410913			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 3	sex		16.58	2*x*	C	**C2C5**	C2C2	C1C1	**C1C1**	**C3C3**
Hori	410914			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 4	sex		15.05	2*x*	C	C1C1	C2C2	C1C1	**C1C1**	**C3C3**
Hori	410915			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 5	sex				C	C1*	C2*	C1*	**C1***	**C3***
Hori	410916			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 6	sex		15.22	2*x*	C	**C2C2**	C2C2	C1C1	C1C1	**C3C3**
Hori	410917			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 7	sex		16.10	2*x*	C	**C1C6**	**C4C6**	**C1C1**	**C1C1**	C4C4
Hori	425899			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 8	sex				C	C1C6	**C1C4**	C1C1	**C1C1**	C3C3
Hori	417179			Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 9	sex				C	C1C6	C2C2	C1C1	**C1C1**	**C6C6**
Hori		763335		Japan, Kagoshima pref., Yakushima Is.	*D . protobissetiana* 10	sex				C	C1C6	C2*	C1*	**C1***	**C3***
Hori	405131			Japan, Tokyo-to, Inagi city	*D . chinensis* 1					E	**E1/E3**	E1*	E2*	**E1/E3**	**E1/E2**
Hori	405220			Japan, Tokyo-to, Hachioji city, Naganuma	*D . chinensis* 2					E	E1/E3	E1*	**E2***	**E1/E3**	**E1/E2**
Hori	405262			Japan, Wakayama pref., Higashimuro county, Kozagawa town. Ichimai-iwa	*D . chinensis* 3					E	E1/E3	E1*	E2*	**E1***	**E1/E2**
Hori	410320			Japan, Miyazaki pref., Nobeoka city, Kitakata town, Shimoshishigawa	*D . chinensis* 4	sex		13.01	2*x*	E	E1E1	E1E1	E2E2	**E1E1**	E2E2
Hori	410321			Japan, Miyazaki pref., Nishiusuki county, Hinokage town	*D . chinensis* 5	sex		13.86	2*x*	E	E1E1	E1E1	E2E2	**E1E1**	E2E2
Hori	410342			Japan, Miyazaki pref., Nobeoka city, Houri river, 100m	*D . chinensis* 6	sex		14.51	2*x*	E	E1E1	E1E1	E2E2	**E1E1**	E2E2
Hori	410350			Japan, Miyazaki pref., Nobeoka city, Kitakata town, Sugawara	*D . chinensis* 7	sex		13.57	2*x*	E	E1E1	E1E1	**E1E1**	**E1E6**	E1E2
Hori	410354			Japan, Miyazaki pref., Nobeoka city, Kitagawa town, Kawauchimvo	*D . chinensis* 8	sex		13.23	2*x*	E	E1E1	**E1E1**	E1E1	**E1E2**	E2E2
Hori	410355			Japan, Miyazaki pref., Nobeoka city, Kitagawa town, Kawauchimvo	*D . chinensis* 9	sex	2*n* =82	13.23	2*x*	E	**E1E1**	**E3E4**	E1E1	**E1E1**	E2E2
Hori	410356			Japan, Miyazaki pref., Hyuga city, Togo town, Shimosannge	*D . chinensis* 10	sex		13.57	2*x*	E	E1E1	E1E1	E1E1	**E1E1**	E2E2
M. Matsumoto	411423			Japan, Hyogo pref., Himeji city	*D . caudipinna* 1	sex				D1	D1D1	**D1D1**	**db1da6**	**D1D1**	**DA10DA10**
M. Matsumoto	411424			Japan, Hyogo pref., Himeji city	*D . caudipinna 2*	sex				D1	D1D1	**D3D7**	**db2da6**	**D1D1**	DA10DA10
M. Matsumoto	411425			Japan, Hyogo pref., Himeji city	*D . caudipinna* 3	sex				D2	D1D1	D5D5	db2da6	**D1D1**	DA10DA10
K. Yamamoto		1190554		Japan, Kanagawa pref., Zushi city, Jinnmuji	*D . caudipinna* 4	sex				D3	D1D1	**D3D8**	**db1db1**	**D1D1**	**DA8DA8**
M. Matsumoto	411078			Japan, Kagoshima pref., Yakushima Is.	*D . caudipinna* 5	sex				D4	D1D1	**D7D2**	db1db1	D1D1	**DA12DA12**
M. Matsumoto	413260			Japan, Kagoshima pref., Yakushima Is.	*D. koidzumiana* 1	sex		17.15	2*x*	D3	D1D1	D7D2	**dd1dd1**	**D1D1**	**DA16DA11**
M. Matsumoto	413262			Japan, Kagoshima pref., Yakushima Is.	*D. koidzumiana* 2	sex				D2	**D1D1**	**D5D5**	**db1dd1**	**D1D1**	**DA10DA11**
M. Matsumoto	413268			Japan, Kagoshima pref., Yakushima Is.	*D. koidzumiana* 3	sex				D3	D1D1	**D7D4**	**db2dd1**	**D1D1**	**DA10DA10**
M. Matsumoto	413349			Japan, Kagoshima pref., Yakushima Is.	*D. koidzumiana* 4	sex				D3	D1D1	D7D2	db1db1	**D1D1**	**DA11DA11**
Hori	405138			Japan, Kanagawa pref., Miura city	*D . pacifica* (α) 1					A3	**A2C2C4**	**A'1C1C2**	A1/C1	**A3C2C3**	**A9/C3**
Hori	405140			Japan, Kanagawa pref., Miura city	*D . pacifica* (α) 2					A3	**A2C1C4**	**A1C1C2**	A3/C1	A2/C4	**A3/C3**
Hori	405143			Japan, Kanagawa pref., Miura city	*D . pacifica* (α) 3					C	**A5C2C4**	**A'1/C2**	A3/C1	A7/C4	A3/C3
Hori	405146			Japan, Kanagawa pref., Miura city	*D . pacifica* (α) 4					A3	**A2/C4**	A'1/C2	A3/C1	A7/C4	**A3/C3**
Hori	405147			Japan, Kanagawa pref., Kamakura city	*D . pacifica* (α) 5					C	A2C2C4	A'1C1C2	**A1/C1**	A8C3C6	A9/C3
Hori	405154			Japan, Kanagawa pref., Zushi city, Mt. Futago	*D . pacifica* (α) 6					C	A5C2C4	A'1/C2	A3/C1	A7/C4	**A3/C3**
Hori	405157			Japan, Kanagawa pref., Zushi city, Mt. Futago	*D . pacifica* (α) 7					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	**A3/C3**
Hori	405158			Japan, Kanagawa pref., Zushi city, Mt. Futago	*D . pacifica* (α) 8					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	**A9/C3**
Hori	405168			Japan, Chiba pref., Sakura city	*D . pacifica* (α) 9					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	**A9/C3**
Hori	405174			Japan, Chiba pref., Sakura city	*D . pacifica* (α) 10					A3	A2/C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	405175			Japan, Chiba pref., Sakura city	*D . pacifica* (α) 11					A2	**A3/C4**	**A3/C2**	**A2/C1**	A4/C4	**A8/C3**
Hori	405176			Japan, Chiba pref., Sakura city	*D . pacifica* (α) 12					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	**A3/C3**
Hori	405179			Japan, Tokyo-to, Inagi city	*D . pacifica* (α) 13					A3	**A3/C1**	**A4C1C3**	A1/C1	**A2C3C4**	**A9/C3**
Hori	405192			Japan, Saitama pref., Hannno city	*D . pacifica* (α) 14					C	A2C2C4	A'1C1C2	A1/C1	A8C3C6	A9/C3
Hori	405205			Japan, Chiba pref., Minamiboso city	*D . pacifica* (α) 15					A3	**A3/C1**	A4C1C3	**A3/C1**	A7/C4	**A3/C3**
Hori	405223			Japan, Shizuoka pref., Kamo county	*D . pacifica* (α) 16					C	A2C2C4	A'1C1C2	**A1/C1**	**A8C3C6**	**A9/C3**
Hori	405236			Japan, Shizuoka pref., Kamo county	*D . pacifica* (α) 17					A2	A3/C4	A3/C2	A2/C1	A4/C4	**A8/C3**
Hori	405240			Japan, Shizuoka pref., Kamo county	*D . pacifica* (α) 18					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	**A3/C3**
Hori	405252			Japan, Wakayama pref., Higashimuro county, Kozagawa town, Aise	*D . pacifica* (α) 19					A3	A2/C4	**A'1/C2**	A3/C1	A7/C4	**A3/C3**
Hori	405254			Japan, Wakayama pref., Higashimuro county, Kozagawa town. Aise	*D . pacifica* (α) 20					A3	A3/C1	**A'1C1C5**	A1/C1	**A3C2C3**	**A9/C3**
Hori	405255			Japan, Wakayama pref., Higashimuro county, Kushimoto town	*D . pacifica* (α) 21					A3	A2C1C4	A1C1C2	**A3/C1**	A2/C4	**A3/C3**
Hori	405269			Japan, Wakayama pref., Higashimuro county, Taiji town	*D . pacifica* (α) 22					A3	A2A6C4	**A5A'1C2**	**A9A10C1**	**A1A2C4**	A3A6C3
Hori	405272			Japan, Wakayama pref., Higashimuro county, Taiji town	*D . pacifica* (α) 23					A3	A2/C4	**A10/C2**	A1/C1	A2/C4	**A1A3C3**
Hori	405280			Japan, Mie pref., Minamimuro county, Kihou town, Ida	*D . pacifica* (α) 24					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	**A9/C3**
Hori	405292			Japan, Mie pref., Kumano city, Hobo town	*D . pacifica* (α) 25					A3	**A2A6C4**	A5A'1C2	**A3A10C1**	**A1A2C4**	**A3A6C3**
Hori	405297			Japan, Mie pref., Kumano city, Hobo town	*D . pacifica* (α) 26					A2	A3/C4	A3/C2	**A2/C1**	A4/C4	**A8/C3**
Hori	405308			Japan, Mie pref., Owase city, Obarano	*D . pacifica* (α) 27					A3	A2/C4	**A'1/C2**	A3/C1	**A7/C4**	**A3/C3**
Hori	406853			Japan, Kanagawa pref., Naka county	*D . pacifica* (α) 28					A3	**A3/C4**	A4/C2	A3/C1	A2/C4	**A3/C3**
Hori	406857			Japan, Tokyo-to, Inagi city	*D . pacifica* (α) 29					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	406859			Japan, Tokyo-to, Hachioji city, Minamiosawa	*D . pacifica* (α) 30					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	406860			Japan, Tokyo-to, Inagi city	*D . pacifica* (α) 31					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	406862			Japan, Saitama pref., Maibara city	*D . pacifica* (α) 32					A3	A2C1C4	A1C1C2	A3/C1	**A2/C4**	A3/C3
Hori	406867			Japan, Kagawa pref., Takamatsu city, Yashima	*D . pacifica* (α) 33					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	406871			Japan, Kagawa pref., Takamatsu city, Yashima	*D . pacifica* (α) 34					A3	A2C2C4	A'1C1C2	**A1/C1**	A3C2C3	A9/C3
Hori	406873			Japan, Shimane pref., Oki county	*D . pacifica* (α) 35					A3	**A2C4C7**	A1C1C2	**A3/C1**	A2/C4	A3/C3
Hori	406874			Japan, Shimane pref., Oki county	*D . pacifica* (α) 36					C	A2C2C4	A'1C1	A1/C1	A3C2C3	A9/C3
Hori	406876			Japan, Osaka-fu, Sennann county	*D . pacifica* (α) 37	apo	2*n* =123			A3	A2A6C4	**A6A'1C2**	**A3A11C1**	**A1A7C4**	**A3/C3**
Hori	406879			Japan, Shimane pref., Matsue city, Nishikawazu town	*D . pacifica* (α) 38					C	A5C2C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406880			Japan, Shimane pref., Matsue city, Shimane town	*D . pacifica* (α) 39					A3	A2C4C7	A1C1C2	**A3/C1**	A2/C4	A3/C3
Hori	406979			Japan, Shizuoka pref., Haibara county	*D . pacifica* (α) 40					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	406980			Japan, Shizuoka pref., Haibara county	*D . pacifica* (α) 41					A2	A3/C4	A3/C2	**A2A3C1**	A4/C4	**A3/C3**
Hori	406881			Japan, Hiroshima pref., Hatsukaichi city, Miyajima	*D . pacifica* (α) 42					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	406885			Japan, Hiroshima pref., Hatsukaichi city, Miyajima	*D . pacifica* (α) 43					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	406887			Japan, Hiroshima pref., Takehara city	*D . pacifica* (α) 44					A3	A2C2C4	A'1/C2	A1/C1	A3C2C3	A9/C3
Hori	406888			Japan, Hiroshima pref., Takehara city	*D . pacifica* (α) 45					A3	A2/C4	A'1/C2	**A3/C1**	A7/C4	A3/C3
Hori	406897			Japan, Hiroshima pref., Inno Is., Mukunoura town	*D . pacifica* (α) 46					A3	**A3A7C4**	A'1/C2	A3/C1	**A8/C3**	A3/C3
Hori	406900			Japan, Hiroshima pref., Inno Is., Sannnosho town	*D . pacifica* (α) 47					A3	A2/C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406849			Japan, Hiroshima pref., Inno Is., Sannnosho town	*D . pacifica* (α) 48					A3	**A3/C4**	A4A'1C1	A3/C1	**A2A7C3**	**A2A5C3**
Hori	406905			Japan, Hiroshima pref., Inno Is., Kagamiura	*D . pacifica* (α) 49					A3	A2C2C4	A'1C1C2	**A1/C1**	A3C2C3	A9/C3
Hori	406909			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Asa town	*D . pacifica* (α) 50					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	406910			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Asa town	*D . pacifica* (α) 51					A3	A2/C4	**A'1/C2**	A3/C1	A7/C4	A3/C3
Hori	406911			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Asa town	*D . pacifica* (α) 52					C	A3/C1	A'1C1	A1/C1	A3C2C3	A9/C3
Hori	406912			Japan, Hiroshima pref., Hiroshima city, Asaminami-ku, Numata town	*D . pacifica* (α) 53					A3	A2/C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406914			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Kabe town	*D . pacifica* (α) 54					A3	A2/C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406917			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Kabe town	*D . pacifica* (α) 55					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	406918			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D . pacifica* (α) 56					A3	A3/C4	**A4A'1C1**	A3/C1	**A2A7C3**	**A3A5C3**
Hori	406919			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D . pacifica* (α) 57					C	A3/C1	**A'1/C1**	A1/C1	A3C2C3	A9/C3
Hori	406923			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D . pacifica* (α) 58					A3	A2/C4	**A'1/C2**	A3/C1	A7/C4	A3/C3
Hori	406928			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D . pacifica* (α) 59					C	**A5C2C4**	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406933			Japan, Tokushima pref., Anann city	*D . pacifica* (α) 60					A3	A2/C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406934			Japan, Tokushima pref., Komatsushima city	*D . pacifica* (α) 61					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	406936			Japan, Tokushima pref., Komatsushima city	*D . pacifica* (α) 62					C	A5C2C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406938			Japan, Tokushima pref., Itano county	*D . pacifica* (α) 63					A3	**A3/C4**	**A4/C2**	A3/C1	A2/C4	A3/C3
Hori	406948			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D . pacifica* (α) 64					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	406949			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D . pacifica* (α) 65					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	406956			Japan, Hiroshima pref., Higashihiroshima city	*D . pacifica* (α) 66					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	406957			Japan, Hiroshima pref., Higashihiroshima city	*D . pacifica* (α) 67					A3	A2/C4	A'1/C2	A3/C1	A7/C4	A3/C3

Appendix2-1
Voucher specimens examined in this study. Any genotypes that were identified by sequencing are in boldface. Otherwise, they the genotypes were deduced from comparisons of band positions in SSCP gels. When ploidy is unknown, the genotype is in brackets. For samples with unknown genome dosage, the unidentified genomes are marked by asterisks.

Coll.	MAK	TNS	TAIF	Locality	no.	reproductive mode	chromosome numbers	DNA amount (pg)	ploidy	*rbcL*	*AK1*	*EST*	*GapCp*	*G6PDH*	*PgiC*
Hori	406958			Japan, Okayama pref., Okayama city, Kita-ku, Ichinomiya	*D. pacifica* (α) 68					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	406966			Japan, Okayama pref., Tamano city	*D. pacifica* (α) 69					C	A5C2C4	A'1/C2	A3/C1	A7/C4	A3/C3
Hori	406970			Japan, Okayama pref., Tamano city	*D. pacifica* (α) 70					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	409222			Japan, Saga pref., Karatsu city, Kyuuragi town	*D. pacifica* (α) 71					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	A9/C3
Hori	409219			Japan, Saga pref., Imari city, Okawauchi town	*D. pacifica* (α) 72		2*n* =82	17.09	2*x*	A3	**A2/C4**	**A'1C1**	A3/C1	A7/C4	A3C3
Hori	409213			Japan, Saga pref., Karatsu city, Kyuuragi town	*D. pacifica* (α) 73			16.77	2*x*	A3	**A3/C4**	A'1/C2	A7/C1	**A8/C4**	A3/C3
Hori	409189			Japan, Kagawa pref., Takamatsu city	*D. pacifica* (α) 74		2*n* =123	24.48	3*x*	A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	**A9/C3**
Hori	409187			Japan, Kagawa pref., Takamatsu city	*D. pacifica* (α) 75			25.65	3*x*	A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	**A9/C3**
Hori	409186			Japan, Nagasaki pref., Higashisonogi county	*D. pacifica* (α) 76					A3	A2/C4	A'1/C2	**A3/C1**	A7/C4	**A3/C3**
Hori	409185			Japan, Kagawa pref., Ayauta county, Ayagawa town	*D. pacifica* (α) 77			25.22	3*x*	A3	A2C2C4	A'1C1C2	**A1/C1**	A3C2C3	A9/C3
Hori	409181			Japan, Kagawa pref., Ayauta county, Ayagawa town	*D. pacifica* (α) 78		2*n* =82	16.02	3*x*	A2	A3/C4	**A3C2**	**A2C1**	**A4C4**	A8C3
Hori	409176			Japan, Nara pref., Yoshino county	*D. pacifica* (α) 79					A3	A2C2C4	A'1C1C2	A1/C1	A3C2C3	**A9/C3**
Hori	409076			Japan, Miyazaki pref., Miyazaki city, Mt. Boroishi	*D. pacifica* (α) 80					A3	A3/C1	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	409073			Japan, Kumamoto pref., Itsuki village	*D. pacifica* (α) 81					A3	A3/C1	A'1/C2	A3/C1	A7/C4	**A9/C3**
Hori	409072			Japan, Kumamoto pref., Yatsushiro city, Izumi town	*D. pacifica* (α) 82			24.01	3*x*	A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	409068			Japan, Kumamoto pref., Uki city	*D. pacifica* (α) 83		2*n* =123	23.42	3*x*	A3	A2A6C4	**A3A'1C2**	**A1/C1**	A7/C4	**A3/C3**
Hori	409060			Japan, Miyazaki pref., Kobayashi city	*D. pacifica* (α) 84					C	A3/C1	A'1C1	**A1/C1**	A3C2C3	**A9C1C3**
Hori	409023			Japan, Chiba pref., Chiba city	*D. pacifica* (α) 85					A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	411419			Cutivated in Tokyo Botanical Garden	*D. pacifica* (α) 86			21.89	3*x*	A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	423572			Japan, Fukui pref., Nanjo county, Minamiechizen town, Kawachi	*D. pacifica* (α) 87			16.26	2*x*	A3	A2/C4	A'1C1	A3/C1	A7/C4	A3/C3
Hori	423575			Japan, Fukui pref., Nanjo county, Minamiechizen town, Kaburaei	*D. pacifica* (α) 88			22.25	3*x*	A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	423576			Japan, Fukui pref., Nanjo county, Minamiechizen town, Kaburaei	*D. pacifica* (α) 89			21.96	3*x*	A3	A2C1C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	423617			Japan, Tokyo-to, Izuoshima Is.	*D. pacifica* (α) 90			16.1	2*x*	A3	A2/C4	A10/C2	A3/C1	A7/C4	A3/C3
Hori	423624			Japan, Fukui pref., Awara city	*D. pacifica* (α) 91			16.56	2*x*	A3	A2/C4	A'1C1	A3/C1	A7/C4	A3C3
Hori	423625			Japan, Fukui pref., Awara city	*D. pacifica* (α) 92			15.94	2*x*	A3	A2/C4	A'1C1	A3/C1	A7/C4	A3C3
Hori	423628			Japan, Fukui pref., Awara city	*D. pacifica* (α) 93			16.73	2*x*	A3	A2/C4	A'1C1	A3/C1	A7/C4	A3C3
Hori	425869			Japan, Fukui pref., Fukui city	*D. pacifica* (α) 94					A3	A2/C4	A1C1C2	A3/C1	A2/C4	A3/C3
Hori	405189			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 1					A3	**A2B2C4**	**A2C1B1**	**A3B1C1**	**A1B1C4**	A3C3B1
Hori	406858			Japan, Tokyo-to, Inagi city	*D. pacifica* (ß) 2			24.88	3*x*	A3	A2B2C4	A2C1B1	A3B1C1	**A1B1C4**	A3C3B1
Hori	409144			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 3					B	A2B2C4	A2C1B1	A3B1C1	A1B1C4	**A3C3B1**
Hori	409143			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 4					A3	A2B2C4	A2C1B1	**A3B1C1**	A1B1C4	**A3C3B1**
Hori	409141			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 5					A3	A2B2C4	A2C1B1	A3B1C1	A1B1C4	**A3C3B1**
Hori	409140			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 6					A3	A2B2C4	A2C1B1	A3B1C1	A1B1C4	**A3C3B1**
Hori	409138			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 7					A3	A2B2C4	A2C1B1	A3B1C1	A1B1C4	**A3C3B1**
Hori	409137			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 8					A3	A2B2C4	A2C1B1	A3B1C1	A1B1C4	**A3C3B1**
Hori	411374			Japan, Tokyo-to, Akiruno city	*D. pacifica* (ß) 9					A3	A2B2C4	A2C1B1	A3B1C1	A1B1C4	A3C3B1
Hori	411375			Japan, Tokyo-to, Nishitama county	*D. pacifica* (ß) 10					A3	A2B2C4	A2C1B1	**A3B1C1**	A1B1C4	A3C3B1
Hori	423537			Japan, Fukui pref., Katsuyama city	*D. pacifica* (ß) 11			21.62	3*x*	B	A2B2C4	A'1C2B1	A3B1C1	A7B1C4	A3C3B1
Hori	423574			Japan, Fukui pref., Nanjo county, Minamiechizen town, Kaburaei	*D. pacifica* (ß) 12			24.8	3*x*	B	A2B2C4	A'1C2B1	A3B1C1	A7B1C4	A3C3B1
Hori	449117			Japan, Saitama pref., Hannno city	*D. pacifica* (ß) 13					A3	A2B2C4	A2C1B1	A3B1C1	A1B1C4	A3C3B1
Hori	405126			Japan, Tokyo-to, Hachioji city, Minamiosawa	*D. pacifica* (γ) 1					A2	A3C1D1	A3C2D3	**A2C1da6**	A4C4D1	**A8C3DA8**
Hori	405137			Japan, Kanagawa pref., Miura city	*D. pacifica* (γ) 2					A3	**A3C1D1**	A'1C2D2	**A3C1db1**	**A2C3dd1**	**A8C3DA10**
Hori	405141			Japan, Kanagawa pref., Miura city	*D. pacifica* (γ) 3					A2	A3C1D1	A3C2D3	**A2C1db1**	A4C4D1	A8C3DA10
Hori	405145			Japan, Kanagawa pref., Kamakura city	*D. pacifica* (γ) 4					A2	A3C1D1	A3C2D3	A2C1db1	A4C4D1	**A8C3DA8**
Hori	405148			Japan, Kanagawa pref., Kamakura city	*D. pacifica* (γ) 5					A2	**A3C4D1**	**A3C2D3**	**A2C1db1**	**A4C4D1**	A8C3DA10
Hori	405149			Japan, Kanagawa pref., Kamakura city	*D. pacifica* (γ) 6					A3	**A2C4D1**	**A'1C2D2**	**A3C1db1**	**A4C4D1**	**A3C3DA7**
Hori	405156			Japan, Kanagawa pref., Zushi city, Mt. Futago	*D. pacifica* (γ) 7					A2	**A3C4D6**	A3C2D6	A2C1db1	A4C4D1	**A8C3DA8**
Hori	405159			Japan, Kanagawa pref., Zushi city, Mt. Futago	*D. pacifica* (γ) 8					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	**A8C3DA10**
Hori	405178			Japan, Chiba pref., Sakura city	*D. pacifica* (γ) 9					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	**A8C3DA8**
Hori	405191			Japan, Saitama pref., Hannno city	*D. pacifica* (γ) 10					A2	A3C4D1	A3C2D6	**A2C1db1**	A4C4D1	**A8C3DA10**
Hori	405208			Japan, Chiba pref., Minamiboso city	*D. pacifica* (γ) 11					A3	A3C4D1	A'1C2D2	A3C1db1	A4C4D1	**A3C3DA7**
Hori	405226			Japan, Shizuoka pref., Kamo county	*D. pacifica* (γ) 12					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	A8C3DA8
Hori	405241			Japan, Shizuoka pref., Shizuoka city	*D. pacifica* (γ) 13					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	**A8C3DA10**
Hori	405250			Japan, Wakayama pref., Higashimuro county, Taiji town	*D. pacifica* (γ) 14					A2	A3C4D1	A3C2D6	**A2C1db3**	A4C4D1	**A8C3DA10**
Hori	405282			Japan, Mie pref., Minamimuro county, Kihou town, Ida	*D. pacifica* (γ) 15					A2	A3C4D1	A3C2D6	A2C1db3	A4C4D1	**A8C3DA10**
Hori	405310			Japan, Tokyo-to, Izuoshima Is.	*D. pacifica* (γ) 16					A2	A2C4D1	A3C2D3	A2C1db1	A4C4D1	**A8C3DA8**
Hori	405318			Japan, Kanagawa pref., Odawara city	*D. pacifica* (γ) 17					A3	A2C4D1	A'1C2D2	**A3C1db1**	A4C4D1	**A3C3DA7**
Hori	406863			Japan, Kagawa pref., Takamatsu city, Yashima	*D. pacifica* (γ) 18					A2	A3C4D1	A3C2D3	A2C1db3	A4C4D1	A8C3DA10
Hori	406875			Japan, Shimane pref., Oki county	*D. pacifica* (γ) 19					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	A8C3DA10
Hori	406882			Japan, Hiroshima pref., Hatsukaichi city, Miyajima	*D. pacifica* (γ) 20					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	A8C3DA10
Hori	406883			Japan, Hiroshima pref., Hatsukaichi city, Miyajima	*D. pacifica* (γ) 21					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	A8C3DA10
Hori	406886			Japan, Hiroshima pref., Takehara city	*D. pacifica* (γ) 22					A2	A3C4D1	A3C2D3	**A2C1db2**	A4C4D1	A8C3DA10
Hori	406892			Japan, Hiroshima pref., Takehara city	*D. pacifica* (γ) 23					A2	A3C4D1	A3C2D3	A2C1db2	A4C4D1	A8C3DA10
Hori	406898			Japan, Hiroshima pref., Inno Is., Mukunoura town	*D. pacifica* (γ) 24					A2	A3C4D1	A3C2D3	**A2C1dd1**	A4C4D1	A8C3DA8
Hori	406916			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Kabe town	*D. pacifica* (γ) 25					A2	A3C4D1	A3C2D3	A2C1dd1	A4C4D1	A8C3DA11
Hori	406922			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D. pacifica* (γ) 26					A2	A3C4D1	A3C2D3	A2C1db2	A4C4D1	A8C3DA10
Hori	406935			Japan, Tokushima pref., Komatsushima city	*D. pacifica* (γ) 27					A2	A3C4D1	A3C2D3	A2C1db2	A4C4D1	**A8C3DA10**
Hori	406942			Japan, Hiroshima pref., Higashihiroshima city	*D. pacifica* (γ) 28					A2	A3C4D1	A3C2D3	A2C1dd1	A4C4D1	A8C3DA11
Hori	406947			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D. pacifica* (γ) 29					A2	A3C4D1	A3C2D3	A2C1db2	A4C4D1	A8C3DA10
Hori	406961			Japan, Okayama pref., Okayama city, Kita-ku, Ichinomiya	*D. pacifica* (γ) 30					A2	A3C4D1	A3C2D3	A2C1db2	A4C4D1	A8C3DA10
Hori	409220			Japan, Saga pref., Imari city, Okawauchi town	*D. pacifica* (γ) 31		2*n* =123	24.83	3*x*	A2	A3C4D1	**A3C2D6**	A2C1db2	A4C4D1	A8C3DA8
Hori	409218			Japan, Saga pref., Takeo city, Yamauchi town	*D. pacifica* (γ) 32			25.15	3*x*	A2	A3C4D1	A3C2D3	A2C1db2	A4C4D1	A8C3DA10
Hori	409209			Japan, Saga pref., Karatsu city, Hizenn town	*D. pacifica* (γ) 33			26.93	3*x*	A2	A3C4D1	A3C2D3	A2C1dd1	A4C4D1	A8C3DA10
Hori	409204			Japan, Kagawa pref., Takamatsu city, Mt. Iwaseo	*D. pacifica* (γ) 34					A2	A3C4D1	A3C2D3	A2C1dd1	A4C4D1	**A8C3DA11**
Hori	409193			Japan, Kagawa pref., Takamatsu city, Kinashi town	*D. pacifica* (γ) 35			27.17	3*x*	A2	A3C4D1	A3C2D3	A2C1dd1	A4C4D1	**A8C3DA11**
Hori	409136			Japan, Saitama pref., Hannno city	*D. pacifica* (γ) 36					A2	A3C4D1	A3C2D3	**A2C1db1**	A4C4D1	**A8C3DA10**
Hori	409071			Japan, Kumamoto pref., Uki city	*D. pacifica* (γ) 37					A2	A3C4D1	A3C2D6	A2C1dd1	A4C4D1	A8C3DA11
Hori	409022			Japan, Chiba pref., Chiba city	*D. pacifica* (γ) 38					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	**A8C3DA10**
Hori	449109			Japan, Tokyo-to, Inagi city	*D. pacifica* (γ) 39					A2	A3C4D1	A3C2D3	A2C1db1	A4C4D1	A8C3DA10
Hori	405166			Japan, Chiba pref., Sakura city	*D. sacrosancta* 1					A3	**A2C4E1**	**A2C2E1**	**A3C1E1**	**A1C4E1**	**A3C3E1**
Hori	405229			Japan, Shizuoka pref., Kamo county	*D. sacrosancta* 2					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	405251			Japan, Wakayama pref., Higashimuro county, Kozagawa town. Aise	*D. sacrosancta* 3					A3	A2C4E1	A2C2E1	**A3C1E1**	A1C4E1	**A3C3E1**
Hori	405258			Japan, Wakayama pref., Higashimuro county, Kozagawa town. Ichimai-iwa	*D. sacrosancta* 4					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	405279			Japan, Wakayama pref., Higashimuro county, Nachikatsuura town. Mt. Nachi	*D. sacrosancta* 5					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E2**
Hori	405286			Japan, Mie pref., Minamimuro county, Kihou town, Ida	*D. sacrosancta* 6					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	405291			Japan, Mie pref., Kumano city, Hadasu	*D. sacrosancta* 7					E	A2C4E1	A2C2E1	A1C1E2	**A7C3E1**	A9C3E2
Hori	405293			Japan, Mie pref., Kumano city, Hobo town	*D. sacrosancta* 8					E	**A3C4E1**	A4C1E1	A3C1E1	A1C4E1	A9C3E1
Hori	405295			Japan, Mie pref., Kumano city, Hobo town	*D. sacrosancta* 9					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E2**
Hori	405319			Japan, Shizuoka pref., Haibara county	*D. sacrosancta* 10					E	A2C4E1	A2C2E1	**A1C1E2**	A7C3E1	**A9C3E2**
Hori	405328			Japan, Tokyo-to, Hachioji city, Todori town	*D. sacrosancta* 11					A3	A2C4E1	A2C2E1	**A3C1E1**	A1C4E1	**A3C3E1**
Hori	406982			Japan, Kagawa pref., Takamatsu city, Yashima	*D. sacrosancta* 12					E	A2C4E1	A2C2E1	**A1C1E2**	A7C3E1	A3C3E2
Hori	407012			Japan, Shizuoka pref., Haibara county	*D. sacrosancta* 13					E	A2C4E1	A2C2E1	A1C1E2	A7C3E1	A9C3E2
Hori	407051			Japan, Shizuoka pref., Haibara county	*D. sacrosancta* 14					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	407025			Japan, Kagawa pref., Takamatsu city, Yashima	*D. sacrosancta* 15					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	A3C3E1
Hori	406984			Japan, Hiroshima pref., Hatsukaichi city, Miyajima	*D. sacrosancta* 16					E	**A2C4E3**	A2C2E1	A3C1E1	A1C4E1	**A9C3E1**
Hori	406985			Japan, Hiroshima pref., Hatsukaichi city, Miyajima	*D. sacrosancta* 17					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	A3C3E1

Appendix2-1
Voucher specimens examined in this study. Any genotypes that were identified by sequencing are in boldface. Otherwise, they the genotypes were deduced from comparisons of band positions in SSCP gels. When ploidy is unknown, the genotype is in brackets. For samples with unknown genome dosage, the unidentified genomes are marked by asterisks.

Coll.	MAK	TNS	TAIF	Locality	no.	reproductive mode	chromosome numbers	DNA amount (p.g.)	ploidy	*rbcL*	*AK1*	*EST*	*GapCp*	*G6PDH*	*PgiC*
Hori	406988			Japan, Hiroshima pref., Takehara city	*D. sacrosancta* 18					A3	A2C4E1	A2C2E1	**A3C1E1**	A1C4E1	A3C3E1
Hori	407020			Japan, Hiroshima pref., Inno Is., Mukunoura town	*D. sacrosancta* 19					E	A3C4E1	**A4C1E1**	A3C1E1	A1C4E1	A9C3E1
Hori	407023			Japan, Hiroshima pref., Inno Is., Kagamiura	*D. sacrosancta* 20					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	406990			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Asa town	*D. sacrosancta* 21					E	A2C4E1	A2C2E1	A1C1E2	**A11C3E1**	A9C3E2
Hori	406992			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Asa town	*D. sacrosancta* 22					E	A2C4E1	A4C1E1	A3C1E1	A1C4E1	**A9C3E1**
Hori	406993			Japan, Hiroshima pref., Hiroshima city, Asaminami-ku, Numata town	*D. sacrosancta* 23					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	406998			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Kabe town	*D. sacrosancta* 24					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	A3C3E1
Hori	406999			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D. sacrosancta* 25					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	407028			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D. sacrosancta* 26					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	407001			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D. sacrosancta* 27					A3	A2C4E1	A2C2E1	**A3C1E1**	A1C4E1	A3C3E1
Hori	407003			Japan, Tokushima pref., Itano county	*D. sacrosancta* 28					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	407004			Japan, Hiroshima pref., Higashihiroshima city	*D. sacrosancta* 29					A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	407032			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D. sacrosancta* 30					E	A2C4E3	A2C2E1	A3C1E1	A1C4E1	**A9C3E1**
Hori	407033			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D. sacrosancta* 31					E	A2C4E3	A2C2E1	A3C1E1	A1C4E1	**A9C3E1**
Hori	407007			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D. sacrosancta* 32					E	A2C4E3	A2C2E1	A3C1E1	A1C4E1	A9C3E1
Hori	407035			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D. sacrosancta* 33					E	A3C4E1	A4C1E1	A3C1E1	A1C4E1	**A9C3E1**
Hori	407010			Japan, Okayama pref., Wake county	*D. sacrosancta* 34					E	A2C4E1	A2C2E1	**A1C1E2**	A7C3E1	**A9C3E2**
Hori	407038			Japan, Okayama pref., Tamano city	*D. sacrosancta* 35					E	A3C4E1	A9C1E1	A3C1E1	**A1C4E1**	A9C3E1
Hori	407039			Japan, Okayama pref., Tamano city	*D. sacrosancta* 36					E	A2C4E1	A4C1E1	A1C1E2	**A7C3E1**	A9C3E2
Hori	407041			Japan, Okayama pref., Tamano city	*D. sacrosancta* 37					E	A2C4E3	A2C2E1	A3C1E1	A1C4E1	**A9C3E1**
Hori	407043			Japan, Okayama pref., Tamano city	*D. sacrosancta* 38					E	A2C4E3	A2C2E1	A3C1E1	A1C4E1	**A9C3E1**
Hori	407044			Japan, Okayama pref., Tamano city	*D. sacrosancta* 39					E	A3C4E1	**A4C1E1**	A3C1E1	A1C4E1	A9C3E1
Hori	407047			Japan, Okayama pref., Tamano city	*D. sacrosancta* 40					E	A3C4E1	**A4C1E1**	A3C1E1	A1C4E1	**A9C3E1**
Hori	409223			Japan, Saga pref., Takeo city, Yamauchi town	*D. sacrosancta* 41		2*n* =123	22.01	3*x*	A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	A3C3E1
Hori	409216			Japan, Saga pref., Takeo city, Yamauchi town	*D. sacrosancta* 42			23.42	3*x*	A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	A3C3E1
Hori	409214			Japan, Saga pref., Takeo city, Yamauchi town	*D. sacrosancta* 43			22.25	3*x*	E	A2C4E1	A2C2E1	A3C1E1	**A8C4E5**	A9C3E2
Hori	409208			Japan, Saga pref., Imari city, Hatatsu town, Koba	*D. sacrosancta* 44			20.98	3*x*	A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	409075			Japan, Miyazaki pref., Miyazaki city, Mt. Boroishi	*D. sacrosancta* 45			21.89	3*x*	A3	A2C4E1	A2C2E1	A3C1E1	**A1C4E4**	**A3C3E1**
Hori	409069			Japan, Kumamoto pref., Uki city	*D. sacrosancta* 46			22.04	3*x*	A3	A2C4E1	A2C2E1	A3C1E1	A1C4E1	**A3C3E1**
Hori	409066			Japan, Miyazaki pref., Kobayashi city	*D. sacrosancta* 47			20.95	3*x*	E	A2C4E3	A2C2E1	**A1C1E2**	**A8C3E4**	**A9C3E2**
Hori	405129			Japan, Tokyo-to, Hachioji city, Shimoyugi	*D. kobayashii* 1					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	405133			Japan, Tokyo-to, Inagi city	*D. kobayashii* 2		2*n* =123	23.42	3*x*	E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	405222			Japan, Tokyo-to, Hachioji city, Naganuma	*D. kobayashii* 3					E	**B1C4E2**	**B1C1E2**	**B1C1E2**	**B1C1E1**	**B1C3E2**
Hori	405333			Japan, Tokyo-to, Nishitama county	*D. kobayashii* 4					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	405334			Japan, Tokyo-to, Nishitama county	*D. kobayashii* 5					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	405351			Japan, Kanagawa pref., Kawasaki city	*D. kobayashii* 6					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	406839			Japan, Chiba pref., Sakura city	*D. kobayashii* 7					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	406840			Japan, Chiba pref., Sakura city	*D. kobayashii* 8					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	406841			Japan, Chiba pref., Sakura city	*D. kobayashii* 9					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	406842			Japan, Nara pref., Yoshino county	*D. kobayashii* 10					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	411373			Japan, Tokyo-to, Akiruno city	*D. kobayashii* 11					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	B1C3E2
Hori	411407			Japan, Aichi pref., Kitashitara county, Tamine	*D. kobayashii* 12					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	B1C3E2
Hori	411408			Japan, Aichi pref., Kitashitara county, Tamine	*D. kobayashii* 13					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	B1C3E2
Hori	412524			Japan, Ibaraki pref., Moriya city	*D. kobayashii* 14					E	B1C4E2	B1C1E2	B1C1E2	B1C1E1	**B1C3E2**
Hori	405130			Japan, Tokyo-to, Hachioji city, Minamiosawa	*D. bissetiana* 1					C	**B1/C4**	B1C1C2	**B1/C1**	B1/C1	**B1/C3**
Hori	405132			Japan, Tokyo-to, Inagi city	*D. bissetiana* 2					C	B1/C4	B1C1C2	B1/C1	B1/C1	**B1C2C3**
Hori	405155			Japan, Kanagawa pref., Zushi city, Mt. Futago	*D. bissetiana* 3					C	**B1C3C4**	**B1C1C2**	B1/C1	**B1/C1**	**B3C2C3**
Hori	405164			Japan, Chiba pref., Sakura city	*D. bissetiana* 4					C	B1C3C4	B1C1C2	B1/C1	B1/C1	**B1C2C3**
Hori	405190			Japan, Saitama pref., Hannno city	*D. bissetiana* 5					C	B1/C4	**B1C2C4**	**B2C1C2**	**B1C1C5**	**B2C1C5**
Hori	405221			Japan, Tokyo-to, Hachioji city, Naganuma	*D. bissetiana* 6					C	B1/C4	B1C1C2	B1/C1	**B1C1C4**	**B1/C3**
Hori	405313			Japan, Tokyo-to, Izuoshima Is.	*D. bissetiana* 7					C	B1C3C4	B1C1C2	B1/C1	B1/C1	**B1C2C3**
Hori	405343			Japan, Tokyo-to, Nishitama county	*D. bissetiana* 8					B	B1/C4	**B1/C1**	B1/C1	B1/C1	**B1/C5**
Hori	405344			Japan, Tokyo-to, Nishitama county	*D. bissetiana* 9					B	B1/C4	B1/C1	B1/C1	B1/C1	**B1/C4**
Hori	406802			Japan, Tokyo-to, Inagi city	*D. bissetiana* 10					C	B1/C4	B1C1C2	B1/C1	B1/C1	B1/C3
Hori	406803			Japan, Tokyo-to, Inagi city	*D. bissetiana* 11					C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	406804			Japan, Nara pref., Yoshino county	*D. bissetiana* 12					B	B1/C4	B1/C1	B1/C1	B1/C1	B1/C3
Hori	406818			Japan, Shizuoka pref., Haibara county	*D. bissetiana* 13					C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	406805			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Asa town	*D. bissetiana* 14					C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	406806			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Asa town	*D. bissetiana* 15					C	B1C3C4	B1C1C2	**B1/C1**	B1/C1	**B1/C2**
Hori	406809			Japan, Hiroshima pref., Hiroshima city, Asakita-ku, Kabe town	*D. bissetiana* 16					C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	406811			Japan, Okayama pref., Okayama city, Mt. Tatsunokuchi	*D. bissetiana* 17					C	B1C3C4	B1C1C2	**B1/C1**	B1/C1	B1C2C3
Hori	406813			Japan, Hiroshima pref., Higashihiroshima city	*D. bissetiana* 18					C	B1C3C4	B1C1C2	B1/C1	**B1/C1**	B1C2C3
Hori	406814			Japan, Hiroshima pref., Kure city, Mt. Yasumi	*D. bissetiana* 19					C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	406817			Japan, Okayama pref., Okayama city, Kita-ku, Ichinomiya	*D. bissetiana* 20					C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	409180			Japan, Kagawa pref., Ayauta county, Ayagawa town	*D. bissetiana* 21		2*n* =123	23.98	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	**B1C2C3**
Hori	409179			Japan, Kagawa pref., Nakatado county, Mannnou town	*D. bissetiana* 22					C	B1/C4	B1C1C2	B1/C1	B1/C1	**B1/C3**
Hori	409174			Japan, Tokyo-to, Inagi city	*D. bissetiana* 23					B	B1/C4	**B1/C2**	B1/C1	B1/C1	**B1/C3**
Hori	409147			Japan, Saitama pref., Hannno city	*D. bissetiana* 24					C	B1C3C4	B1C1C2	B1/C1	B1/C1	**B1C2C3**
Hori	409146			Japan, Saitama pref., Hannno city	*D. bissetiana* 25					C	B1/C4	B1/C2	B1/C1	B1/C1	**B1/C3**
Hori	409145			Japan, Saitama pref., Hannno city	*D. bissetiana* 26					C	B1C3C4	B1C1C2	B1/C1	B1/C1	**B1C2C3**
Hori	409224			Japan, Ibaraki pref., Mt. Tsukuba	*D. bissetiana* 27					C	B1/C4	B1C1C2	B1/C1	B1/C1	**B1/C3**
Hori	409225			Japan, Ibaraki pref., Mt. Tsukuba	*D. bissetiana* 28					B	**B1/C3**	B1/C1	**B1/C1**	B1/C1	**B1/C3**
Hori	409077			Japan, Kumamoto pref., Yatsushiro city, Nihonnsugi pass	*D. bissetiana* 29					C	B1C3C4	B1C1C2	**B1/C1**	B1/C1	B1C2C3
Hori	409062			Japan, Miyazaki pref., Kobayashi city	*D. bissetiana* 30					C	B1/C4	B1/C2	B1/C1	B1/C1	B1/C3
Hori	409019			Japan, Chiba pref., Chiba city	*D. bissetiana* 31					C	B1/C4	B1C1C2	**B1/C1**	B1/C1	**B1/C3**
Hori	411379			Japan, Tokyo-to, Akiruno city	*D. bissetiana* 32					B	B1/C4	B1/C1	**B1/C1**	B1/C1	B1/C3
Hori	423538			Japan, Fukui pref., Ohno city, Kamishita	*D. bissetiana* 33			24.2	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423540			Japan, Fukui pref., Ohno city, Iouji	*D. bissetiana* 34			24.48	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423543			Japan, Akita pref., Daisenn city, Kyouwa	*D. bissetiana* 35		2*n* =123	28.69	3*x*	B	B1/C3	B1/C1	B1/C1	B1/C1	B1/C3
Hori	423545			Japan, Akita pref., Daisenn city, Kyouwa	*D. bissetiana* 36			28.97	3*x*	B	B1/C3	B1/C1	B1/C1	B1/C1	B1/C3
Hori	423549			Japan, Akita pref., Oga city, Kitaura	*D. bissetiana* 37			29.67	3*x*	B	B1/C4	B1/C2	B1/C1	B1/C1	B1/C3
Hori	423551			Japan, Miyagi pref., Sendai city, Taihaku-ku	*D. bissetiana* 38			26.02	3*x*	B	B1/C4	B1/C1	B1/C1	B1/C1	B1/C3
Hori	423553			Japan, Miyagi pref., Sendai city, Taihaku-ku	*D. bissetiana* 39			25.49	3*x*	C	B1/C4	B1C1C2	B1/C1	B1C1C4	B1/C3
Hori	423556			Japan, Miyagi pref., Kakuda city, Oda, Gonnbara forest road	*D. bissetiana* 40			26.18	3*x*	B	B1/C3	B1/C1	B1/C1	B1/C1	B1/C3
Hori	423561			Japan, Miyagi pref., Kakuda city, Oda, Mt. Nabemori	*D. bissetiana* 41			25.49	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423564			Japan, Akita pref., Oga city, Tsubaki	*D. bissetiana* 42			25.62	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423601			Japan, Miyagi pref., Igu county, Marumori town	*D. bissetiana* 43			28.44	3*x*	C	B1/C4	B1C1C2	B1/C1	B1C1C4	B1/C3
Hori	423602			Japan, Miyagi pref., Igu county, Marumori town	*D. bissetiana* 44			27.6	3*x*	C	B1/C4	B1C1C2	B1/C1	B1C1C4	B1/C3
Hori	423603			Japan, Miyagi pref., Igu county, Marumori town	*D. bissetiana* 45			23.42	3*x*	C	B1/C4	B1C1C2	B1/C1	B1C1C4	B1/C3
Hori	423604			Japan, Miyagi pref., Igu county, Marumori town	*D. bissetiana* 46			23.42	3*x*	C	B1/C4	B1C1C2	B1/C1	B1C1C4	B1/C3
Hori	423605			Japan, Miyagi pref., Shiroishi city	*D. bissetiana* 47			26.54	3*x*	C	B1/C4	B1C1C2	B1/C1	B1C1C4	B1/C3
Hori	423606			Japan, Miyagi pref., Shiroishi city	*D. bissetiana* 48			27.6	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423607			Japan, Miyagi pref., Shiroishi city	*D. bissetiana* 49			27.08	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423609			Japan, Miyagi pref., Shiroishi city	*D. bissetiana* 50			26.77	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3?
Hori	423611			Japan, Akita pref., Nikaho city	*D. bissetiana* 51			27.32	3*x*	B	B1/C4	B1/C1	B1/C1	B1/C4	B1/C3
Hori	423619			Japan, Fukui pref., Awara city	*D. bissetiana* 52			23.42	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423620			Japan, Fukui pref., Awara city	*D. bissetiana* 53			26.12	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423622			Japan, Fukui pref., Awara city	*D. bissetiana* 54			28.44	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423623			Japan, Fukui pref., Awara city	*D. bissetiana* 55			25.93	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	423626			Japan, Fukui pref., Awara city	*D. bissetiana* 56			25.09	3*x*	C	B1C3C4	B1C1C2	B1/C1	B1/C1	B1C2C3
Hori	416655			Japan, Tokyo-to, Bonin Is.	*D. insularis* 1	a	2*n* =82		2*x*	M	**M1M2**	**M**	**B4B4**	**M1M1**	M1M1
Hori	425900			Japan, Tokyo-to, Bonin Is.	*D. insularis* 2	a				M	**M1***	M	**B4B4**	**M1***	**M1***
Hori	406836			Japan, Tokyo-to, Bonin Is.	*D. insularis* var. *chichisimensis* 1	a				A1	**A2C4M1**	**A'1C2M**	**A3C1B4**	**A1C4M1**	**A3C3M1**
Hori	416656			Japan, Tokyo-to, Bonin Is.	*D. insularis* var. *chichisimensis* 2	a	2*n* =123		3*x*	A1	A2C4M1	A'1C2M	A3C1B4	A1C4M1	**A3C3M1**
Hori	407057			Japan, Kagoshima pref., Yakushima Is.	*D. sordidipes*						F1F1	**F5F7**	**F1F1**	F1F1	**F2F2**
Hori	410918			Japan, Kagoshima pref., Yakushima Is.	*D. sordidipes*						F1F1	F2F3	**F2F2**	**F1F1**	**F6F7**
Hori	410919			Japan, Kagoshima pref., Yakushima Is.	*D. sordidipes*						F1F1	F3F6	**F2F4**	F1F4	**F8F9**
Hori	410920			Japan, Kagoshima pref., Yakushima Is.	*D. sordidipes*						**F1F1**	**F2F3**	**F4F4**	**F1F2**	**F2F2**
Hori	423478			Japan, Kagoshima pref., Yakushima Is.	*D. sordidipes*						F1F1	**F7F7**	F1F1	F1F1	F2F2
Hori	423482			Japan, Kagoshima pref., Yakushima Is.	*D. sordidipes*						F4F5	F2F3	**F2F5**	F2F2	**F4F5**
Hori	406981			Japan, Tokyo-to, Inagi city	*D. sabaei*										
Hori	411422			Japan, Kumamoto pref., Aso city, Ichinomiya town	*D. handeliana*										
Li-Yaung Kuo			Kuo2370	Taiwan	*D. hasseltii*										
Hori	425871			Taiwan, Taipei city, Mt. Chihtan	*D. polita*										
Hori	411418			Japan, Aichi pref., Kitashitara county, Syounokoro	*D. tokyoensis*										
Hori	411421			Cutivated in Tokyo Botanical Garden	*D. monticola*										
Hori	405321			Japan, Yamanashi pref., Minamiarupusu city	*D. expansa*										
Hori	405219			Japan, Tokyo-to, Hachioji city, Naganuma	*D. gymnophylla*										
Hori	434066			Japan, Kanagawa pref., Zushi city, Jinnmuji	*Polystichum lepidocaulon*										
Hori	435336			Japan, Niigata pref., Minamiuonuma county	*P. retroso-paleaceum*										
Hori	434041			Japan, Kanagawa pref., Zushi city, Jinnmuji	*Arachniodes exilis*										

Chapter 3.

Revised classification of the species within the *Dryopteris varia* complex (Dryopteridaceae) in Japan

3.1. Introduction

The results of Chapter 2 suggested that classification of the *Dryopteris varia* complex, especially that of the apogamous species, can be revised according to the information for their genome constitutions. This is because the hybridization cycle did not seem to have repeated many times among the members of the *D. varia* complex, and genome constitution of each apogamous species was a simple combination of two or three genomes of the diploid sexual species. Although many Japanese pteridologists have considered classification of the apogamous species within the *D. varia* complex as impossible to perform (Tagawa 1959, Lin *et al*. 1995, Iwatsuki 1995), it is possible if the species are recognized according to their genomic constitutions.

Moreover, the results of Chapter 2 clearly showed that the former classification of the apogamous species of the *Dryopteris varia* complex (Iwatsuki 1992) had issues, especially for *D. pacifica*, because three genotypes (α, β, and γ types) were recognized within this species. The α type consists of the genomes of *D. varia* (A) + *D. protobissetiana* (C), the β type consists of *D. varia* (A) + *D. saxifraga* (B) + *D. protobissetiana* (C), and the γ type consists of *D. varia* (A)+ *D. protobissetiana* (C) + *D. caudipinna* (D). The scientific name of *Dryopteris pacifica* (Nakai) Tagawa also needs

revision. This name was originally described as *Polystichum pacificum* by Nakai (1925) from Japan. Tagawa (1959) recombined it to *Dryopteris*. Christ (1912), however, previously had published this name from the Samoan Islands for a different species of *Dryopteris*. Christ (1912) commented that *D. pacifica* was similar to *Dryopteris dissecta*, and *D. dissecta* now belongs to *Tectaria* (Xing *et al.* 2013). Therefore, *Dryopteris pacifica* of Nakai (1925) must be an illegitimate name, and new names are required for the Japanese *D. pacifica*.

In Chapter 3, species classification of the *Dryopteris varia* complex will be revised according to the genome constitution of each species, as elucidated in Chapter 2. Firstly, diploid sexual taxa with distinct chloroplasts and nuclear genomes are treated as independent species. Next, apogamous species are recognized according to differences in the combinations of the genomes. In other words, apogamous cytotypes with different nuclear genome constitutions are classified as independent species. There were several genome constitutions for *D. pacifica* (α), ACC, AAC, AC or A/C. The same might be true also for *D. bissetiana*, BCC or BBC, although two alleles of B were not recognized because of low genetic variation in *D. saxifraga* (B). However, in this study, I have treated these genotypes as belonging to the same species because it is nearly impossible to distinguish them morphologically. Autopolyploid apogamous cytotypes were classified as the same species as the diploid sexual cytotypes sharing the same genome because there were no significant differences in morphological characteristics. I summarized the taxonomic treatment of 11 species within the *D. varia* complex (*D. bissetiana*, *D. chichisimensis*, *D. erythrovaria*, *D. hikonensis*, *D. insularis*, *D. kobayashii*, *D. protobissetiana*, *D. sacrosancta*, *D. saxifraga*, *D. subhikonensis*, and *D.*

varia.) with their reticulate relationships according to their genome constitutions in Figure 3-1.

For this study, herbarium specimens of the *Dryopteris varia* complex deposited at MAK, MBK, PE, and TNS were examined (Appendix 3-1). The voucher specimens whose genomic constitutions had been fully elucidated were useful especially for describing their morphology and geographic distribution.

3.2. Key to the Japanese species of the *Dryopteris varia* complex (*Dryopteris* subg. *Erythrovariae* sect. *Variae*)

1a. Sori born only on upper part of lamina……. …….*D. insularis*

1b. Sori born on whole lamina……. 2

2a. Scales deflected……. *D. saxifraga*

2b. Scales ascending…….3

3a. Lamina papyraceous; center of indusia often red …… *D. erythrovaria*

3b. Lamina herbaceous or coriaceous; center of indusia translucent …… ..4

4a. Lamina herbaceous …….. 5

4b. Lamina coriaceous …….. 6

5a. Lamina narrowly triangular (width / length = 2/3–1/2); apex of pinnae curved, obtuse ……. …….*D. kobayashii*

5b. Lamina pentagonal (width / length = 3/4–2/3); apex of pinnae straight, acute ……..... *D. sacrosancta*

6a. Scales on pinna rachis flat……..…. *D. varia*

6b. Scales on pinna rachis bullate……..….7

7a. Scales sub-sparsely covered on rachis; endemic to the Bonin Islands……

…….*D. chichisimensis*

7b. Scales densely covered on rachis; not distributed in the Bonin Islands…..……8

8a. Scales on upper petiole bullate…….……*D. protobissetiana*

8b. Scales on upper petiole flat…….……..…….9

9a. Margin of apex of upper pinnae entire…….……*D. bissetiana*

9b. Margin of apex of upper pinnae deeply–shallowly serrated…….……..….10

10a. Margin of apex of upper pinnae deeply serrated; indusia ciliate or entire…….……..... *D. hikonensis*

10b. Margin of apex of upper pinnae shallowly serrated; indusia entire

……. *D. subhikonensis*

3.3. Taxonomic Treatment

Dryopteris bissetiana (Baker) C. Chr., *Ind. Fil.* 245. 1905. Type: Japan, Miyanoshita (may be Kanagawa pref.) (J. Bisset, May 24, 1876, K) in *J. Bot, British and Foreign* 15 (180): 366. 1877 (*Nephrodium bissetianum* Baker). **Figure 3-2a.**

Polypodium setosum Thunb., *Fl. Jap.* 337. 1784

Aspidium setosum (Thunb.) Sw., in *Schrad. J. Bot.* 1800-2: 39. 1801

Dryopteris setosa (Thunb.) Akasawa, in *Bull. Kochi. Wom. Univ.* 7: 27. 1959

Dryopteris varia subsp. *setosa* (Thunb.) Sugimoto, *Keys Herb. Pl. Jap. Pterid.* 405. 1966

Nephrodium bissetianum Baker, in *J. Bot.* 1877: 366

Polystichum bissetianum (Bak.) Nakai, in *Bot. Mag. Tokyo* 45: 102. 1931

Dryopteris thunbergii Koidz., in *Bot. Mag. Tokyo* 38: 106. 1924

Dryopteris saxifragivaria Nakai in J. Jap. Bot. 18: 286. 1942.

Dryopteris varia var. *setosa* (Thunb.) Ohwi, in *Fl. Jap. Pterid.* 88: 1957.

Diagnosis. ***Dryopteris bissetiana*** (Baker) C. Chr. is an apogamous species of hybrid origin between *D. protobissetiana* and *D. saxifraga*. This species is similar to *D. protobissetiana*, *D. hikonensis*, and *D. subhikonensis*. However, *D. bissetiana* differs from these in having pinnules with entire margin, entire indusial, and gradually narrowing lamina. This species is sometimes also similar to *D. saxifraga*. Such intermediate form has been identified as *D. saxifragivaria* Nakai; however, both *D. bissetiana* and *D. saxifragivaria* are of hybrid origin between *D. protobissetiana* and *D. saxifraga*. They share the same genomes from the two parental diploid sexual species. Therefore, this study treated them as the same species, *D. bissetiana*.

Plants terrestrial, evergreen, rhizome erect, or slightly ascending; leaves cespitose; scales dense on rhizome, petiole, pinna stalks, rachises, and pinna rachises; petiole 10–40 cm long; scales lanceolate, ascending or deflected, filiform at apex; scales on basal petiole black, transpicuous; base of scales on basal petiole narrow; base of scales on upper petiole spread; base of scales on rachises and pinna rachises bullate; lamina bipinnate, occasionally tripinnate at base, narrowly triangular, gradually narrowing to apex, 20–50 cm long, 10–30 cm wide, dark green or whitish green, soft coriaceous in texture, surface shiny or dull, recurved at margin; pinnules entire at apical margin; lowest basiscopic pinnules on lowest pinna elongated but not markedly more than second one; sori round, born between the margin and the costa; indusia reniform or circular, entire at margin, transpicuous, approximately 1.5–1.8 mm in diameter; 32 spores per sporangium; chromosome number $2n = 123$, triploid apogamous.

Notes. From South Korea, Lee et al. (2006) reported diploid apogamous *Dryopteris bissetiana*. Lee & Park (2013) reported several sequences of nuclear *PgiC* from *D. bissetiana*. However, all of their sequences nested within the clade of diploid sexual *D. saxifraga* (Clade B). Their sample might not be the diploid apogamous *D. saxifraga* because its chloroplast DNA coincided with that of *D. protobissetiana*. Therefore, it seems possible that they failed to select the nuclear *PgiC* sequences belonging to *D. protobissetiana* (Clade C) from their samples of *D. bissetiana*. So, far, only the triploid apogamous cytotype has been found from *D. bissetiana* in Japan, even though as many as 25 samples have been cytologically analyzed using ploidy analysis or chromosomal observations.

Lee et al. (2006) distinguished *Dryopteris saxifragivaria* Nakai from *D. bissetiana*, which has intermediate morphological characteristics between *D. bissetiana* and *D. saxifraga*, in Korea. However, continuous morphological variations are observed between *D. bissetiana* and *D. saxifragivaria* in Japan, and it is difficult to distinguish them morphologically. I concluded that they belong to the same species (*D. bissetiana*) because they share the same genomic constitutions (the genomes from diploid sexual *D. saxifraga* and *D. protobissetiana*).

Habitat and distribution. Growth occurs both in deciduous and evergreen broad-leaved forests of Japan (Hokkaido, Honshu, Shikoku, Kyusyu), Korea, and the mainland of China. The distribution map for Japan is shown in Figure 3-2b.

Japanese name. Yama-itachishida.

Dryopteris chichisimensis Nakai ex H. Ito, in *J. Bot. Mag. Tokyo* 49: 435. 1935. Type: Japan, the Bonin Islands, Chichijima Island, Mt. Tsutsujiyama (T. Nakai, July 4, 1932, TI) **Figure 3-3a**

Dryopteris insularis var. *chichisimensis* (Nakai ex H. Ito) H. Ito, in *Nakai et Honda, Nova Fl. Jap.* 4: 57. 1939.

Diagnosis. ***Dryopteris chichisimensis*** Nakai ex H. Ito is an apogamous species of hybrid origin between *D. hikonensis* and *D. insularis*. Its genome constitution consists of those from *D. varia, D. protobissetiana* and *D. insularis*. This species is hardly distinguished from *D. hikonensis* based only on morphological traits. Therefore, it is recommended to check nuclear DNA constitution when reporting new localities of this species. However, *D. chichishimensis* often differs from *D. hikonensis* in scales on petiole being sparser.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome and pinna rachises, sub-sparse on petiole, pinna stalks, rachises; petiole 10–40 cm long; scales lanceolate, filiform at apex; base of scales on basal petiole, upper petiole, and rachises narrow; base of scales on pinna rachises bullate; lamina bipinnate, occasionally tripinnate at base, wide triangular, gradually narrowing to apex, 10–30 cm long, 20–50 cm wide, dark green, soft

coriaceous in texture, surface shiny, flat at margin; pinnules deeply serrated at apical margin; lowest basiscopic pinnules on lowest pinna elongated markedly a little more than second one; sori round, born between the margin and the costa; indusia reniform or circular, ciliate at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 32 spores per sporangium; chromosome number 2*n* = 123, triploid apogamous.

Habitat and distribution. Subtropical wet evergreen forests. Japan (the Ogasawa Islands, including the Kazan-retto Islands). The distribution map for Japan is shown in Figure 3-3b.

Japanese name. Chichijima-Itachishida.

Notes. Iwatsuki (1995) commented on the distribution of this species in Izu-Islands without indicating a voucher specimen. On the other hand, I have found the specimens of this species, newly collected, from Kita-iwoto Island in addition to Chichijima Island in MAK. Previous reports have called this species Chichijima-Benishida, as its Japanese name. However, it does not have an affinity to Benishida (the *D. erythrosora* complex), but rather to Itachishida (the *D. varia* complex). Therefore, a new Japanese name, Chichijima-Itachishida is proposed in this study.

Dryopteris erythrovaria K. Hori et N. Murak., sp. nov. TYPE: Japan, Tokyo, Inagi City, Momura, approximately 100 m altitude, on soil cliff near dry road in forests, K. Hori *2478*, collected on June 18, 2016 (holotype, MAK). **Figure 3-4a**

Diagnosis. ***Dryopteris erythrovaria*** K. Hori et N. Murak. is an apogamous species of hybrid origin between *D. hikonensis* and *D. caudipinna*. Its genome consists of those from *D. varia*, *D. protobissetiana*, and *D. caudipinna*. This species is characterized by the combination of large papyraceous lamina and red indusia. Large papyraceous lamina is one of the characteristics of *D. caudipinna*. Red indusia are also one of the characteristics of *D. caudipinna*, though it sometimes has transpicuous indusia. The above mentioned genome constitution might be the reason why *D. erythrovaria* sometimes has transpicuous indusial, like the other members of the *D. varia* complex.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, petiole, pinna stalks, rachises, and pinna rachises; petiole 20–50 cm long; scales lanceolate, filiform at apex; scales on basal petiole black; base of scales on basal petiole narrow; base of scales on upper petiole and rachises spread; base of scales on pinna rachises bullate; lamina bipinnate to tripinnatifid, wide triangular, sub-abruptly narrowing to apex, 30–80 cm long, 20–40 cm wide, dark green or yellowish green, papyraceous in texture, surface shiny or dull, flat at margin; pinnules deeply serrated at apical margin; lowest basiscopic pinnules on lowest pinna

elongated markedly a little more than second one; sori round, born between the margin and the costa; indusia red to white in center or transpicuous, reniform or circular, almost entire or rarely ciliate at margins, approximately 1.5–1.8 mm in diameter; 32 spores per sporangium; chromosome number 2*n* = 123, triploid apogamous.

Habitat and distribution. Warm temperate evergreen forests. Japan (Honshu, Shikoku, Kyusyu), Korea (Cheju-Island) and eastern parts of mainland China (Anhui, Zhejiang Provinces). The distribution map for Japan is shown in Figure 3-4b.

Japanese name. Beni-O-Itachishida.

Dryopteris hikonensis (H. Ito) Nakaike, in *New Fl. Jp.*: 841. 1992. Type: Japan, Shiga pref. (H. January 15, 1933, TI). **Figure 3-5a**

Polystichum pacificum Nakai in *Bot. Mag. Tokyo* 39: 119. 1925 (illegitimate name)

Polystichum hololepis var. *hikonensis* H. Ito in *J. Jap. Bot.* 10: 451. 1934

Dryopteris bissetiana var. *hikonensis* (H. Ito) in *Bot. Mag. Tokyo* 50: 36. 1936

Dryopteris varia subsp. *hikonensis* (H. Ito) Sugimoto, *Keys Herb. Pl. Jap. Pterid.* 281. 404. 1966

Dryopteris bissetiana var. *typica* H. Ito in *Nakai et Honda, Fl. Jap.* 4: 55. 1939.

Dryopteris fuyangensis Ching & P. S. Chiu in *Bot. Res. Academia Sinica* 2: 26–7, t. 9, f. 4. 1987

Dryopteris immixta Ching in *Fl. Tsinling.* 2: 225–226, pl. 41, f. 1–2. 1974

Dryopteris lungjingensis Ching & P. S. Chiu [*"luntsingensis"*] in *Bot. Res. Academia Sinica* 2: 27–28, pl. 10. 1987

Dryopteris paravaria Ching & P. S. Chiu in *Bot. Res. Academia Sinica* 2: 22–23, t. 8, f. 3. 1987

Dryopteris pudouensis Ching in Bull. in *Bull. Bot. Res. Harbin* 3(3): 11–12, f. 9. 1983

Dryopteris quadrifida Ching ex K. H. Shing & J. F. Cheng in *Jiangxi Sci.* 8(3): 49. 1990

Dryopteris shanghaiensis Ching & P. S. Chiu in *Bot. Res. Academia Sinica* 2: 24, t. 9, f. 1. 1987

Dryopteris tieanzuensis Ching & P. S. Chiu in *Bot. Res. Academia Sinica* 2:

24–25, t. 9, f. 2. 1987.

Dryopteris yushanensis Ching & P. S. Chiu in *Bot. Res. Academia Sinica* 2: 28–29, t. 10, f. 2. 1987

Diagnosis. ***Dryopteris hikonensis*** (H. Ito) Nakaike is an apogamous species of hybrid origin between *D. varia* and *D. protobissetiana*. This species is different from *D. varia* in having slightly bullate scales on rachises. It is also different from *D. protobissetiana* in having flat scales on petiole, and most basiscopic pinnules are not very markedly elongated.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, petiole, pinna stalks, rachises, and pinna rachises; petiole 10–40 cm long; scales lanceolate, ascending, filiform at apex; scales on basal petiole black to blackish brown; base of scales on basal petiole, upper petiole, and rachises spread; base of scales on pinna rachises bullate; lamina bipinnate, occasionally tripinnate at base, wide triangular, not very abruptly narrowing to apex, 20–50 cm long, 10–30 cm wide, dark, yellowish or whitish green, soft coriaceous in texture, surface shiny or dull, flat at margin; pinnules deeply serrated at apical margin; lowest basiscopic pinnules on lowest pinna elongated markedly a little more than second one; sori round, between the margin and the costa; indusia reniform or circular, entire to ciliate at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 32 spores per

sporangium; chromosome number 2*n* = 82 (diploid apogamous) or 2*n* = 123 (triploid apogamous).

Habitat and distribution. Warm temperate to subtropical evergreen forests. Japan (Honshu, Shikoku, Kyusyu, Ryukyu), Korea, and mainland of China. The distribution map for Japan is shown in Fig. 3-5b.

Japanese name. O-Itachishida.

Dryopteris insularis Kodama, *Icon. Pl. Koisik.* 2: t. 49. 1914. Type: Japan (the Bonin Islands, Tokyo pref.) (TI, Specimen number, collector, and date are not cited). **Figure 3-6a**

Dryopteris insularis var. *typical* H. Ito, in *Nakai et Honda, Nova Fl. Jap.* 4: 57. 1939

Dryopteris varia var. *insularis* (Kodama) H. Ohba, in *Sci. Rep. Tohoku Univ.* (B) 36: 113. 1971.

Diagnosis. ***Dryopteris insularis*** Kodama is characterized by having sori borne only on upper part of lamina and glandular ciliate at margins of indusia. Its genome is different from another sexual species and apogamous species.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome and pinna rachises, not very dense on petiole, pinna stalks, and rachises; petiole 20–35 cm long; scales brown, lanceolate, ascending, filiform at apex; base of scales on basal petiole, upper petiole, and rachises narrow; scales on pinna rachises bullate; lamina bipinnate to tripinnatifid, wide triangular, gradually narrowing to apex, 35–45 cm long, 25–35 cm wide, whitish green, soft coriaceous in texture, surface shiny, flat at margin; pinnules obtuse, finely serrated at apical margin; lowest basiscopic pinnules on lowest pinna elongated but not markedly more than second one; sori round, borne on upper part of lamina and expand downwardly, soriferous pinnae more or less contracted, between margin and the costa on pinnules; indusia reniform, glandular ciliate at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 32 spores per sporangium; chromosome number 2*n* = 82, diploid apogamous.

Habitat and distribution. Subtropical dry evergreen forests. Japan (Bonin Islands). The distribution map for Japan is shown in Figure 3-6b.

Japanese name. Munin-Itachishida.

Notes. In previous studies, this species has been called as Munin-Benishida. However, as is similar in the case of *Dryopteris chichisimensis*, this species does not have an affinity to Benishida (the *D. erythrosora* complex), but instead definitely belongs to the group of Itachishida (the *D. varia* complex). Thus, a new Japanese name is proposed in this study.

Dryopteris kobayashii Kitagawa, in *Rep. First Sci. Exped. Manchoukuo* 4 (2): 56–58, f. 11. 1935. Type: China, Fengtian, Hsiao-ping-tao. (M. Kobayahi, n 39. October 9, 1932, TI). **Figure 3-7a**

Diagnosis. ***Dryopteris kobayashii*** Kitagawa is an apogamous species of hybrid origin between *D. bissetiana* and *D. chinensis*. Its genome consists of those from *D. saxifraga*, *D. protobissetiana* and *D. chinensis*. This species is very similar in gross morphology to *D. sacrosancta*, but is distinguishable by its narrowly triangular lamina, curved pinnae with obtuse apex and always-whitish green young pinnule.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, sparse on petiole, pinna stalks, rachises, and pinna

rachises; petiole 10–40 cm long; scales lanceolate, filiform at apex; base of scales on basal petiole, upper petiole narrow; base of scales on rachises spread; base of scales on pinna rachises bullate; lamina bipinnate to tripinnatifid, narrowly triangular, gradually narrowing to apex, 20–40 cm long, 10–20 cm wide, yellowish green, herbaceous in texture, surface dull, flat at margin; pinnules entire or shallowly serrated or entire at apical margin; lowest basiscopic pinnules on lowest pinna longest but not markedly elongated more than second one; sori round, born between the margin and the costa; indusia transpicuous, reniform or circular, entire at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 32 spores per sporangium; chromosome number $2n = 123$, triploid apogamous.

Habitat and distribution. Warm temperate evergreen forests. Japan (Honshu, Shikoku, Kyushu), Korea, and northeastern part of mainland China. The distribution map for Japan is shown in Figure 3-7b.

Japanese name. Ryoto-Itachishida.

Dryopteris protobissetiana K. Hori et N. Murak., in *Acta Phytotaxa. Geobot.* (2015). Type: Japan, Kagoshima pref., Yakushima Island, Mt. Myojo, 300 m alt, K. Hori *Dpaci 913* (holotype, MAK). **Figure 3-8a**

Diagnosis. ***Dryopteris protobissetiana*** K. Hori et N. Murak. is most similar to *D. bissetiana* (Baker) C. Chr. in having slightly bullate scales and a dark green lamina surface, but differs from it in having flat and serrated margins at apexes of upper pinnae and flat lamina margins.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, petiole, pinna stalks, rachises, and pinna rachises; petiole 10–30 cm long; base of scales on basal petiole and upper petiole spread; base of scales on rachises and pinna rachises bullate; lamina bipinnate, occasionally tripinnate at base, narrowly triangular, gradually narrowing to apex, 10–40 cm long, 10–20 cm wide, dark green, soft coriaceous in texture, surface shiny, flat at margin; pinnules finely serrated at apical margin; lowest basiscopic pinnules on lowest pinna elongated but not markedly more than second one; sori round, born between the margin and the costa or relatively nearer to the margin than to the costa; indusia reniform or circular, entire or erose at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 64 spores per sporangium; chromosome number $2n = 82$, diploid sexual.

Habitat and distribution. Warm temperate evergreen forests. Japan (southern part of Yakushima Island, Kagoshima pref., Kyushu). The distribution map for Japan is shown in Figure 3-8b.

Japanese name. Moto-Itachishida.

Dryopteris sacrosancta Koidz., in *Bot. Mag. Tokyo* 38: 108. 1924. Type: Japan, Hiroshima pref., Miyajima Island. (Faurie, November 1913, TI). **Figure 3-9a**

Polystichum sacrosanctum (Koidz.) Koidz., in *Bot. Mag. Tokyo* 43: 388. 1929

Polystichum bissetianum var. *sacrosanctum* (Koidz.) Nakai, in *Bot. Mag. Tokyo* 45: 103. 1931

Dryopteris bissetiana var. *sacrosancta* (Koidz.) H. Ito, in *Bot. Mag. Tokyo* 50: 36. 1936

Dryopteris varia subsp. *sacrosancta* (Koidz.) Sugimoto, *Keys Herb. Pl. Jap. Pterid.* 281. 405. 1966

Dryopteris bissetiana var. *tenuifrons* H. Ito, in *Bot. Mag. Tokyo* 50: 37. 1936

Dryopteris varia var. *sacrosancta* (Koidz.) Ohwi, *Fl. Jap. Pterid.* 88. 1957.

Diagnosis. ***Dryopteris sacrosancta*** Koidz. is an apogamous species of hybrid origin between *D. hikonensis* and *D. chinensis*. Its genome consists of those from *D. varia*, *D. protobissetiana* and *D. chinensis*. This species and *D. kobayashii* are very similar in morphology by having herbaceous yellowish green lamina. The scales of these two species are sparser than the other members of the *D. varia* complex. However, this species is distinguished from *D. kobayashii* by having widely triangular lamina,

pinnae with straightly acute apex, and sometimes reddish-brown young pinnule.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, sparse on petiole, pinna stalks, rachises, and pinna rachises; petiole 10–40 cm long; scales lanceolate, filiform at apex; scales on basal petiole black, transpicuous at margin; base of scales on basal petiole, upper petiole, rachises, and pinna rachises spread; lamina bipinnate to tripinnatifid, pentagonal, sub-abruptly narrowing to apex, 20–50 cm long, 10–30 cm wide, yellowish green, herbaceous in texture, surface weakly shiny or dull, flat at margin; pinnules finely serrated at apical margin; lowest basiscopic pinnules on lowest pinna elongated markedly a little more than second one; sori round, born between the margin and the costa; indusia transpicuous, reniform or circular, entire at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 32 spores per sporangium; chromosome number $2n = 123$, triploid apogamous.

Habitat and distribution. Warm temperate evergreen forests. Endemic to Japan (Honshu, Shikoku, Kyusyu). The distribution map is shown in Figure 3-9b.

Japanese name. Hime-Itachishida.

Dryopteris saxifraga H. Ito, in *Bot. Mag. Tokyo*. 50: 125 (1936). Type: Japan, Shizuoka pref. or Yamanashi pref., Mt. Hujisan (B. Hayata F3, TI). **Figure 3-10a**

Dryopteris varia subsp. *saxifraga* (H. Ito) Sugimoto, *Keys Herb. Pl. Jap. Pterid.* 282. 405. 1966

Dryopteris varia var. *saxifraga* (H. Ito) H. Oba, in *Sci. Rep. Tohoku Univ.* (B) 36: 111. 1971.

Diagnosis. ***Dryopteris saxifraga*** H. Ito is characterized by having pinnules with entire margin and oblong lamina. This species is a sexual diploid and has a distinct genome.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, petiole, pinna stalks, rachises, and pinna rachises; petiole 5–20 cm long; scales lanceolate, deflected, filiform at apex; base of scales on basal petiole spread; base of scales on upper petiole, rachises, and pinna rachises bullate; lamina bipinnate, occasionally tripinnate at base, oblong, gradually narrowing to apex, 5–30 cm long, 5–15 cm wide, whitish green, soft coriaceous in texture, surface dull, recurved at margin; pinnules obtuse, entire or sinuate at apical margin; lowest

basiscopic pinnules on lowest pinna elongated but not markedly more than second one; sori round, born between the margin and the costa; indusia reniform or circular, entire or erose at margins, transpicuous, approximately 1.5–2.0 mm in diameter; 64 spores per sporangium; chromosome number 2*n* = 82, diploid sexual.

Notes. From South Korea, Lee & Park (2006) reported the triploid apogamous cytotype of *Dryopteris saxifraga*. Lee & Park (2013) reported sequences of nuclear *PgiC* from the triploid apogamous *D. saxifraga*, which made a clade with those of diploid sexual *D. saxifraga* in their *PgiC* molecular tree. However, *D. bissetiana* is sometimes similar to *D. saxifraga* in gross morphology because *D. bissetiana* is of hybrid origin between *D. saxifraga* and *D. protobissetiana*. Their plant samples were confirmed as triploid apogamous. On the other hand, so far, only the diploid sexual cytotype has been found from *D. saxifraga* in Japan. These facts suggested that triploid apogamous *D. saxifraga* in Lee & Park (2013) might be *D. bissetiana*.

Habitat and distribution. Deciduous broad-leaved forests. Japan (Hokkaido, Honshu, Shikoku, Kyusyu), Korea, and the mainland of northeastern China. The distribution map for Japan is shown in Figure 3-10b.

Japanese name. Iwa-Itachishida.

Dryopteris subhikonensis K. Hori et N. Murak., sp. nov. TYPE: Japan, Saitama, Han-nou City, Shirako, 200 m, on soil cliff near dry road in forests, K. Hori *2270*, collected on July 2, 2016 (holotype, MAK). **Figure 3-11a**

Diagnosis. ***Dryopteris subhikonensis*** K. Hori et N. Murak. is an apogamous species of hybrid origin between *D. hikonensis* and *D. saxifraga* or between *D. bissetiana* and *D. varia*. Its genome consists of those from *D. protobissetiana, D. saxifrage,* and *D. varia*. This species is hardly distinguished from *D. hikonensis* based only on their morphological traits; therefore, it is recommended to check nuclear DNA constitution when reporting new localities of this species. However, *D. subhikonensis* is usually different from *D. hikonensis* in having shallowly serrated or entire pinnules, as well as entire indusia. *D. hikonensis* is commonly observed in the forests along the Pacific Ocean and Seto Inland sea, whereas *D. subhikonensis* is mainly observed in the mountains in southeastern Honshu and in the forests along the Japan sea.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, petiole, pinna stalks, rachises, and pinna rachises; petiole 10–40 cm long; scales lanceolate, filiform at apex; base of scales on basal petiole and upper petiole narrow; base of scales on rachises spread; base of scales on

pinna rachises bullate; lamina bipinnate, occasionally tripinnate at base, wide triangular, sub-abruptly narrowing to apex, 20–50 cm long, 10–30 cm wide, dark green, soft coriaceous in texture, surface weakly shiny, flat at margin; pinnules shallowly serrated or entire at apical margin; lowest basiscopic pinnules on lowest pinna elongated markedly a little more than second one; sori round, born between the margin and the costa; indusia reniform or circular, entire at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 32 spores per sporangium; triploid apogamous (ploidy level was checked by ploidy analysis, the chromosome number was not determined).

Habitat and distribution. Warm temperate evergreen forests. Japan (South eastern Honshu and southern part of Japan sea side). The distribution map for Japan is shown in Figure 3-11b.

Japanese name. Iwa-O-Itachishida.

Dryopteris varia (L.) Kuntze **Figure 3-12a**

Fraser-Jenkins (1986) designated holotypes of *Lastrea opaca* Hook. and

Nephrodium coriaceum C. Hope as the lectotype, because the holotype of *Polypodium varium* L. was lost.

Lastrea opaca Hook., in *Hooker's J. Bot.* 9: 339 (1857).–*Aspidium opacum* (Hook.) Benth., *Fl. Hongk.*: 456 (1861). Type: Hong Kong and mainland N.W. of Hong Kong, *J. C. Bowman* (K!–lectotype)

Nephrodium coriaceum C. Hope, in *J. Bot., Lond*. 28: 328 (1890). Type: India, Kapili Hot Springs, North Cachar Hills, Assam, 1000ft, February 1890, *Gustav Mann* (K - lectotype; BM, DD, E, K, P, PE - isolectotypes).

Polypodium varium L., *Sp. Pl.* 2: 1090. 1753

Aspidium varium (Linnaeus) Sweet in *Schrad. J. Bot.* 1800–2: 35.1801

Nephrodium varium (L.) C. Presl, *Reliq. Haenk.* 1. 36. 1825

Polystichum varium (L.) C. Presl, *Abh. Königl. Böhm. Ges. Wiss., ser.* 5. 1851

Lastrea varia (L.) Moore, *Ind. Fil.* 107. 1858

Aspidium opacum (Hooker) Bentham., *Fl. Hongk.* 456. 1861

Dryopteris yabei Hayata, *Mat. Fl. Formos.* 424. 1911

Polystichum hololepis Hayata, *Ic. Pl. Formos.* 5: 332. 1915

Dryopteris matsuzoana Koidz., in *Bot. Mag. Tokyo* 39: 15. 1925

Dryopteris ogawai H. Ito in Nakai, *Ic. Pl. As. Or.* 1: 18. Pl. 9. 1935

Dryopteris yabei var. *ogawai* (H. Ito) H. Ito, in *Bot. Mag. Tokyo* 50: 128. 1935

Dryopteris yabei var. *hololepis* (Hayata) H. Ito, in *Bot. Mag. Tokyo* 50: 128. 1936

Dryopteris yabei var. *matsuzoana* (Koidz.) H. Ito, in *Bot. Mag. Tokyo* 50: 128. 1936

Dryopteris yabei form. *ogawai* (H. Ito) H. Ito, in Nakai et Honda, *Nova Fl. Jap.* 4: 59. 1939

Dryopteris yabei form. *typica* H. Ito, in Nakai et Honda in *Nova Fl. Jap.* 4: 59. 1939

Dryopteris sinobissetiana Ching & Z. Y. Liu in Bull., in *Bot. Res., Harbin* 4(4): 8–9, f. 36. 1984

Dryopteris caudifolia Ching & P. S. Chiu, in *Bot. Res. Academia Sinica* 2: 21–22, t. 8, f. 2. 1987

Dryopteris lingii Ching, in *Bot. Res. Academia Sinica* 2: 23–24, t. 8, f. 4. 1987

Dryopteris glabrescens Ching & P. S. Chiu ex K. H. Shing & J. F. Cheng, in *Jiangxi Sci.* 8(3): 48. 1990

Dryopteris pseudobissetiana Ching ex K. H. Shing & J. F. Cheng, in *Jiangxi Sci*. 8(3): 49. 1990

Diagnosis. ***Dryopteris varia*** (L.) Kuntze is very variable in morphology; however, it can be characterized by having abruptly narrowed lamina at apex, hard texture of lamina, and flat scales. Intraspecific cytological variation is observed in this species, but the genome constitution is different from the other members of the *D. varia* complex.

Plants terrestrial, evergreen, rhizome erect or slightly ascending, leaves cespitose; scales dense on rhizome, petiole, pinna stalks, rachises, and pinna rachises; petiole 10–40 cm long; scales lanceolate, ascending, filiform at apex; base of scales on basal petiole narrow; base of scales on upper petiole, rachises, and pinna rachises spread; lamina bipinnate, occasionally tripinnate at base, pentagonal to wide triangular, abruptly narrowing to apex, 20–40 cm long, 10–30 cm wide, dark, yellowish or whitish green, hard coriaceous in texture, surface shiny to dull, flat at margin; pinnules acute, entire, shallowly, finely or deeply serrated at apical margin; lowest basiscopic pinnules on lowest pinna elongated markedly more than second one; sori round, between the margin and the costa; indusia reniform or circular, entire to ciliate at margins, transpicuous, approximately 1.5–1.8 mm in diameter; 64 or 32 spores per sporangium; chromosome number $2n = 82$ (diploid sexual, known only from Taiwan) or 123 (triploid apogamous).

Notes. Tsai & Shieh (1975, 1985) reported the tetraploid sexual cytotype of *Dryopteris varia* from Taiwan. However, so far, tetraploid cytotype has never been found, even though Ebihara et al. (2014) and I (the present study) cytologically reexamined four and five individuals of *D. varia*, respectively, from the same localities in Taiwan, respectively. Lin et al. (1995) reported one individual of the "diploid apogamous" form from Mie pref., Japan. However, they did not indicate voucher specimen, and it is impossible to confirm the information. Hirabayashi (1966, 1967) also reported the diploid apogamous cytotype from Mie pref. and Wakayama pref., Japan. He did not indicate voucher specimen. Taxonomy of the *D. varia* complex is confused, and the plants of *D. hikonensis* might be often misidentified as *D. varia* because they are similar in gross morphology.

Habitat and distribution. Warm temperature to subtropical evergreen forests. Japan (Honshu, Shikoku, Kyushu, Ryukyu), Korea, the mainland of China, Taiwan, India, Thailand, Indochina, and the Philippines. The distribution map for Japan is shown in Figure 3-12b.

Japanese name. Nankai-Itachishida.

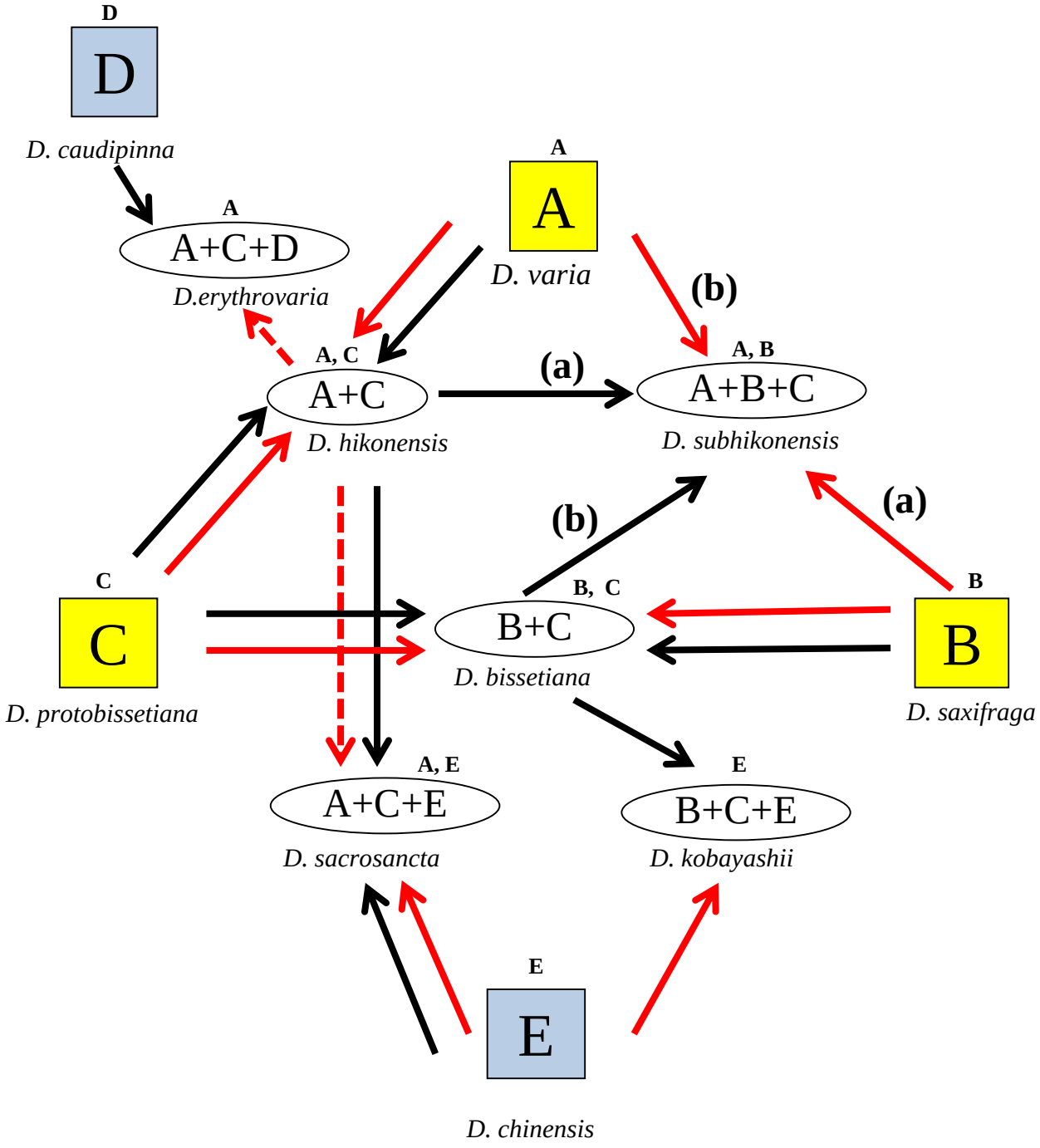

Figure 3-1. The reticulogram of the *Dryopteris varia* complex and revised scientific name of each species. This figure is the same as Figure 1-3 except that revised scientific names for the three types of *D. pacifica* (α, β, γ) are indicated as *D. hikonensis*, *D. subhikonensis* and *D. erythrovaria*, respectively.

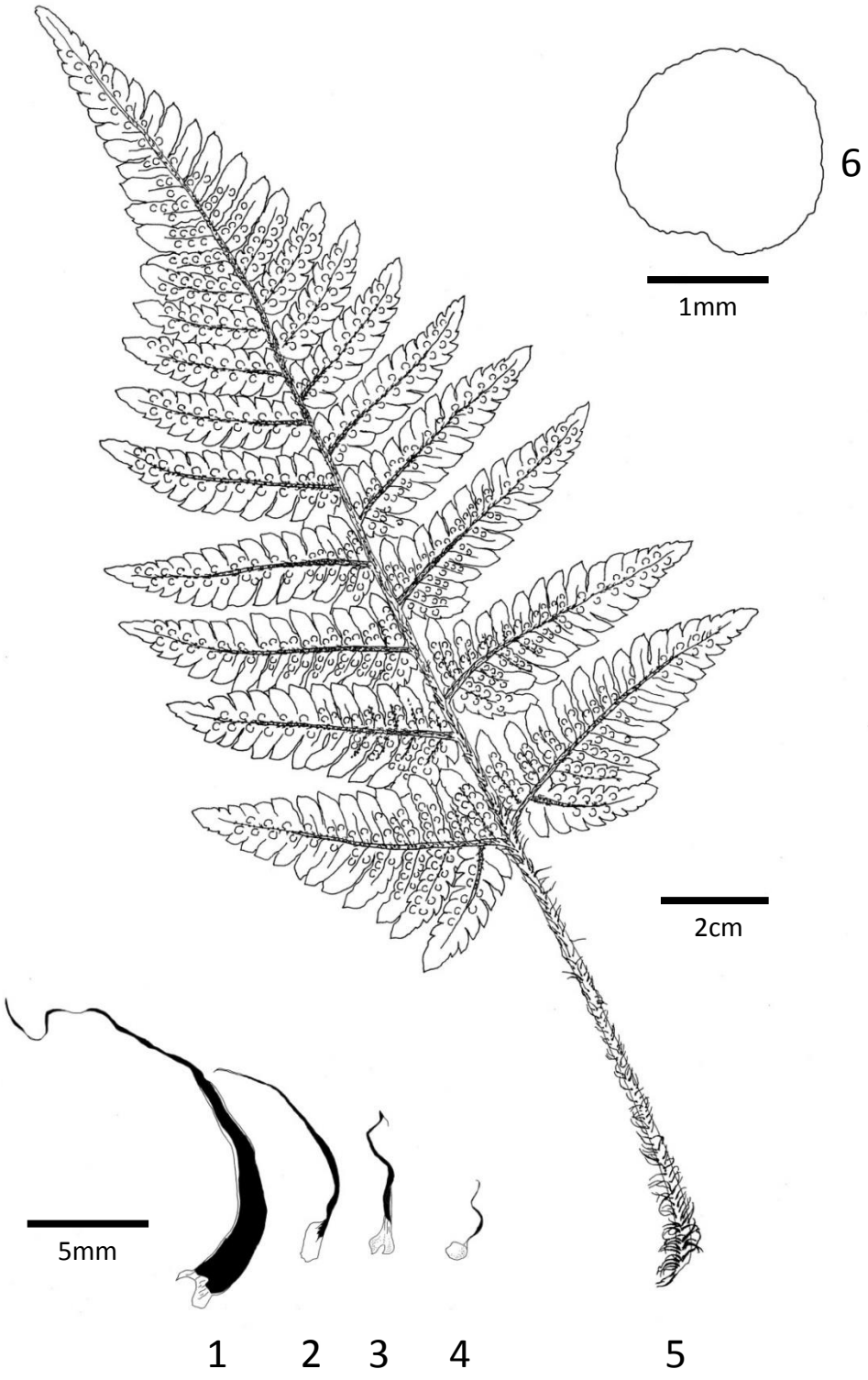

Figure 3-2a. ***Dryopteris bissetiana*** **(Baker) C.Chr.** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from the specimen of *MAK 449107*).

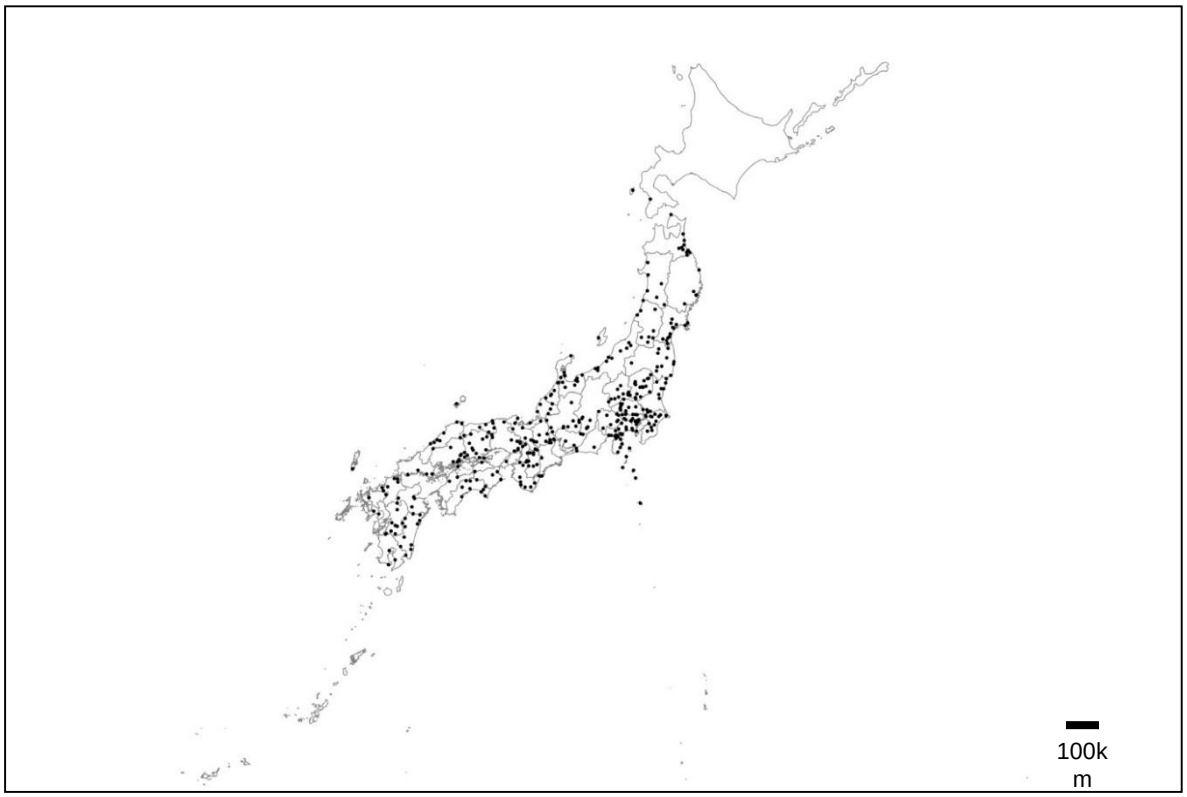

Figure 3-2b. The distribution map of *Dryopteris bissetiana* (Baker) C.Chr. in Japan.

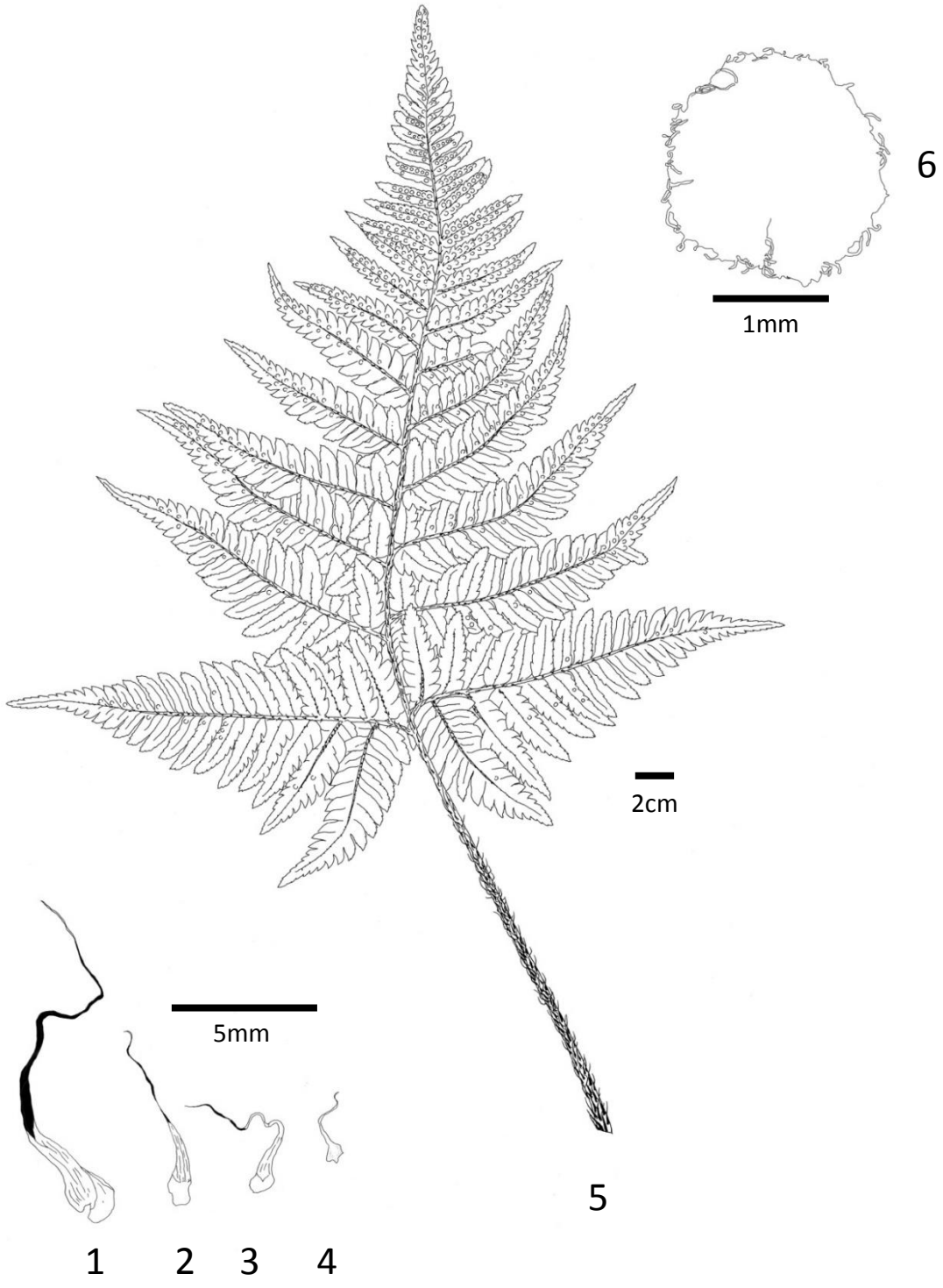

Figure 3-3a. ***Dryopteris chichisimensis*** **Nakai ex H. Ito** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from the specimen of *MAK 449110*).

Figure 3-3b. The distribution map of *Dryopteris chichisimensis* Nakai ex H. Ito in Japan.

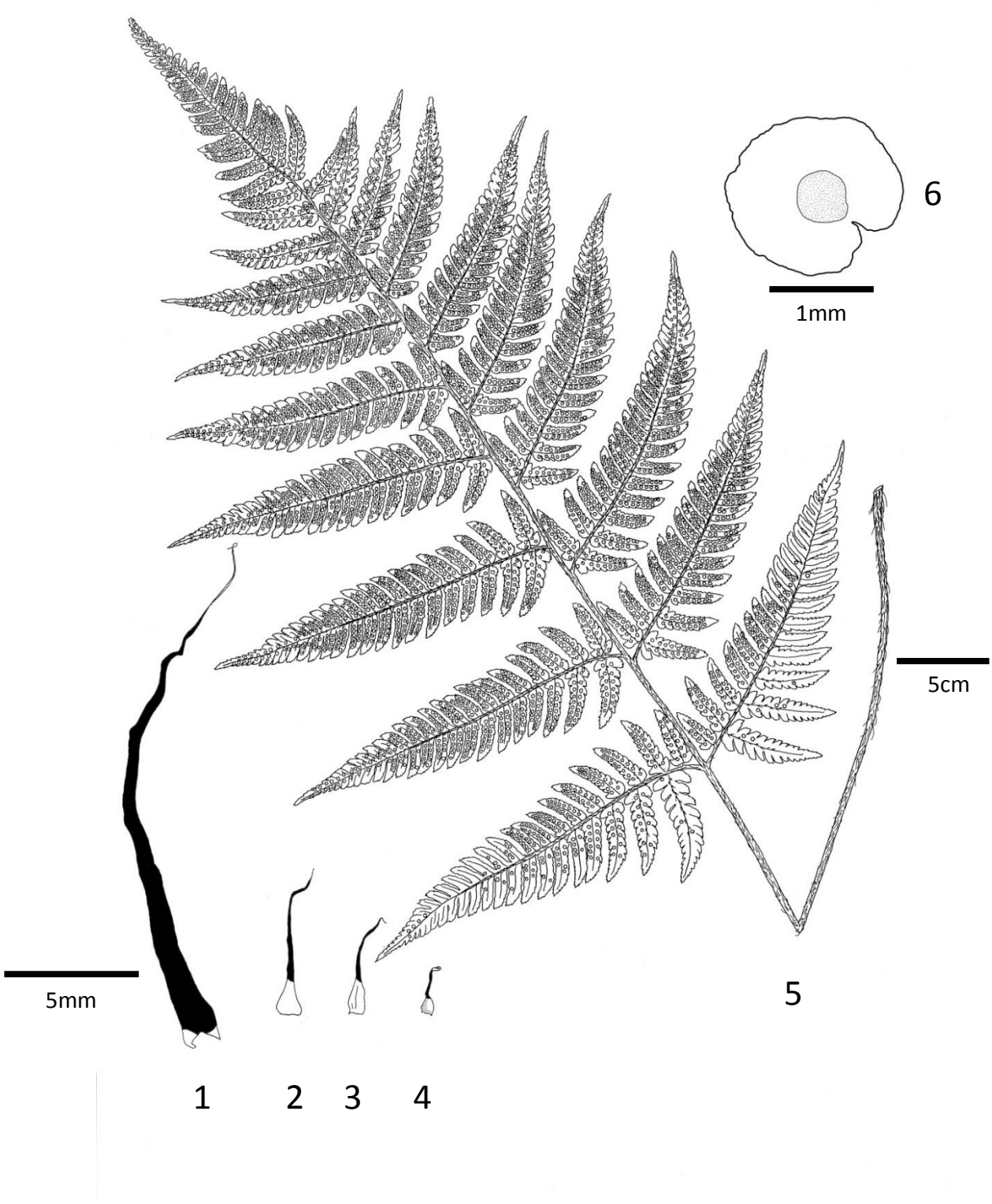

Figure 3-4a. *Dryopteris erythrovaria* K. Hori et N. Murak. 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from type specimen of *MAK 449109*).

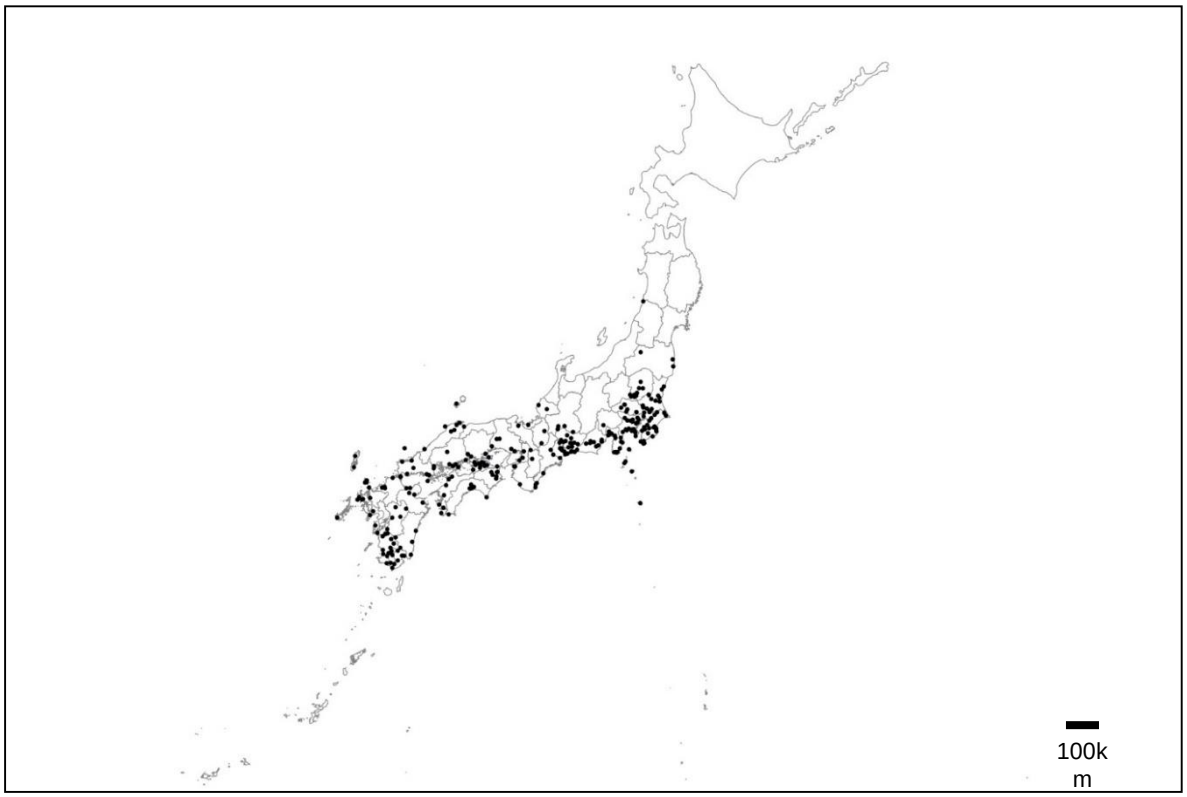

Figure 3-4b. The distribution map of *Dryopteris erythrovaria* K.Hori et N.Murak. in Japan.

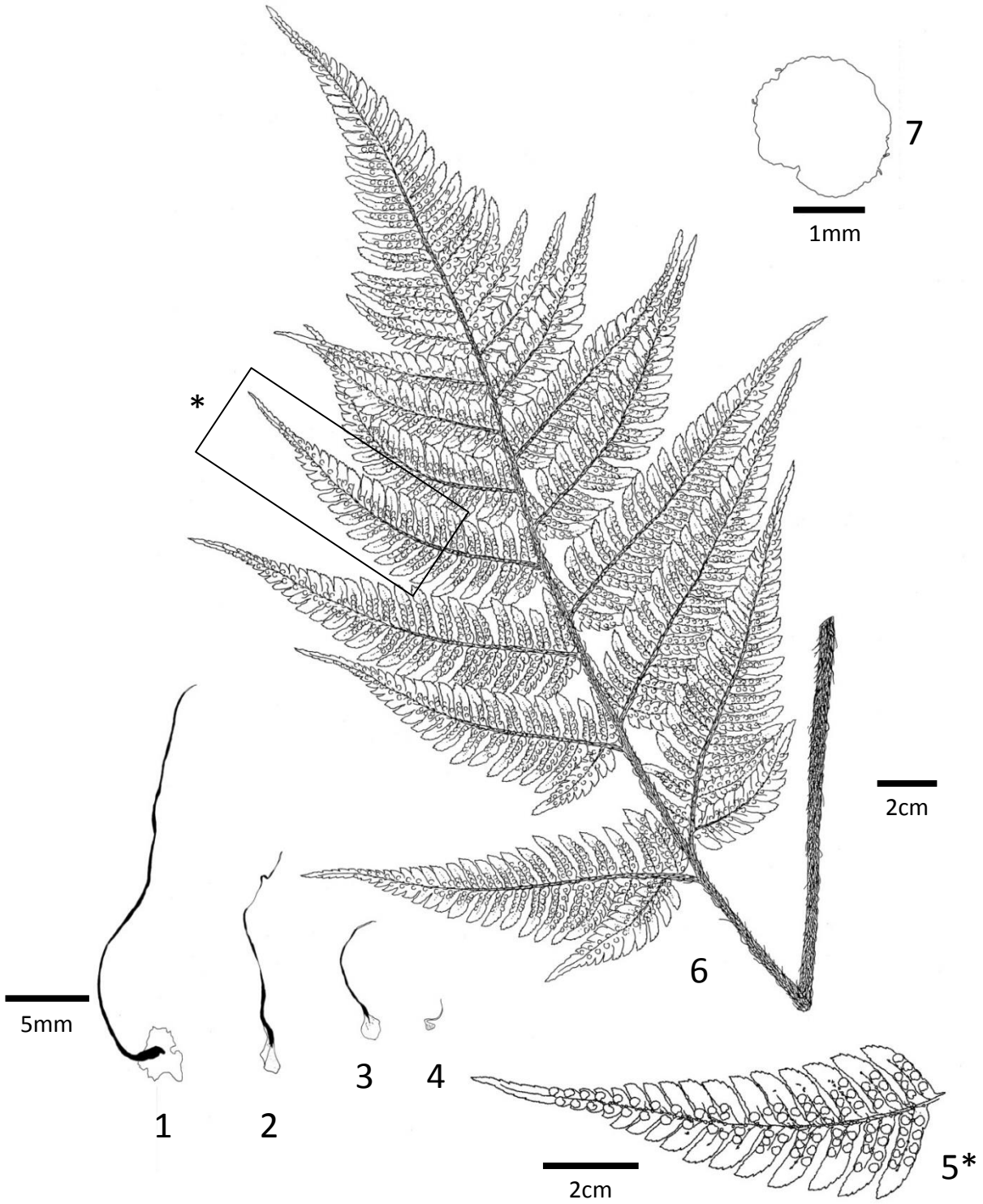

Figure 3-5a. ***Dryopteris hikonensis*** **(H. Ito) Nakaike** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of upper pinnae. 6: abaxial surface of frond. 7: indusium. (drawn from the specimen of *MAK 449113*).

Figure 3-5b. The distribution map of *Dryopteris hikonensis* (H. Ito) Nakaike in Japan.

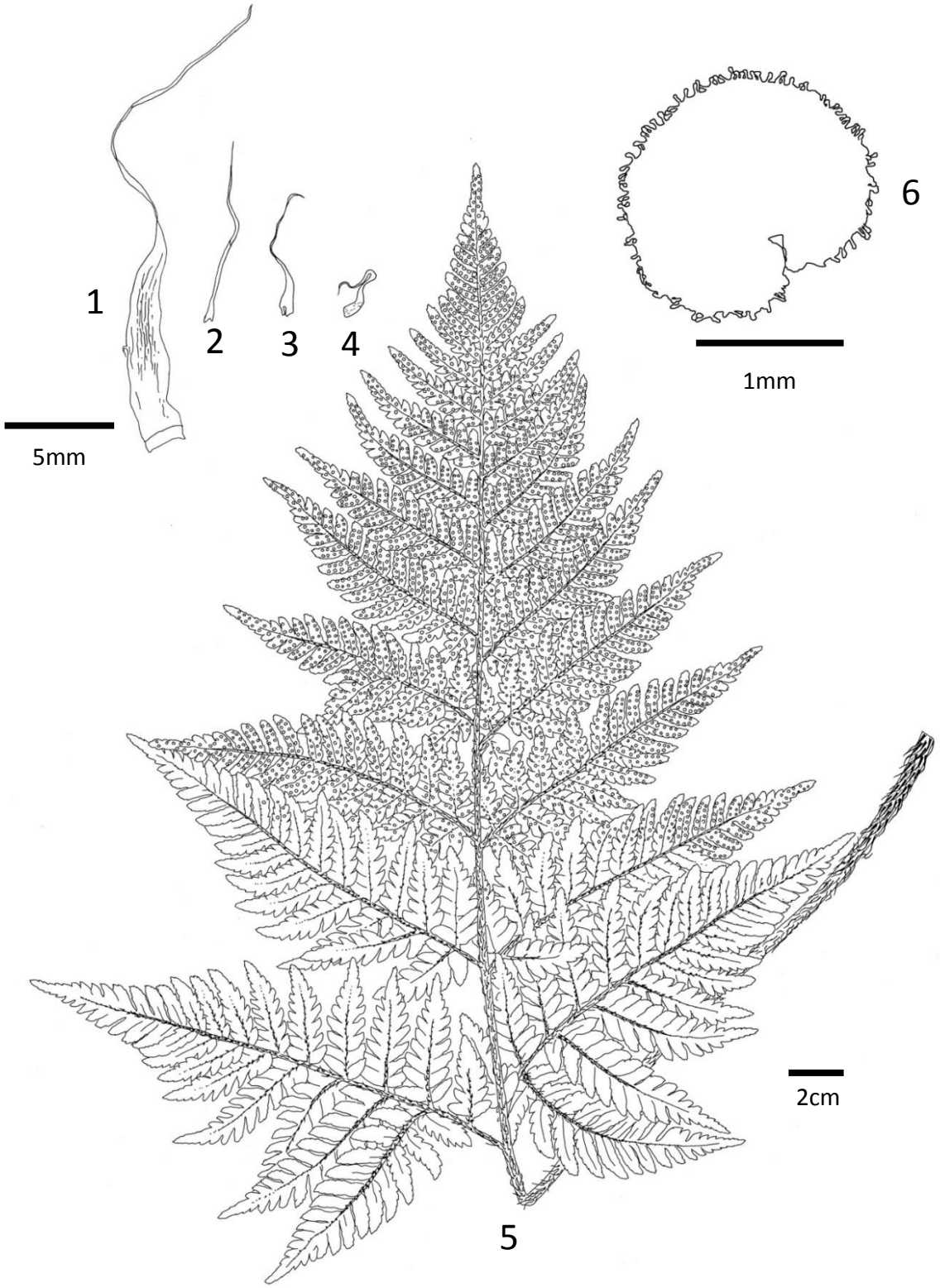

Figure 3-6a. ***Dryopteris insularis*** **Kodama** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from the specimen of *MAK 449111*).

Figure 3-6b. The distribution map of *Dryopteris insularis* Kodama in Japan.

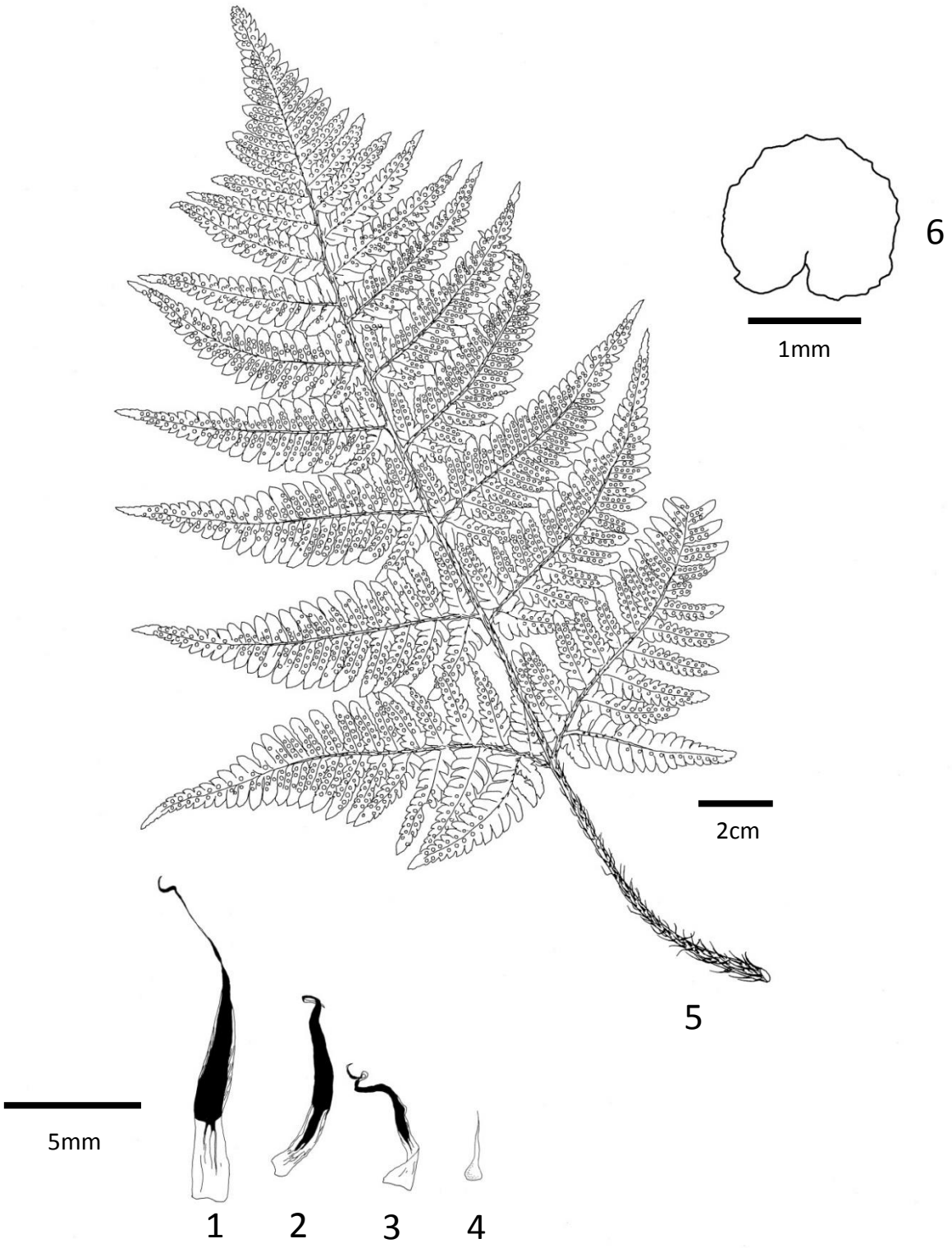

Figure 3-7a. ***Dryopteris kobayashii*** **Kitagawa** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from the specimen of *MAK 449112*).

Figure 3-7b. The distribution map of *Dryopteris kobayashii* Kitagawa in Japan.

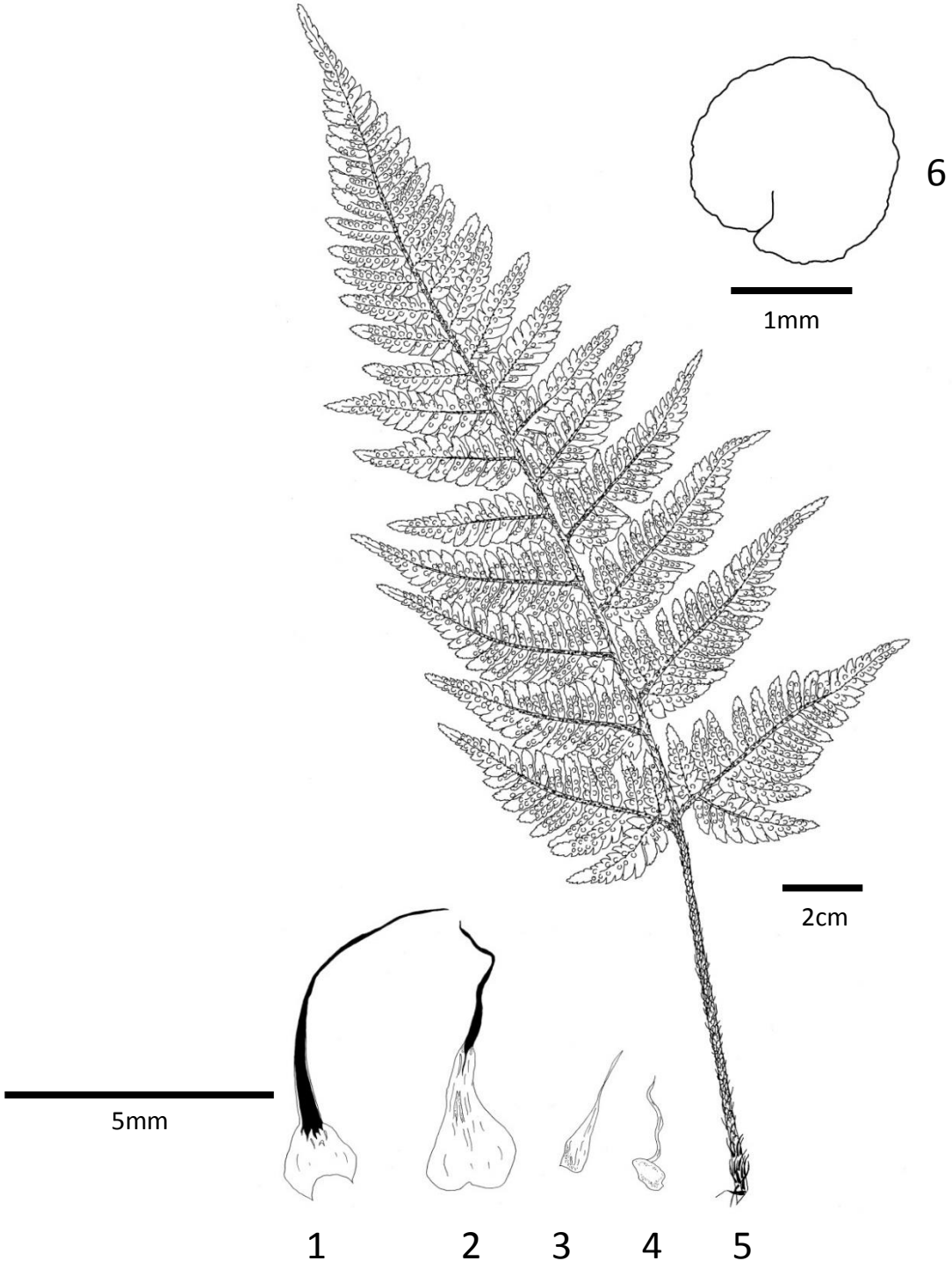

Figure 3-8a. ***Dryopteris protobissetiana*** **K. Hori et N. Murak.** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from type specimen of *MAK 449114*).

Figure 3-8b. The distribution map of *Dryopteris protobissetiana* K.Hori et N.Murak. in Japan.

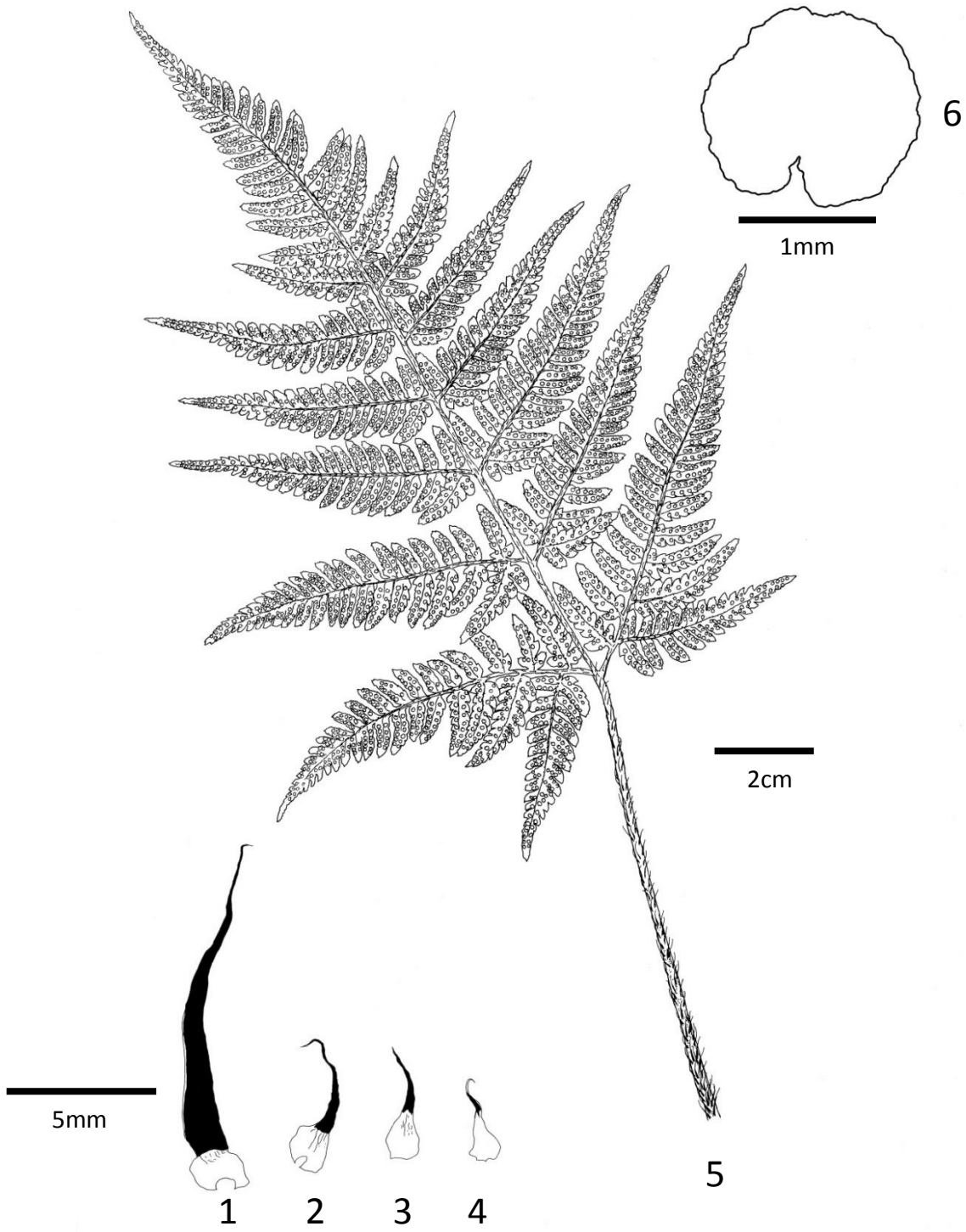

Figure 3-9a. *Dryopteris sacrosancta* Koidz. 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from the specimen of *MAK 449115*).

Figure 3-9b. The distribution map of *Dryopteris sacrosancta* Koidz. in Japan.

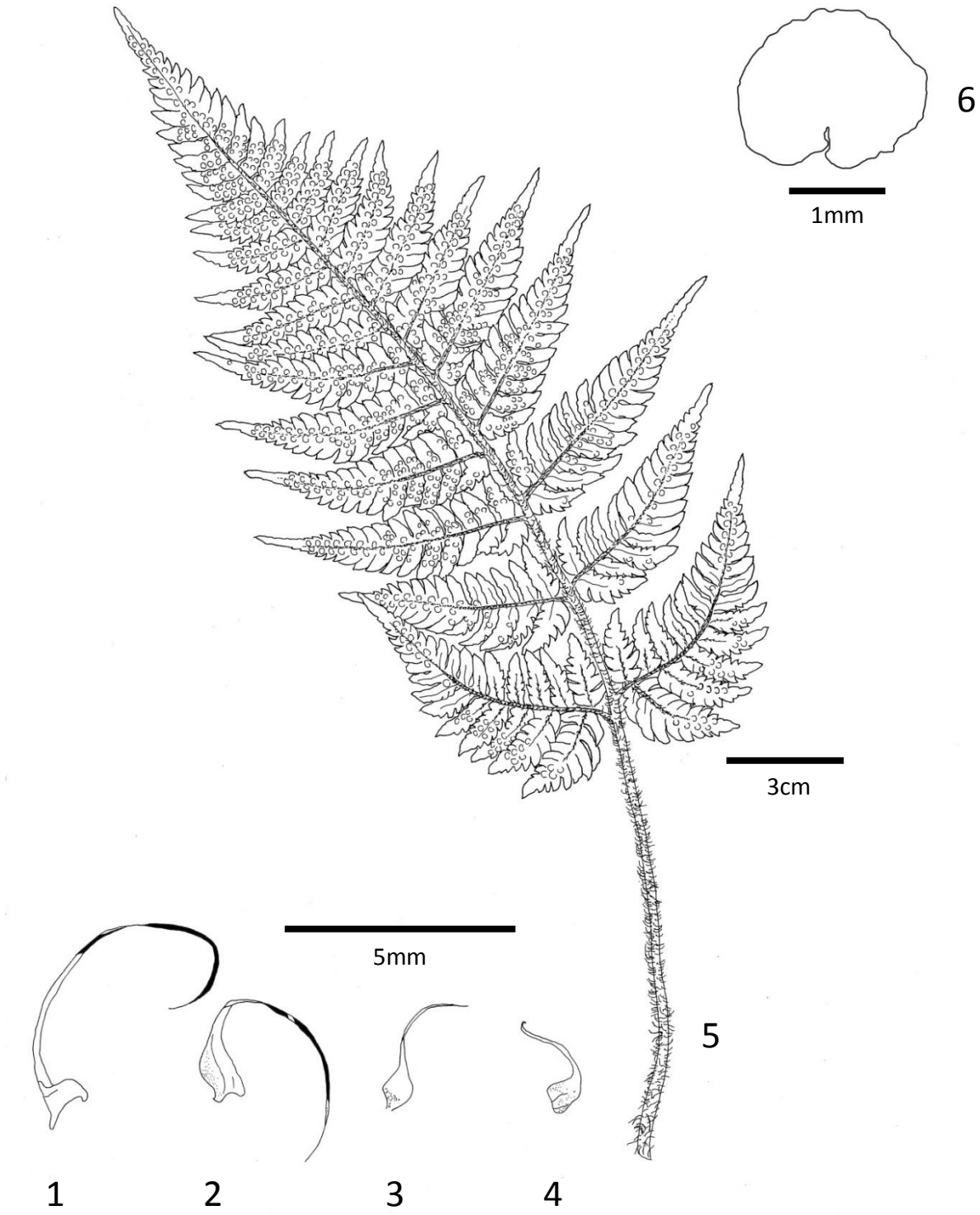

Figure 3-10a. ***Dryopteris saxifraga*** **H. Ito** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from the specimen of *MAK 449116*).

Figure 3-10b. The distribution map of *Dryopteris saxifraga* H. Ito in Japan.

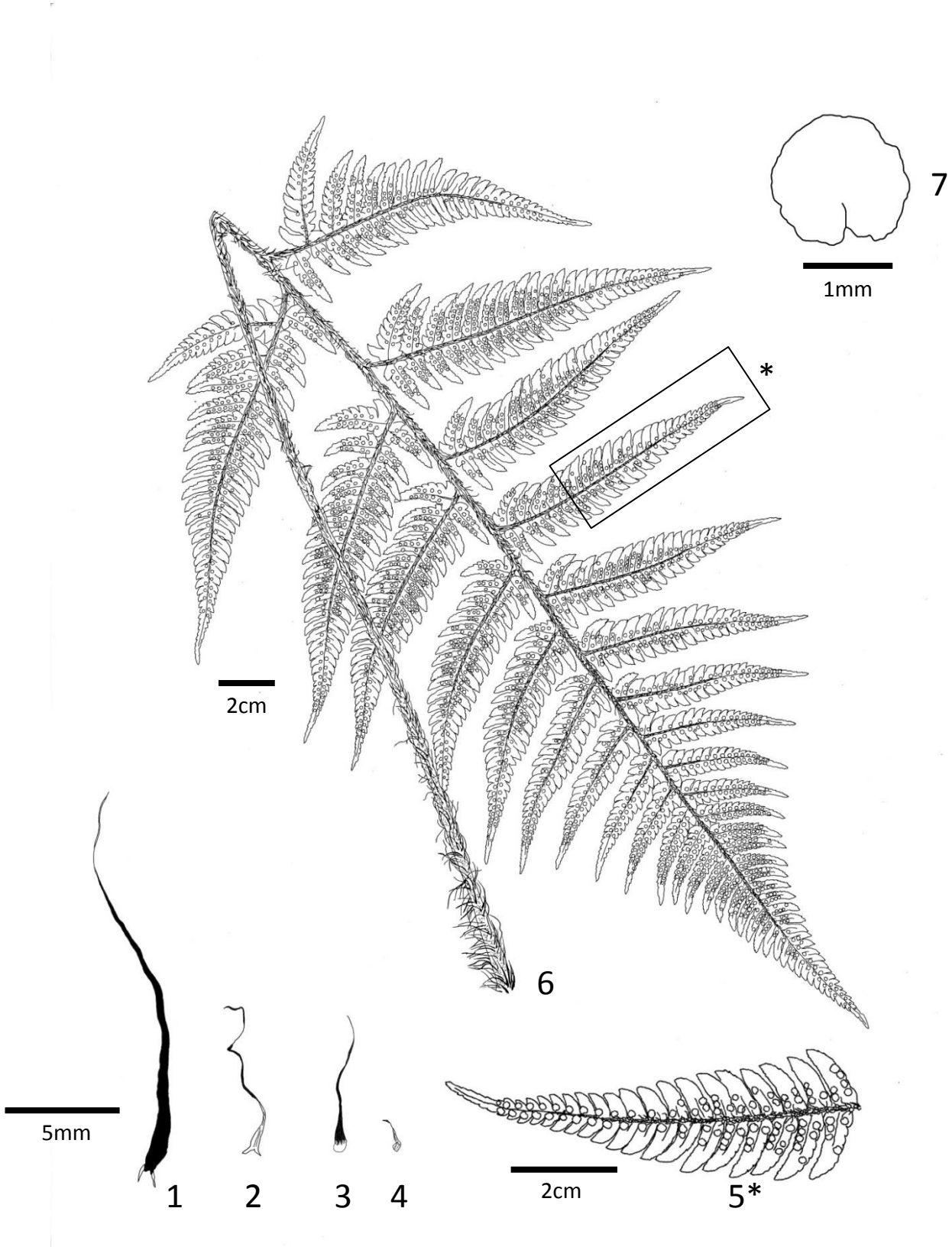

Figure 3-11a. ***Dryopteris subhikonensis*** **K.Hori et N.Murak.** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of upper pinnae. 6: abaxial surface of frond. 7: indusium. (drawn from type specimen of *MAK 449117*).

Figure 3-11b. The distribution map of *Dryopteris subhikonensis* K. Hori. et N. Murak. in Japan.

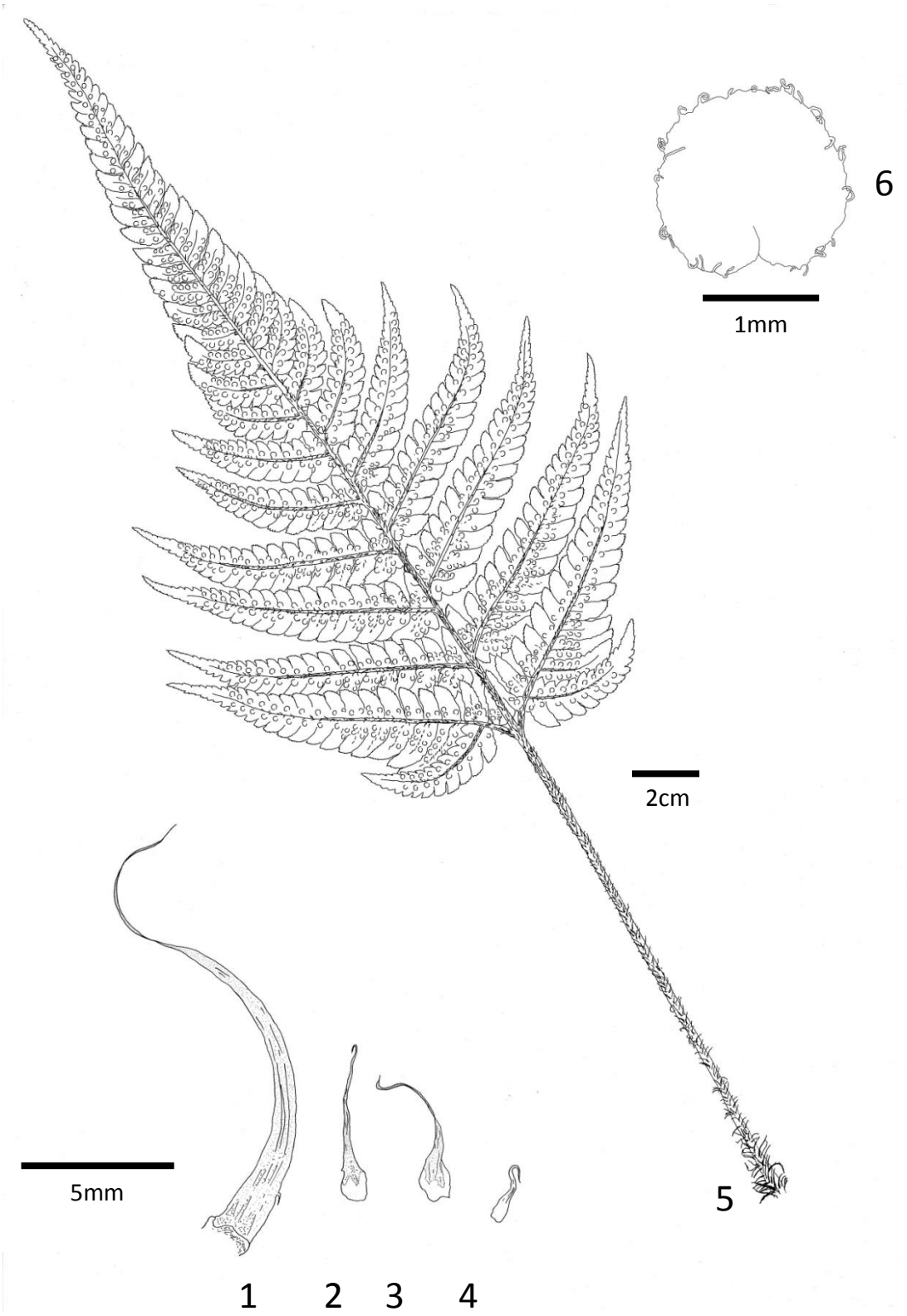

Figure 3-12a. ***Dryopteris varia*** **(L.) Kuntze** 1-4: Morphology of the scales on basal petioles, upper petioles, rachis and pinna rachises. 5: abaxial surface of frond. 6: indusium. (drawn from the specimen of *MAK449120*).

Figure 3-12b. The distribution map of *Dryopteris varia* (L.) Kuntze in Japan.

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
Japan									
D. bissetiana	709002				1998	7	20	H. Marui	Aichi pref., Inuyama city, Tsugao
D. bissetiana	817794				1977	1	12	F. Miyamoto	Aichi pref., Chiryuu city, Koubou town
D. bissetiana	439181				1978	8	23	M. Fukuhara	Aichi pref., Kitashitara county, Toyone village, Sakauba
D. bissetiana	439175				1981	4	12	K. Inukai	Aichi pref., Toyokawa city, Zaika town
D. bissetiana	439572				1981	5	31	K. Inukai	Aichi pref., Toyohashi city, Ishinomaki town, Oike
D. bissetiana	449345				1983	12	11	K. Inukai	Aichi pref., Toyohashi city, Hosoya town
D. bissetiana	1246309				2010	6	27	M. Hyodo	Ehime pref., Imabari city, Hakata town, Kitaura
D. bissetiana	573313				1981	12	31	M. Hyodo	Ehime pref., Iyo county, Toyobe town, Asou
D. bissetiana	1246857				1989	4	3	M. Hyodo	Ehime pref., Saijo city, Yoshii
D. bissetiana	512507				1974	10	5	N. Wadao	Ibaraki pref., Kitaibaraki city, Sekimoto town
D. bissetiana	512486				1974	10	24	M. Yasu	Ibaraki pref., Takahagi city, Takado
D. bissetiana	512512				1981	7	10	E. Fukushi	Ibaraki pref., Hitachi city, Kawarago
D. bissetiana	512470				1979	7	8	M. Yasu	Ibaraki pref., Hitachiota city, Hase
D. bissetiana	512509				1982	7	31	M. Yasu	Ibaraki pref., Kuji county, Oogo town, Fukuroda
D. bissetiana	512492				1980	9	21	M. Yasu	Ibaraki pref., Mito city, Kanamachi town
D. bissetiana	512484				1975	6	4	M. Yasu	Ibaraki pref., Higashiibaraki county, Amigake
D. bissetiana	512503				1975	8	27	Y. Nakazaki	Ibaraki pref., Ryuugasaki city, Kitakata town
D. bissetiana	512479				1975	8	27	M. Yasu	Ibaraki pref., Toride city, Shimotakai
D. bissetiana	1039387				1959	10	19	H. Ito	Okayama pref., Niimi city, Ikura
D. bissetiana	512095				1982	9	18	Y. Obata	Okayama pref., Bizenn city, Katomoto
D. bissetiana	512099				1982	10	11	Y. Obata	Okayama pref., Kurashiki city, Asabara
D. bissetiana	461784				1983	3	8	Y. Obata	Okayama pref., Tamano city, Hirooka
D. bissetiana	512050				1982	10	13	Y. Obata	Okayama pref., Souja city, Minagi
D. bissetiana	512101				1982	9	22	Y. Obata	Okayama pref., Takahashi city, Shimomura
D. bissetiana	448643				1984	2	20	Y. Obata	Okayama pref., Ibara city, Ooe town, Kajikusa
D. bissetiana	1246785				1984	7	1	Y. Obata	Okayama pref., Aida county, Nishiawakura village, Kageishi
D. bissetiana	1246850				1992	6	28	Y. Obata	Okayama pref., Maniwa county, Shinnjo village
D. bissetiana	1246933				1983	9	18	T. Saito	Okayama pref., Katsuta county, Nagi town
D. bissetiana	1246948				1983	4	18	Y. Obata	Okayama pref., Tsuyama city, Yokoyama
D. bissetiana	493191				1981	10	18	Y. Oda	Iwate pref., Shimohei county,Tanohata village, Kitayamazaki
D. bissetiana	493190				1980	7	26	E. Fukushi	Iwate pref., Kamaishi city, Nitta
D. bissetiana	493255				1972	10	18	K. Inoue	Iwate pref., Kamaishi city, Kojirahama
D. bissetiana	493189				1980	7	26	E. Fukushi	Iwate pref., Kamaishi city, Hashino village, Nakamura
D. bissetiana	493202				1980	11	30	M. Suzuki	Iwate pref., Higashiiwai county, Daito town, Oohara
D. bissetiana	1253266				2014	11	15	T. Ogi	Gifu pref., Ibi county, Ibigawa town, Sakauchikawakami
D. bissetiana	1253098				2013	7	27	T. Ogi	Gifu pref., Nakatsugawa city, Yamaguchi
D. bissetiana	504124				1982	9	10	H. Nagase	Gifu pref., Takayama city, Enako town
D. bissetiana	444972				1983	9	23	N. Nimura	Gifu pref., Kamo county, Shirakawa town
D. bissetiana	446030				1983	10	30	K. Inukai	Gifu pref., Kaji county, Mitaka town
D. bissetiana	446027				1983	10	23	K. Inukai	Gifu pref., Kamo county, Sakahogi town
D. bissetiana	506318				1979	10	14	Y. Yamamoto	Gifu pref., Fuwa county, Tarui town
D. bissetiana	506320				1977	7	22	Y. Yamamoto	Gifu pref., Yourou county, Yourou town
D. bissetiana	446031				1983	11	6	K. Inukai	Gifu pref., Ena city, Higashino
D. bissetiana	1249962				2013	7	18	Y. Akagi	Miyazaki pref., Nishiusuki county, Hinokage town
D. bissetiana	1039042					5	20	I. Taki	Miyazaki pref., Kobayashi city, Shimobaba
D. bissetiana	556734				1982	9	12	T. Minamitani	Miyazaki pref., Nobeoka city, Hasekawauchi
D. bissetiana	556348				1982	10	4	T. Minamitani	Miyazaki pref., Miyazaki city, Kagamizu
D. bissetiana	556751				1976	2	22	T. Minamitani	Miyazaki pref., Miyakonojo city, Sekinoo fall
D. bissetiana	1263319				2013	7	10	Y. Akagi	Miyazaki pref., Nishiusuki county, Gokase town
D. bissetiana	1263317				2014	9	7	Y. Akagi	Miyazaki pref., Higashiusuki county,Shiiba village
D. bissetiana	1263684				2015	5	21	Y. Akagi	Miyazaki pref., Nobeoka city, Kitagawa town
D. bissetiana	1263677				2014	2	12	Y. Akagi	Miyazaki pref., Higashiusuki county,Shiiba village
D. bissetiana	1263676							Y. Akagi	Miyazaki pref., Higashiusuki county, Kadokawa town
D. bissetiana	1263663				1970	8	31	Y. Akagi	Miyazaki pref., Nichinann city, Kitasato town
D. bissetiana	1263662				2014	3	21	Y. Akagi	Miyazaki pref., Kushima city, Nukumi
D. bissetiana	572334				1972	5	28	Y. Ueno	Miyagi pref., Oshika county, Onagawa town
D. bissetiana	572361				1980	10	24	M. Takeichi	Miyagi pref., Miyagi county, Matsushima town
D. bissetiana	572565				1982	8	29	O. Asano	Miyagi pref., Kurokawa county, Yamato town
D. bissetiana	572362				1980	10	26	M. Takeichi	Miyagi pref., Miyagi county, Rifu town
D. bissetiana	572566				1982	8	8	O. Asano	Miyagi pref., Natori city, Uematsu
D. bissetiana	572346				1982	7	22	K. Syogo	Miyagi pref., Watari county, Watari town
D. bissetiana	572355				1977	7	30	K. Takahashi	Miyagi pref., Igu county, Marumori town
D. bissetiana	572343				1975	7	10	Y. Ueno	Miyagi pref., Shiroishi city, Masuoka
D. bissetiana	1168670				1999	9	11	S. Mori	Miyagi pref., Kakuda city, Oda
D. bissetiana	592838				1991	7	23	S. Tsugaru	Kyoto pref., Kameoka city, Asahi town
D. bissetiana	603750				1993	7	8	S. Tsugaru	Kyoto pref., Ayabe city, Koyaoka town
D. bissetiana	538003				1977	2	14	T. Nakaike	Kyoto pref., Souraku county, Minamiyamashiro village
D. bissetiana	442821				1982	8	6	Y. Tsujimoto	Kyoto pref., Souraku county, Kasagi town
D. bissetiana	538001				1982	6	7	T. Nakaike	Kyoto pref., Yosa county, Ine town
D. bissetiana	442819				1982	8	10	A. Komaki	Kyoto pref., Miyazu city, Takiba
D. bissetiana	538014				1982	6	6	T. Nakaike	Kyoto pref., Fukuchiyama city, Shimoamatsu
D. bissetiana	1246866				1985	7	12	Y. Kimitsuka	Kyoto pref., Kyoto city, Sakyo-ku, Mt. Kibune
D. bissetiana	734691				1968	1	14	T. Yamanaka	Kumamoto pref., Kuma county, Kuma village
D. bissetiana	223006				1968	5	13	M. Shiroto	Kumamoto pref., Minamata city, Yude
D. bissetiana	332427				1974	10	10	H. Tomita	Kumamoto pref., Kuma county, Itsuki village
D. bissetiana	516599				1978	8	20	J. Beppu	Kumamoto pref., Kikuchi county, Otsu town
D. bissetiana	517659				1981	8	16	J. Beppu	Kumamoto pref., Kuma county, Itsuki village
D. bissetiana	574094				1979	9	16	T. Kariyazaki	Kumamoto pref., Hitoyoshi city, Oobata
D. bissetiana	516613				1971	2	21	J. Beppu	Kumamoto pref., Yatsushiro city, Higashi town
D. bissetiana	574093				1961	11	13	M. Shiroto	Kumamoto pref., Minamata city, Ichiwatase
D. bissetiana	1039381				1955	8	14	T. Satomi	Gunnma pref., Tomioka city, Ichinomiya town
D. bissetiana	350015				1975	10	19	T. Satomi	Gunnma pref., Kannra county, Shimonita town
D. bissetiana	350336				1976	9	17	T. Satomi	Gunnma pref., Kannra county, Nannboku village
D. bissetiana	446734				1983	7	12	S. Waku	Gunnma pref., Oura county, Itakura town
D. bissetiana	568551				1982	7	4	T. Nakaike	Gunnma pref., Kiryuu city, Umeda
D. bissetiana	568550				1982	7	4	T. Nakaike	Gunnma pref., Kiryuu city, Tennjinn
D. bissetiana	568560				1982	6	12	B. Sasaki	Gunnma pref., Maebashi city, Izumisawa town
D. bissetiana	568557				1980	10	10	A. Yamamoto	Gunnma pref., Shibukawa city, Soboshima
D. bissetiana	446865				1983	7	19	S. Waku	Gunnma pref., Kiryuu city, Hirosawa town
D. bissetiana	826434				1969	9	23	Y. Jotani	Gunnma pref., Takasaki city, Ishihara town
D. bissetiana	448599				1982	7	4	B. Sasaki	Gunnma pref., Kiryuu city, Hirosawa
D. bissetiana	1246802				1987	10	8	S. Waku	Gunnma pref., Tone county, Syowa town
D. bissetiana	500570				1975	10	14	T. Takeda	Hiroshima pref., Fukuyama city, Yamano town
D. bissetiana	500599				1977	8	11	T. Takeda	Hiroshima pref., Fukuyama city, Goubunn town
D. bissetiana	500613				1975	7	9	T. Takeda	Hiroshima pref., Fuchu city, Aratani town
D. bissetiana	500594				1975	9	10	T. Takeda	Hiroshima pref., Fuchu city, Oguni
D. bissetiana	500580				1978	3	29	T. Takeda	Hiroshima pref., Mihara city, Fuka town
D. bissetiana	500581				1976	3	11	T. Takeda	Hiroshima pref., Mihara city, Kihara town
D. bissetiana	500585				1975	5	31	T. Takeda	Hiroshima pref., Mihara city, Suba town
D. bissetiana	500794				1976	6	15	T. Takeda	Hiroshima pref., Miyoshi city, Awaya
D. bissetiana	500588				1976	3	16	T. Takeda	Hiroshima pref., Takehara city, Konashi
D. bissetiana	450477				1982	8	3	S. Mitani	Kagawa pref., Takamatsu city, Nakayama town
D. bissetiana	450475				1982	8	25	S. Mitani	Kagawa pref., Kannnonnji city, Awai town
D. bissetiana	450480				1982	2	7	S. Mitani	Kagawa pref., Syoudo county, Tonosyou town
D. bissetiana	725564				1983	11	20	K. Yamaoka	Kochi pref., Takaoka county, Ochi town
D. bissetiana	715828				2000	7	7	K. Nishimura	Kochi pref., Kochi city, Fudeyama
D. bissetiana	511849				1979	5		T. Miyazaki	Kochi pref., Takaoka county, Nakatosa town
D. bissetiana	541787				1982	9	1	Y. Yamamoto	Kochi pref., Aki county, Umaji village, Yanase
D. bissetiana	541796				1982	10	12	Y. Yamamoto	Kochi pref., Aki county, Yasuda town
D. bissetiana	541801				1981	11	15	K. Yamaoka	Kochi pref., Nagaoka couny, Ootoyo town
D. bissetiana	445976				1983	2	26	Y. Yamamoto	Kochi pref., Aki city, Shimoyama
D. bissetiana	541778				1981	11	9	Y. Yamamoto	Kochi pref., Nagaoka county, Motoyama town
D. bissetiana	541777				1981	12	4	Y. Yamamoto	Kochi pref., Tosa county, Ookawa village
D. bissetiana	446347				1983	11	20	K. Yamaoka	Kochi pref., Takaoka county, Ochi town
D. bissetiana	1246936				1983	6	14	Y. Yamamoto	Kochi pref., Muroto city, Kiragawa town
D. bissetiana	518754				1975	8	20	J. Tannno	Saga pref., Imari city, Higashiyamashiro town
D. bissetiana	518751				1975	9	14	Y. Kurashige	Saga pref., Tosu city, Tateshi
D. bissetiana	518753				1981	11	14	Y. Kurashige	Saga pref., Imari city, Higashyamashiro town
D. bissetiana	1023950				1993	10	31	T. Iwata	Saitama pref., Chichibu city, Urayama
D. bissetiana	1026017				1984	11	10	T. Iwata	Saitama pref., Hannno city, Kosyuu
D. bissetiana	1023912				1999	4	26	T. Iwata	Saitama pref., Chichibu city, Maita

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. bissetiana	1023968				1990	10	29	T. Iwata	Saitama pref., Higashimatsuyama city, Iwadono
D. bissetiana	1023969				1990	10	21	T. Iwata	Saitama pref., Chichibu city, Kuna
D. bissetiana	1026061				1983	10	31	T. Iwata	Saitama pref., Chichibu county, Minano town
D. bissetiana	1026068				1983	8	26	T. Iwata	Saitama pref., Chichibu county, Nagatoro town
D. bissetiana	527328				1982	11	9	M. Shimosegawa	Saitama pref., Hasuda city, Komasaki
D. bissetiana	483988				1983	7	31	M. Shimosegawa	Saitama pref., Wako city, Shimoniikura
D. bissetiana	527318				1981	7	11	H. Enndou	Saitama pref., Tokorozawa city, Honngou
D. bissetiana	527322				1981	2	10	S. Ojima	Saitama pref., Honnjo city, Chiyoda
D. bissetiana	441662				1980	9	21	S. Yamauchi	Mie pref., Yokkaichi city, Isaka town
D. bissetiana	441743				1982	8	17	M. Kawazoe	Mie pref., Kuwana city, Shichi
D. bissetiana	441746				1982	8	11	M. Kawazoe	Mie pref., Mie county, Komono town, Chikusa
D. bissetiana	441668				1979	8	5	S. Yamauchi	Mie pref., Suzuka city, Ogisu town
D. bissetiana	441669				1981	9	13	K. Ohora	Mie pref., Kumano city, Asuka town
D. bissetiana	441663				1981	3	8	K. Ohora	Mie pref., Kumano city, Atashika town
D. bissetiana	450215				1984	3	4	T. Nakaike	Mie pref., Kumano city, Nikishima town
D. bissetiana	1246837				1984	8	20	K. Kada	Mie pref., Yokkaichi city, Isaka town, Dougayama
D. bissetiana	1248688				2005	12	1	J. Sato	Yamagata pref., Yonezawa city, Tateyama
D. bissetiana	1248521				1981	8	14	J. Sato	Yamagata pref., Nishiokitama county, Oguni town
D. bissetiana	507416				1981	7	27	N. Sakawa	Yamagata pref., Mogami county, Kanayama town
D. bissetiana	936146				1983	4	13	N. Sakawa	Yamagata pref., Yamagata city, Monndenn
D. bissetiana	507196				1982	9	14	N. Sakawa	Yamagata pref., Higashiokitama county, Takahata town
D. bissetiana	447764				1983	10	22	N. Sakawa	Yamagata pref., Nannyou city, Wada
D. bissetiana	507190				1972	8	21	N. Kato	Yamagata pref., Akumi county, Yuza town, Fukura
D. bissetiana	507366				1980	8	23	N. Kato	Yamagata pref., Sakata city, Hirooka
D. bissetiana	507193				1960	7	4	N. Kato	Yamagata pref., Tsuruoka city, Sannze
D. bissetiana	1266039				1975	3	22	T. Yamada	Yamaguchi pref., Ube city, Kamiube
D. bissetiana	1246291				1991	11	3	H. Masaki	Yamaguchi pref., Houfu city, Hisakane
D. bissetiana	1246294				1991	1	27	H. Masaki	Yamaguchi pref., Yanai city, Hizumi
D. bissetiana	1246296				1991	1	22	H. Masaki	Yamaguchi pref., Hikari city, Mii
D. bissetiana	1246905				1981	10	11	H. Enndou	Yamanashi pref., Minamitsuru county
D. bissetiana	1246906				1981	8	6	H. Enndou	Yamanashi pref., Kitatsuru county, Tabayama village
D. bissetiana	1246899				1980	11	23	H. Enndou	Yamanashi pref., Otsuki city, Saruhashi
D. bissetiana	1246904				1981	10	11	H. Enndou	Yamanashi pref., Tsuru city, Ohira
D. bissetiana	1246922				1981	8	17	A. Yamamoto	Yamanashi pref., Kofu city, Mitake
D. bissetiana	1246911				1981	8	29	E. Fukushi	Yamanashi pref., Minamitsuru county, Narusawa village
D. bissetiana	1246915				1981	10	24	M. Nakagawa	Yamanashi pref., Minamikoma county, Minobe town
D. bissetiana	388519				1976	4	4	T. Tatebe	Shiga pref., Inugami county, Taga town
D. bissetiana	448336				1981	7	11	T. Tatebe	Shiga pref., Gamou county, Hino town
D. bissetiana	448331				1980	12	2	T. Tatebe	Shiga pref., Oumiyahata city, Shima town
D. bissetiana	448334				1977	3	11	T. Tatebe	Shiga pref., Otsu city, Kiryuutsuji
D. bissetiana	448335				1977	2	12	T. Tatebe	Shiga pref., Otsu city, Kozeki town
D. bissetiana	448340				1981	2	26	T. Tatebe	Shiga pref., Otsu city, Zezeikenouchi town
D. bissetiana	349881				1975	3	20	M. Yokota	Shiga pref., Otsu city, Sonoyama
D. bissetiana	734265				1983	8	28	T. Yamanaka	Kagoshima pref., Yubisuki city, Mt. Kaimonndake
D. bissetiana	523092				1980	6	1	K. Takesako	Kagoshima pref., Kanoya city, Takakuma valley
D. bissetiana	461060				1985	7	1	M. Kawabata	Kagoshima pref., Kagoshima city, Yoshino town
D. bissetiana	480396				1977	10	5	J. Takada	Akita pref., Akita city, Kanaashi
D. bissetiana	929646				1963	11	10		Niigata pref., Shibata city, Higashiakatani
D. bissetiana	170810				1965	9	26	T. Iwano	Niigata pref., Kashiwazaki city, Yoneyama
D. bissetiana	929752				1963	10	11		Niigata pref., Shibata city, Yonekura
D. bissetiana	542939				1979	6	10	T. Takahashi	Niigata pref., Kamo city, Kamijo
D. bissetiana	542936				1982	8	3	S. Sugesawa	Niigata pref., Nagaoka city, Miyamoto
D. bissetiana	542935				1952	6	7	T. Iwano	Niigata pref., Kashiwazaki city, Shiiya
D. bissetiana	572862				1983	8	23	S. Tomitori	Niigata pref., Joetsu city, Takasumi
D. bissetiana	572863				1983	9	11	Y. Hasegawa	Niigata pref., Joetsu city, Haizuka
D. bissetiana	1246774				1984	7	28	M. Saito	Niigata pref., Joetsu city, Gochi
D. bissetiana	1246867				1985	5	10	I. Ito	Niigata pref., Gosenn city, Sadori
D. bissetiana	287207				1971	7	28	H. Komaki	Kanagawa pref., Hiratsuka city, Kamikichisawa
D. bissetiana	323242				1975	1	26	M. Sakakibara	Kanagawa pref., Ashigarashimo county, Hakone town
D. bissetiana	450390				1981	10	10	A. Yamamoto	Kanagawa pref., Kawasaki city, Takatsu-ku
D. bissetiana	450359				1982	7	10	E. Fukushi	Kanagawa pref., Yokohama city, Kanagawa-ku
D. bissetiana	450373				1974	11	3	A. Sato	Kanagawa pref., Yokosuka city, Nagaura town
D. bissetiana	439084				1982	11	21	E. Fukushi	Kanagawa pref., Kawasaki city, Aso-ku
D. bissetiana	450378				1982	1	3	T. Sato	Kanagawa pref., Fujisawa city, Nishimatano
D. bissetiana	450368				1979	8	8	J. Moriya	Kanagawa pref., Isehara city, Koyasu
D. bissetiana	450389				1982	5	8	K. Tanaka	Kanagawa pref., Kamakura city, Koshigoe
D. bissetiana	450385				1982	3	22	K. Tanaka	Kanagawa pref., Odawara city, Nebukawa
D. bissetiana	440116				1980	10	5	M. Neichi	Aomori pref., Hachinohe city, Shirahama
D. bissetiana	929639				1984	9	22	T. Sato	Shizuoka pref., Fuji city, Iwamoto
D. bissetiana	1138412				1956	8	13	T. Akiyama	Shizuoka pref., Numazu city, Oohira
D. bissetiana	946531				1938	8	23		Shizuoka pref., Susono city, Sano
D. bissetiana	510852				1979	9	2	Y. Kobayashi	Shizuoka pref., Sunntou county, Oyama town
D. bissetiana	445153				1983	5	21	A. Suzuki	Shizuoka pref., Tagata county, Kannnami town
D. bissetiana	510835				1977	11	13	A. Suzuki	Shizuoka pref., Kamo county, Higashiizu town
D. bissetiana	510388				1982	9	27	T. Yamamoto	Shizuoka pref., Shimoda city, Suzaki
D. bissetiana	444473				1982	5	3	T. Hosokura	Shizuoka pref., Numazu city, Ashidaka
D. bissetiana	444474				1974	8	14	T. Hosokura	Shizuoka pref., Gotennba city, Innno
D. bissetiana	574519				1983	9	17	A. Suzuki	Shizuoka pref., Mishima city, Sano
D. bissetiana	1246763				1986	1	19	T. Hosokura	Shizuoka pref., Shimotaga, Nakagawa
D. bissetiana	1246770				1986	8	20	T. Hosokura	Shizuoka pref., Ito city, Futo
D. bissetiana	1246872				1991	8	7	T. Hosokura	Shizuoka pref., Kakegawa city, Sayoshika
D. bissetiana	1246897				1987	5	31	T. Hosokura	Shizuoka pref., Fuji city, Hina
D. bissetiana	559110				1990	6	19	S. Tsugaru	Ishikawa pref., Komatsu city, Kanahira town
D. bissetiana	495906				1976	9	5	S. Komaki	Ishikawa pref., Kahoku county, Tsubata town
D. bissetiana	495881				1975	8	8	S. Komaki	Ishikawa pref., Suzu city, Takaya
D. bissetiana	495901				1979	3	21	S. Komaki	Ishikawa pref., Nanao city, Sano
D. bissetiana	495897				1979	8	12	S. Komaki	Ishikawa pref., Hakui city, Sugaike
D. bissetiana	495882				1981	8	20	S. Komaki	Ishikawa pref., Kahoku county, Tsubata town
D. bissetiana	495826				1975	9	10	K. Yoneyma	Ishikawa pref., Kanazawa city, Nukadani town
D. bissetiana	495824				1977	11	3	K. Yoneyma	Ishikawa pref., Komatsu city, Iwabuchi
D. bissetiana	495813				1978	10	8	K. Yoneyma	Ishikawa pref., Kaga city, Sou
D. bissetiana	1249151				1993	1	13	T. Kuramata	Chiba pref., Sakura city, Odake
D. bissetiana	550593				1976	12	12	Y. Kimitsuka	Chiba pref., Tyoshi city, Nojiri
D. bissetiana	447743				1983	10	30	K. Nakamura	Chiba pref., Tyoshi city, Saruda town
D. bissetiana	533474				1981	3	4	Y. Koike	Chiba pref., Katori county Tako town
D. bissetiana	533483				1976	10	19	Y. Koike	Chiba pref., Katori county Tako town
D. bissetiana	533492				1977	6	19	Y. Kimitsuka	Chiba pref., Narita city, Shimokatsuta
D. bissetiana	533497				1980	12	28	Y. Kimitsuka	Chiba pref., Tougane city, Matsunogou
D. bissetiana	533462				1983	1	2	K. Nakamura	Chiba pref., Mobara city, Honnnou
D. bissetiana	533467				1980	11	2	M. Aida	Chiba pref., Abiko city, Ichibushinndenn
D. bissetiana	533489				1977	11	6	Y. Kimitsuka	Chiba pref., Sakura city, Jounai
D. bissetiana	533464				1982	10	17	K. Nakamura	Chiba pref., Funabashi city, Kanasugi town
D. bissetiana	550724				1977	1	15	Y. Kimitsuka	Chiba pref., Ichihara city, Koorimoto
D. bissetiana	533501				1982	12	30	K. Nakamura	Chiba pref., Tyousei county, Nagatsuka town
D. bissetiana	447745				1983	10	23	K. Nakamura	Chiba pref., Ichihara city, Yonehara
D. bissetiana	533477				1977	12	3	H. Kobayashi	Chiba pref., Kimitsu city, Atago
D. bissetiana	533488				1980	1	6	Y. Kimitsuka	Chiba pref., Noda city, Hanai
D. bissetiana	533463				1981	10	11	K. Nakamura	Chiba pref., Kamagaya city, Nakazawa
D. bissetiana	582344				1982	6	28	H. Mitsuhashi	Osaka pref., Kawachinagano city, Kobuka
D. bissetiana	582338				1982	8	26	A. Komaki	Osaka pref., Minoo city, Minoo
D. bissetiana	445043				1983	3	20	H. Mizuno	Osaka pref., Toyono county, Nose town
D. bissetiana	582339				1981	10	10	A. Komaki	Osaka pref., Takatsuki city, Hagitani
D. bissetiana	582349				1980	6	23	H. Masago	Osaka pref., Izumisano city, Oogi
D. bissetiana	936151				1982	8	22	T. Suzuki	Osaka pref., Yao city, Koudachi
D. bissetiana	1202202				2009	8	6	H. Tsuji	Oita pref., Hita city, Nakatsue village
D. bissetiana	1247395				1940	11	10	M. Hadano	Oita pref., Beppu city, Mt. Tsurumiyama
D. bissetiana	521304				1976	7	14	Y. Maruno	Oita pref., Beppu city, Torii
D. bissetiana	516652				1982	8	11	Y. Takaoka	Oita pref., Takeda city, Mt. Mitake
D. bissetiana	516590				1979	1	6	H. Inoue	Oita pref., Hita city, Kushigawa
D. bissetiana	768810				2007	10	6		Nagasaki pref., Tsushima city, Izuhara town
D. bissetiana	1246302				2012	9	6	S. Yamada	Nagasaki pref., Unnzenn city, Chijiwa town
D. bissetiana	388511				1976	6	27	F. Ueno	Nagasaki pref., Oomura city, Imamura

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. bissetiana	114112				1954	7	1	M. Muramatsu	Nagano pref., Shimoina county, Yasuoka village
D. bissetiana	555971				1977	8	9	T. Nakaike	Nagano pref., Suwa county, Fujimi town
D. bissetiana	421055				1981	10	31	H. Tsutsumi	Nagano pref., Kamiina county, Nakagawa village
D. bissetiana	421032				1981	6	26	H. Tsutsumi	Nagano pref., Shimoina county, Yasuoka village
D. bissetiana	555964				1969	4	12	H. Okubara	Nagano pref., Kiso county, Ookuwa village
D. bissetiana	555975				1982	10	17	H. Tsutsumi	Nagano pref., Shimoina county, Neba village
D. bissetiana	420554				1981	5	8	H. Tsutsumi	Nagano pref., Kiso county, Minamikiso town
D. bissetiana	421035				1981	7	23	H. Tsutsumi	Nagano pref., Iida city, Chiyono pond
D. bissetiana	932565				1980	11	3	M. Fukuhara	Nagano pref., Kiso county, Ookuwa village
D. bissetiana	500848				1983	2	27	A. Tanaka	Tottori pref., Iwami county, Iwami town
D. bissetiana	500849				1983	2	27	A. Tanaka	Tottori pref., Iwami county, Iwami town
D. bissetiana	501257				1981	10	18	A. Tanaka	Tottori pref., Yazu county, Wakasa town
D. bissetiana	501264				1980	10	2	A. Tanaka	Tottori pref., Tottori city, Oobata
D. bissetiana	500846				1982	12	12	A. Tanaka	Tottori pref., Yazu county, Chizu town
D. bissetiana	501261				1982	1	8	A. Tanaka	Tottori pref., Touhaku county, Misasa town
D. bissetiana	500838				1981	9	22	M. Aoto	Tottori pref., Hino county, Nichinann town
D. bissetiana	501263				1981	11	1	A. Tanaka	Tottori pref., Sakaiminato city, Takenouchi town
D. bissetiana	501259				1981	9	17	M. Aoto	Tottori pref., Hino county, Nichinann town
D. bissetiana	1245687				2014	9	28	H. Sawae	Shimane pref., Goutsu city, Hazumi town
D. bissetiana	1250203				2014	11	27	H. Sawae	Shimane pref., Hamada city, Kanagi town
D. bissetiana	1251080				2.013	12	1	K. Shibata	Shimane pref., Ooda city, Tomiyama
D. bissetiana	1251319				2014	8	31	K. Shibata	Shimane pref., Ouchi county, Kawamoto town
D. bissetiana	569393				1982	8	22	A. Minami	Shimane pref., Goutsu city, Atoichi town
D. bissetiana	449703				1981	11	1	A. Minami	Shimane pref., Hamada city, Ubuyu town
D. bissetiana	443929				1983	5	1	A. Minami	Shimane pref., Oki county, Nishinoshima town
D. bissetiana	1256809				2015	9	13	M. Yanaura	Shimane pref., Matsue city, Shimane town
D. bissetiana	449699				1982	8	22	A. Minami	Shimane pref., Goutsu city, Atoichi town
D. bissetiana	1024462				2011	6	28		Tokyo pref., Kouzushima Island, Mt. Chichibu
D. bissetiana	9511740				1998	7	6		Tokyo pref., Minato-ku, Shirokanedai
D. bissetiana	817744				1978	4	16	K. Inoue	Tokyo pref., Nishitama county, Okutama town
D. bissetiana	523258				1981	1	24	Y. Kimitsuka	Tokyo pref., Ota-ku, Ikegami
D. bissetiana	523420				1981	9	27	B. Sasaki	Tokyo pref., Itabashi-ku, Takashimadaira
D. bissetiana	523377				1980	9	29	A. Yamamoto	Tokyo pref., Setagaya-ku, Todoriki valley
D. bissetiana	523413				1980	9	30	M. Mitsuhashi	Tokyo pref., Fuchu city, Shiraitodai
D. bissetiana	523421				1980	11	30	B. Sasaki	Tokyo pref., Machida city, Onoji town
D. bissetiana	523415				1980	10	4	M. Mitsuhashi	Tokyo pref., Hachioji city, Takao town
D. bissetiana	523384				1980	10	4	H. Enndou	Tokyo pref., Oume city, Kaminariki
D. bissetiana	523373				1957	8	26	S. Wataga	Tokyo pref., Hachioji city, Kamionngata
D. bissetiana	523418				1979	7	28	I. Kumada	Tokyo pref., Izu-oshima Island, Okada
D. bissetiana	523417				1975	6	8	R. Kurokawa	Tokyo pref., Mikurajim Island, Kawada
D. bissetiana	1246047				1983	4	17	T. Nakaike	Tokyo pref., Oume city, Kurosawa
D. bissetiana	1246765				1984	10	13	E. Fukushi	Tokyo pref., Nishitama county, Okutama town
D. bissetiana	1039322				1957	10	13	T. Inobe	Tokushima pref., Myozai county, Kamiyama town
D. bissetiana	476239				1979	9	8	T. Nakayama	Tokushima pref., Tokushima city, Bizann town
D. bissetiana	1246305				1982	8	22	T. Nakayama	Tokushima pref., Anann city, Tsubaki town
D. bissetiana	1039284				1957	3	20	K. Ogawa	Tochigi pref., Utsunomiya city, Nozawa town
D. bissetiana	799557				1987	8	7	F. Matsumura	Tochigi pref., Kanuma city, Tochikubo
D. bissetiana	799560				1985	7	25	H. Ogura	Tochigi pref., Nikko city, Takigahara
D. bissetiana	799538				1981	9	20	M. Sakurai	Tochigi pref., Otawara city, Minaikanemaru
D. bissetiana	799549				1987	9	5	T. Noguchi	Tochigi pref., Ashikaga city, Yamashita town
D. bissetiana	799550				1987	6	28	T. Noguchi	Tochigi pref., Tochigi city, Kashiwagura town
D. bissetiana	799552				1987	6	28	Y. Sugita	Tochigi pref., Sano city, Koshidoko pass
D. bissetiana	525021				1980	8	13	M. Usui	Tochigi pref., Nasu county, Nasu town
D. bissetiana	525176				1981	7	16	M. Usui	Tochigi pref., Shioya county, Takanezawa town
D. bissetiana	525151				1982	7	11	M. Usui	Tochigi pref., Haga county, Mashiko town
D. bissetiana	525014				1980	8	27	T. Tashiro	Tochigi pref., Yaita city, Kawasakisori town
D. bissetiana	525019				1980	8	28	M. Usui	Tochigi pref., Utsunomiya city, Nagaoka town
D. bissetiana	525023				1980	9	12	M. Usui	Tochigi pref., Oyama city, Tyuuou town
D. bissetiana	525170				1980	10	22	M. Usui	Tochigi pref., Nikko city, Yamauchi
D. bissetiana	525172				1981	10	7	M. Usui	Tochigi pref., Nikko city, Nishiokorogawa
D. bissetiana	444703				1983	4	2	J. Kato	Tochigi pref., Otawara city, Kitaowagu
D. bissetiana	1186222				2002	7	21	K. Kawabata	Nara pref., Gojou city, Kuruno town
D. bissetiana	592568				1971	6	20	Y. Tsujimoto	Nara pref., Yoshino county, Kawakami village
D. bissetiana	592009				1976	10	24	Y. Kimitsuka	Nara pref., Tennri city, Takimoto
D. bissetiana	592402				1977	12	28	Y. Tsujimoto	Nara pref., Nara city, Syouryakuji temple
D. bissetiana	592008				1976	10	25	Y. Kimitsuka	Nara pref., Yoshino county, Tennkawa village
D. bissetiana	1256713				2015	5	27	F. Kasetani	Nara pref., Uda county, Mitsue village
D. bissetiana	511909				1975	5	15	Y. Tsujimoto	Nara pref., Yamatokouriyama city, Yatayama town
D. bissetiana	552800				1982	11	6	T. Oshima	Toyama pref., Shimoniikawa county, Asahi town
D. bissetiana	552383				1982	9	11	T. Oshima	Toyama pref., Kurobe city, Makurano
D. bissetiana	552761				1981	8	29	T. Oshima	Toyama pref., Uotsu city, Miyazu
D. bissetiana	552762				1981	9	15	T. Oshima	Toyama pref., Nakaniikawa county, Kamiichi town
D. bissetiana	552798				1982	9	23	T. Oshima	Toyama pref., Himi city, Nakada
D. bissetiana	537329				1983	8	10	T. Oshima	Toyama pref., Takaoka city, Ishitsutsumi
D. bissetiana	1246954				1978	8	19	T. Oshima	Toyama pref., Himi city, Sugata
D. bissetiana	1246957				1980	5	28	I. Yamamoto	Toyama pref., Tonami city, Iguridani
D. bissetiana	1246961				1978	7	16	T. Oshima	Toyama pref., Uotsu city, Hirasawa
D. bissetiana	507463				1981	9	13	Y. Saito	Fukui pref., Katsuyama city, Kitadani town
D. bissetiana	507527				1977	7	15	Y. Saito	Fukui pref., Ono city, Kamishita
D. bissetiana	507465				1976	6	26	Y. Saito	Fukui pref., Imadate county, Ikeda town
D. bissetiana	507524				1977	6	4	Y. Saito	Fukui pref., Fukui city, Kouchi
D. bissetiana	507501				1981	7	9	S. Watanabe	Fukui pref., Nyuu county, Echizenn town
D. bissetiana	507523				1971	10	17	S. Watanabe	Fukui pref., Tsuruga city, Okuasou
D. bissetiana	507519				1978	9	3	S. Watanabe	Fukui pref., Obama city, Wakasahime shrine
D. bissetiana	557488				1968	5	4	S. Kobayashi	Fukuoka pref., Kitakyuusyuu city, Kokuraminami-ku
D. bissetiana	557487				1977	10	6	S. Kobayashi	Fukuoka pref., Kitakyuusyuu city, Yahatanishi-ku
D. bissetiana	557483				1965	8	12	S. Tsutsui	Fukuoka pref., Kasuya ounty, Sasaguri town
D. bissetiana	557486				1967	12	24	S. Tsutsui	Fukuoka pref., Fukuoka city, Nishi-ku
D. bissetiana	557474				1967	10	15	S. Tsutsui	Fukuoka pref., Fukuoka city, Sawara-ku
D. bissetiana	443134				1968	8	18	S. Tsutsui	Fukuoka pref., Kitakyuusyu city, Kokuraminami-ku
D. bissetiana	1221546				1982	9	15	Y. Kobayashi	Fukushima pref., Nihonnmatsu city, Matsuoka
D. bissetiana	572159				1981	2	2	M. Yasu	Fukushima pref., Futaba county, Tomioka town
D. bissetiana	506776				1978	8	10	Y. Sugano	Fukushima pref., Souma county, Iidate village
D. bissetiana	506865				1980	12	2	Y. Kaneda	Fukushima pref., Tamura county, Miyakoji village
D. bissetiana	570926				1970	10	1	Y. Yuzawa	Fukushima pref., Iwaki city, Onahama
D. bissetiana	506853				1979	3	27	Y. Kaneda	Fukushima pref., Ishikawa county, Hirata village
D. bissetiana	506900				1977	7	17	Y. Kaneda	Fukushima pref., Fukushima city, Kuroiwa town
D. bissetiana	506730				1978	9	30	Y. Kaneda	Fukushima pref., Ishikawa county, Tamagwa village
D. bissetiana	506884				1977	10	20	Y. Kaneda	Fukushima pref., Higashishirakaa county, Tanakura town
D. bissetiana	506899				1977	7	30	Y. Kaneda	Fukushima pref., Minamiaizu county, Tadami town
D. bissetiana	444760				1980	10	10	M. Yasu	Fukushima pref., Futaba county, Naraha town
D. bissetiana	738482				1980	12	31	T. Yamada	Hyogo pref., Ashiya city, Yamaashiya town
D. bissetiana	756427				1999	11	13	K. Ushijima	Hyogo pref., Kawabe county, Inagawa town
D. bissetiana	568606				1977	11	20	T. Shiraiwa	Hyogo pref., Sannda city, Kogaki
D. bissetiana	568769				1977	4	3	T. Shiraiwa	Hyogo pref., Nishinomiya city, Jurinnji town
D. bissetiana	568770				1964	11	22	T. Shiraiwa	Hyogo pref., Koube city, Hyogo-ku
D. bissetiana	568764				1976	8	18	Y. Kobayashi	Hyogo pref., Toyooka city, Taki
D. bissetiana	568612				1978	8	29	T. Shiraiwa	Hyogo pref., Sayou county, Sayou town
D. bissetiana	445044				1983	3	30	H. Mizuno	Hyogo pref., Nishiwaki city, Sumiyoshi town
D. bissetiana	568618				1980	9	6	K. Mune	Hyogo pref., Sumoto city, Nakagawara town
D. bissetiana	444294				1974	9	5	Y. Kimitsuka	Hokkaido, Hiyama county, Esashi town
D. bissetiana	518465				1971	12	12	H. Masago	Wakayama pref., Shinnguu city, Takada
D. bissetiana	518499				1980	8	16	H. Masago	Wakayama pref., Higashimuro county, Kozagawa town
D. bissetiana	518505				1978	4	30	H. Masago	Wakayama pref., Ito county, Kouya town
D. bissetiana	518475				1974	8	9	H. Masago	Wakayama pref., Nishimuro county, Susami town
D. bissetiana	518477				1978	5	7	H. Masago	Wakayama pref., Ito county, Katsuragi town
D. bissetiana	518489				1976	5	11	H. Masago	Wakayama pref., Hidaka county, Inami town
D. bissetiana	518485				1977	10	23	H. Masago	Wakayama pref., Tanabe city, Kamiakitsu
D. bissetiana	518461				1977	8	31	H. Masago	Wakayama pref., Nishimuro county, Shirahama town
D. bissetiana	443063				1976	3	6	Y. Kimitsuka	Wakayama pref., Hashimoto city, Yagurawaki
D. bissetiana	817742				1957	7	18	Y. Jotani	Tokyo pref., Toshima Island
D. bissetiana	817739				1932	8	17	Y. Jotani	Tokyo pref., Miyakejima Island

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. bissetiana	523422				1976	9	5	T. Yasui	Tokyo pref., Hachijojima Island
D. bissetiana	1092966				1964	9	3	H. Ito	Niigata pref., Sado Island, Aikawa town
D. bissetiana	1223542				2014	9	4	Y. Horii	Miyagi pref., Kakuda city, Oyama, Mt. Nabemori
D. bissetiana	1221844				1991	4	17	M. Neichi	Miyagi pref., Ishinomaki city, Momoura
D. bissetiana	1221846				2005	10	31	M. Neichi	Miyagi pref., Shiogama city, Ichimoriyama
D. bissetiana	1221847				2003	7	7	M. Neichi	Miyagi pref., Oohira village, Komaba
D. bissetiana	1221854				2014	8	17	Y. Horii	Akita pref., Sennboku city, Kakunodate town
D. bissetiana	530553				1989	7	23	Y. Horii	Akita pref., Yuzawa city, Minase town
D. bissetiana	480481				1981	10	14	S. Fujiwara	Akita pref., Ogachi county, Ugo town
D. bissetiana	480478				1982	10	17	T. Kikuchi	Akita pref., Honjo city, Ishizawakyo
D. bissetiana	511094				1987	8	15	A. Yamamoto	Akita pref., Noshiro city, Oogara
D. bissetiana	1167721				1997	12	20	S. Mori	Aomori pref., Hachinohe city, Korekawatanaka
D. bissetiana	440095				1980	9	14	N. Saito	Aomori pref., Kamikita county, Rokunohe town
D. bissetiana	440089				1980	10	12	M. Takezawa	Aomori pref., Towada city, Kirida
D. bissetiana	440108				1980	8	23	A. Takahashi	Aomori pref., Sannnohe county, Gonohe town
D. bissetiana	440086				1980	4	27	M. Neichi	Aomori pref., Misawa city, Shiogama
D. bissetiana	440117				1980	5	16	A. Takahashi	Aomori pref., Kamikita county, Rokkasyo village
D. bissetiana	1254041				1978	4	17	K. Takahashi	Aomori pref., Hachinohe city, Kanehama
D. bissetiana	1254038				2005	10	31	K. Takahashi	Aomori pref., Shimokita county, Ooma town
D. bissetiana	1254042				1978	12	17	K. Matsunaga	Aomori pref., Hachinohe city, Nanngou-ku
D. bissetiana	1264250				2015	10	27	T. Sudou	Aomori pref., Misawa city, Fukaya
D. bissetiana	1246946				1983	7	17	M. Neichi	Hokkaido, Okushiri town, Monai river
D. bissetiana		449107			2013	1	30	S. Mitani	Japan, Kagawa pref., Ayauta county, Ayagawa town
D. chichisimensis		416656			2015	2	22	H. Kato	Chichijima Island of Bonin Island
D. chichisimensis		274405			1993	7	10	T. Yasui	Kitaioto Island
D. erythrovaria	439277				1976	7	18	M. Fukuhara	Aichi pref., Atsumi county, Atsumi town, Irakomisaki
D. erythrovaria	439264				1980	11	23	K. Inukai	Aichi pref., Atsumi county, Atsumi town, Yamada
D. erythrovaria	439276				1976	3	21	M. Fukuhara	Aichi pref., Atsumi county, Tawara town, Katahama
D. erythrovaria	439287				1982	6	27	K. Inukai	Aichi pref., Okazaki City, Chiharazawa town
D. erythrovaria	1247532				1980	9	21	M. Muramatsu	Aichi pref., Okazaki city, Hobo town
D. erythrovaria	1247534				1980	8	15	K. Inukai	Aichi pref., Nukata county, Nukata town, Ishihara
D. erythrovaria	439275				1978	4	16	M. Fukuhara	Aichi pref., Gamagouri city, Aira town
D. erythrovaria	1247533				1980	8	12	K. Inukai	Aichi pref., Shinjo city, Mt. Funatsuki
D. erythrovaria	582531				1983	5	15	K. Inukai	Aichi pref., Nishikamo county, Obara town, Tougou
D. erythrovaria	439284				1979	4	22	M. Fukuhara	Aichi pref., Chita county, Higashiura town, Ishihama
D. erythrovaria	439279				1980	10	12	M. Fukuhara	Aichi pref., Chita county, Minamichita town, Iwaya
D. erythrovaria	817562				1979	3	30	H. Miyazaki	Aichi pref., Chita county, Minamichita town, Yamami
D. erythrovaria	439282				1981	4	12	M. Fukuhara	Aichi pref., Chita county, Minamichita town, Shijojima
D. erythrovaria	1247457				1979	9	16	K. Inukai	Aichi pref., Chita county, Mihama town, Kouwa
D. erythrovaria	439278				1979	8	30	K. Inukai	Aichi pref., Chita county, Mihama town, Noma
D. erythrovaria	439285				1982	5	23	K. Inukai	Aichi pref., Chita city, Ookusa
D. erythrovaria	817601				1977	1	12	F. Miyamoto	Aichi pref., Chiryuu city, Koubou town
D. erythrovaria	439288				1982	11	3	K. Inukai	Aichi pref., Toukai city, Oota town
D. erythrovaria	439283				1974	12	15	M. Fukuhara	Aichi pref., Hatazu county, Hatazu town, Hgashibata
D. erythrovaria	439267				1982	1	3	K. Inukai	Aichi pref., Toyohashi city, Ikobe town
D. erythrovaria	439268				1980	3	15	K. Inukai	Aichi pref., Toyohashi city, Unoya town, Fumonnji temple
D. erythrovaria	439273				1979	6	2	K. Inukai	Aichi pref., Toyoda city, Ishigusu town, Kamigiri
D. erythrovaria	439274				1980	3	20	K. Inukai	Aichi pref., Toyoda city, Oobata town
D. erythrovaria	582535				1983	3	31	K. Inukai	Aichi pref., Ichinomiya city, Kisogawa towm, Satokomaki
D. erythrovaria	555869				1980	10	6	M. Hyoudou	Ehime pref., Iyo county, Futami town, Houshi
D. erythrovaria	554951				1981	5	24	Y. Kimizuka	Ehime pref., Ochi county, Hakata town, Kiuraminami
D. erythrovaria	1247433				2012	3	3	M. Hyoudou	Ehime pref., Imabari city, Kamiura town, Sakari
D. erythrovaria	554954				1978	6	18	Y. Miyoshi	Ehime pref., Matsuyama city, Taisanji town
D. erythrovaria	1248439				1989	5	28	Y. Kimizuka	Ehime pref., Nakajima town, Hachimannguu
D. erythrovaria	1037735				1960	11	23	H. Ishikawa	Ehime pref., Doi town, Nakanogawa
D. erythrovaria	555870				1980	8	31	M. Hyoudou	Ehime pref., Higashiuwa county, Uwa town, Akema
D. erythrovaria	1247567				1981	12	26	A. Minami	Ehime pref., Minamiuwa county, Goso town, Hunanokawa
D. erythrovaria	555883				1981	10	6	M. Hyoudou	Ehime pref., Kitauwa county, Tsushima town, Suge
D. erythrovaria	512231				1980	11	2	Y. Kimizuka	Ibaraki pref., Iwai cty, Arakyo
D. erythrovaria	512461				1981	10	11	Y. Kimizuka	Ibaraki pref., Iwai city, Yahagi
D. erythrovaria	512459				1970	6	14	Y. Nakazaki	Ibaraki pref., Hitachioota city, Kanasagou
D. erythrovaria	512243				1978	3	25	M. Yasu	Ibaki pref., Kashima city, Hokota town, Iina
D. erythrovaria	512456				1964	3	7	Y. Nakazaki	Ibaraki pref., Hitachioota city, Mt. Mayumiyama
D. erythrovaria	512439				1979	8	18	Y. Koike	Ibaraki pref., Niihari county, Yasato town, Ichinosawa
D. erythrovaria	512228				1973	8	18	N. Wada	Ibaraki pref., Niihari county, Yasato town, Mt. Itajiki
D. erythrovaria	512242				1977	4	29	M. Yasu	Ibaraki pref., Nishiibaraki county, Iwase town, Mt. Amamakiyama
D. erythrovaria	512444				1974	5	9	N. Wada	Ibaraki pref., Nishiibaraki county, Iwase town, Tomiyakannnonn
D. erythrovaria	1037629				1971	4	27	H. Ito	Ibaraki pref., Mt. Tsukubasann
D. erythrovaria	572898				1982	12	3	M. Yasu	Ibaraki pref., Higashiibaraki county, Ibaraki town, Nakaishizaki
D. erythrovaria	572899				1982	8	20	M. Yasu	Ibaraki pref., Higashiibaraki county, Ogawa town, Miyata
D. erythrovaria	512236				1972			M. Yasu	Ibaraki pref., Hitachi city, Namegawaoka
D. erythrovaria	512443				1981	5	19	M. Yasu	Ibaraki pref., Ryuugasaki city, Izumi
D. erythrovaria	512460				1975	8	27	Y. Nakazaki	Ibaraki pref., Ryuugasaki city, Kitagata town
D. erythrovaria	522002				1977	11	23	T. Saito	Okayama pref., Ihara city, Dousokei
D. erythrovaria	521957				1977	1	15	T. Saito	Okayama pref., Asaguchi county, Kamogata town
D. erythrovaria	521965				1982	6	17	Y. Obata	Okayama pref., Setouchi city, Ushimado
D. erythrovaria	1017543				1936	4	2	T. Nomura	Gifu pref., Mt. Kinnkazann
D. erythrovaria	600244				1981	2	25	Y. Yamamoto	Gifu pref., Minokamo city, Hirohashi
D. erythrovaria	556441				1971	12	27	S. Tsutsui	Miyazaki pref., Ebino city, Tetsuyama
D. erythrovaria	556274				1974	3	31	T. Minamitani	Miyazaki pref., Koyu county, Tonou town, Mt. Osuzu
D. erythrovaria	1039342				1951	6	12	I. Taki	Miyazaki pref., Mt. Oohatayama
D. erythrovaria	1263299				2015	8	27	Y. Akagi	Miyazaki pref., Nichinann city, Nanngou town, Ikenami
D. erythrovaria	588721				1991	7	26	S. Tsugaru	Kyoto-fu, Maizuru city, Nishiooura, Oohanyuu, Mt. Kunimiyama
D. erythrovaria	518777				1978	7	2	J. Beppu	Kumamoto pref., Aso county, Namino village, Sakanoue
D. erythrovaria	518648				1963	6	9	M. Shiroto	Kumamoto pref., Ashikita county, Ashikita town, Sajiki
D. erythrovaria	574079				1983	1	9	M. Ishizaka	Kumamoto pref., Shimomasuki county, Matsuhashi town, Magarino
D. erythrovaria	518772				1978	7	23	J. Beppu	Kumamoto pref., Kikuchi city, Anakawa pass
D. erythrovaria	518646				1993	11	20	K. Kai	Kumamoto pref., Kammasuki county, Yabe town, Uchidaijinn
D. erythrovaria	1247608				1978	3	26	M. Shiroto	Kumamoto pref., Minamata city, Kugino, Samukawa
D. erythrovaria	1018985				1959	8	16	T. Yamanaka	Kumamoto pref., Minamata city, Yude
D. erythrovaria	517870				1960	8	19	T. Yamanaka	Kumamoto pref., Amakusa county, Amakusa town, Fukuregi
D. erythrovaria	535687				1976	11	16	T. Takeda	Hiroshima pref., Takata county, Yoshida town, Kooriyamajou
D. erythrovaria	1247843				1976	12	21	T. Takeda	Hiroshima pref., Ootake city, Kasayama
D. erythrovaria	518622				1976	3	6	T. Takeda	Hiroshima pref., Fukuyama city, Tajiri
D. erythrovaria	1247841				1976	5	4	T. Takeda	Hiroshima pref., Toyota county, Akitsu town, Komatsubara
D. erythrovaria	518631				1975	12	20	T. Takeda	Hiroshima pref., Toyota county, Kinoe town
D. erythrovaria	518672				1975	5	25	S. Mitani	Kagawa pref., Ayauta county, Ayanami town, Oomiya shrine
D. erythrovaria	345216				1974	12	15	S. Mitani	Kagawa pref., Yashima
D. erythrovaria	520410				1976	1	15	S. Mitani	Kagawa pref., Shoudo county, Kankakei
D. erythrovaria	210683				1968	12	4	Y. Magi	Kagawa pref., Kannonnji city, Kamara shrine
D. erythrovaria	210684				1984	9	29	Y. Kimizuka	Kagawa pref., Marugame city, Hiroshima
D. erythrovaria	1264269				1976	1	7	S. Mitani	Kagawa pref., Takamatsu city, Nishiueda town, Fujio shrine
D. erythrovaria	520409				1976	6	20	S. Mitani	Kagawa pref., Takamatsu city, Nakayama town
D. erythrovaria	520413				1975	4	25	M. Arai	Kagawa pref., Sakaide city, Goshikidai
D. erythrovaria	1046297				1982	2	7	S. Mitani	Kagawa pref., Shoudo county, Tonosho town
D. erythrovaria	443181				1974	12	1	S. Mitani	Kagawa pref., Ookawa county, Nagao town, Kametsuru park
D. erythrovaria	520388				1981	5	25	Y. Kimizuka	Kagawa pref., Nakatado county, Tadotsu town
D. erythrovaria	520401				1975	4	15	M. Nii	Kagawa pref., Nakatado county, Mannnou town
D. erythrovaria	295875				1983	3	27	K. Yamaoka	Kouchi pref., Kami county, Tosayamada town, Aburaishi
D. erythrovaria	1219416				1981	12	6	K. Yamaoka	Kouchi pref., Kouchi city, Kaira
D. erythrovaria	500914				1974	10	22	T. Nakaike	Kouchi pref., Sakihama town
D. erythrovaria	511871				1973	5	29	Y. Kimizuka	Kouchi pref., Muroto city, Higashidera
D. erythrovaria	511865				1983	1	4	K. Yamaoka	Kouchi pref., Muroto city, Nahari town, Sugawa
D. erythrovaria	511866				1971	7	24	T. Inobe	Kouchi pref., Syukuge city, Okinoshima town
D. erythrovaria	1037667				1951	3	31	H. Ito	Kouchi pref., Ashizurimsaki
D. erythrovaria	1039254				1983	10	5	K. Yamaoka	Kouci pref., Nanngoku city, Nakanokawa
D. erythrovaria	446351				1980	12	7	K. Yamaoka	Kouchi pref., Hata county, Ootsuki town
D. erythrovaria	1045641				2011	8	30	Y. Inoue	Saga pref., Imari city, Fukuno
D. erythrovaria	1119507				1977	5	1	Y. Kurashige	Saga pref., Higashimatsura county, Chinnzei town, Matsushima
D. erythrovaria	1023871				1986	8	22	T. Iwata	Saitama pref., Minano town, Minoyama
D. erythrovaria	1026497				1982	9	20	M. Shimosegawa	Saitama pref., Yorii town
D. erythrovaria	448034				1990	10	7	T. Iwata	Saitama pref., Oosato county, Yorii town, Sueno

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. erythrovaria	1026488				1991	11	24	T. Iwata	Saitama pref., Chichibu county, Ogano town, nagatome
D. erythrovaria	1026468				1994	10	25	T. Iwata	Saitama pref., Chichibu city, Kagemori
D. erythrovaria	1026517				1965	11	7	H. Ito	Saitama pref., Iruma county, Moroyama town, Kamakita Lake
D. erythrovaria	1092502				1986	11	9	T. Iwata	Saitama pref., Hannnou city, Kusumizaka
D. erythrovaria	293141				1973	1	25	T. Moriya	Saitama pref., Hiki county, Ogawa town, Suguro
D. erythrovaria	293507				1973	1	13	T. Magofuku	Saitama pref., Hiki county, Ogawa town, Kiroko
D. erythrovaria	1037677				1951	8	24	T. Magofuku	Mie pref., Ujiyamada city, Naiguu
D. erythrovaria	1037678				1951	12	30	T. Magofuku	Mie pref., Matsuzaka city, Hatadono shrine
D. erythrovaria	723365				1981	7	17	K. Seto	Mie pref., Matsuzaka city, Kannomihatadono shrine
D. erythrovaria	817516				1982	3	8	S. Yamauchi	Mie pref., Daiou town, Azena
D. erythrovaria	1247463				1979	7	7	S. Yamauchi	Mie pref., Toba city, Imaura town
D. erythrovaria	441922				1957	2	10	Y. Higuchi	Mie pref., Owase city, Kuki
D. erythrovaria	441907				1990	7	20	N. Sakawa	Yamagata pref., Yuza town, Shimoonoso
D. erythrovaria	569339				1977	6	19	K. Oka	Yamaguchi pref., Ube city, Hirose
D. erythrovaria	1264267				1980	3	8	A. Minami	Yamaguchi pref., Iwakuni city, Yamasemizuguchi
D. erythrovaria	569418				1979	10	6	A. Minami	Yamaguchi pref., Kuamge county, Kamiseki town, Hourijima
D. erythrovaria	535686				1989	12	17	H. Masaki	Yamaguchi pref., Hikari city, Iwayashita
D. erythrovaria	1204147				1989	10	15	A. Minami	Yamaguchi pref., Kamiseki town, Uwajima
D. erythrovaria	1204148				1980	11	3	A. Minami	Yamaguchi pref., Ootsu county, Yuya town, Ookawajiri
D. erythrovaria	276117				1965	5	5	K. Oka	Yamaguchi pref., Hagi city, Kasayama
D. erythrovaria	566898				1977	4	21	K. Oka	Yamaguchi pref., Hagi city, Mishima
D. erythrovaria	569421				1936	9	13	S. Funo	Yamanashi pref., Minamikoma county, Minobu village, Mt. Minobusann
D. erythrovaria	1247603				1981	9	2	S. Yamauchi	Shiga pref., Kouga county, Kounann town, Mt. Iwaoyama
D. erythrovaria	441926				1982	11	15	T. Tatebe	Shiga pref., Hikone city, Miyata town, Nishiyama
D. erythrovaria	1184921				2008	2	11	A. Ebihara	Kagoshima pref., Aira county, Aira town, Hiramatsu
D. erythrovaria	520907				1979	8	19	K. Takesako	Kagoshima pref., Aira county, Makizono town, Sasanodann
D. erythrovaria	520904				1961	1	15	T. Yamanaka	Kagoshima pref., Isa county, Hishikari town, Kusumoto
D. erythrovaria	734751				1982	8	8	K. Takesako	Kagoshima pref., Kimotsuki county, Aira town, Nishidake
D. erythrovaria	520858				1978	11	5	K. Takesako	Kagoshima pref., Kimotsuki county, Nejime town
D. erythrovaria	520838				1976	8	2	K. Takesako	Kagoshima pref., Kimotsuki county, Sata town, Hariyama
D. erythrovaria	520852				1985	8	18	T. Yamanaka	Kagoshima pref., Kimtosuki county, Sata town, Takenoura
D. erythrovaria	1045649				1958	11	2	H. Ito	Kagoshima pref., Tarumi city, Mt. Takakumayama
D. erythrovaria	520905				1976	9	26	K. Takesako	Kagoshima pref., Yubisuki city, Kimondaira
D. erythrovaria	520840				1977	7	3	K. Takesako	Kagoshima pref., Kagoshima county, Sakurajima Island, Sakurajima town, Shirahama
D. erythrovaria	520839				1980	2	23	K. Kawahara	Kagoshima pref., Kagoshima city, Higashisakurajima town, Yuno
D. erythrovaria	520829				1981	10	10	K. Kawahara	Kagoshima pref., Kagoshima city, Hirakawa town, Mt. Eboshidake
D. erythrovaria	520863				1977	3	6	K. Kawahara	Kagoshima pref., Imizu county, Nagashima town, Hirao
D. erythrovaria	520813				1982	9	15	Y. Hashiguchi	Kagoshimapref., Imizu city, Kamichishiki town
D. erythrovaria	520902				1976	7	29	K. Takesako	Kagoshima pref., Soo county, Takarabe town
D. erythrovaria	520851				1982	8	9	M. Kawabata	Kagoshima pref., Soo county, Shibushi town, Natsui
D. erythrovaria	1247469				1981	5	31	K. Takesako	Kagoshima pref., Soo county, Shibushi town, Maniwa
D. erythrovaria	520900				1976	8	18	K. Takesako	Kagoshima pref., Soo county, Shibushi town, Houmann
D. erythrovaria	520859				1980	11	2	K. Takesako	Kagoshima pref., So county, Oosumi town, Sakamoto
D. erythrovaria	520896				1977	7	26	K. Takesako	Kagoshima pref., Soo county, Ariake town, Ootorikyou
D. erythrovaria	520836				1979	5	3	K. Takesako	Kagoshima pref., Hioki city, Fukiage town, Tashirono
D. erythrovaria	520837				1974	2	3	K. Takesako	Kagoshima pref., Hioki city, Higashiichiki town
D. erythrovaria	520857				1991	5	26	M. Kawabata	Kagoshima pref., Ibusuki county, Ei town, Orio
D. erythrovaria	561485				1981	10	2	K. Kawahara	Kagoshima pref., Ibusuki county, Ei town, Nakamura
D. erythrovaria	1018265				1936	5	3	T. Nomura	Kanagawa pref., Fujisawa city, Enoshima Island
D. erythrovaria	450096				1982	1	24	T. Sato	Kanagawa pref., Yokosuka city, Koyasu
D. erythrovaria	450076				1982	11	20	C. Hino	Kanagawa pref., Yokohama city, Minatokita Ward
D. erythrovaria	450092				1981	5	18	T. Sato	Kanagwa pref., Kamakura city, Imaizumi
D. erythrovaria	941836				1946	6	9	T. Date	Kanagawa pref., Kamakura city, Komachi
D. erythrovaria	457374				1965	11	28	C. Okawa	Kanagawa pref., Naka county, Ooiso town, Mt. Komayama
D. erythrovaria	450090				1982	4	29	K. Tanaka	Kanagawa pref., Odawara city, Kuno
D. erythrovaria	184105				1966	10		T. Ichikawa	Kanagawa pref., Zushi city, Jinnmuji
D. erythrovaria	450094				1971	1	20	Y. Sasaki	Kanagawa pref., Kawasaki city, Higashitakane
D. erythrovaria	450087				1981	11	14	K. Tanaka	Kanagawa pref., Naka county, Ooiso town, Nishikoiso
D. erythrovaria	450093				1970	6	13	Y. Sasaki	Kanagawa pref., Fujisawa city, Oobajoushi
D. erythrovaria	450077				1981	11	22	J. Moriya	Kanagawa pref., Hiratsuka city, Tsuchiyatashiro
D. erythrovaria	1248447				1985	7	30	Y. Kimizuka	Shizuoka pref., Ito city, Matsubara
D. erythrovaria	510871				1975	11	9	T. Shimakawa	Shizuoka pref., Ito city, Kawana
D. erythrovaria	1160628				2002	7	15	S. Matsumoto	Shizuoka pref., Shimoda city, Suzaki
D. erythrovaria	929383				1982	3	21	T. Sato	Shizuoka pref., Shimoda city, Touji
D. erythrovaria	929371				1982	9	5	T. Sato	Shizuoka pref., Kamo county, Kawazu town, Midaka
D. erythrovaria	510894				1981	12	12	T. Kawana	Shizuoka pref., Kamo county, Higashiizu town, Atsukawa
D. erythrovaria	510883				1981	8	18	T. Sato	Shizuoka pref., Kamo county, Minamiizu town, Aoichi
D. erythrovaria	510234				1982	9	2	T. Sato	Shizuoka pref., Kamo county, Minamiizu town, Tyougano
D. erythrovaria	510860				1980	10	17	T. Sato	Shizuoka pref., Hamamatsu city, Tenryuu Ward, Haruno town, Makino
D. erythrovaria	510239				1982	11	27	T. Sugino	Shizuoka pref., Syuuchi county, Mori town, Mikura
D. erythrovaria	437457				1975	1	17	Y. Kimizuka	Shizuoka pref., Numazu city, Mt. Kanukiyama
D. erythrovaria	929368				1982	8	13	T. Sato	Shizuoka pref., Numazu city, Ashidaka
D. erythrovaria	510236				1982	9	5	T. Sugino	Shizuoka pref., Haibata county, Kawane town, Sasamado
D. erythrovaria	447771				1983	11	18	Y. Kimizuka	Shizuoka pref., Nishiizu town, Dougashima
D. erythrovaria	510246				1953	5	10	T. Sugino	Shizuoka pref., Shizuoka city, Shimomura
D. erythrovaria	510874				1982	4	29	M. Nakagawa	Shizuoka pref., Shizuoka city, Kamisuke
D. erythrovaria	510873				1982	9	5	H. Suzuki	Shizuoka pref., Tenryuu city, Tsuki
D. erythrovaria	510242				1962	9	23	K. Satake	Shizuoka pref., Shimada city, Shimogusa
D. erythrovaria	510241				1976	8	6	T. Sugino	Shizuoka pref., Fujieda city, Ichinose
D. erythrovaria	41334				1929	2		H. Asukayama	Shizuoka pref., Atami city, Hatsushima Island
D. erythrovaria	929380				1978	3	26	Y. Kimizuka	Shizuoka pref., Fujinomiya city, Yamamiya
D. erythrovaria	929356				1984	9	24	T. Sato	Shizuoka pref., Fuji city, Kuwasaki
D. erythrovaria	929358				1976	9	12	F. Gonnda	Shizuoka pref., Fuji city, Kowadakubo
D. erythrovaria	544994				1953	6	10	T. Nakaike	Chiba pref., Awa county, Shirahama town
D. erythrovaria	1247365				1983	1	15	K. Nakamura	Chiba pref., Isumi county, Misaki town, Mikado
D. erythrovaria	533657				1983	1	7	K. Nakamura	Chiba pref., Isumi county, Misaki town, Shiigi
D. erythrovaria	799525				1981	1	16	K. Sato	Chiba pref., Ichinomiya town, Mt. Gundariyama
D. erythrovaria	533559				1981	9	20	M. Aida	Chiba pref., Abiko city, Fusa
D. erythrovaria	545011				1976	10	30	Y. Koike	Chiba pref., Tateyama city, Awa shrine
D. erythrovaria	318417				1973	2	26	Y. Saito	Chiba pref., Tateyama city, Hamada
D. erythrovaria	817533				1978	11	18	F. Miyamoto	Chiba pref., Kimitsu city, Sasagawa
D. erythrovaria	544998				1977	7	3	Y. Kimizuka	Chiba pref., Katori county, Tounosyou town, Shimoiida
D. erythrovaria	1037676				1953	12	13	H. Ito	Chiba pref., Mt. Mitsuishisann
D. erythrovaria	979462				1950	8	2	H. Ito	Chiba pref., Yotsukaidou city, Nagaoka
D. erythrovaria	979459				1983	12	25	T. Akiba	Chiba pref., Yotsukaidou city, Monoi
D. erythrovaria	533663				1983	12	12	T. Akiba	Chiba pref., Ichihara city, Anegasaki shrine
D. erythrovaria	533655				1982	12	30	K. Nakamura	Chiba pref., Ichihara city, Ookura
D. erythrovaria	1249541				1982	12	30	K. Nakamura	Chiba pref., Ichikawa city, Mt. Mamasann
D. erythrovaria	533541				1982	3	2	Y. Saito	Chiba pref., Katsuura city, Okitsu
D. erythrovaria	544993				1979	11	12	Y. Kimizuka	Chiba pref., Matsudo city, Hiragahondo temple
D. erythrovaria	533551				1980	1	15	Y. Koike	Chiba pref., Tomisato city, Kunou
D. erythrovaria	1247369				1982	12	28	K. Nakamura	Chiba pref., Sannmu city, Narutou
D. erythrovaria	39441				1979	9	15	Y. Koike	Chiba pref., Mt. Kiyosumiyama
D. erythrovaria	533552				1932	2		S. Asano	Chiba pref., Choshi city, Saruda town
D. erythrovaria	545001				1976	2	7	Y. Kimizuka	Chiba pref., Chousei county, Ichinomiya town, Torami
D. erythrovaria	1247379				1982	12	30	K. Nakamura	Chiba pref., Chousei county, Osakabe
D. erythrovaria	817538				1983	9	14	T. Iwata	Chiba pref., Minamibousou city, Chikura town, Ookawa
D. erythrovaria	1026540				1960	1	31	T. Iwata	Chiba pref., Yachimata city, Toyama
D. erythrovaria	1247367				1977	4	30	Y. Kimizuka	Chiba pref., Mobara city, Kaminagayoshi
D. erythrovaria	533659				1983	1	2	K. Nakamura	Chiba pref., Mobara city, Honnou
D. erythrovaria	533549				1987	8	19	M. Ono	Chiba pref., Kisarazu city, Takakura
D. erythrovaria	1247374				1983	1	4	K. Nakamura	Chiba pref., Kisarazu city, Kazusakiyokawa
D. erythrovaria	1037743				1982	10	7	K. Kawahara	Osaka-fu, Sakai city, Ootori shrine
D. erythrovaria	580829				1983	8	24	A. Komaki	Osaka-fu, Sennnann county, Kumatori town, Shichiyama
D. erythrovaria	102708				1932	1	31	Y. Sanno	Osaka-fu, Nakakawachi county, Mt. Tamateyama
D. erythrovaria	1046294				1952	7	15	Y. Sanno	Oita pref., Usa city, Usahachimann
D. erythrovaria	1247425				1977	4	17	Y. Takaoka	Oita pref., Usa city, Takanamiyashiki
D. erythrovaria	1247415				1978	4	23	Y. Takaoka	Oita pref., Usuki city, Tsukumijima Island
D. erythrovaria	1247424				1976	3	28	Y. Takaoka	Oita pref., Hayami county, Hiji town, Toyooka
D. erythrovaria	1247389				1978	7	2	Y. Takaoka	Oita pref., Nakatsu city, Oosada, Kazura shrine
D. erythrovaria	388540				1976	5	11	H. Haneda	Nagasaki pref., Iki county, Iki Island, Ashibe town
D. erythrovaria	337171				1965	11	2	T. Shinagawa	Nagasaki pref., Iki county, Iki Island, Gounoura town, Katabaru, Fure

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. erythrovaria	575563				1980	12	18	S. Tsutsui	Nagasaki pref., Iki county, Iki Island, Ishida town, Inndouji
D. erythrovaria	575551				1972	9	23	S. Kobayashi	Nagasaki pref., Kamiagata county, Tsushima Island, Kamiagata town, Mt. Mitake
D. erythrovaria	1247429				1982	9	5	B. Matsubayashi	Nagasaki pref., Tsushima city, Tsushima Island, Izuhara town, Tatera
D. erythrovaria	345357				1976	3	15	F. Ueno	Nagasaki pref., Oomura city, Hidorigou
D. erythrovaria	388526				1976	5	13	H. Haneda	Nagasaki pref., Minamimatsura county, Tamanoura town, Arakawa
D. erythrovaria	447968				1984	2	5	A. Minami	Nagasaki pref., Hirado city, Hirado Island, Tsuji town
D. erythrovaria	575561				1977	6	2	S. Tsutsui	Nagasaki pref., Hirado city, Hirado Island, Himosashi town
D. erythrovaria	446986				1983	12	21	S. Tsutsui	Nagasaki pref., Kitamatsura county, Kosaza town, Kamanage
D. erythrovaria	446987				1983	12	21	S. Tsutsui	Nagasaki pref., Kitamatsura county, Kosaza town, Town
D. erythrovaria	444411				1971	11	12	A. Tanaka	Tottori pref., Yonago city, Shiroyama
D. erythrovaria	443921				1932	12	11	A. Minami	Shimane pref., Oki county, Oki Island, Nishinoshima town, Beppu
D. erythrovaria	1184913				2013	5	30	H. Sawae	Shimane pref., Masuda City, Nishihirabara town, Shimohirahara
D. erythrovaria	449640				1979	9	23	K. Inoue	Shimane pref., Oki county, Oki Island, Ama town, Hishiura
D. erythrovaria	449677				1982	1	15	A. Minami	Shimane pref., Matsue city, Tasumi town
D. erythrovaria	448712				1977	4	11	K. Oka	Shimane pref., Oki county, Oki Island, Nishinoshima town, Mt. Takuhiyama
D. erythrovaria	1264266				1976	8	21	K. Oka	Shimane pref., Izumo city, Taisya town, Izumoooyashiro
D. erythrovaria	446207				1983	2	11	A. Minami	Shimane pref., Unnnann city, Mitoya town, Tonogouchi
D. erythrovaria	1092503				1964	5	10	Y. Moriyama	Shimane pref., Izumo city, Taisya town, Hinomisaki
D. erythrovaria	817503				1977	9	4	H. Yamamoto	Tokyo-to, Izuoshima Island
D. erythrovaria	523069				1931	5	3	E. Fukushi	Tokyo-to, Inagi city, Yanokuchi
D. erythrovaria		449109			2016	6	18	K. Hori	Tokyo-to, Inagi city, Momura
D. erythrovaria	9511761				1934	10	28	T. Sato	Tokyo-to, Minato Ward, Shirokanedai
D. erythrovaria	523261				1981	4	1	Y. Jotani	Tokyo-to, Miyake Island
D. erythrovaria	817512				1980	10	16	Y. Jotani	Tokyo-to, Shikine Island
D. erythrovaria	121178				1933	8	25	Y. Jotani	Tokyo-to, Niijima Island
D. erythrovaria	523265				1935	7	14	A. Yamamoto	Tokyo-to, Machida city, Miwa town
D. erythrovaria	523065				1980	11	30	B. Sasaki	Tokyo-to, Machida city, Onoji town
D. erythrovaria	523068				1980	2	5	C. Azegami	Tokyo-to, Machida city, Aihara town
D. erythrovaria	523070				1955	8	28	S. Toga	Tokyo-to, Hachioji city, Ozu town, Usui
D. erythrovaria	817517				1953	6	7	Y. Kawasaki	Tokyo-to, Hachijojima Island
D. erythrovaria	476189				1930	12	30	K. Abe	Tokushima pref., Katsuura county, Katsuura town
D. erythrovaria	476014				1975	7	30	K. Abe	Tokushima pref., Kamiyama town, Ookubo
D. erythrovaria	1039325				1960	8	10	T. Inobe	Tokushima pref., Tokushima city, Hachimann town, Mayyama
D. erythrovaria	476013				1964	11	2	K. Abe	Tokushima pref., Naka county, Naka town
D. erythrovaria	476010				1973	9	5	K. Abe	Tokushima pref., Yoshinogawa city, Suijin fall
D. erythrovaria	1037627				1951	6	26	T. Inobe	Tokushima pref., Myouzai county, Kamiyama town, Jinnryo
D. erythrovaria	337201				1975	2	11	H. Kinoshita	Tokushima pref., Naruto city, Kitanada town, Awata
D. erythrovaria	384450				1976	6	12	T. Waku	Tochigi pref., Sano city, Tanuma town
D. erythrovaria	525093				1980	11	3	T. Tashiro	Tochigi pref., Shioya county, Shioya town, Sanukikannnonn
D. erythrovaria	1247638				1984	6	16	M. Usui	Tochigi pref., Tochigi city, Mt. Kikamoyama
D. erythrovaria	525092				1980	10	5	T. Tashiro	Tochigi pref., Sano city, Mt. Karasawayama
D. erythrovaria	586138				1983	7	12	M. Usui	Tochigi pref., Utsunomiya city, Kamikanai town
D. erythrovaria	525090				1976	3	20	T. Waku	Tochigi pref., Kanuma city, Iwayama
D. erythrovaria	554431				1982	10	12	M. Usui	Tochigi pref., Ashikaga city, Nishiba town
D. erythrovaria	799526				1987	9	5	H. Ogura	Tochigi pref., Ashikaga city, Asakura town
D. erythrovaria	799530				1987	9	5	T. Noguchi	Tochigi pref., Itakura city, Itakura
D. erythrovaria	554430				1982	9	30	M. Usui	Tochigi pref., Tochigi city, Shiriuchi
D. erythrovaria	511908				1978	12	27	Y. Tsujimoto	Nara pref., Takaichi county, Asuka village, Inabuchi
D. erythrovaria	511907				1982	5	20	T. Suzuki	Nara pref., Nara city, Oshikuma town
D. erythrovaria	507926				1959	9	27	T. Miyauchi	Fukui pref., Imadate county, Ikeda town, Matsugaya
D. erythrovaria	507972				1977	8	6	Y. Saito	Fukui pref., Obama city, Mt. Iimoriyama
D. erythrovaria	507928				1976	8	8	Y. Saito	Fukui pref., Fukui city, Godaishi fall
D. erythrovaria	1037724				1983	8	4	N. Ogami	Fukuoka pref., Chikushino city, Futsukaichi
D. erythrovaria	587661				1947	10	20	S. Toyama	Fukuoka pref., Fukuoka city, Nishi Ward, Oronoshima Island
D. erythrovaria	557394				1968	12	31	S. Tsutsui	Fukuoka pref., Fukuoka city, Minami Ward, Mt. Kounosuyama
D. erythrovaria	557643				1982	2	10	K. Takatsu	Fukuoka pref., Kitakyuusyuu city, Yahatanishi Ward, Asakawa
D. erythrovaria	557634				1975	4	5	T. Inoue	Fukuoka pref., Kitakyuusyuu city, Moji Ward, Ootsumi
D. erythrovaria	557635				1981	6	7	S. Kobayashi	Fukuoka pref., Kitakyuusyuu city, Moji Ward, Hisyakuda
D. erythrovaria	541607				1983			T. Yamada	Fukushima pref., Kitakata city, Keitoku town
D. erythrovaria	444859				1978	11	3	M. Yasu	Fukushima pref., Futaba county, Hirono town
D. erythrovaria	184816				1978	11	3	M. Yasu	Fukushima pref., Futaba county, Oohisa village
D. erythrovaria	1247688				1976	3	21	T. Shiraiwa	Hyogo pref., Himeji city, Yasutomi town, Seki
D. erythrovaria	1247663				1977	9	18	T. Shiraiwa	Hyogo pref., Shisou city, Yamasaki town
D. erythrovaria	1247685				1979	6	17	T. Shiraiwa	Hyogo pref., Koube city, Nishi Ward, Oshibedani town, Sakae
D. erythrovaria	449254				1983	10	23	T. Shiraiwa	Hyogo pref., Akou city, Nishiune
D. erythrovaria	1247657				1951	6	17	M. Inada	Hyogo pref., Sayou county, Sayou town, Mt. Funakoshiyama
D. erythrovaria	36950				1930	12	27	Y. Kurimoto	Wakayama pref., Shinguu city, Hirokado
D. erythrovaria	518568				1969	1	6	H. Masago	Wakayama pref., Nishimuro county, Shirahama town, Katata
D. erythrovaria	1037723				1951	10	25	E. Maeda	Wakayama pref., Higashimuro county, Shimosato town
D. erythrovaria	518468				1969	1	6	H. Masago	Wakayama pref., Higashimuro county, Taiji town
D. erythrovaria	518576				1977	8	21	T. Oka	Wakayama pref., Wakayama city, Isao, Narutakifudou
D. hikonensis	439262				1980	1	5	K. Inukai	Aichi pref., Okazaki City, Iwato town
D. hikonensis	439271				1979	11	3	K. Inukai	Aichi pref., Inuyama City, Zenshino
D. hikonensis	439286				1980	3	16	K. Inukai	Aichi pref., Tokoname City, Segi Town
D. hikonensis	582524				1979	2	12	M. Muramatsu	Aichi pref., Shinjo City, Nanakubo
D. hikonensis	439290				1982	10	30	M. Muramatsu	Aichi pref., Seto City, Kamihanda river
D. hikonensis	439330				1982	12	30	K. Inukai	Aichi pref., Nishio City, Kamihasumi town
D. hikonensis	1247538				1980	9	20	K. Inukai	Aichi pref., Chita county, Minamichita town, Utsumi
D. hikonensis	1247537				1982	5	23	K. Inukai	Aichi pref., Chita City, Ookusa
D. hikonensis	439294				1979	12	30	K. Inukai	Aichi pref., Chiryuu City, Kobo town
D. hikonensis	582533				1983	5	29	K. Inukai	Aichi pref., Handa City, Itayama town
D. hikonensis	1247456				1982	3	22	K. Inukai	Aichi pref., Toyohashi City, Takatsuka town
D. hikonensis	1247539				1983	5	3	K. Inukai	Aichi pref., Toyoda City, Kanou town
D. hikonensis	439265				1980	11	23	M. Muramatsu	Aichi pref., Kitashitara county, Toei town
D. hikonensis	439269				1979	12	15	K. Inukai	Aichi pref., Nagoya City, Naka Ward, Sannnomaru
D. hikonensis	1246311				2008	12	14	M. Hyodo	Ehime pref., Iyo City, Futami town, Kaminada
D. hikonensis	1247612				1985	9	9	Y. Kimizuka	Ehime pref., Uwajima City, Toshima
D. hikonensis	555867				1980	10	12	M. Hyodo	Ehime pref., Kita county, Uchigo town, Iogi
D. hikonensis	555906				1981	10	11	M. Hyodo	Ehime pref., Matsuyama City, Sugesawa
D. hikonensis	554956				1981	1	16	Y. Kimizuka	Ehime pref., Araihama City, Daijoin
D. hikonensis	1247434				2012	1	21	M. Hyodo	Ehime pref., Saiyo City, Akehama town, Tanohamaoosaki
D. hikonensis	1246310				2013	1	6	M. Hyodo	Ehime pref., Ozu City, Hijikawa town, Myogadani
D. hikonensis	554957				1979	8	30	M. Hyodo	Ehime pref., Yahatahama City, Nakatsugawa
D. hikonensis	512447				1974	12	28	M. Yasu	Ibaraki pref., Inashiki county, Miura village
D. hikonensis	512450				1974	10	13	M. Yasu	Ibaraki pref., Takahagi City, Shimada
D. hikonensis	512241				1975	8	27	M. Yasu	Ibaraki pref., Toride City, Omonnma
D. hikonensis	512451				1974	11	11	Y. Nakazaki	Ibaraki pref., Migawa town, Sakurayama
D. hikonensis	512462				1982	3	24	E. Fukushi	Ibaraki pref., Ryuugasaki City, Itabashi
D. hikonensis	448642				1984	2	20	Y. Obata	Okayama pref., Ihara City, Ooe town, Kajikusa
D. hikonensis	522004				1982	5	16	Y. Morimoto	Okayama pref., Kume county, Kumenann town, Matama
D. hikonensis	512654				1980	11	14	Y. Obata	Okayama pref., Tamano City, Tama
D. hikonensis	512650				1982	11	23	K. Yasuhara	Okayama pref., Oda county, Yagake town, Satoyamada
D. hikonensis	388690				1976	1	31	T. Watanabe	Okayama pref., Niimi City, Karamatsumakabe
D. hikonensis	521946				1980	11	2	K. Yasuhara	Okayama pref., Asaguchi county, Satosho town, Shinjo
D. hikonensis	512097				1980	11	14	Y. Obata	Okayama pref., Kurashiki City, Kojima, Kaminomachi
D. hikonensis	521962				1981	8	14	T. Saito	Okayama pref., Tsuyama City, Higashiichinomiya, Nakayama shrine
D. hikonensis	541666				1983	8	28	Y. Obata	Okayama pref., Bizenn City, Mitsuishi
D. hikonensis	344161				1977	7	9	M. Takara	Okinawa pref., Naha City, Syuri, Mt. Benngatake
D. hikonensis	506319				1979	11	24	Y. Yamamoto	Gifu pref., Annpachi county, Wanouchi town, Sato
D. hikonensis	446024				1983	10	23	K. Inukai	Gifu pref., Kamo county, Sakahogi town, Katsuyama
D. hikonensis	444962				1978	10	23	M. Fukuhara	Gifu pref., Kagamihara City, Unuma town, Igi
D. hikonensis	600216				1977	9	28	Y. Yamamoto	Gifu pref., Seki City, Shizuno
D. hikonensis	1248438				1984	5	20	K. Inukai	Gifu pref., Gifu City, Mitabora
D. hikonensis	446023				1983	11	6	K. Inukai	Gifu pref., Nakatsugawa City, Agi
D. hikonensis	1253366				2013	11	2	T. Ogiyama	Gifu pref., Ibi county, Ibigawa town, Otsuhara
D. hikonensis	556284				1982	9	12	T. Minamitani	Miyazaki pref., Nobeoka City, Uwada town
D. hikonensis	556729				1976	1	3	T. Minamitani	Miyazaki pref., Miyazaki City, Kaeda river
D. hikonensis	556432				1981	12	1	K. Minamitani	Miyazaki pref., Kushima City, Toimisaki
D. hikonensis	1263314				2013	12	22	Y. Akagi	Miyazaki pref., Koyu county, Kishiro town, Nakanomata
D. hikonensis	556122				1982	3	21	T. Minamitani	Miyazaki pref., Kobayashi City, Higashikata, Obara
D. hikonensis	556281				1982	11	20	T. Yoshida	Miyazaki pref., Nishiusuki county, Gokase town, Sannkasyo
D. hikonensis	556436				1961	10	7	I. Taki	Miyazaki pref., Seito City, Katauchi

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. hikonensis	1263305				2013	7	13	Y. Akagi	Miyazaki pref., Miyakonojo City, Yasuhisa town, Yuyadani
D. hikonensis	556288				1981	10	11	T. Minamitani	Miyazaki pref., Higashiusuki county, Morozuka village, Nanatsuyama
D. hikonensis	556298				1982	9	20	T. Minamitani	Miyazaki pref., Hyuuga City, Hiraiwa
D. hikonensis	556745				1981	11	3	K. Minamitani	Miyazaki pref., Nichinann City, Futo
D. hikonensis	572345				1982	3	6	K. Shogo	Miyazaki pref., Yamamoto town, Sakamoto
D. hikonensis	537957				1982	6	6	T. Nakaike	Kyoto-fu, Miyazu City, Hioki
D. hikonensis	654485				1933	4	18	Y. Araki	Kyoto-fu, Kyoto City, Sakyo Ward, Mt. Daimonnji
D. hikonensis	442741				1982	8	11	A. Komaki	Kyoto-fu, Maizuru City, Mihama pass
D. hikonensis	1246945				1983	8	8	T. Nakaike	Kyoto-fu, Fukuchiyama City, Nagatano
D. hikonensis	1247221				2014	1	19	M. Watababe	Kumamoto pref., Ashikita county, Ashikita town, Yoneda
D. hikonensis	574088				1982	9	12	M. Ishizaka	Kumamoto pref., Udo City, Shimoamida town
D. hikonensis	734626				1968	1	14	T. yamanaka	Kumamoto pref., Kuma county, Kuma village
D. hikonensis	574081				1982	8	1	M. Ishizaka	Kumamoto pref., Tamana county, Nannkann town, Kushige
D. hikonensis	574080				1982	8	1	M. Ishizaka	Kumamoto pref., Arao City, Shimoyama
D. hikonensis	517865				1977	10	16	M. Ishizaka	Kumamoto pref., Yamaga City, Ishi
D. hikonensis	517861				1981	9	20	J. Beppu	Kumamoto pref., Kamimasuki county, Masuki town
D. hikonensis	517869				1963	3	3	M. Shiroto	Kumamoto pref., Minamata City, Yunode
D. hikonensis	518645				1978	5	8	M. Shiroto	Kumamoto pref., Amakusa county, Reihoku town, Tororo
D. hikonensis	518643				1982	5	21	S. Watanabe	Kumamoto pref., Yatsushiro City, Futamihonn town, Kobusegi
D. hikonensis	1045261				1968	12	2	H. Ito	Hiroshima pref., Hatsukaichi City, Miyajima town
D. hikonensis	518621				1976	5	9	Y. Nishimura	Hiroshima pref., Kure City, Nishisouzuke town
D. hikonensis	518651				1975	5	31	T. Takeda	Hiroshima pref., Mihara City, Sougou town
D. hikonensis	520524				1976	6	15	T. Takeda	Hiroshima pref., Miyoshi City, Awaya
D. hikonensis	518632				1976	3	16	T. Takeda	Hiroshima pref., Takehara City, Konashi
D. hikonensis	520520				1975	7	9	T. Takeda	Hiroshima pref., Fuchu City, Araya town
D. hikonensis	518660				1975	9	30	T. Takeda	Hiroshima pref., Fukuyama City, Kamo town, Kusuda
D. hikonensis	257029				1969	11	2	Y. Maki	Kagawa pref., Kannnonnji City, Awai town
D. hikonensis	520411				1974	9	7	S. Mitani	Kagawa pref., Takamatsu City, Mineyama
D. hikonensis	520389				1976	1	31	S. Mitani	Kagawa pref., Shodo county, Dosho town, Fuchisaki shrine
D. hikonensis	520408				1975	11	16	S. Mitani	Kagawa pref., Nakatado county, Kotohira town, Mt. Kotohira
D. hikonensis	511867				1982	10	26	Y. Yamamoto	Kochi pref., Aki county, Kitagawa village, Nishinotani
D. hikonensis	511820				1981	3	22	T. Miyazaki	Kochi pref., Takaoka county, Nakatosa town, yatabe
D. hikonensis	511819				1979	7	22	T. Miyazaki	Kochi pref., Kochi City, Mt. Godaisann
D. hikonensis	511838				1983	1	4	K. yamaoka	Kochi pref., Muroto City, Sakihama
D. hikonensis	446318				1983	10	26	K. yamaoka	Kochi pref., Syukuge City, Nagahata
D. hikonensis	511857				1979	3	4	K. yamaoka	Kochi pref., Suzaki City, Awakaigann
D. hikonensis	541773				1982	10	10	K. yamaoka	Kochi pref., Nagaoka county, Ootoyo town, Kawaguchi
D. hikonensis	511856				1979	2	18	K. yamaoka	Kochi pref., Tosa City, Kitabara
D. hikonensis	511840				1981	11	28	K. yamaoka	Kochi pref., Tosashimizu City, Misaki
D. hikonensis	446344				1983	10	5	K. yamaoka	Kochi pref., Nanngoku City, Nakanokawa
D. hikonensis	544921				1981	11	15	Y. Kurashige	Saga pref., Imari City, Imari town
D. hikonensis	518755				1975	7	13	M. Iwamura	Saga pref., Kashima City, Iida
D. hikonensis	517442				1969	11	23	Y. Kurashige	Saga pref., Taku City, Kitataku town, Minenosu
D. hikonensis	517381				1976	7	17	Y. Kurashige	Saga pref., Tosu City, Yubi town
D. hikonensis	518757				1976	8	20	J. Tannno	Saga pref., Karatsu City, Sashi
D. hikonensis	518758				1979	2	11	N. Niwaki	Saga pref., Takeo City, Asahi town, Nakano
D. hikonensis	444542				1981	7	11	H. Endou	Saitama pref., Tokorozawa City, Honngou
D. hikonensis	1026538				1983	10	31	T. Iwata	Saitama pref., Chichibu county, Minano town, Kanazawa
D. hikonensis	1023882				1995	10	1	T. Iwata	Saitama pref., Chichibu City, Kuna, Hiranita
D. hikonensis	444536				1982	11	14	S. Kobayashi	Saitama pref., Iruma county, Ogose town, Mugihara
D. hikonensis	444540				1978	5	7	Y. Kobayashi	Saitama pref., Iruma county, Moroyama town
D. hikonensis	444530				1981	8	2	S. Kobayashi	Saitama pref., Hannou City, Azu
D. hikonensis	587728				1983	10	29	M. Shimoseagwa	Saitama pref., Hiki county, Yoshimi town, Kuroiwa
D. hikonensis	441929				1981	3	8	K. Oohora	Mie pref., Kumano City, Atashika town, Atashika
D. hikonensis	441717				1982	7	27	J. Kawazoe	Mie pref., Kuwana City, Kuwabe
D. hikonensis	441916				1980	10	10	S. Yamauchi	Mie pref., Mie county, Komono town, Komono
D. hikonensis	441711				1982	11	21	S. Yamauchi	Mie pref., Yokkaichi City, Miyazuma town
D. hikonensis	441941				1976	10	15	Y. Kimizuka	Mie pref., Shima county, Shima town, Wagu
D. hikonensis	449932				1983	1	13	S. Yamauchi	Mie pref., Taki county, Taki town, Hase
D. hikonensis	1247461				1976	10	14	Y. Kimizuka	Mie pref., Toba City, Uramura town
D. hikonensis	441944				1982	6	27	Y. Nakamura	Mie pref., Minamimuro county, Kihou town, Ida
D. hikonensis	345244				1972	11	4	T. Date	Mie pref., Owase City, Kuki
D. hikonensis	441943				1979	6	11	Y. Kimizuka	Mie pref., Nabari City, Nakachiyama
D. hikonensis	1246815				1989	12	28	H. Masaki	Yamaguchi pref., Shimomatsu City, Kawachiyoshiwara
D. hikonensis	1247435				1980	8	10	A. Minami	Yamaguchi pref., IwakuniCity, Rokurochitakahata
D. hikonensis	1247589				1980	3	17	A. Minami	Yaaguchi pref., Kumage county, Kamiseki town, Yahima
D. hikonensis	1246986				1966	5	29	K. Oka	Yamaguchi pref., Hagi City, Mishima
D. hikonensis	445656				1983	10	16	K. Oka	Yamaguchi pref., Bouhu City, Tominonoda
D. hikonensis	535250				1989	1	25	A. Minami	Yamaguchi pref., Yanai City, Hizumioodani
D. hikonensis	545313				1990	6	17	T. Kurihara	Yamaguchi pref., Yamaguchi City, Kinnkei fall
D. hikonensis	1246903				1981	8	18	A. Yamamoto	Yamanashi pref., Ootsuki City, Sasago town, Hunahashisawa
D. hikonensis	1246919				1981	10	17	M. Nakagawa	Yamanashi pref., Minamikoma county, Nannbu town, Narushima
D. hikonensis	448324				1981	3	5	T. Tatebe	Shiga pref., Inugami couty, Taga town, Kawasou
D. hikonensis	1248444				1985	7	12	Y. Kimizuka	Shiga pref., Ootsu City, Sakamotohonnmachi
D. hikonensis	520849				1977	7	29	M. Shiroto	Kagoshima pref., Akune City, Takamatsu
D. hikonensis	114391				1904			T. Makino	Kagoshima pref., Amami Island
D. hikonensis	580904				1982	8	18	T. Nakaike	Kagoshima pref., Kumage county, Yakusima town, Nagata
D. hikonensis	586115				1982	8	18	T. Nakaike	Kagoshima pref., Kumage county, Yakushima town, Kurio
D. hikonensis	520911				1959	10	11	M. Kawabata	Kagoshima pref., Kumage county, Yakushima town, Yudomari
D. hikonensis	586116				1982	8	16	T. Nakaike	Kagoshima pref., Kuamge coutny, Yakushima town, Hirauchi
D. hikonensis	520862				1977	1	27	T. Nakaike	Kagoshima pref., Kumage county, Nakatane town, Atakaiso
D. hikonensis	447978				1983	12	27	K. Kawahara	Kagosima pref., Kuamge county, Minamitane town, Kohirayama
D. hikonensis	520823				1981	10	24	K. Kawahara	Kagoshima pref., Yubisuki City, Higashikata Mt. Uomidake
D. hikonensis	520886				1976	8	5	K. Takesako	Kagoshima pref., Kanoya City, Tennjinn town
D. hikonensis	523101				1977	7	3	K. Takesako	Kagoshima pref., Kagoshima county, Sakurajima Island Shirahama
D. hikonensis	98617				1976	8	25	F. Miyamoto	Kagoshima pref., Kagoghima county, Toshima village, Nakanoshima Island
D. hikonensis	51421				1963	3	11	S.Sako	Kagoshima pref., Kagoshima county, Toshima village, Takarajima Island
D. hikonensis	367559				1978	8	30	Y. Sasaki	Kagoshima pref., Kumage county, Yakushima town, Kuchinoerabujima Island
D. hikonensis	1247474				1985	1	17	M. Kawabata	Kagoshima pref., Kagoshima City, Shimofukumoto town, Gonngenngao
D. hikonensis	580860				1977	8	9	M. Shiroto	Kagoshima pref., Imizu couty, Nagashima town, Jokawauchi
D. hikonensis	580858				1982	9	11	K. Takesako	Kagoshima pref., Tarumi City, Takano
D. hikonensis	508003				1986	5	5	T. Kariyasaki	Kagoshima pref., Tanegashima Island, Nishinoomote City, Furuta
D. hikonensis	450078				1981	11	9	J. Moriya	Kanagaw pref., Isehara City, Higashitomioka
D. hikonensis	450074				1974	10	6	T. Sato	Kanagawa pref., Yokosuka City, Nagaura town
D. hikonensis	450095				1981	1	11	J. Sato	Kanagawa pref., Ykohama City, Asahi Ward, Yasashi town
D. hikonensis	1247633				1983	11	11	F. Miyamaoto	Kanagawa pref., Yokohama City, Midori Ward, Sannho town
D. hikonensis	450085				1981	10	11	A. Yamamoto	Kanagawa pref., Yokohama City, Midori Ward, Nagatsuta town, Fukada
D. hikonensis	754495				1977	12	4	C. Okawa	Kanagawa pref., Kamakura Cty, Juunisyo
D. hikonensis	667184				1990	8	30	T. Fujii	Kanagawa pref., Miura City, Misak town, Koajiro
D. hikonensis	345791				1976	1	8	N. Tashiro	Kanagawa pref., Odawara City, Haneo
D. hikonensis	450068				1976	7	18	Y. Kimizuka	Kanagawa pref., Hadano City, Oohadano
D. hikonensis	450064				1982	4	10	E. Fukushi	Kanagawa pref., Kawasaki City, Nakahara Ward, Todoriki
D. hikonensis	439083				1982	11	21	E. Fukushi	Kanagawa pref., Kawasaki City, Asou Ward, Kurokawa
D. hikonensis	450079				1976	1	6	T. Date	Kanagawa pref., Ashigarashimo county, Manazuru town, Manatsurumisaki
D. hikonensis	450062				1982	5	22	K. Tanaka	Kanagawa pref., Ashigarashimo county, Hakone town, Senngokubara
D. hikonensis	323438				1975	1	26	M. Sakakibara	Kanagawa pref., Ashigarashimo county, Hakone town, Hatajuku
D. hikonensis	450066				1979	11	3	Y. Kimizuka	Kanagawa pref., Ashigarakami county, Yamakita town
D. hikonensis	450075				1981	11	28	T. Oka	Kanagawa pref., Fujisawa City, Kawan
D. hikonensis	450082				1980	10	19	A. Yamamoto	Kanagawa pref., Minamiashigra City, Daiyuuzann
D. hikonensis	287161				1971	7	25	H. Komaki	Kanagawa pref., Hiratsuka City, Kamiyoshizawa
D. hikonensis	510870				1981	10	2	T. Shimakawa	Shizuoka pref., Ito City, Tomidomatsuo
D. hikonensis	1112399							S. Matsumoto	Shizuoka pref., Shimoda City, Suzaki
D. hikonensis	510868				1979	7	22	A. Suzuki	Shizuoka pref., Shimoda City, Renndaiji
D. hikonensis	510884				1981	11	6	T. Sato	Shizuoka pref., Kamo coutny, Matsuzaki town, Funada
D. hikonensis	509884				1974	12	30	T. Sato	Shizuoka pref., Kakegawa City, Kakegawa park
D. hikonensis	510886				1980	9	12	T. Sato	Shizuoka pref., Kosei City, Oota
D. hikonensis	1018264				1936	6	9	T. Nomura	Shizuoka pref., Gotennba City, Itazuma
D. hikonensis	437454				1973	9	9	I. Yamashita	Shizuoka pref., Syuuchi county, Mori town
D. hikonensis	445161				1983	9	19	T. Hosokura	Shizuoka pref., Sunntou county, Oyama town, Subashiri
D. hikonensis	510867				1975	1	17	Y. Kimizuka	Shizuoka pref., Numazu City, Mt. Kanuki
D. hikonensis	510891				1980	9	23	T. Sato	Shizuoka pref., Fukuroi City, Muramatsu
D. hikonensis	510823				1982	9	5	T. Sugino	Shizuoka pref., Shimada City, Kamiaiga

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. hikonensis	510865				1982	10	24	T. Shimakawa	Shizuoka pref., Atami City, Izumi
D. hikonensis	929384				1985	5	4	T. Sato	Shizuoka pref., Fujinomiya City, Saori
D. hikonensis	445164				1983	8	22	T. Sato	Shizuoka pref., Fuji City, Kuwasaki
D. hikonensis	929381				1983	9	24	T. Sato	Shizuoka pref., Fuji City, Kamiya
D. hikonensis	496014				1975	8	25	S. Komaki	Ishikawa pref., Hakui county, Shika town, Yaguradani
D. hikonensis	533536				1980	6	15	O. Kawana	Chiba pref., Awa county, Kyonann town, Ichiibara
D. hikonensis	544997				1977	7	24	Y. Kimizuka	Chiba pref., Isumi county, Ootaki town, Horikiri
D. hikonensis	444068				1982	11	14	Y. Koayashi	Chiba pref., Abiko City, Tennnoudai
D. hikonensis	545010				1981	10	11	K. Nakamura	Chiba pref., Kamagaya City, Nakazawa
D. hikonensis	533485				1980	11	20	H. Endou	Chiba pref., Kamogawa City, Futomi
D. hikonensis	533544				1980	6	29	O. Kawana	Chiba pref., Tateyama City, Okada
D. hikonensis	533540				1981	11	1	O. Kawana	Chiba pref., Kimitsu City, Kururi
D. hikonensis	533553				1976	7	14	Y. Koike	Chiba pref., Katori couty, Tako town, Tsugiura
D. hikonensis	1247373				1983	1	4	K. Nakamura	Chiba pref., Ichihara City, Anesaki shrine
D. hikonensis	1264270				1987	1	15	K. Yashiro	Chiba pref., Katsuura City, Suginotani
D. hikonensis	1264271				1986	4	26	K. Yashiro	Chiba pref., Narita City, Goube
D. hikonensis	721921				2003	3	15	K. Yashiro	Chiba pref., Chiba City, Midori Ward, Hirakawa town
D. hikonensis	533664				1982	10	17	K. Nakamura	Chiba pref., Funabashi City, Kanesugi town
D. hikonensis	817431				1959	8	30	Y. Jotani	Chiba pref., Choshi City, Inuboemisaki
D. hikonensis	545009				1982	12	30	K. Nakamura	Chiba pref., Chosei county, Nagara town, Osakabe
D. hikonensis	545000				1980	12	28	Y. Kimizuka	Chiba pref., Tougane City, Douuniwa
D. hikonensis	533548				1979	9	10	O. Kawana	Chiba pref., Futtsu City, Shimobyakko
D. hikonensis	533661				1983	1	2	K. Nakamura	Chiba pef., Mobara City, Kaminagayoshi
D. hikonensis	936278				1983	4	4	K. Nakamura	Chiba pref., Noda City, Nakane
D. hikonensis	1247636				1984	6	2	Y. Kimizuka	Chiba pref., Nagareyama City, Hiregasaki
D. hikonensis	936251				1982	8	29	A. Komaki	Osak-fu, Kawachinagano City, Kannshinn temple
D. hikonensis	936247				1978	3	15	Y. Kimizuka	Osaka-fu, Kouno City, Kisaichi
D. hikonensis	528331				1976	8	22	K. Watanabe	Osaka-fu, Takatsuka City, Honnzannji temple
D. hikonensis	528329				1981	9	18	A. Komaki	Osaka-fu, Mishima county, Shimamoto town, Syakudai
D. hikonensis	528330				1981	9	16	A. Komaki	Osaka-fu, Suita City, Yamadahigashi
D. hikonensis	528328				1982	7	15	T. Suzuki	Osaka-fu, Daitou City, Tatsuma
D. hikonensis	1247627				1984	9	28	Y. Kimizuka	Osaka-fu, Ikeda City, Kibe town, Mt. Satsukiyama
D. hikonensis	582347				1982	5	30	T. Suzuki	Osaka-fu, Higashiosak City, Nukadatani
D. hikonensis	582345				1982	4	29	T. Suzuki	Osaka-fu, Kashiwara City, Aotani
D. hikonensis	936261				1982	8	22	T. Suzuki	Osaka-fu, Yao City, Koudachi
D. hikonensis	528325				1980	4	3	A. Komaki	Osaka-fu, Toyono county, Nose town, Tennou
D. hikonensis	527994				1977	4	6	Y. Kimizuka	Osaka-fu, Izumi City, Matsuodera temple
D. hikonensis	936245				1977	4	6	Y. Kimizuka	Ooita pref., Kitsuki City, Funabe
D. hikonensis	1247398				1947	9		M. Hadano	Ooita pref., Saeki City, Aoyama
D. hikonensis	1247404				1940	9	15	M. Hadano	Ooita pref., Ooita City, Mt. Takasakiyama
D. hikonensis	445652				1983	11	3	H. Inoue	Ooita pref., Takeda City, Kannda
D. hikonensis	1204649				2008	1	7	H. Tsuji	Ooita pref., Nakatsu City, Yamakuni town, Morizane
D. hikonensis	509608				1977	6	4	S. Tsutsui	Ooita pref., Hita City, Akibaru town
D. hikonensis	575562				1980	12	19	S. Tsutsui	Nagasaki pref., Iki county, Ashibe town, Moroyoshi, Nakafure
D. hikonensis	337172				1969	8	6	T. Shinagawa	Nagasaki pref., Iki county, Gounoura town, Tsubofure
D. hikonensis	337176				1965	12	2	T. Shinagawa	Nagasaki pref., Iki county, Gounoura town, Katabarufure
D. hikonensis	575521				1978	2	25	S. Tsutsui	Nagasaki pref., Shimoagata county, Izuhara town, Higake
D. hikonensis	575655				1979	7	18	S. Tsutsui	Nagasaki pref., Shimoagata county, Mitsushima town, Ashiura
D. hikonensis	575522				1978	2	24	S. Tsutsui	Nagasaki pref., Shimoagata county, Mitsushima town, Sumo
D. hikonensis	1247431				2011	9	14	S. Yamada	Nagasaki pref., Isahaya City, Oba town, Seseda
D. hikonensis	447967				1984	2	4	A. Minami	Nagasaki pref., Sasebo City, Satomi town
D. hikonensis	575657				1979	7	20	S. Tsutsui	Nagasaki pref., Kamiagata county Kamiagata town, Eko
D. hikonensis	768809				2007	10	6	F. Kengo	Nagasaki pref., Tsushima City, Izuhara City, Naiinn river
D. hikonensis	575552				1975	0	0	F. Ueno	Nagasaki pref., Oomura City, Hidomaru town
D. hikonensis	575547				1980	3	9	B. Matsubayashi	Nagasaki pref., Nagasaki City, Mogi town, Chiji
D. hikonensis	1247430				2009	4	19	S. Yamada	Nagasaki pref., Shimabara City, Shinnyama
D. hikonensis	447096				1983	12	26	A. Minami	Nagasaki pref., Higashisonogi county, Kawatana town, Kobagou
D. hikonensis	1247432				2010	7	20	S. Yamada	Nagasaki pref., Minamishimabara City, Kazusa town, Mt. Iwatoyama
D. hikonensis	447091				1983	12	25	A. Minami	Nagasaki pref., Hirato City, Ookubo
D. hikonensis	447090				1983	12	26	A. Minami	Nagasaki pref., Kitamatsura county, Saza town, Shikatamenn
D. hikonensis	555969				1982	8	6	H. Tsutsumi	Nagano pref., Shimoina county, Yasuoka village, Tamoto
D. hikonensis	500931				1981	12	26	A. Tanaka	Tottori pref., Iwami county, Iwami town
D. hikonensis	500922				1981	11	1	A. Tanaka	Tottori pref., Sakaiminato City, Takeuchi town, Yogo shrine
D. hikonensis	500930				1978	10	1	A. Tanaka	Tottori pref., Tottori City, Fukui
D. hikonensis	500927				1982	1	8	A. Tanaka	Tottori pref., Touhaku county, Misasa town, Misasa
D. hikonensis	500765				1981	10	21	M. Aoto	Tottori pref., Hino county, Hino town, Nota
D. hikonensis	500940				1982	12	12	A. Tanaka	Tottori pref., Yazu county, Chizu town, Yamane
D. hikonensis	500936				1981	11	22	A. Tanaka	Tottori pref., Yonago City, Innda town
D. hikonensis	443934				1981	8	4	H. Endou	Shimane pref., Oki county, Oki Island, Nishinoshima town
D. hikonensis	1251046				2013	10	6	K. Shibata	Shimane pref., Unnnann City, Daitou town, Kamikuno
D. hikonensis	1245631				2014	7	31	Hiroshi Sawae	Shimane pref., Masuda City, Asaka town
D. hikonensis	1245686				2014	9	28	Hiroshi Sawae	Shimane pref., Goutsu City, Hazumi town, Honngou
D. hikonensis	1247360				1983	8	31	A. Minami	Shimane pref., Kanoashi county, Tsuwano town, Yamashita
D. hikonensis	384241				1977	8	27	H. Yamamoto	Shimane pref., Izumo City, Ottatate town, Tachikuekyo
D. hikonensis	1251314				2014	8	30	K. Shibata	Shimane pref., Matsue City, Shime town, Nonami
D. hikonensis	1251291				2014	9	13	K. Shibata	Shimane pref., Oota City, Ooya town
D. hikonensis	1250176				2014	10	30	Hiroshi Sawae	Shimane pref., Hamada City, Asahi town, Honngou
D. hikonensis	523259				1981	8	14	Y. Kimizuka	Tokyo-to, Minato Ward, Shiba
D. hikonensis	121152				1932	8	18	Y. Jotani	Tokyo-to, Miyake Island
D. hikonensis	523067				1980	10	13	M. Mitsuhashi	Tokyo-to, Koganei City, Maebara town
D. hikonensis	523260				1979	7	11	Y. Kimizuka	Tokyo-to, Shikinejima Island
D. hikonensis	1024493				2011	6	30	A. Ebihara	Tokyo-to, Niijima Island
D. hikonensis	523256				1976	8	17	Y. Kimizuka	Tokyo-to, Kouzushima Island
D. hikonensis	444426				1983	1	29	H. Endou	Tokyo-to, Setagaya Ward, Dennennchofu
D. hikonensis	523064				1980	10	2	M. Mitsuhashi	Tokyo-to, Nishitama county, Okutama town, Koshisawa
D. hikonensis	523063				1981	10	27	H. Endou	Tokyo-to, Nishitama county, Mizuho town, Sayamakyuuryo
D. hikonensis	345341				1976	11	27	N. Sahashi	Tokyo-to, Aogashima Island
D. hikonensis	817901				1975	10	1	Y. Jotani	Tokyo-to, Izuooshima Island
D. hikonensis	738480				1980	12	14	T. Yamada	Tokyo-to, Machida City, Aira town
D. hikonensis	604828				1955	8	28	C. Okawa	Tokyo-to, Hachioji City, Mt. Takaosann
D. hikonensis	362111							T. Tanaka	Tokyo-to, Hachijojima Island
D. hikonensis	523066				1978	10	15	M. Mitsuhashi	Tokyo-to, Fuchu City, Nishifu town
D. hikonensis	523062				1975	4	15	K. Tamaki	Tokyo-to, Toshima Island
D. hikonensis	1247447				1979	8	2	T. Nakayama	Tokushima pref., Anann city, Suii town
D. hikonensis	1247448				1971	1	10	T. Nakayama	Tokushima pref., Umibe county, Mugi town, Hegawa
D. hikonensis	1039426				1960	11	3	T. Inobe	Tokushima pref., Tokushima City, Mayuyama
D. hikonensis	337200				1975	2	11	S. Kinoshita	Tokushima pref., Naruto City, Kitanada town, Awata
D. hikonensis	446892				1983	11	25	M. Usui	Tochigi pref., Shioya county, Takanesawa town, Iimuro
D. hikonensis	799529				1987	9	6	T. Noguchi	Tochigi pref., Ashikaga City, Tsukiya town
D. hikonensis	511904				1979	6	11	Y. Kimizuka	Nara pref., Uda county, Soni village, Kake
D. hikonensis	511902				1976	10	25	Y. Kimizuka	Nara pref., Yoshino county, Tennkawa village, Yamakamikaa
D. hikonensis	1186217				2002	7	21	K .Kawabata	Nara pref., Gojo City, Kitayama town
D. hikonensis	1256715				1994	12	24	F. Kasetani	Nara pref., Tennri City, Yanamoto, Mt. Ryuuou
D. hikonensis	511905				1976	10	24	Y. Kimizuka	Nara pref., Nara City, Yagyuu town, Yagyuu
D. hikonensis	1246938				1983	10	1	T. Oshima	Toyama pref., Shimoshinnkawa county, Asahi town, Oohira
D. hikonensis	552737				1979	6	24	T. Oshima	Toyama pref., Himi City, Sugata
D. hikonensis	507903				1978	9	3	S. Watanabe	Fukui pref., Obama City, Wakasa
D. hikonensis	507924				1980	8	8	S. Watanabe	Fukui pref., Ooi county, Takahama town, Kunoura
D. hikonensis	443142				1982	5	16	T. Kimura	Fukuoka pref., Onnga county, Ashiya town, Yamaga
D. hikonensis	557390				1977	10	15	S. Kobayashi	Fukuoka pref., Kurume City, Goi town, Mt. Takara
D. hikonensis	592541				1982	9	19	S. Kobayashi	Fukuoka pref., Yukuhashi City, Minoshima
D. hikonensis	557642				1971	9	7	S. Kobayashi	Fukuoka pref., Munataka City, Akama
D. hikonensis	443146				1978	2	12	S. Tsutsui	Fukuoka pref., Oomuta City, Mt. Amagi
D. hikonensis	557389				1968	3	31	S. Tsutsui	Fukuoka pref., Chikushi county, Nakagawa town, Narutake
D. hikonensis	557392				1966	12	4	S. Tsutsui	Fukuoka pref., Tagawa county, Kawara town, Mt. Kawaradake
D. hikonensis	557453				1982	8	14	S. Kobayashi	Fukuoka pref., Yame county, Hirokawa town, Niishiro
D. hikonensis	592538				1982	12	12	S. Tsutsui	Fukuoka pref., Iizuka City, Dainichiji
D. hikonensis	557395				1975	3	2	C. Nagata	Fukuoka pref., Fukuoka City, Higashi Ward, Kashii
D. hikonensis	557638				1975	10	5	S. Kobayashi	Fukuoka pref., Kitakyuusyuu City, Moji Ward, Komorie
D. hikonensis	572179				1980	10	26	M. Yasu	Fukushima pref., Iwaki City, Yoshima town
D. hikonensis	572178				1977	12	11	M. Yasu	Fukushima pref., Iwaki City, Obama
D. hikonensis	385008				1978	7	24	M. Saito	Fukuoka pref., Iwaki City, Numanouchi

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. hikonensis	506689				1976	8	8	T. Nakaike	Fukuoka pref., Iwaki City, Ichikawabe town, Shibahara
D. hikonensis	1247687				1978	3	30	T. Shiraiwa	Hyogo pref., Ashiya City, Kouza fall
D. hikonensis	1247672				1976	7	21	T. Shiraiwa	Hyogo pref., Kasai City, Sakamoto town
D. hikonensis	1247678				1977	11	28	T. Shiraiwa	Hyogo pref., Takasago City, Amida town, Jitoku
D. hikonensis	1247691				1977	11	20	T. Shiraiwa	Hyogo pref., Sannda City, Kogaki
D. hikonensis	1247675				1977	1	30	T. Shiraiwa	Hyogo pref., Miki City, Hosokawa town
D. hikonensis	1247649				1976	10	4	T. Shiraiwa	Hyogo pref., Sumoto City, Awajishima Island, Shimouchizenn
D. hikonensis	1247671				1975	11	3	T. Shiraiwa	Hyogo pref., Sumoto City, Awajishima Island,Yura
D. hikonensis	445041				1983	5	9	H. Mizuno	Hyogo pref., Koube City, Nishi Ward, Tamatsu town, Nakatsu
D. hikonensis	1247693				1980	9	28	T. Shiraiwa	Hyogo pref., Koube City, Kita Ward, Oosawa town, Nakaoosawa
D. hikonensis	448589				1981	9	27	A. Komaki	Hyogo pref., Kawanishi City, Tada
D. hikonensis	756422				2000	9	20	K. Ushijima	Hyogo pref., Kawabe county, Inagawa town, Nibe
D. hikonensis	434993				1977	2	11	T. Nakaike	Hyogo pref., Aioi City, Yamate
D. hikonensis	1247669				1980	4	3	Y. Kimizuka	Hyogo pref., Himeji City, Katsuhara Ward, Aboshi town, Mt. Asahi
D. hikonensis	1247653				1979	8	23	M. Hayashi	Hyogo pref., Toyooka City, Kamikage
D. hikonensis	518540				1978	5	7	H. Masago	Wakayama pref., Ito county, Katsuragi town, Hoshikawa
D. hikonensis	518590				1978	8	29	H. Masago	Wakayama pref., Ito county, Kouya town, Mt. Kouyasann
D. hikonensis	345221				1976	1	31	H. Masago	Wakayama pref., Kainann City, Minamino, Kamifukaya
D. hikonensis	518593				1978	4	23	H. Masago	Wakayama pref., Hashimoto City, Yamauchi
D. hikonensis	503858				1980	11	3	H. Masago	Wakayama pref., Shinnguu City, Takada
D. hikonensis	318748				1971	8	14	H. Masago	Wakayama pref., Nishimuro county, Shirahama town
D. hikonensis	503890				1982	6	3	H. Masago	Wakayama pref., Tanabe City, Inari town, Hiraiwa
D. hikonensis	518466				1980	8	16	H. Masago	Wakayama pref., Higashimuro county, Kozagawa town, Ogawa
D. hikonensis	518575				1976	7	18	H. Masago	Wakayama pref., Hidaka county, Inami town, Kabora
D. hikonensis	518571				1977	10	10	H. Masago	Wakayama pref., Arita county, Yuasa town, Yamada
D. hikonensis		409072			2013	3	26	Y. Inoue	Japan, Kumamoto pref., Yatsushiro city, Izumi town
D. insularis		425900			2015	2	22	H. Kato	Chichijima Island of Bonin Island, Mt. Chibusayama
D. insularis		276778			1994	3	19	M. Ito	Chichijima Island of Bonin Island
D. kobayashii		405133			2012	6	25	K. Hori	Japan, Tokyo-to, Inagi city
D. kobayashii	738120				2009	6	13	A. Ebihara	Aichi pref., Shinjo city, Mt. Houraiji
D. kobayashii	385751				1980	1	1	M. Muramatsu	Aichi pref., Kitashitara county, Toyone village
D. kobayashii	1223566				1988	9	13	K. Inukai	Aichi pref., Higashikamo county, Asuke town
D. kobayashii	1223578				1988	10	15	K. Inukai	Aichi pref., Nishikamo county, Fujioka town
D. kobayashii	360958				1972	9	23	T. Yasumi	Ibaraki pref., Kuji county, Kanasagou village
D. kobayashii	361107				1966	8	8	T. Yasumi	Ibaraki pref., Mt. Tsukuba
D. kobayashii	360913				1975	6	4	M. Yasu	Ibaraki pref., Kashima county, Asahi village
D. kobayashii	360343				1977	8	3	Y. Yamamoto	Gifu pref., Mizuho city, Hannbara
D. kobayashii	359903				1974	9	18	Y. Yamamoto	Gifu pref., Ena county, Yamaoka town
D. kobayashii	1223543				1987	8	15	T. Ogiyama	Gifu pref., Nakatsugawa city, Nakatsugawa
D. kobayashii	1223545				1984	9	11	T. Ogiyama	Gifu pref., Kaji city, Mt. Shimabuki
D. kobayashii	1223555				1985	7	29	T. Ogiyama	Gifu pref., Toki city, Nakagiirioohira
D. kobayashii	1223569				1984	10	10	T. Ogiyama	Gifu pref., Kaji county, Mitaka town
D. kobayashii	1037799				1973	6	14	Y. Ueno	Miyagi pref., Shiroishi city, Ootakasawa, Omachi
D. kobayashii	1037800				1973	8	22	Y. Ueno	Miyagi pref., Shiroishi city, Ootakasawa, Misawa
D. kobayashii	1253950				2015	9	19	M. Date	Miyagi pref., Higashimatsushima city, Miyatojima Island
D. kobayashii	360297				1966	9	26	T. Satoi	Gunnma pref., Kannra county, Nakaosaka
D. kobayashii	364877				1955	7	10	T. Wakana	Gunnma pref., Hokuchi village, Shimohakoda
D. kobayashii	342812				1974	11	8	T. Satoi	Gunnma pref., Maebashi city, Sakamoto
D. kobayashii	39439				1932	11	16	Y. Tanaka	Gunnma pref., Mt. Myogi
D. kobayashii	172871				1966	9	4	M. Kurihara	Gunnma pref., Takasaki city, Kannnonnyama
D. kobayashii	1037773				1953	10	28	T. Satoi	Gunnma pref., Kannra county, Osaka village
D. kobayashii	1223567				1986	7	17	S. Waku	Gunnma pref., Seta county, Azuma village
D. kobayashii	360251				1977	8	7	T. Takeda	Hiroshima pref., Yamagata county, Geihoku town
D. kobayashii	360835				1975	11	16	H. Koike	Saga pref., Fujitsu county, Tara town
D. kobayashii	1024350				1993	10	31	T. Iwata	Saitama pref., Chichibu city, Urayama
D. kobayashii	1024339				2001	9	5	T. Iwata	Saitama pref., Hannou city, Honngou
D. kobayashii	365127				1977	9	22	S. Kobayashi	Saitama pref., Kodama county, Misato village
D. kobayashii	360431				1977	8	20	T. Waku	Saitama pref., Urawa city, Inumagata
D. kobayashii	363827				1976	1	23	S. Kobayashi	Saitama pref., Hannou city, Kusumi
D. kobayashii	363953				1976	4	6	M. Sakakibara	Saitama pref., Hannou city, Agano
D. kobayashii	1037782				1951	10	1	G. Kato	Yamgata pref., Akumi county, Yusa town
D. kobayashii	1223571				1985	11	23	Y. Kobayashi	Yamanashi pref., Minamikoma county, Minobu town
D. kobayashii	1223579				1981	10	24	M. Nakagawa	Yamanashi pref., Minamikoma county, Hayakawa town
D. kobayashii	362513				1976	10	3	Takewo Date	Yamanashi pref., Kofu city, Hirase town
D. kobayashii	362936				1976	7	11	T. Waku	Shiga pref., Otsu city, Sakamoto
D. kobayashii	384055				1978	6	24	T. Oka	Kanagawa pref., Yokohama city, Asahi-ku
D. kobayashii	363107				1958	6	9	N. Iida	Kanagawa pref., Ashigarashimo county, Hakone town
D. kobayashii	362918				1975	7	31	A. Osawa	Kanagawa pref., Hadano city, Fudagake
D. kobayashii	345770				1970	1	15	Y. Akita	Kanagawa pref., Aikou county, Mimase
D. kobayashii	345815				1951	8	6	J. Wada	Kanagawa pref., Hadano city, Minoge
D. kobayashii	1037820				1952	6	22	Y. Kawasaki	Kanagawa pref., Ooyama
D. kobayashii	926962				1985	10	3	T. Satoi	Shizuoka pref., Fujinomiya city, Nebara
D. kobayashii	349988				1968	10	23	T. Nakaike	Shizuoka pref., Susono city, Suyama
D. kobayashii	349898				1959	9	16	J. Sugimoto	Shizuoka pref., Gotennba city, Inno
D. kobayashii	348157				1976	6	27	T. Sugino	Shizuoka pref., Iwata county, Tatsuyama village
D. kobayashii	815278				1978	9	3	F. Miyamoto	Shizuoka pref., Iwata county, Misakubo town
D. kobayashii	1016872				1978	12	24	T. Nakamura	Shizuoka pref., Kamo county, Higashiizu town
D. kobayashii	1223582				1993	1	13	T. Kuramata	Chiba pref., Sakura city, Odake
D. kobayashii	1223548				1993	4	10	T. Kuramata	Chiba pref., Yachiyo city, Kamikouya
D. kobayashii	1223552				1987	8	27	K. Yashiro	Chiba pref., Sakura city, Kamikatsuta
D. kobayashii	1223554				1987	11	23	K. Yashiro	Chiba pref., Tomisato town, Yamato
D. kobayashii	1223572				1988	7	14	A. Ozaki	Chiba pref., Sakura city, Iizuka
D. kobayashii	1264719				1986	12	16	K. Yashiro	Chiba pref., Katori county, Tako town
D. kobayashii	1271984				2016	11	5	I. Yamazumi	Osaka pref., Minamikawachi county, Chihaya
D. kobayashii	359267				1976	10	24	M. Ishizuka	Oita pref., Kokonoe town, Takigami
D. kobayashii	421068				1981	7	17	H. Tsutsumi	Nagano pref., Minamishinano village, Tagumasawa
D. kobayashii	362747				1967	10	6	H. Okubara	Nagano pref., Kiso county, Minamikiso town
D. kobayashii	421067				1981	10	31	H. Tsutsumi	Nagano pref., Ookuwa village, Atera
D. kobayashii	362648				1970	6	2	T. Nagai	Nagano pref., Iida city, Imamiya shrine
D. kobayashii	362603				1974	9	22	T. Oka	Nagano pref., Shimoina county, Tooyama river, Kitamatazawa
D. kobayashii	745569				2001	8	25	N. Kurosaki	Shimane pref., Ouchi county, Yamato village
D. kobayashii	345738				1976	10	6	M. Mitsuhashi	Tokyo pref., Oume city, Nekabu
D. kobayashii	1037757				1953	11		H. Ito	Tokyo pref., Okutama town, Hikawa
D. kobayashii	817656				1976	6	12	K. Kobayashi	Tokyo pref., Nishitama county, Okutama town, Umesawa
D. kobayashii	1223573				1983	3	20	A. Yamamoto	Tokyo pref., Machida city, Honnmachida
D. kobayashii	1223581				1960	3	13	C. Azegami	Tokyo pref., Hachioji city, Motohachioji
D. kobayashii	363557				1971	10	24	K. Akimoto	Tokyo pref., Hachioji city, Kamionngata town
D. kobayashii	363332				1963	2	17	C. Azegami	Tokyo pref., Hachioji city, Takiyamajoshi
D. kobayashii	363512				1975	7	20	T. Nakaike	Tokyo pref., Nishitama county, Hinohara village
D. kobayashii	363242				1963	10	20	T. Hashimoto	Tokyo pref., Kitatama county, Yamato town
D. kobayashii	363422				1975	10	12	M. Mitsuhashi	Tokyo pref., Nishitama county, Okutama town, Mt. Kawagokeyama
D. kobayashii	359127				1977	6	12	T. Waku	Tochigi pref., Sano city, Mt. Karasawa
D. kobayashii	365802				1978	8	27	T. Waku	Tochigi pref., Kamitsuga county, Awano town
D. kobayashii	1223558				2014	7	19	R. Sonobe	Tochigi pref., Nasushiobara city, Nemuro
D. kobayashii	360746				1977	8	28	T. Waku	Tochigi pref., Nasukarasuyama town
D. kobayashii	361152				1976	9	19	T. Waku	Tochigi pref., Shiobara town, Tokurasawa
D. kobayashii	359086				1977	4	17	T. Waku	Tochigi pref., Ashikaga city, Mt Gyodou
D. kobayashii	1250137				2015	6	21	K. Fukuda	Tochigi pref., Kanuma city, Shirakuwada
D. kobayashii	1256714				2006	7	23	F. Kasetani	Nara pref., Yoshino county, Tennkawa village
D. kobayashii	366535				1978	7	8	T. Waku	Fukushima pref., Haramachi city, Shinntagawa valley
D. kobayashii	360886				1976	8	9	T. Nakaike	Fukushima pref., Iwaki city, Obama town
D. kobayashii	181154				1967	8	12	M. Yasu	Fukushima pref., Futaba county, Oohisa village
D. kobayashii	385523				1979	12	6	T. Waku	Fukushima pref., Futaba county, Namie town
D. kobayashii	1264259				1976	8	9	T. Nakaike	Fukushima pref., Iwaki city, Onahama
D. kobayashii	359085				1949	10	23	M. Inada	Hyogo pref., Koube city, Mt. Maya
D. kobayashii			37424		1979	7	29	Y. Yamamoto	Kochi pref., Agawa county, Ikekawa village
D. protobissetiana	410906				2013	8	7	K. Hori	Japan, Kagoshima pref., Yakushima Island
D. sacrosancta	926968				1976	11	17	M. Fukuhara	Aichi pref., Gamagoori city, Sagara town
D. sacrosancta	926967				1975	9	14	A. Suzuki	Aichi pref., Seto city, Shirasaka town
D. sacrosancta	926966				1976	8	8	K. Inukai	Aichi pref., Toyota city, Nakagane town
D. sacrosancta	926960				1976	1	15	M. Fukuhara	Aichi pref., Okazaki city, Maiki town
D. sacrosancta	926961				1974	10	6	H. Miyazaki	Aichi pref., Inuyama city, Tsugao

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. sacrosancta	926938				1975	8	24	M. Fukuhara	Aichi pref., Nagoya city, Midori-ku
D. sacrosancta	979126				1977	10	27	T. Waku	Aichi pref., Chita county, Minamichita town
D. sacrosancta	742657				1983	12	11	K. Inukai	Aichi pref., Toyohashi city, Hosoya town
D. sacrosancta	738504				1985	9	9	Y. Kimitsuka	Ehime pref., Uwajima city, Tojima
D. sacrosancta	1018642				1961	6	20	H. Ishikawa	Ehime pref., Araihama city, Daijouin
D. sacrosancta	1160658				2010	9	29	M. Hyodo	Ehime pref., Imabari city, oonishi town
D. sacrosancta	1223564				1978	5	3	H. Ishikawa	Ehime pref., Araihama city, Hunaki
D. sacrosancta	1037699				1963	7	14	Y. Nomura	Ehime pref., yahatahama city, Kamakura
D. sacrosancta	1037691				1985	9	9	Y. Kimitsuka	Ehime pref., Uwajima city, Tojima
D. sacrosancta	1037692				1981	8	30	M. Hyodo	Ehime pref., Ozu city, Niiya
D. sacrosancta	1037706				1976	11	15	T. Waku	Ibaraki pref., Ishioka city, Takahama
D. sacrosancta	1037707				1960	3	6	I. Akasawa	Okayama pref., Kasaoka city, Ooshima
D. sacrosancta	1037710				2014	8	26	H.Mizote	Okayamapref., Akaiwa city, Yoshii town
D. sacrosancta	1037711				2014	8	13	H.Mizote	Okayama pref., Takahashi city, Tamagawa town
D. sacrosancta	294082				1960	3	6	I. Akasawa	Okayama pref., Kasaoka city, Ooshima
D. sacrosancta	1222489				1977	2	11	M.Ishizuka	Okayama pref., Bizenn city, Mitsuishi
D. sacrosancta	1222494				1978	7	14	Y. Obata	Okayama pref., Wake county, Wake town
D. sacrosancta	381602				1976	12	30	K. Nakamura	Okayama pref., Kurashiki city, Tamashimamichiguchi
D. sacrosancta	1111295				1976	12	30	K. Nakamura	Okayama pref., Oda county, Yagake town
D. sacrosancta	192566				1976	12	28	K. Nakamura	Okayama pref., Ihara city, Nishiebara town
D. sacrosancta	926988				1980	11	14	Y. Obata	Okayama pref., Tamano city, Tama
D. sacrosancta	926983				1986	3	13	Y. Obata	Okayama pref., Kasaoka city, Nabeshima
D. sacrosancta	1037734				1986	8	9	A. Minami	Miyazaki pref., Nobeoka city, Daikyou river
D. sacrosancta	1037729				1950	5	20	I. Taki	Miyazaki pref., Kobayashi city, Shimobaba
D. sacrosancta	817660				1976	10	3	T. Muroya	Miyazaki pref., Nobeoka city, Yasui
D. sacrosancta	817658				1975	4	30	T. Muroya	Miyazaki pref., Hyuuga city, Mt. Hira
D. sacrosancta	817669				1978	7	30	T. Minamitani	Miyazaki pref., Koyu county, Kawanami town
D. sacrosancta	817665				1978	7	30	T. Minamitani	Miyazaki pref., Saito city, Tonokoori town
D. sacrosancta	1037750				1977	7	17	T. Minamitani	Miyazaki pref., Higashiusuki county, Shiiba village
D. sacrosancta	1037780				1976	2	22	T. Minamitani	Miyazaki pref., Miyakonojo city, Sekinoo
D. sacrosancta	1057878				2014	5	23	Y. Akagi	Miyazaki pref., Nishiusuki county, Hinokage town
D. sacrosancta	1024394				2014	2	20	Y. Akagi	Miyazaki pref., Nichinann city, Aburatsu
D. sacrosancta	276115				1974	6	15	N. Yoshikawa	Kyoto pref., Kyoto city, Sakyo-ku
D. sacrosancta	293589				1971	6	26	S. Mitsuta	Kyoto pref., Uji city, Shirakawaguchi
D. sacrosancta	144801				1976	9	19	N. Yoshikawa	Kyoto pref., Jouyo city, Kuseshibagahara
D. sacrosancta	148220				1977	2	14	M. Ishizuka	Kyoto pref., Souraku county, Minamiyamashiro village
D. sacrosancta	1037819				1971	6	5	J. Beppu	Kumamoto pref., Kuma county, Mizukami village
D. sacrosancta	1037793				1977	1	30	J. Beppu	Kumamoto pref., Kikuchi city, Hirose
D. sacrosancta	1037785				1975	6	1	J. Beppu	Kumamoto pref., Kamimashiki county, Mihune town
D. sacrosancta	363017				1974	12	22	J. Beppu	Kumamoto pref., Hitoyoshi city, Yatake
D. sacrosancta	359717				1977	1	22	J. Beppu	Kumamoto pref., Uto city, Amizu
D. sacrosancta	359918				1972	5	18	J. Beppu	Kumamoto pref., Yatsushiro city, Shikigawachi
D. sacrosancta	360392				1971	6	5	J. Beppu	Kumamoto pref., Minamata city, Yude
D. sacrosancta	362582				1980	9	14	T. Tamaru	Hiroshima pref., Asaminami-ku, Gionn
D. sacrosancta	363584				1976	3	1	T. Takeda	Hiroshima pref., Fukuyama city, Goubunn
D. sacrosancta	366484				1975	7	5	T. Takeda	Hiroshima pref., Fuchu city, Aratani town
D. sacrosancta	363314				1975	6	15	T. Takeda	Hiroshima pref., Mihara city, Kihara town
D. sacrosancta	363134				1976	6	1	T. Takeda	Hiroshima pref., Syoubara city, Nanazuka
D. sacrosancta	363071				1976	6	15	T. Takeda	Hiroshima pref., Miyoshi city, Awaya
D. sacrosancta	360632				1976	3	16	T. Takeda	Hiroshima pref., Takehara city, Konashi
D. sacrosancta	362072				1977	1	5	T. Takeda	Hiroshima pref., Kure city, Aga town, Mt. Yasumiyama
D. sacrosancta	360298				1956	10	14	K. Oka	Hiroshima pref., Ootake city, Kuba
D. sacrosancta	359490				1975	3	2	M. Arai	Kagawa pref., Nakatado county, Kotohira town
D. sacrosancta	359441				1976	1	7	S. Mitani	Kagawa pref., Takamatsu city, Nishiueda town
D. sacrosancta	360702				1976	3	5	S. Mitani	Kagawa pref., Kannnonnji city, Awai town
D. sacrosancta	361442				2000	7	7	K. Nishimura	Kochi pref., Kochi city, Fudeyama
D. sacrosancta	363450				1977	2	6	K. Yamaoka	Kochi pref., Aki county, Umaji village
D. sacrosancta	368309				1973	1	14	K. Yamaoka	Kochi pref., Aki city, Inokuchi
D. sacrosancta	362620				1976	7	12	T. Kouzai	Kochi pref., Nagaoka county, Motoyama town
D. sacrosancta	362532				1976	6	14	K. Yamaoka	Kochi pref., Nanngoku city, Dateno
D. sacrosancta	362892				1976	4	18	T. Kouzai	Kochi pref., Kochi city, Harihara
D. sacrosancta	362775				1976	3	25	T. Kouzai	Kochi pref., Takaoka county, Sakawa town
D. sacrosancta	362667				1977	5	29	K. Yamaoka	Koch pref., Suzaki city, Ikenotani
D. sacrosancta	361468				1977	1	23	T. Kouzai	Kochi pref., Syukuge city, Katashima
D. sacrosancta	359463				1974	10	21	T. Nakaike	Kochi pref., Tosashimizu city, Kubotsu
D. sacrosancta	361962				1980	6	22	K. Yamaoka	Kochi pref., Muroto city, Sakihama
D. sacrosancta	361202				1975	8	12	H. Koike	Saga pref., Takeo city, Nishikawanobori town
D. sacrosancta	361286				2014	3	11	Y. Inoue	Saga pref., Imari city, Hadatsu town
D. sacrosancta	359352				1975	3	21	M. Koike	Saga pref., Taku city, Kitataku town
D. sacrosancta	360116				1978	5	5	K. Kobayashi	Mie pref., Owase city, Kuki
D. sacrosancta	360048				1952	6	4	T. Magofuku	Mie pref., Kuwana city, Masuo
D. sacrosancta	364195				1991	1	20	K. Ohora	Mie pref., Kumano city, Atashika
D. sacrosancta	360167				1976	10	16	Y. Kimitsuka	Mie pref., Toba city, Momotori
D. sacrosancta	359859				1978	3	12	S. Yamauchi	Mie pref., Yokkaichi city, Midorigaoka town
D. sacrosancta	359778				1953	8	7	T. Magofuku	Mie pref., Ise city, Yamatohimenomiya
D. sacrosancta	361024				1978	3	6	R. ito	Mie pref., Owase city, Minamiura
D. sacrosancta	360771				1976	5	2	A. Nakashima	Mie pref., Minamimuro county, Mihama town
D. sacrosancta	363645				1984	8	18	S. Kiryuu	Mie pref., Yokkaichi city, Nishisakabe town
D. sacrosancta	363096				1976	8	11	H. Masaki	Yamaguchi pref., Yanai city, Heigunn
D. sacrosancta	367454				1976	2	11	H. Masaki	Yamaguchi pref., Kumage county, Tabuse town
D. sacrosancta	361068				1975	12	7	K. Oka	Yamaguchi pref., Hagi city, Sannmi
D. sacrosancta	359262				1956	1	15	K. Oka	Yamaguchi pref., Yamaguchi city, Yoshiki
D. sacrosancta	361338				1975	3	22	T. Yamada	Yamaguchi pref., Ube city, Kawakami
D. sacrosancta	361600				1975	9	3	K. Oka	Yamaguchi pref., Mine city, Higashiatsu town
D. sacrosancta	361645				1975	12	25	K. Oka	Yamaguchi pref., Shimonoseki city, Fukue
D. sacrosancta	361690				2013	10	27	H. Akimaru	Yamaguchi pref., Abu county, Abu town
D. sacrosancta	363859				1976	6	13	M. Yokota	Yamaguchi pref., Iwakuni city, Tennnoo
D. sacrosancta	366566				1980	4	5	A. Minami	Yamaguchi pref., Kumage county, Kaminoseki town, Yashima Island
D. sacrosancta	362104				1981	10	10	A. Minami	Yamaguchi pref., Iwakuni city, Hashirajima Island
D. sacrosancta	362284				1991	8	7	A. Minami	Yamaguchi pref., Kumage couty, Kaminoseki town, Iwaishima Island
D. sacrosancta	363733				1985	11	23	H. Obata	Yamanashi pref., Minamikoma county, Uchibuna
D. sacrosancta	360703				1963	2	22	T. Yamanaka	Kagoshima pref., Imizu city, Kamiokawauchi
D. sacrosancta	367391				1976	8	26	K. Takesako	Kagoshima pref., Kanoya city, Shimotakakuma town
D. sacrosancta	362115				1976	8	1	K. Takesako	Kagoshima pref., Tarumi city, Kaminomiya
D. sacrosancta	362205				1967	12	3	M. Hiki	Kagoshima pref., Yubisuki city, Lake Ikeda
D. sacrosancta	362430				1973	6	17	K. Takesako	Kagoshima pref., Kagoshima city, Hirakawa town
D. sacrosancta	362691				1977	1	10	K. Takesako	Kagoshima pref., Makurazkai city, Mt. Kunimidake
D. sacrosancta	359634				1985	1	17	M. Kawabata	Kagoshima pref., Kagoshima city, Shimofukumoto town
D. sacrosancta	362925				1951	8	4	T. Kawasaki	Kanagawa pref., Ashigarashimo county, Manazuru town
D. sacrosancta	361950				1974	1	13	T. Waku	kanagawa pref., Minamiashigara city, Kousaiji
D. sacrosancta	361987				1954	11	21	S. Oya	Kanagawa pref., Miura county, Hayama town
D. sacrosancta	362085				1957	1	20	N. Iida	Kanagawa pref., Odawara city, Kazamatsuri
D. sacrosancta	362098				1984	5	5	K. Tanaka	Kanagawa pref., Isehara city, Hyuga river
D. sacrosancta	362055				1985	12	31	A. Ozaki	Kanagawa pref., Yokohama city, Midori-ku
D. sacrosancta	366778				1983	11	12	F. Konta	Shizuoka pref., Fuji city, Goudo
D. sacrosancta	359915				1986	4	12	T. Sato	Shizuoka pref., Numazu city, Ashitaka
D. sacrosancta	359983				1973	4	4	M. Sakakibara	Shizuoka pref., Shimoda city, Renndaiji
D. sacrosancta	362974				1967	2	25	T. Shimakawa	Shizuoka pref., Kamo county, Higashiizu town
D. sacrosancta	363019				1964	9	16	G. Yuyama	Shizuoka pref., Gotennba city, Innno
D. sacrosancta	363379				1976	4	1	T. Sugino	Shizuoka pref., Kakegawa city, Nissaka
D. sacrosancta	363694				1976	2	8	T. Sugino	Shizuoka pref., Fukuroi city, Kasui
D. sacrosancta	1250218				2002	11	24	S. Matsumoto	Shizuoka pref., Shimoda city, Suzaki
D. sacrosancta	1046288				1983	9	10	T. Hosokura	Shizuoka pref., Susono city, Imasato
D. sacrosancta	1247355				1987	3	18	K. Yashiro	Chiba pref., Narita city, Oomuro
D. sacrosancta	1037631				1981	8	9	I. Ueda	Chiba pref., Tougane city, Tannnoo
D. sacrosancta	1037642				1993	3	6	T. Kuramata	Chiba pref., Funabashi city, Kanahori town
D. sacrosancta	1037658				1961	6	24	T. Kodama	Osaka pref., Kashiwara city, Honndou
D. sacrosancta	1037657				1974	3	28	T. Oka	Osaka pref., Minoo city, Minoo park
D. sacrosancta	436775				1984	9	28	Y. Kimitsuka	Osaka pref., Ikeda city, Kibe town
D. sacrosancta	1039274				1976	9	5	M. Hadano	Oita pref., Saiki city, Kariu
D. sacrosancta	535685				1951	9	26	M. Hiki	Oita pref., Beppu city, Otobara
D. sacrosancta	344224				1976	9	5	M. Hadano	Oita pref., Tsukumi city, Tokuura

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. sacrosancta	345710				1977	3	27	Y. Maruno	Oita prf., Oita city, Kawarauchi
D. sacrosancta	345864				1977	1	14	K. Oka	Oita pref., Usa city, Saigi
D. sacrosancta	345939				1976	3	28	Y. Takaoka	Oita pref., Hayami county, Hiji town
D. sacrosancta	346416				1977	1	14	K. Oka	Oita pref., Usa city, Yamaguchi
D. sacrosancta	348472				1977	6	4	S. Tsutsui	Oita pref., Hita city, Akibaru
D. sacrosancta	348791				1977	1	15	Y. Maruno	Oita pref., Usuki city, Fujikawachi
D. sacrosancta	350153				1983	11	4	A. Minami	Oita pref., Takeda city, Koubaru
D. sacrosancta	350197				1940	8	0	M. Hadano	Oita pref., Beppu city, Tsurumi
D. sacrosancta	350511				1978	8	10	S. Tsutsui	Nagasaki pref., Shimabara city, Shiratani
D. sacrosancta	350606				1976	2	21	F. Ueno	Nagasaki pref., Omura city, Mizoroku town
D. sacrosancta	350831				1934	6	4	T. Baba	Nagasaki pref., Higashisonogi county, Hasami town
D. sacrosancta	350959				1961	1	5	B. Matsubayashi	Nagasaki pref., Nagasaki city, Utsustugawa
D. sacrosancta	351310				1984	2	4	A. Minami	Nagasaki pref., Hirado city, Fukagawa town
D. sacrosancta	351323				1984	2	4	A. Minami	Nagasaki pref., Sasebo city, Satoyoshi town
D. sacrosancta	351418				2010	9	6	F. Konta	Nagano pref., Shimoina county, Anann town
D. sacrosancta	359958				1962	12	25	A. Tanaka	Tottori pref., Tottori city, Yoshioka
D. sacrosancta	363404				2005	11	23	M.Yagiura	Shimane pref., Izumo city, Nishihayashigi town
D. sacrosancta	367882				2014	1	23	H. Sawae	Shimane pref., Masuda city, Iiura town
D. sacrosancta	384263				2013	4	6	M. Yagiura	Shimane pref., Yasugi city, Yasugi town
D. sacrosancta	385603				1976	4	23	K. Oka	Shimane pref., Gotsu city, Tsuchi town
D. sacrosancta	395562				2015	3	12	H. Sawae	Shimane pref., Kanoashi county, Tsuwano town
D. sacrosancta	396060				2013	10	6	K. Shibata	Shimane pref., Yasugi city, Tayori town
D. sacrosancta	396061				2014	8	31	K. Shibata	Shimane pref., Ouchi county, Kawamoto town
D. sacrosancta	466696				1938	4	26	T. Sato	Tokyo pref., Hachioji city, Kamikawa town, Mt. Imaguma
D. sacrosancta	466740				1972	12	17	T. Nakaike	Tokyo pref., Hachioji city, Motohachioji
D. sacrosancta	466773				1982	12	2	H. Endo	Tokyo pref., Hachioji city, Tobuki town
D. sacrosancta	466776				1977	6	27	S. Mitani	Tokushima pref., Katsuura county, Katsuura town
D. sacrosancta	535222				1960	8	12	T. Inobe	Tokushima pref., Tokushima city, Hachimann town
D. sacrosancta	535329				1960	6	26	K. Abe	Tokushima pref., Tokushima city, Hachimann town
D. sacrosancta	1171527				1970	1	4	T. Nakayama	Tokushima pref., Anann city, Tachibana town
D. sacrosancta	1222477				1971	8	28	T. Nakayama	Tokushima pref., Anann city, Kamodani
D. sacrosancta	1222482				1957	9	22	K. Ogawa	Tochigi pref., Otawara city, Fukuhara town
D. sacrosancta	1222484				2013	8	28	K. Kawabata	Nara pref., Yoshino county, Totsugawa village
D. sacrosancta	1222486				1972	10	21	Y. Tsujimoto	Nara pref., Nara city, Kasugayama
D. sacrosancta	1222491				1977	9	18	K. Ohora	Nara pref., Yoshino county, Totsugawa village
D. sacrosancta	1222496				1978	3	27	Y. Tsujimoto	Nara pref., Ikoma city, Takayama town
D. sacrosancta	1222497				2002	10	12	K. Kawabata	Nara pref., Yoshino county, Yoshino town
D. sacrosancta	1222499				1982	9	19	Y. Tsujimoto	Nara pref., Gojo city, Kouzuke town
D. sacrosancta	1222506				1968	10	13	S. Tsutsui	Fukuoka pref., Buzenn city, Iwaya
D. sacrosancta	1222507				1976			T. Shinnriki	Fukuoka pref., Kitakyuusyuu city, Moji-ku
D. sacrosancta	1222509				1969	1	24	S. Kobayashi	Fukuoka pref., Kitakyuusyuu city, Kokurakita-ku
D. sacrosancta	1222515				1976	7	18	T. Shinnriki	Fukuoka pref., Kitakyuusyuu city, Wakmatsu-ku
D. sacrosancta	1222519				1976	8	1	H. Inoue	Fukuoka pref., Tsukushino city, Futsukaichi
D. sacrosancta	1222523				1975	3	2	S. Tsutsui	Fukuoka pref., Omuta city, Mt. Miikeyama
D. sacrosancta	1222524				1975	4	22	S. Tsutsui	Fukuoka pref., Fukuoka city, Minami park
D. sacrosancta	1222525				1964	12	4	S. Tsutsui	Fukuoka pref., Tsukushi county, Nakagawa town
D. sacrosancta	1222527				1976	7	29	S. Kurashige	Fukuoka pref., Omuta city, Miike
D. sacrosancta	1222544				1976	9	15	T. Shiraiwa	Fukuoka pref., Sannda city, Aimoto
D. sacrosancta	1222547				1978	5	20	T. Shiraiwa	Hyogo pref., Kasai city, Kawachi town
D. sacrosancta	1222553				1976	3	7	T. Shiraiwa	Hyogo pref., Sumoto city, Yura
D. sacrosancta	1222557				1977	2	20	T. Shiraiwa	Hyogo pref., Himeji city, Kitahirano town
D. sacrosancta	1222563				1976	11	21	T. Shiraiwa	Hyogo pref., Akou county, Kamigoori town
D. sacrosancta	1222566				1983	8	16	T. Shiraiwa	Hyogo pref., Koube city, Suma-ku
D. sacrosancta	1222572				1977	2	11	T. Nakaike	Hyogo pref., Aioi city, Yamate
D. sacrosancta	1222578				1976	10	17	T. Shiraiwa	Hyogo pref., Koube city, Nada-ku
D. sacrosancta	1222589				1990	11	3	K. Ohora	Wakayama pref., Shinnguu city, Takada
D. sacrosancta	1222619				1977	1	15	H. Masago	Wakayama pref., Higashimuro county, Kozagawa town
D. sacrosancta	1222625				1976	1	11	H. Masago	Wakayama pref., Nishimuro county, Susami town
D. sacrosancta	1222630				1976	1	31	H. Masago	Wakayama pref., Kainann city, Minaminogami
D. sacrosancta	1222635				1976	1	13	H. Masago	Wakayama pref., Tanabe city, Shinnjo town
D. sacrosancta	1223591				1977	7	31	T. Oka	Wakayama pref., Wakayama city, Kata
D. sacrosancta	1223593				1976	7	31	H. Masago	Wakayama pref., Hidaka county, Hidaka town
D. sacrosancta	1223604				1978	1	22	H. Masago	Wakayama pref., Wakayama city, Miidera
D. sacrosancta	350219				2007	10	9	Kengo Fuse	Nagasaki pref., Tsushima city, Mitsushima town
D. sacrosancta		409216			2013	1	7	Y. Inoue	Japan, Saga pref., Takeo city, Yamauchi town
D. saxifraga		423584			2014	9	4	Y. Horii	Japan, Akita pref., Sennboku city, Dakigaeri velley
D. saxifraga	927053				1978	8	23	M. Fukuhara	Aichi pref., Kitashitara county, Toyone village
D. saxifraga	927051				1979	4	29	M. Muramatsu	Aichi pref., Higashikamo county, Asuke town
D. saxifraga	927050				1979	4	30	M. Muramatsu	Aichi pref., Kitashitara county, Shitara town
D. saxifraga	927049				1980	1	1	M. Muramatsu	Aichi pref., Kitashitara county, Toyone village
D. saxifraga	927048				1981	9	12	M. Hyodo	Aichi pref., Kamiukena county, Yanadani village
D. saxifraga	927042				1979	9	16	M. Hyodo	Aichi pref., Kamiukena county, Kuma town
D. saxifraga	927347				1980	9	21	M. Yasu	Ibaraki pref., Mito city, Sakado town
D. saxifraga	1183285				1977	11	3	M. Yasu	Ibaraki pref., Takahagi city, Shimana
D. saxifraga	1183395				1976	6	26	M. Yasu	Ibaraki pref., Kuji county, Daigo town
D. saxifraga	1225781				1978	7	8	M. Yasu	Ibaraki pref., Kitaibaraki city, Ookita valley
D. saxifraga	927044				1977	6	12	M. Yasu	Ibaraki pref., Hitachi city, Mt. Takasuzu
D. saxifraga	1799				1969	7	6	Y. Nakazaki	Ibaraki pref., Kuji county, Daigo town
D. saxifraga	1169026				1976	7	4	H. Fujita	Ibaraki pref., Kuji county, Daigo town
D. saxifraga	1025143				2014	7	13	H. Mizote	Okayama pref., Tsuyama city, Aba
D. saxifraga	1025220				1986	8	31	Y. Obata	Okayama pref., Maniwa county, Tyuuka vlillage
D. saxifraga	1025162				1984	10	15	Y. Obata	Okayama pref., Atetsu county, Oosa town
D. saxifraga	1025186				2013	8	23	H. Suzuki	Iwate pref., Kamaishi city, Toni town
D. saxifraga	1025205				1980	8	13	Y. Nakajima	Iwate pref., Morioka city, Oshida
D. saxifraga	1025206				1978	10	24	T. Omori	Iwate pref., Hanamaki city, Toyosawa
D. saxifraga	1025178				1980	7	28	Y. Kaneda	Iwate pref., Ofunato city, Hikoroichi town
D. saxifraga	100294				1979	8	4	K. Inoue	Iwate pref., Ninohe county, Ashiro town
D. saxifraga	286009				1967	8	27	T. Takahashi	Iwate pref., Kitakamicity, Kuchinai town
D. saxifraga	115551				1980	9	6	E. Fukushi	Iwate pref., Shimohei county, Tarou town
D. saxifraga	89445				1980	7	13	E. Fukushi	Iwate pref., Ninohe county, Ichinohe town
D. saxifraga	213544				1980	7	26	Y. Kaneda	Iwate pref., Rikuzenntakada city, Yahagi town
D. saxifraga	89990				1980	8	24	Y. Oda	Iwate pref., Shimohei county, Iwaizumi town
D. saxifraga	317502				1968	7	30	Y. Oda	Iwate pref., Kunohe county, Ymagata village
D. saxifraga	58779				1980	7	26	T. Nakaike	Iwate pref., Kamaishi city, Kasshi town
D. saxifraga	1221849				1980	7	23	T. Nakaike	Iwate pref., Higashiiwai county, Daito town
D. saxifraga	89462				1980	9	15	Y. Oda	Iwate pref., Kunohe county, Noda village
D. saxifraga	9510716				1980	9	23	Y. Oda	Iwate pref., Kuji city, Ookawame town
D. saxifraga	9510409				1981	9	16	Y. Oda	Iwate pref., Kunohe couny, Yamagata village
D. saxifraga	1025227				1980	7	20	E. Fukushi	Iwate pref., Shimohei county, Iwaizumi town
D. saxifraga	1025231				1980	7	6	M. Takezawa	Iwate pref., Kunohe county, Karumai town
D. saxifraga	1025226				1980	10	12	M. Neichi	Iwate pref., Ninohe city, Kinntaichi
D. saxifraga	1025225				1980	7	13	E. Fukushi	Iwate pref., Ninohe city, Ichinohe town
D. saxifraga	1025222				1979	11	3	Y. Nakajima	Iwate pref., Shiba county, Shiba town
D. saxifraga	1025230				1980	9	15	Y. Nakajima	Iwate pref., Shiba county, Yahaba town
D. saxifraga	1025228				1981	10	4	Y. Nakajima	Iwate pref., Hanamaki city, Hanamakionnsenn
D. saxifraga	1221453				1979	10	17	K. Kudo	Iwate pref., Ichinoseki city, Takizawa
D. saxifraga	931256				1981	10	11	Y. Oda	Iwate pref., Shimohei county, Tanobata village
D. saxifraga	707089				2014	11	8	T. Ogiyama	Gifu pref., Ena city, Yamaoka town
D. saxifraga	1800				1986	9	21	H. Miyazaki	Gifu pref., Ibi county, Bannnai village
D. saxifraga	817465				1983	5	18	T. Ogiyama	Gifu pref., Ena county, Yamaoka town
D. saxifraga	784688				1983	10	10	H. Nagase	Gifu pref., Takayama city, Taki town
D. saxifraga	817651				1982	10	3	H. Nagase	Gifu pref., Takayama city, Yamaguchi town
D. saxifraga	817632				1980	9	21	Y. Yamamoto	Gifu pref., Gunnjo county, Shiratori town
D. saxifraga	817314				1978	9	10	Y. Yamamoto	Gifu pref., Motosu county, Neo village
D. saxifraga	1039049				2014	9	14	T. Ogiyama	Gifu pref., Ibi county, Ibigawa town
D. saxifraga	1039045				2014	11	8	T. Ogiyama	Gifu pref., Ena city, Misato town
D. saxifraga	1039043				2015	8	29	T. Ogiyama	Gifu pref., Nakatsugawa city, Akigawa
D. saxifraga	1039041				1988	9	23	T. Ogiyama	Gifu pref., Gunnjo county, Minami village
D. saxifraga	425898				1989	8	16	T. Ogiyama	Gifu pref., Kamo county, Yaozu town
D. saxifraga	820526				1999	1	1	T. Ogiyama	Gifu pref., Gunnjo county, Minami village
D. saxifraga	821432				2007	5	4	T. Ogiyama	Gifu pref., Gero city, Kanayama town
D. saxifraga	817653				2013	12	14	T. Ogiyama	Gifu pref., Ena city, Iiji town

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. saxifraga	817635				1972	5	6	Y. Akagi	Miyazaki pref., Nishiusuki county, Takachiho town
D. saxifraga	817634				2014	10	30	Y. Akagi	Miyazaki pref., Higashiusuki county, Morozuka village
D. saxifraga	817648				2009	4	19	Y. Akagi	Miyazaki pref., Saito city, Ohae
D. saxifraga	817640				1981	6	14	K. Matsunaga	Miyagi pref., Kurokawa county, Yamato town
D. saxifraga	817644				1981	6	20	-	Miyagi pref., Tamatsukuri county, Naruko town
D. saxifraga	817633				1981	5	1	Y. Ueno	Miyagi pref., Natori city, Natori new town
D. saxifraga	817637				1975	3	16	Y. Ueno	Miyagi pref., Shiroishi city, Shirakawa
D. saxifraga	817636				1973	8	12	K. Takahashi	Miyagi pref., Yamato town, Mt. Atagago
D. saxifraga	1039065				1976	6	20	K. Takahashi	Miyagi pref., Kesennnuma city, Kamihigashigawane
D. saxifraga	1039053				1975	6	14	Y. Ueno	Miyagi pref., Shiroishi city, Saikawa
D. saxifraga	1039047				1982	8	13	M. Takeichi	Miyagi pref., Katta county, Zaou town
D. saxifraga	1039035				1976	7	27	K. Takahashi	Miyagi pref., Kurokawa county, Yamato town
D. saxifraga	1091976				1994	7	20	J. Beppu	Kumamoto pref., Kamimasuki county, Seiwa village
D. saxifraga	1092975				1982	8	15	T. Kariyazaki	Kumamoto pref., Kikuchi valley
D. saxifraga	1092972				1984	8	7	T. Kariyazaki	Kumamoto pref., Aso county, Minamioguni town
D. saxifraga	1092985				1974	2	22	J. Beppu	Kumamoto pref., Yatsushiro county, Izumi village
D. saxifraga	1092984				1994	7	20	J. Beppu	Kumamoto pref., Kamimasuki county, Seiwa village
D. saxifraga	799534				1971	7	18	Y. Mizuta	Gunnma pref., Numata city, Mt. Kaba
D. saxifraga	1025172				1965	8	11	T. Satomi	Gunnma pref., Tano county, Ueno vilage
D. saxifraga	1025173				1965	9	20	T. Satomi	Gunnma pref., Kannra county, Nannmoku village
D. saxifraga	1025187				1986	3	22	S. Ishiguro	Gunnma pref., Tone county, Kawaba village
D. saxifraga	1025191				1980	8	6	N. Gotoshi	Gunnma pref., Tano county, Ueno vilage
D. saxifraga	1025192				1981	11	10	N. Gotoshi	Gunnma pref., Kitagunnma county, Ikaho town, Mt. Haruna
D. saxifraga	1025195				1984	8	2	S. Ishiguro	Gunnma pref., Usui county, Matsuida town
D. saxifraga	1025196				1983	8	2	A. Yamamoto	Gunnma pref., Agatsuma county, Kuni village
D. saxifraga	1025197				1983	8	1	A. Yamamoto	Gunnma pref., Gunnma county, Kurabuchi village, Kawaura
D. saxifraga	1025200				1984	9	25	S. Waku	Gunnma pref., Seta county, Kurohone village
D. saxifraga	1025201				1985	9	19	S. Waku	Gunnma pref., Agatsuma county, Nakanojo town, Shima
D. saxifraga	1025203				1973	9	12	T. Takeda	Hiroshima pref., Hiba county, Saijo town, Mt. Hiba
D. saxifraga	1025204				1981	9	15	Y. Yamamoto	Kochi pref., Nagaoka county, Ootoyo town
D. saxifraga	1025213				1994	5	29	T. Iwata	Saitama pref., Chichibu city, Urayama
D. saxifraga	480472				1982	11	30	S. Kobayashi	Siatama pref., Hannnou city, Oogawara
D. saxifraga	493241				1973	9	9	Y. Higuchi	Mie pref., Iinami county, Iidaka town
D. saxifraga	493239				1980	3	3	S. Yamauchi	Mie pref., Ise city, Izumodani
D. saxifraga	495920				1959	7	30	N. Kato	Yamagata pref., Nishitagawa county, Mt. Atsumidake
D. saxifraga	572161				1959	6	28	N. Kato	Yamagata pref., Tenndou city, Takatakifudo
D. saxifraga	1221458				1982	9	16	N. Sakawa	Yamagata pref., Higashine city, Yokosawa
D. saxifraga	1221459				1979	10	10	N. Sakawa	Yamagata pref., Ymaagat city, Mt. Senohara
D. saxifraga	1221462				1981	8	9	N. Sakawa	Yamagata pref., Sagae city, Sanegawa river
D. saxifraga	1221466				1981	8	6	N. Sakawa	Yamagata pref., Tenndou city, Wakamatsukannnonn
D. saxifraga	1221479				1981	8	2	N. Sakawa	Yamagata pref., Yamagata city, Mt. Chitose
D. saxifraga	1221481				1981	8	21	N. Sakawa	Yamagata pref., Kaminoyama city, Mt. Zaou
D. saxifraga	444280				1976	9	2	N. Kato	Yamagata pref., Mogami county, Mamurogawa town
D. saxifraga	449277				1972	4	29	N. Kato	Yamagata pref., Akumi county, Hirata town
D. saxifraga	444284				1979	5	12	N. Kato	Yamagata pref., Mogami county, Tozawa village
D. saxifraga	444283				1977	6	28	N. Kato	Yamagata pref., Mogami county, Mamurogawa town
D. saxifraga	444968				1981	8	14	J. Sato	Yamagata pref., Nishiokitama county, Oguni town
D. saxifraga	333448				1980	8	10	J. Sato	Yamagata pref., Nagai city, Mt. Kamio
D. saxifraga	572294				1982	10	3	N. Kato	Yamagata pref., Akumi county, Yusa town
D. saxifraga	572297				2007	8	2	J. Sato	Yamagata pref., Yonezawa city, Sekine
D. saxifraga	572298				2013	9	9	T. Namegata	Yamagata pref., Yonezawa city, Yanazawa
D. saxifraga	569844				1967	8	12	S. Serizawa	Yamanashi pref., Mt. Fuji, Aokigahara
D. saxifraga	568785				1981	8	6	H. Endou	Yamanashi pref., Kitatsuru county, Tanabayama village
D. saxifraga	572897				1990	7	22	S. Ishiguro	Yamanashi pref., Higashiyamanashi county, Yamato village
D. saxifraga	554938				1982	8	22	M. Nakagawa	Yamanashi pref., Mt. Fuji, Aokigahara
D. saxifraga	572305				1981	8	18	A. Yamamoto	Yamanashi pref., Ennzann city
D. saxifraga	572299				1986	7	27	S. Ishiguro	Yamanashi pref., Otsuki city, Nakamura
D. saxifraga	1221482				1974	9	9	S. Kurata	Yamanashi pref., Kofu city, Kurobera
D. saxifraga	506696				1968	11	3	T. Shiraiwa	Shiga pref., Shiga county, Shiga town
D. saxifraga	506697				1975	10		K. Takesako	Kagoshima pref., Aira county, Makizono town
D. saxifraga	555295				2014	9	4	Y. Horii	Akita pref., Sennboku ity, Kakunodate town
D. saxifraga	1221545				1978	7	15	R. Kamata	Akita pref., Akita city, Mt. Ohira
D. saxifraga	1221483				1976	9	10	T. Nakaike	Akita pref., Sennboku county, Tazawako town
D. saxifraga	507149				1981	10	1	S. Okita	Akita pref., Kabe county, Kabe town
D. saxifraga	510905				1976	9	12	T. Nakaike	Akita pref., Akita city, Nibetsu
D. saxifraga	518460				1979	10	10	M. Hatakeyama	Akita pref., Kitaakita county, Moriyoshi town
D. saxifraga	574092				1982	9	24	S. Fujiwara	Akita pref., Ogachi county, Ogachi town
D. saxifraga	1221456				1976	10	17	S. Okita	Akita pref., Sennboku county, Oota town
D. saxifraga	1221463				1982	9	15	T. Kikuchi	Akita pref., Kazuno city, Hanawa
D. saxifraga	1221464				1981	9	13	T. Kikuchi	Akita pref., Kazuno county, Kosaka town
D. saxifraga	1221465				1979	10	14	T. Kikuchi	Akita pref., Oodate city, Yukisawa
D. saxifraga	1221467				1981	5	30	T. Kikuchi	Akita pref., Kitaakita county, Ani town
D. saxifraga	1221468				1960	8	4	M. Hatakeyama	Akita pref., Yamamoto county, Fujisato town
D. saxifraga	1221469				1977	10	16	T. Kikuchi	Akita pref., Odate city, Ezurisawa
D. saxifraga	1221470				1979	8	6	M. Hatakeyama	Akita pref., Kitaakita county, Kamioani village
D. saxifraga	1221474				1962	7	1	F. Konta	Niigata pref., Shibata city, Akaya
D. saxifraga	1221519				1981	11	14	M. Saito	Niigata pref., Shibata city, Mt. Niou
D. saxifraga	1221531				1982	1	3	N. Sato	Kanagawa pref., Ashigarakami county, Yamakita town
D. saxifraga	1221532				1981	5	31	N. Sato	Kanagawa pref., Ashigarashimo county, Hakone town
D. saxifraga	1221542				1987	8	13	A. Yamamoto	Aomori pref., Shimokita county, Higashidoori village
D. saxifraga	506690				1980	9	15	E. Fukushi	Aomori pref., Shimokita county, Oma town
D. saxifraga	506695				1980	11	3	M. Neichi	Aomori pref., Hachinohe city, Sasago
D. saxifraga	506698				1980	10	10	M. Sudo	Aomori pref., Towada city, Shimokirida
D. saxifraga	506699				1980	10	12	A Takahashi	Aomori pref., Sannnohe county, Sannnohe town
D. saxifraga	506700				1982	10	3	M. Sudo	Aomori pref., Aomori city, Mie fall
D. saxifraga	506705				1979	9	14	M. Saito	Aomori pref., Nakatsugaru county, Nishimeya village
D. saxifraga	506706				1981	8	8	M. Saito	Aomori pref., Minamitsugaru county, Oowani town
D. saxifraga	506707				1976	9	26	M. Sudo	Aomori pref., Kamikita county, Nananohe town
D. saxifraga	506712				1980	9	14	E. Fukushi	Aomori pref., Shimokita county, Sai village
D. saxifraga	506666				1974	5	18	M. Sudo	Aomori pref., Shimokita county, Higashidoori village
D. saxifraga	470222				1984	8	10	M. Sudo	Aomori pref., Towada city, Fukamochi
D. saxifraga	470221				1985	6	30	M. Sudo	Aomori pref., Mutsu city, Okuyagenn
D. saxifraga	470220				1984	8	10	M. Sudo	Aomori pref., Towada city, Houryou
D. saxifraga	470218				1985	6	15	T. Sato	Shizuoka pref., Fuji city, Sekotsuji
D. saxifraga	470217				1984	7	15	T. Sato	Shizuoka pref., Fujinomiya city, Mt. Tyojagatake
D. saxifraga	470216				1983	8	22	T. Sato	Shizuoka pref., Fujinomiya city, Mt. Fuji
D. saxifraga	555296				1984	10	23	T. Sato	Shizuoka pref., Fujinomiya city, Nebara
D. saxifraga	523408				1987	8	3	T. Hosokura	Shizuoka pref., Fuji city, Imamiya
D. saxifraga	523407				1981	9	30	A. Suzuki	Shizuoka pref., Susono city, Kurozuka
D. saxifraga	523406				1982	9	12	T. Yamamoto	Shizuoka pref., Kamo county, Kawazu town
D. saxifraga	523405				1982	9	19	M. Nakagawa	Shizuoka pref., Fujinomiya city, Kamiide
D. saxifraga	523411				1972	9	24	I. Yamashita	Shizuoka pref., Iwata county, Sakuma town
D. saxifraga	500762				1975	8	10	T. Shimakawa	Shizuoka pref., Ito city, Mt. Yahazu
D. saxifraga	495915				1980	6	19	A. Suzuki	Shizuoka pref., Mishima city, Yamanakashinndenn
D. saxifraga	493250				1982	9	25	T. Yamamoto	Shizuoka pref., Kamo county, Matsuzaki town
D. saxifraga	492338				1978	8	26	T. Sugino	Shizuoka pref., Numazu city, Mt. Aitaka
D. saxifraga	493232				1983	8	3	T. Hosokura	Shizuoka pref., Fujinomiya city, Mt. Fuji
D. saxifraga	493236				1983	9	19	T. Hosokura	Shizuoka pref., Gotennba city, Innno
D. saxifraga	493238				1958	9	21	Y. Jotani	Shizuoka pref., Fujinomiya city
D. saxifraga	495914				1976	5	20	S. Komaki	Ishikawa pref., Hakui county, Shika town
D. saxifraga	572177				1981	6	4	S. Komaki	Ishikawa pref., Suzu city, Houryuu town, Ushima
D. saxifraga	572174				2010	9	12	I. Yamazumi	Osaka pref., Minamikawachi county, Chihaya-akasaka village
D. saxifraga	572172				1977	4	29	M. Arakane	Oita pref., Kusu county, Kokonoe town
D. saxifraga	572171				1955	11	6	M. Arakane	Oita pref., Beppu city, Inoseto
D. saxifraga	572169				1960	7	29	M. Hatano	Oita pref., Oita county, Shounai town
D. saxifraga	572167				2009	8	2	S. Matsumoto	Nagano pref., Matsumoto city, Shimashimadani
D. saxifraga	572165				1948	8	29	M. Muramatsu	Nagano pref., Shimoina county, Ooshika village
D. saxifraga	493275				1948	8	29	M. Muramatsu	Nagano pref., Shimoina county, Ooshika village
D. saxifraga	493233				1977	8	7	T. Nakaike	Nagano pref., Shiojiri city, Sakurasawa
D. saxifraga	493230				1981	8	2	K. Otsuka	Nagano pref., Azumi village, Norikurakougenn
D. saxifraga	493247				1976	7	28	H. Okubara	Nagano pref., Matsumoto city, Mikatayama
D. saxifraga	493252				1981	10	4	H. Okubara	Nagano pref., Kamiminochi county, Shinano town

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. saxifraga	493256				1965	8	28	H. Okubara	Nagano pref., Kiso county, Ookuwa village
D. saxifraga	493242				1962	7	27	H. Okubara	Nagano pref., Kiso county, Kiso village
D. saxifraga	493235				1982	8	4	H. Tsutsumi	Nagano pref., Saku city, Kabutoiwayama
D. saxifraga	480415				1983	7	20	H. Okubara	Nagano pref., Kiso county, Narakawa village
D. saxifraga	507160				1981	5	8	H. Tsutsumi	Nagano pref., Shimoina county, Achi village
D. saxifraga	586124				1981	8	11	H. Tsutsumi	Nagano pref., Iijima town, Yotagirigawa valley
D. saxifraga	480421				1981	10	5	H. Tsutsumi	Nagano pref., Suzaka city, Yonago
D. saxifraga	480414				1981	10	23	H. Tsutsumi	Nagano pref., Hase village, Mikobuchi
D. saxifraga	480475				1981	10	11	H. Tsutsumi	Nagano pref., Shimoina county, Ooshika village
D. saxifraga	480419				1981	5	9	H. Tsutsumi	Nagano pref., Shimoina county, Anann town
D. saxifraga	510906				1957	8	12	H. Tanioka	Tottori pref., Tottori city, Saigou village
D. saxifraga	510904				1971	11	23	A. Tanaka	Tottori pref., Yazu county, Wakasa town
D. saxifraga	552747				1958	7	27	A. Tanaka	Tottori pref., Yazu county, Chizu town
D. saxifraga	554207				2008	9	11	H. Sawae	Shimane pref., Masuda city, Hikimichoushiso
D. saxifraga	582523				1990	9	23	M.Yagiura	Shimane pref., Iiishi county, Iinann town, Tonnbara
D. saxifraga	582522				2003	8	23	M.Yagiura	Shimane pref., Mino county, Hikimi town, Mikazura
D. saxifraga	512068				1996	9	6	S. Matsumoto	Tokyo metroplitan., Nishitama county, Okutama town, Nippara
D. saxifraga	507153				1964	8	8	Y. Ishii	Tokyo metroplitan., Nishitama county, Hinohara village
D. saxifraga	507165				1978	4	16	K. Inoue	Tokyo metroplitan., Nishitama county, Okutama town, Unazawa
D. saxifraga	444552				1985	8	27	H. Ogura	Tochigi pref., Nasu county, Nasu town
D. saxifraga	444545				1924	7	2	N/A	Tochigi pref., Nikko city, Tosyouguu
D. saxifraga	444549				1975	8	29	T. Tashiro	Tochigi pref., Kanuma city, Mt. Ozakusann
D. saxifraga	450015				1957	8	19	K. Ogawa	Tochigi pref., Kamitsuga county, Ashio town
D. saxifraga	444271				1995	4	29	F. Kasetani	Nara pref., Yoshino county, Tennkawa village
D. saxifraga	447559				1977	8	3	T. Oshima	Toyama pref., Uotsu city, Toradani
D. saxifraga	543044				1990	5	6	Y. Saito	Fukui pref., Ono city, Minamirokuroshi
D. saxifraga	438741				1980	7	22	S. Watanabe	Fukui pref., Ono city, Nakamizudani
D. saxifraga	444862				1980	6	29	S. Watanabe	Fukui pref., Ono city, Kmanoko
D. saxifraga	444274				1980			S. Watanabe	Fukui pref., Katsuyama city, Mt. Toritate
D. saxifraga	450098				1979	10	9	S. Watanabe	Fukui pref., Mikata county, Mihama town
D. saxifraga	444269				2008	7	12	Y. Saito	Fukui pref., Ono city, Izumi
D. saxifraga	444293				1974	5	6	N. Ogami	Fukuoka pref., Buzenn city, Mt. Inugatake
D. saxifraga	444292				1975	5	4	S. Tsutsui	Fukuoka pref., Yame county, Yabe village
D. saxifraga	444290				1978	7	2	M. Yasu	Fukushima pref., Date county, Reizann town
D. saxifraga	444289				1980	7	6	M. Yasu	Fukushima pref., Souma county, Kashima town
D. saxifraga	444285				1981	7	4	Y. Kaneda	Fukushima pref., Minamiaizu county, Shimosato town
D. saxifraga	444281				1981	8	30	Y. Kaneda	Fukushima pref., Fukushima city, Shirotsu
D. saxifraga	444286				1979	4	24	Y. Kaneda	Fukushima pref., Yama county, Nishiaizu town
D. saxifraga	447561				1982	8	16	Y. Sugano	Fukushima pref., Date county, Tsukidate village
D. saxifraga	440105				1979	8	23	Y. Sugano	Fukushima pref., Futaba county, Namie town
D. saxifraga	440111				1980	12	2	Y. Kaneda	Fukushima pref., Tamura county, Funabiki town
D. saxifraga	440110				1980	12	3	Y. Kaneda	Fukushima pref., Iwaki city, Shimotowada
D. saxifraga	440091				1980	12	2	Y. Kaneda	Fukushima pref., Futaba county, Kuzuo village
D. saxifraga	448330				1980	12	3	Y. Kaneda	Fukushima pref., Futaba county, Tomioka town
D. saxifraga	448030				1980	12	2	Y. Kaneda	Fukushima pref., Tamura county, Miyakoji village
D. saxifraga	441740				1980	11	3	Y. Kaneda	Fukushima pref., Kawanuma county, Yanaizu town
D. saxifraga	441741				1979	6	25	Y. Kaneda	Fukushima pref., Aizuwakamatsu city, Takizawa town
D. saxifraga	541800				1979	7	23	Y. Kaneda	Fukushima pref., Tamura county, Miharu town
D. saxifraga	446346				1979	3	27	Y. Kaneda	Fukushima pref., Higashishirakawa county, Furudono town
D. saxifraga	440103				1979	3	27	Y. Kaneda	Fukushima pref., Iwaki city, Yamadama town
D. saxifraga	440099				1979	8	19	Y. Kaneda	Fukushima pref., Aizuwakamatsu city, Higashiyama town
D. saxifraga	440113				1979	3	27	Y. Kaneda	Fukushima pref., Ishikawa county, Hirada village
D. saxifraga	444966				1978	9	24	Y. Kaneda	Fukushima pref., Tamura county, Miharu town
D. saxifraga	440106				1983	10	9	T. Okubo	Fukushima pref., Ishikawa county, Ishikawa town
D. saxifraga	440114				1982	10	23	M. Yasu	Fukushima pref., Kooriyama city, Suzugauchi
D. saxifraga	932567				1977	10	18	Y. Yuzawa	Fukushima pref., Iwaki city, Yotsukura town
D. saxifraga	441681				1982	9	9	M. Yasu	Fukushima pref., Ishikawa county, Ishikawa town
D. saxifraga	441693				1980	6	8	M. Yasu	Fukushima pref., Tamura county, Mt. Ootakine
D. saxifraga	1168795				1981	7	19	M. Yasu	Fukushima pref., Higashishirakawa county, Furudono town
D. saxifraga	420635				1981	10	4	M. Yasu	Fukushima pref., Higashishirakawa county, Yamatsuri town
D. saxifraga	421015				1981	3	28	M. Yasu	Fukushima pref., Souma city, Ochiai
D. saxifraga	421014				1977	5	22	M. Yasu	Fukushima pref., Higashishirkawa county, Furudono town
D. saxifraga	439333				1978	11	3	M. Yasu	Fukushima pref., Futaba county, Naraha town
D. saxifraga	421012				1972	11	13	N. Wada	Fukushima pref., Iwaki city, Kawamae
D. saxifraga	439335				1978	6	25	Y. Hayashi	Hyogo pref., Mikata county, Mikata town
D. saxifraga	421023				1979	7	29	T. Shiraiwa	Hyogo pref., Kita Ward, Mt. Rokkosann
D. saxifraga	421008				1994	6	5	K. Horie	Hokkai-do, Asahikawa city, Kamui
D. saxifraga	421017				1985	8	5	K. Tsuchikura	Hokkai-do, Kushiro county, Kushiro town, Kamibeppo
D. saxifraga	421021				1947	10	8	T. Sasaki	Hokkai-do, Ishikari city, Nakamanabetsu, Mt. Ishigaki
D. saxifraga	445042				1971	11	23	Y Takahashi	Hokkai-do, Samani county, Samani town
D. saxifraga	440088				1984	7	8	Y. Aida	Hokkai-do, Kato county, Shikaoi town
D. saxifraga	440097				1986	6	8	Y. Aida	Hokkai-do, Suttsu county, Kuromatsunai town
D. saxifraga	440093				1984	7	15	Y. Aida	Hokkai-do, Asyoro county,Asyoro town
D. saxifraga	440094				1984	8	11	Y. Aida	Hokkai-do, Hiroo county, Hiroo town
D. saxifraga	1249965				1982	8	11	Y. Aida	Hokkai-do, Ashibetsu city, Okuashibetsu
D. saxifraga	1253339				1981	11	28	M. Nakajim	Hokkai-do, Kushiro county, Kushiro town, Kamibeppo
D. saxifraga	1253134				1980	7	8	M. Ishizuka	Hokkai-do, Tomakomai city, Mt. Taruyama
D. saxifraga	1254043				1983	9	15	S. Aida	Hokkai-do, Yuufutsu county, Shimukappu village
D. saxifraga	1261930				1983	9	4	S. Aida	Hokkai-do, Yuubari city, Noborikawa
D. saxifraga	1261932				1981	10	8	A. Matsumoto	Hokkai-do, Kawakami county, Teshikaga town
D. saxifraga	1261934				1983	9	15	Y. Aida	Hokkai-do, Yuufutsu county, Hobetsu town
D. saxifraga	1261936				1963	9	20	Y. Takahashi	Hokkai-do, Saru county, Biratori town
D. saxifraga	1261937				1982	9	19	Y. Aida	Hokkai-do, Bibai city, Sakae town
D. saxifraga	1261938				1983	10	2	Y. Aida	Hokkai-do, Yuufutsu county, Atsuma town, Takaoka
D. saxifraga	1264249				1980	9	15	K. Kuratsu	Hokkai-do, Kameta county, Todohokke village
D. saxifraga	1264248				1981	9	15	T. Takahashi	Hokkai-do, Hakodate city, Mt. Mimori
D. saxifraga	1264247				1981	9	23	T. Takahashi	Hokkai-do, Hakodate city, Gabino
D. saxifraga	1246779				2004	10	30	C. Takeda	Hokkai-do, Uraga county, Uraga town
D. saxifraga	1246787				2004	11	2	C. Takeda	Hokkai-do, Hidaka county, Shinnhidakara town
D. saxifraga	1246824				2007	10	11	C. Takeda	Hokkai-do, Shiraoi county, Shiraoi town
D. saxifraga	1246840				2011	7	5	C. Takeda	Hokkai-do, Sapporo city, Kiyota-ku, Ariake
D. saxifraga	1246841				2012	8	16	C. Takeda	Hokkai-do, Noboribetsu city, Kawakami town
D. saxifraga	1246842				2014	8	16	C. Takeda	Hokkai-do, Matsumae county, Fukushima town
D. saxifraga	829532				2015	9	21	C. Takeda	Hokkai-do, Chitose city, Poropinai
D. saxifraga	572168				1980	11	9	B. Matsubayashi	Nagasaki pref., Minamitakaki county, Obama town, Mt. Unnzenn
D. subhikonensis	114193				1985	6	16	K. Inukai	Aichi pref., Okazaki City, Okuden town, Mt. Murazumi
D. subhikonensis	195959				1988	10	9	K. Inukai	Aichi pref., Nishikamo county, Obara village, Okusa
D. subhikonensis	196132				1982	9	18	Y. Obata	Okayama pref., Okayama City, Ichinomiya, Kibitsuhiko shrine
D. subhikonensis	268414				1982	12	2	Y. Obata	Okayama pref., Maniwa county, Chuka village, Hatsuwa
D. subhikonensis	421037				1982	6	27	Y. Obata	Okayama pref., Niimi City, Iwayama
D. subhikonensis	421058				1960	8	8	I .Akasawa	Okayama pref., Takahashi City, Mt. Gagyuu
D. subhikonensis	421059				1961	8	8	I .Akasawa	Okayama pref., Kamikawa county, Kawakami town, Takayamaichi
D. subhikonensis	421060				1985	6	9	Y. Obata	Okayama pref., Aida county, Higashiawakura village, Mt. Ushiroyama
D. subhikonensis	441909				1985	7	14	Y. Obata	Okayama pref., Aida county, Sakuto town, Hogidani
D. subhikonensis	444409				1978	12	10	M. Fukuhara	Gifu pref., Tajimi City, Simoichinokura town
D. subhikonensis	444531				1977	7	26	Y. Yamamoto	Gifu pref., Kaji City, Owaki
D. subhikonensis	444535				1981	3	17	Y. Yamamoto	Gifu pref., Kamo county, Hichiso town, Murokane
D. subhikonensis	444537				1979	11	30	Y. Yamamoto	Gifu pref., Gifu City, Kuronoinuzuka
D. subhikonensis	444538				1997	9	25	H. Marui	Gifu pref., Minokamo City, Yamanoue town, Tabata
D. subhikonensis	445038				1984	10	18	H. Miyazaki	Gifu pref., Kamo county, Yaozu town, Kamisinndenn
D. subhikonensis	446165				1990	7	12	H. Miyazaki	Gifu pref., Yamagata county, Miyama town, Kannzaki
D. subhikonensis	446179				1985	10	19	N. Nimura	Gifu pref., Masuda county, Gero town, Kadoharafukaya
D. subhikonensis	446733				1984	9	24	T. Ogiyama	Gifu pref., Ena City, Kushihara village, Mt. Takado
D. subhikonensis	448325				2001	10	8	T. Ogiyama	Gifu pref., Mugi county, Horado village, Oono
D. subhikonensis	448624				2010	6	6	T. Ogiyama	Gifu pref., Ena City, Kamiyahagi town, Hiraiwa
D. subhikonensis	449307				1983	9	25	K. Shogo	Miyagi pref., Watari county, Watari town, Nagatoro
D. subhikonensis	496020				1982	6	7	T. Nakaike	Kyoto-fu, Miyazu City, Yura
D. subhikonensis	496021				1982	6	6	T. Nakaike	Kyoto-fu, Amata county, Yakuno town, Sue
D. subhikonensis	500661				1982	6	6	T. Nakaike	Kyoto-fu, Fukuchiyama City, Umedani
D. subhikonensis	500767				1982	6	7	T. Nakaike	Kyoto-fu, Maizuru City, Magura
D. subhikonensis	500768				1982	6	7	T. Nakaike	Kyoto-fu, Maizuru City, Nakada
D. subhikonensis	500769				1987	1	18	C. Satonaka	Kyoto-fu, Kyoto City, Sakyo Ward, Kibune

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. subhikonensis	500921				1983	8	8	T. Nakaike	Kyoto-fu, Amata county, Sannwa town, Nakaide
D. subhikonensis	500932				1983	11	14	K. Mashita	Gunnma pref., Tano county, Onishi town, Inume
D. subhikonensis	506302				1973	4	19	N. Sudoushi	Gunnma pref., Ota City, Kanayama
D. subhikonensis	506313				1983	10	29	S. Ojima	Gunnma pref., Kannra county, Shimonita town, Abuta
D. subhikonensis	506321				1982	5	23	T. Nakaike	Gunnma pref., Tano county, Onishi town, Yashio
D. subhikonensis	507420				1973	4	25	N. Sudoushi	Gunnma pref., Tano county, Ojima town, Chorakuji temple
D. subhikonensis	507472				1974	10	1	T. Takeda	Hiroshima pref., Hiba county, Saijo town, Hogaya
D. subhikonensis	507499				1973	12	12	T. Takeda	Hiroshima pref., Saeki county, Miyajima Island
D. subhikonensis	507821				2013	10	11	K. Kuwata	Hiroshima pref., Jinnsekikougen town, Fukunaga
D. subhikonensis	507836				1982	11	9	M. Shimosegawa	Saitama pref., Hasuda City, Komasaki
D. subhikonensis	507971				1982	11	23	S. Kobayashi	Saitama pref., Chichibu county, Yoshida town, Kamisato
D. subhikonensis	518656				1954	8	6	T .Kawasaki	Saitama pref., Iruma county, Ogose town, Mt. Koshigami
D. subhikonensis	538065				1991	9	15	T. Iwata	Saitama pref., Chichibu City, Kuna
D. subhikonensis	568604				1983	10	9	T. Iwata	Saitama pref., Hannnou City, Higashiagano
D. subhikonensis	817416				1987	11	17	T. Iwata	Saitama pref., Chichibu City, Tochiya
D. subhikonensis	840423				1985	5	29	T. Iwata	Saitama pref., Honjo City, Kodama town, Mase pass
D. subhikonensis	1023868				1985	3	18	S. Ojima	Saitama pref., Honjo City, Wakaizumi park
D. subhikonensis	1023878				2013	10	4	H. Akimaru	Yamaguchi pref., Hagi City, Susa
D. subhikonensis	1023879				1981	10	10	S. Yamauchi	Shiga pref., Kannzaki county, Eigenji town, Kanzaki river
D. subhikonensis	1023887				1980	6	25	T. Tatebe	Shiga pref., Hikone City, Oka town
D. subhikonensis	1023963				1979	6	6	F. Miyamoto	Shiga pref., Ika county, Kinomoto town, Furuhashi
D. subhikonensis			446180		2016	7	21	K. Hori	Shiga pref., Takashima City, Katsuno
D. subhikonensis	1026065				1989	7	22	Y. Horii	Akita pref., Kisakata town, Osugo
D. subhikonensis	1026467				1975	9	21	S. Komaki	Ishikawa pref., Hakui City, Yanagida
D. subhikonensis	1026469				1979	2	12	S. Komaki	Ishikawa pref., Nanao City, Furushiro
D. subhikonensis	1026474				1981	8	11	S. Komaki	Ishikawa pref., Kahoku county, Tsuhata town, Minaminakajo
D. subhikonensis	1026485				1981	7	5	M. Honda	Ishikawa pref., Komatsu City, Shimoosugi
D. subhikonensis	1026489				1990	8	26	T. Kawahara	Ishikawa pref., Kanazawa City, Shibotakasaka town
D. subhikonensis	1026491				1981	8	15	I. Ueda	Chiba pref., Chiba City, Sawa town
D. subhikonensis	1026507				1981	11	9	H. Tsutsumi	Nagano pref., Kiso county, Minamikiso town, Mt. Hosono
D. subhikonensis	1026509				1981	7	3	H. Tsutsumi	Nagano pref., Shimoina county, Tenryuu village, Fukushima
D. subhikonensis			446181		2016	7	20	K. Hori	Shizuoka pref., Hamamatsu City, Tenryuu Ward, Sakuma town, Urakawa
D. subhikonensis	1026511				1983	3	29	A. Tanaka	Tottori pref., Yazu county, Yazu town, Youro
D. subhikonensis	1026513				1980	9	7	M. Aoto	Tottori pref., Hino county, Ebu town, Matano
D. subhikonensis	1026521				1980	10	2	M. Aoto	Tottori pref., Hino county, Nitinann town, Tyaya
D. subhikonensis	1039271				1981	11	3	A. Tanaka	Tottori pref., Touhaku county, Misasa town, Anagamo
D. subhikonensis	1039354				1981	10	18	T. Iwanaga	Tottori pref., Yazu county, Motigase town, Kanaya
D. subhikonensis	1039395				1983	2	11	A. Minami	Shimane pref., Unnnan City, Daito town, Nakayuishi
D. subhikonensis	1039396				1983	2	11	A. Minami	Shimane pref., Unnnan City, Mitoya town, Tonogouchi
D. subhikonensis	1044934				1981	11	3	A. Minami	Shimane pref., Matsue City, Tamayu town, Oodani
D. subhikonensis	1045039				2015	6	27	Y. Shinohara	Shimane pref., Goutsu City, Matsukawa town, Oota
D. subhikonensis	1092955				1993	7	11	T. Yamada	Tokyo-to, Nishitama county, Okutama town, Kori
D. subhikonensis	1246817				1968	10	11	K. Inoue	Tokyo-to, Nishitama county, Okutama town, Mt. Kariyose
D. subhikonensis	1246942				1971	11	14	M. Miyamaoto	Tokyo-to, Nishitama county, Okutama town, Nippara
D. subhikonensis	1247576				1988	8	30	Y. Kobayashi	Tokyo-to, Oume City, Nariki
D. subhikonensis			406858		2012	11	30	K. Hori	Tokyo-to, Inagi City, Momura
D. subhikonensis	1247586				1962	6	2	K. Ogawa	Tochigi pef., Tochigi City, Mt. Boroishi
D. subhikonensis	1247602				1984	8	5	T. Oshima	Toyama pref., Oyabe City, Komoridani
D. subhikonensis	1247626				1969	6	23	S. Watanabe	Fukui pref., Asuwa county, Miyama town, Ashimi
D. subhikonensis	1248435				1979	10	10	S. Watanabe	Fukui pref., Mikata county, Mihama town, Ooyabu
D. subhikonensis	1248440				1949	9	23	Y. Saito	Fukui pref., Katsuyama City, Osoawa town, Mt. Mimuro
D. subhikonensis	1248445				1983	8	20	T. Shiraiwa	Hyogo pref., Mikata county, Muraoka town, Hikage
D. subhikonensis	1253057				1976	5	25	M. Hayashi	Hyogo pref., Kisaki county, Kasumi town, Hayato
D. subhikonensis	1256344				1987	11	11	M. Saito	Niigata pref., Sado City, Aikawa
D. subhikonensis	1257635				1988	8	6	M. Saito	Niigata pref., Sado City, Ogi town
D. subhikonensis			446174		2016	7	3	K. Hori	Kanagawa pref., Sagamihara City, Midori Ward, Obara
D. subhikonensis			446786		2016	6	27	K. Hori	Kanagawa pref., Odawara City, Kuno
D. subhikonensis			449117		2016	7	2	K. Hori	Japan, Saitama pref., Hannno city
D. varia	927039				1986	1	3	K. Inukai	Aichi pref., Chita county, Mihama town, Noma
D. varia	927035				1977	10	27	T. Waku	Aichi pref., Chita county, Minamichita town, Utsumi
D. varia	763911				1988	4	24	H. Miyazaki	Aichi pref., Toyohashi city, Unoya town, Fumonnji temple
D. varia	735003				1988	3	31	H. Miyazaki	Aichi pref., Chita county, Mihama town, Kiriyama
D. varia	1110317				1976	10	12	K. Oka	Ehime pref., Yahatahama city, Sirado town, Kubota
D. varia	1183397				1976	10	11	K. Oka	Ehime pref., Uwajima city, Shiroyama
D. varia	1221986				1978	7	2	Y. Miyoshi	Ehime pref., Matsuyama city, Gogoshima Island
D. varia	388543				1980	10	26	H. Hyodo	Ehime pref., Iyo county, Futami town, Houshi
D. varia	1222643				1970	12	29	T. Kunai	Ehime pref., Uwa county, Ipponnmatsu town, Masaki
D. varia	385541				2012	3	3	H. Hyodo	Ehime pref., Imabari city, Kamiura town, Sakari
D. varia	504206				2014	2	5	H. Hyodo	Ehime pref., Oozu city, Sugeta town
D. varia	504205				1960	7	5	H. Ishikawa	Ehime pref., Araihama city
D. varia	508029				2008	12	14	H. Hyodo	Ehime pref., Iyo city, Futami town, Kaminada
D. varia	514327				1978	1	27	T. Saito	Okayama pref., Bizenn city, Ootaki, Fukuoji temple
D. varia	508025				1965	12	26	T. Ymanaka	Miyazaki pref., Nichinann city, Sakatani
D. varia	508013				1969	6	8	T. Muroya	Miyazaki pref., Nichinann city, Oodoutsu, Shiozuru
D. varia	507980				1976	7	30	Y. Akagi	Miyazaki pref., Nichinann city, Ibii river
D. varia	507978				1986	8	9	A. Minami	Miyazaki pref., Higashiusuki county, Kitagawa town
D. varia	504182				1975	2	16	T. Muroya	Miyazaki pref., Kushima city, Ooyadori
D. varia	1219443				1976	1	7	K. Minamitani	Miyazaki pref., Nichinann city, Obijoshi
D. varia	1219446				1975	11	18	K. Minamitani	Miyazaki pref., Minaminaka county, Kitasato town, Inohae
D. varia	1219457				1976	7	18	Y. Akagi	Miyazaki pref., Saito city, tyausubara
D. varia	1219460				1976	3	1	T. Minamitani	Miyazaki pref., Higashimorokata county, Takaoka town, Kusumi
D. varia	1219475				1943	11	3	M. Nagasawa	Miyazaki pref., Kadokawa town
D. varia	1219481				2013	8	23	Y. Akagi	Miyazaki pref., Nobeoka city, Kitagawa town, Moriya river
D. varia	1219485				2013	9	5	Y. Akagi	Miyazaki pref., Nobeoka city, Kumanoe town
D. varia	1219486				2015	3	4	Y. Akagi	Miyazaki pref., Nobeoka city, Miyanaga town
D. varia	1219487				2014	5	9	Y. Akagi	Miyazaki pref., Higashiusuki county, Kadokawa town
D. varia	1219414				2013	6	4	Y. Akagi	Miyazaki pref., Higashiusuki vounty, Morotsuka village
D. varia	1219426				2013	10	26	Y. Akagi	Miyazaki pref., Koyu county, Nishimera village
D. varia	1219427				2013	3	30	Y. Akagi	Miyazaki pref., Koyu county, Kijo town, Ishikawauchi
D. varia	1219428				2013	10	1	Y. Akagi	Miyazaki pref., Koyu county, Kawaminami town
D. varia	360016				2014	3	19	Y. Akagi	Miyazaki pref., Higashimorokata county, Aya town
D. varia	360447				2014	3	23	Y. Akagi	Miyazaki pref., Kobayashi city, Nojiri town
D. varia	360562				2013	7	25	Y. Akagi	Miyazaki pref., Miyazaki city, Kiyotake town, Minase river
D. varia	360517				2013	7	31	Y. Akagi	Miyazaki pref., Nichinann city, Nanngou town
D. varia	360472				2013	7	30	Y. Akagi	Miyazaki pref., Kushima city, Honnjou
D. varia	360382				1979	8	4	M. Ishizaka	Kumamoto pref., Kikuchi county, Otsu town
D. varia	360202				1978	4	2	M. Ishizaka	Kumamoto pref., Uto city, Amizu town
D. varia	359932				1977	1	4	J. Beppu	Kumamoto pref., Kumamoto city, Mt. Kinnbou
D. varia	359012				1965	11	11	M. Shiroto	Kumamoto pref., Minamata city, Ookubo
D. varia	362336				1964	10	18	M. Shiroto	Kumamoto pref., Minamata city, Fukagawa
D. varia	360308				1975	3	9	S. Kurashige	Kumamoto pref., Shimomashiki county, Toyou town
D. varia	363325				1976	8	7	J. Beppu	Kumamoto pref., Yatsushiro county, Touyou village
D. varia	363966				1959	11	20	M. Shiroto	Kumamoto pref., Minamata city, Hyakukenn town
D. varia	363190				1958	11	20	M. Shiroto	Kumamoto pref., Minamata city, Yude
D. varia	362425				1971	2	27	J. Beppu	Kumamoto pref., Yatsushiro city, Higashi town
D. varia	363010				1976	8	6	J. Beppu	Kumamoto pref., Yatsushiro county, Izumi village, Mt. Yayamadake
D. varia	362380				1977	1	2	S. Tsutsui	Kumamoto pref., Tamana county, Gyokutou town, Urata
D. varia	362875				1972	7	15	M. Hayagami	Kumamoto pref., Amakusa county, Himedo town
D. varia	362335				1979	7	22	M. Ishizaka	Kumamoto pref., Yamaga city, Fudoiwa
D. varia	362290				1977	1	6	M. Otomasu	Kumamoto pref., Hitoyoshi city, Otsuka
D. varia	362785				1976	8	5	S. Kurata	Kumamoto pref., Yatsushiro county, Touyou village
D. varia	362155				1961	6	1	M. Shiroto	Kumamoto pref., Minamata city, Kuzuwatari
D. varia	363911				1958	11	6	M. Shiroto	Kumamoto pref., Minamata city, Yude
D. varia	360445				1961	12	3	M. Shiroto	Kumamoto pref., Minamata city, Fukagawa
D. varia	360400				1965	11	11	M. Shiroto	Kumamoto pref., Minamata city, Ookubo
D. varia	360715				2015	7	16	M. Watanabe	Kumamoto pref., Amakusa city, Itsuwa town
D. varia	360265				1976	9	21	Y. Nishimura	Hiroshima pref., Kure city, Nigata town
D. varia	360220				1977	1	5	T. Takeda	Hiroshima pref., Kure city, Aga town, Mt. Yasumi
D. varia	363920				1977	4	30	T. Takeda	Hiroshima pref., Toyota county, Kinoe town
D. varia	361255				1975	6	10	T. Takeda	Hiroshima pref., Toyota county, Setoda town, Fukuda
D. varia	361161				1974	4	20	T. Takeda	Hiroshima pref., Mihara city, Saizaki town, Kuwaki
D. varia	360951				1976	3	1	T. Takeda	Hiroshima pref., Fukuyama city, Goubun town

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. varia	363460				1975	12	17	M. Yokota	Hiroshima pref., Aki county, Gamagari town
D. varia	359446				1980	1	27	S. Mitani	Kagawa pref., Shouzu county, Tonosho town
D. varia	359536				1976	5	23	S. Mitani	Kagawa pref., Sakaide city, Mt. Iino
D. varia	360805				1974	12	15	S. Mitani	Kagawa pref., Takamatsu city, Yashima
D. varia	374190				1975	5	11	S. Mitani	Kagawa pref., Takamatsu city, Mt. Mineyama
D. varia	359487				1980	2	3	S. Mitani	Kagawa pref., Mitoyo county, Mino town
D. varia	346302				1985	12	26	T. Miyazaki	Kochi pref., Takaoka county, Nakatosa town
D. varia	371466				1978	1	15	K. Yamaoka	Kochi pref., Tosashimizu city, Kubotsu
D. varia	371493				1976	4	4	T. Kouzai	Kochi pref., Takaoka county, Ochi town
D. varia	366907				1976	2	21	Y. Kougami	Kochi pref., Tosa city, Izuma
D. varia	366354				1976	7	4	T. Kouzai	Kochi pref., Aki city, Iogi
D. varia	361883				1978	9	10	T. Kouzai	Kochi pref., Muroto city, Hane town, Nakakawachi
D. varia	346260				1978	3	21	K. Tamaki	Kochi pref., Hata county, Ootsuki town, Kashiwajima
D. varia	347284				1978	3	22	K. Tamaki	Kochi pref., Tosashimizu city, Shimokawaguchi
D. varia	368370				1977	5	28	T. Kouzai	Kochi pref, Tosashimizu city, Matsuo
D. varia	346296				1979	3	4	K. Yamaoka	Kochi pref., Takaoka county, Nakatosa town
D. varia	346253				1974	10	21	T. Nakaike	Kochi pref., Tosashimizu city, Kubotsu
D. varia	346282				1976	6	13	T. Kouzai	Kochi pref., Muroto city, Kiragawa town, Nichinann
D. varia	346310				1977	12	28	K. Yamaoka	Kochi pref., Tosa city, Usa town
D. varia	346309				1977	12	28	K. Yamaoka	Kochi pref., Tosa city, Takaoka town
D. varia	346366				1972	12	23	S. Mitsuta	Kochi pref., Takaoka county, Ochi town
D. varia	361391				1982	8	30	N. Niwaki	Saga pref., Fujitsu county, Ureshino town, Yunoda
D. varia	359257				1975	11	2	Y. Kurashige	Saga pref., Ogi county, Ushizu town
D. varia	359437				1975	4	27	H. Koike	Saga pref., Kashima city, Mikawachi
D. varia	359482				1976	5	2	T. Baba	Saga pref., Fujitsu county, Ureshino town, Kogui
D. varia	359347				1975	3	21	M. Koike	Saga pref., Taku city, Kitataku town
D. varia	359752				1973	10	4	S. Komaki	Mie pref., Kumano city
D. varia	359797				1976	5	2	Y. Nakajima	Mie pref., Minamimuro county, Mihama town
D. varia	359662				1975	11	22	A. Yamamoto	Mie pref., Owase city, Oosoneura
D. varia	363640				1989	1	8	K. Seto	Mie pref., Kitamuro county, Kiinagashima
D. varia	363550				1967	10	19	H. Ito	Mie pref., Owase city, Yukuno beach
D. varia	900768				1978	3	27	R. Ito	Mie pref., Owase city, Hayata
D. varia	359682				1979	10	14	A. Minami	Yamaguchi pref., Kumage county, Kaminoseki town
D. varia	359772				1988	9	19	H. Masaki	Yamaguchi pref., Shimomatsu city, Kannnonn
D. varia	359916				1977	9	6	A. Minami	Yamaguchi pref., Hikari city, Ushima Island
D. varia	360124				1990	8	7	A. Minami	Yamaguchi pref., Iwakuni city, Hashirajima Island
D. varia	360222				1976	4	11	H. Masaki	Yamaguchi pref., Boufu city, Nojimaakazaki
D. varia	345235				1980	8	14	A. Minami	Yamaguchi pref., Kumage county, Kaminoseki town
D. varia	361930				1982	2	14	A. Minami	Yamaguchi pref., Yanai city, Heigunn
D. varia	361885				1953	9	28	K. Oka	Yamaguchi pref., Kaminoseki town, Iwaishima Island
D. varia	361791				1964	10	19	S. Miyake	Yamaguchi pref., Boufu city, Mukoujima Island
D. varia	361746				1989	1	25	A. Minami	Yamaguchi pref., Yanai city, Hizumi
D. varia	361705				1956	11	10	T. Hashimoto	Shiga pref., Hino town, Mt. Yotsugase
D. varia	361615				1985	8	18	T. Yamanaka	Kagoshima pref., Kimotsuki county, Sata town
D. varia	360627				1960	1	24	T. Yamanaka	Kagoshima pref., Imizu city, Ayukawa
D. varia	361480				1975	4	4	M. Hioki	Kagoshima pref., Kajiki city, Takaida
D. varia	361435				1985	1	21	M. Kawabata	Kagoshima pref., Kagoshima city, Hirakawa town
D. varia	361345				1986	10	26	T. Kariyazaki	Kagoshima pref., Minamitane town, Shimama
D. varia	361841				1987	4	17	T. Kariyazaki	Kagoshima pref., Minamitane town, Nojiri
D. varia	361526				1988	6	15	T. Kariyazaki	Kagoshima pref., Nishinoomote city, Sumiyoshi
D. varia	360331				1986	10	12	T. Kariyazaki	Kagoshima pref., Nishinoomote city, Waseda river
D. varia	359997				1984	3	4	K. Kawahara	Kagoshima pref., Yubisuki city, Mt. Uomidake
D. varia	360267				1980	3	16	M. Shiroto	Kagoshima pref., Akune ciy, Kuronohama
D. varia	359401				1985	1	21	M. Kawabata	Kagoshima pref., Kagoshima city, Hirakawa town, Mt. Eboshidake
D. varia	360175				1977	8	5	K. Takesako	Kagoshima pref., Soo county, Shibushi town, Mt. Gozaisyodake
D. varia	334578				1975	10	25	N. Miyaji	Kagoshima pref., Nishinoomote city, Furuta
D. varia	1219419				1977	1	27	T. Nakaike	Kagoshima pref., Kumage county, Nakatane town, Adakaiso
D. varia	1219424				1974	3	23	K. Takesako	Kagoshima pref., Satsuma county, Shimokoshiki village
D. varia	1219429				1977	1	29	K. Takesako	Kagoshima pref., Kawanabe county, Bounotsu town, Jinnnoo
D. varia	1219431				1977	2	6	K. Takesako	Kagoshima pref., Kawanabe county, Kasasa town
D. varia	1219434				1975	2	26	K. Kawahara	Kagoshima pref., Imizu county, Nagashima town
D. varia	1219436				1977	1	16	K. Takesako	Kagoshima pref., Kaseda city, Mt. Nagaya
D. varia	360069				1959	9	10	T. Yamanaka	Kagoshima pref., Tarumi city, Enoshima
D. varia	360427				1973	7	8	K. Takesako	Kagoshima pref., Kagoshima county, Yoshida town
D. varia	360337				1973	12		K. Takesako	Kagoshima pref., Aira city, Kamou town
D. varia	360063				1976	8	2	K. Takesako	Kagoshima pref., Kimotsuki county, Sata town
D. varia	397423				1976	8	4	K. Takesako	Kagoshima pref., Kimotsuki county, Nejime town
D. varia	362470				1985	1	21	M. Kawabata	Kagoshima pref., Kagoshima city, Hirakawa town
D. varia	362110				1976	7	21	K. Takesako	Kagoshima pref., Aira city, Fukuyama town, Isowaki
D. varia	360846				1986	5	10	T. Kariyazaki	Kagoshima pref., Nishinoomote city, Tennnyogakura
D. varia	361210				1976	3	31	A. Yamamoto	Kagoshima pref., Satsuma county, Shimokoshiki village
D. varia	363469				1959	8	28	M. Shiroto	Kagoshima pref., Kimotsuki county, Sata town
D. varia	1219466				1940	5	23	K. Ochi	Kagoshima pref., Ibusuki city, Yamakawa town
D. varia	1219471				1982	9	25	K. Kawahara	Kagoshima pref., Ibusuki city, Higashikata

Appendix 3-1. Additional specimens of the *Dryopteris varia* complex are examined. Only the name of the first collecter is shown.

Species	TNS	MAK	MBK	PE	Year	Month	Date	Coll.	Locality
D. varia	1219474				1978	8	2	N. Tashiro	Kanagawa pref., Odawara city, Haneo
D. varia	1219476				1947	7	29	T. Date	Kanagawa pref., Ashigarashimo county, Manazuru town
D. varia	1219483				1978	8	20	F. Miyamoto	Kanagawa pref., Zushi city, Hayama town, Mt. Futago
D. varia	360672				1974	8	3	T. Hosokura	Shizuoka pref., Numazu city, Nishiura
D. varia	360537				1968	6	30	T. Sugino	Shizuoka pref., Kakegawa city, Sakashita
D. varia	508021					11	12	I. Yamashita	Shizuoka pref., Syuuchi county, Mori town, Kappu
D. varia	532871				1967	12	27	T. Nakaike	Shizuoka pref., Minamiizu town, Nagatsumyo
D. varia	1219413				1975	11	2	I. Sasamoto	Shizuoka pref., Higashiizu town, Katase shrine
D. varia	385567				1976	2	9	T. Shimakawa	Shizuoka pref., Ito city, Tomido-osawa
D. varia	371494				1976	12	12	T. Yamamoto	Shizuoka pref., Kamo county, Nishiizu town, Arari
D. varia	366613				1985	2	12	T. Hosokura	Shizuoka pref., Numazu city, Oohirayama
D. varia	345234				1973	11	25	T. Sugino	Shizuoka pref., Tennryuu city, Gyoujaiwa
D. varia	345236				1970	4	1	T. Sugino	Shizuoka pref., Kakegawa city, Oowada
D. varia	345753				1976			T. Yamamoto	Shizuoka pref., Kamo county, Matsuzaki town, Ishibu
D. varia	345836				1986	1	19	T. Hosokura	Shizuoka pref., Atami city, Shimotaga
D. varia	345219				1989	3	1	T. Hosokura	Shizuoka pref., Yaizu city, Hamatoume
D. varia	345222				1989	7	13	T. Hosokura	Shizuoka pref., Numazu city, Nishiuraenashi
D. varia	345228				1984	3	7	T. Hosokura	Shizuoka pref., Numazu city, Mt. Kanukiyama
D. varia	345232				1982	10	16	M. Nakagawa	Shizuoka pref., Fuji city, Kiwadakubo
D. varia	368212				1983	8	19	T. Hosokura	Shizuoka pref., Fuji city, Kamiya town
D. varia	367857				1977	3	26	Y. Kimizuka	Shizuoka pref., Atami city, Atamikou
D. varia	367706				1973	9	9	T. Sugino	Shizuoka pref., Syuuchi county, Mori town, Kappu
D. varia	361838				1976	12	5	T. Yamamoto	Shizuoka pref., Kamo county, Nishiizu town, Ishiki
D. varia	346295				1954	3	2	J. Sugimoto	Shizuoka pref., Kamo county, Nishiizu town, Arari
D. varia	366545				1951	5	24	H. Ito	Shizuoka pref., Shimoda city
D. varia	345231				1956	11	14	S. Kurata	Shizuoka pref., Kamo county, Nishiizu town, Arari
D. varia	346330				1976	2	7	Y. Kimizuka	Chiba pref., Katsuura city, Tona
D. varia	346289				1976	3	7	K. Nakamura	Chiba pref., Futtsu city, Mt. Nokogiri
D. varia	346324				1971	5	4	N. Sahashi	Chiba pref., Awa county, Chikura town, Mt. Takatsukayama
D. varia	346338				1975	10	25	I. Ueda	Chiba pref., Kamogawa city, Emi
D. varia	508022				1976	9	26	Y. Kimizuka	Chiba pref., Tateyama city, Higashinagata
D. varia	1219408				1974	11	17	M. Sakakibara	Chiba pref., Minamibousou city, Chikura town, Mt. Takatsukayama
D. varia	817303				1949	4	14	K. Seto	Osaka pref., Sakai city
D. varia	817319				1950	8		T. Kodama	Osaka pref., Habikino city, Furuichi town
D. varia	1039499				1975	9	15	Y. Takaoka	Oita pref., Saeki city, Shiroyama
D. varia	817307				1976	9	5	M. Hadano	Oita pref., Tsukumi city, Tokuura
D. varia	147177				1976	4	4	Y. Maruno	Oita pref., Oita city, Kaku
D. varia	114061				1977	6	4	S. Tsutsui	Oita pref., Hita city, Akibaru town
D. varia	89375				2006	2	18	H. Tsuji	Oita pref., Saiki city, Kamaemorizakiura
D. varia	89352				1985	7	23	A. Minami	Nagasaki pref., Nisisonogi county, Nobosaki town, Mt. Gongenn
D. varia	1039514				1976	5	29	B. Matsubayashi	Nagasaki pref., Nagasaki city, Sannwa town
D. varia	1039513				1962	1	3	S. Ohtani	Nagasaki pref., Nagasaki city, Tomachi
D. varia	1039512				1973	9	16	S. Tsutsui	Nagasaki pref., Nagasaki city, Mogi
D. varia	1039508				1972	7	28	B. Matsubayashi	Nagasaki pref., Kitamatsura county, Ojika Island.
D. varia	1039502				1977	6	8	S. Tsutsui	Nagasaki pref., Minamitakaki conty, Obama town, Obama
D. varia	1039498				1977	6	3	S. Tsutsui	Nagasaki pref., Hirato city, Wakamiya
D. varia	1039493				1977	1	9	S. Tsutsui	Nagasaki pref., Matsura county, Kamigotou town, Aosagaura
D. varia	1039465				1961	9	25	T. Shinagawa	Nagasaki pref., Gounoura town
D. varia	1039467				2015	2	14	S. Yamada	Nagasaki pref., Nagasaki city, Kayaki town
D. varia	1039472				2009	1	23	S. Yamada	Nagasaki pref., Shimabara city, Shinnyama
D. varia	1039475				2008	1	7	S. Yamada	Nagasaki pref., Unnzenn city, Mizuho town
D. varia	817472				2012	12	17	S. Yamada	Nagasaki pref., Minamishimabara city, Kitaarima town
D. varia	817468				2010	7	20	S. Yamada	Nagasaki pref., Minamishimabara city, Nishiarie town
D. varia	9515574				2014	8	7	M. Yagiura	Shimane pref., Izumo city, Taisya town
D. varia	726246				2014	9	1	M. Yagiura	Shimane pref., Matsue city, Mihozeki town
D. varia	215528				1950	9	25	K. Takahashi	Shimane pref., Hikawa county, Taisya town
D. varia	817310				1937	2	21	K. Hayashi	Tokyo pref., Miyakejima Island.
D. varia	1039476				1958	7	20	Y. Jotani	Tokyo pref., Aogashima Island.
D. varia	1039466				1979	9	8	T. Nakayama	Tokushima pref., Tokushima city, Mayuyama
D. varia	1039468				1970	1	3	T. Nakayama	Tokushima pref., Anann city, Mt. Tiryuuji
D. varia	1039470				1977	8	21	T. Nakayama	Tokushima pref., Kaifu county, Mugi town, Debajima
D. varia	1039474				1960	10	27	T. Inobe	Tokushima pref., Komatsu city, Tano town
D. varia	1039473				1960	11	3	T. Inobe	Tokushima pref., Tokushima city, Mayuyama
D. varia	1039483				1994	9	26	Y. Tsujimoto	Nara pref., Yoshino county, Totsukawa village, Tano
D. varia	817471				1968	1	7	S. Tsutsui	Fukuoka pref., Kasuya county, Hiayama town, Ino
D. varia	1039505				1976	10	10	S. Kobayashi	Fukuoka pref., Kitakyuusyuu city, Moji
D. varia	1039491				1977	2	20	S. Tsutsui	Fukuoka pref., Itoshima county, Shima town
D. varia	1039488				1975	4	26	S. Tsutsui	Fukuoka pref., Yame county, Kurogi town
D. varia	111214				1977	1	16	K. Mune	Hyogo pref., Sumoto city, Nakatsugawagumi
D. varia	89354				1977	7	14	H. Masago	Wakayama pref., Nishimuro county, Shirahama town
D. varia	89305				1977	1	16	H. Masago	Wakayama pref., Arita city, Miyazaki town
D. varia	1092526				1976	8	31	H. Masago	Wakayama pref., Hidaka county, Hidaka town
D. varia	115206				1976	3	28	H. Masago	Wakayama pref., Hidaka county, Inami town
D. varia	213756				1978	5	7	H. Masago	Wakayama pref., Naka county, Uchida town
D. varia	115204				1976	1	6	H. Masago	Wakayama pref., Nishimuro county, Shirahama town
D. varia	276116				1976	1	15	A. Nakashima	Wakayama pref., Shinguu city, Mitarai
D. varia	272324				1976	4	18	A. Nakashima	Wakayama pref., Higashimuro county, Nachikatsuura town
D. varia	262585				1976	1	28	H. Masago	Wakayama pref., Higashimuro county, Kozagawa town
D. varia	115203				1977	1	15	H. Masago	Wakayama pref., Higashimuro county, Kozagawa town
D. varia	310132				1976	2	1	H. Masago	Wakayama pref., Hidaka county, Minamibegawa village
D. varia	1092530				1972	3	25	H. Masago	Wakayama pref., Tanabe city, Nagano, Kaminagase
D. varia	1247222				1977	7	14	H. Masago	Wakayama pref., Tanabe city, Shinnjo town, Miyanodani
D. varia	1249960				1977	1	16	H. Masago	Wakayama pref., Arita city, Yuasa town, Obama
D. varia	817530				1972	9	23	H. Masago	Wakayama pref., Arita city, Miyahara town, Hata
D. varia	1261467				1972	3	20	H. Masago	Wakayama pref., Gobo city, Hachimannyama
D. varia	1261466				1977	1	16	H. Masago	Wakayama pref., Arita city, Hatsushima town
D. varia	1261465				1977	8	24	A. Nakashima	Wakayama pref., Higashimuro county, Nachikatsuura town
D. varia	1261464				1977	4	29	H. Masago	Wakayama pref., Hidaka county, Hidaka town
D. varia	1261463				1972	1	14	A. Nakashima	Wakayama pref., Higashimuro county, Nachikatsuura town
D. varia	1261462				1977	1	15	H. Masago	Wakayama pref., Higashimuro county, Kozagawa town
D. varia	1261460				1978	1	22	H. Masago	Wakayama pref., Wakayama city, Kmiidera
D. varia	360447				1977	7	10	M. Takara	Okinawa pref., Okinawa city, Chibana
D. varia	1219468				1982	11	7	M. Takara	Okinawa pref., Kunigami county, Kinn town
D. varia	1219471				1981	1	18	M. Takara	Okinawa pref., Nakagami county, Nishihara town. Tanabaru
D. varia	128612				1953	2	3	T. Amano	Okinawa pref., Kumejima Island
D. varia	504204				1985	10	15	T. Kariyazaki	Kagoshima pref., Minamitane town, Kaminaka
D. varia	514326				1987	6	8	T. Kariyazaki	Kagoshima pref., Nishinomote city, Takenoda
D. varia	508031				1984	2	24	T. Kariyazaki	Kagoshima pref., Minamitane town, Nishinohirano
D. varia	817478				1976	12	23	F. Miyamoto	Kagoshima pref., Kumage county, Yakushima Island, Takenokawa river
D. varia	726061				1959	8	8	M. Hutoh	Kagoshima pref., Amami-oshima Island, Ugenn village, Yuwan
D. varia	366613				1978	8	29	N. Sasaki	Kagoshima pref., Kuchinoerabu Island
D. varia	1039469				1963	7	30	H. Ito	Shimane pef., Oki Islands
China									
D. bissetiana				01000733	1981	10	9	Li Jian-Xiu	Shangdong Prov., Mt. Meng
D. bissetiana				01870698	2008	11	21	Zhang Xian-Chun	Hubei Prov., Yichang city
D. bissetiana				01138918	1998	10	16	Zhang Xian-Chun	Anhui Prov., Huangshan city, Mt. Huangshan
D. erythrovaria	749946				1993	10	24	T. Nakaike	Anhui Prov., She County, Mt. Qinling
D. erythrovaria				00945880	1956	10	28	Ying Ru Lu	Zhejiang Prov., Mt. Qingwang
D. erythrovaria				00945884	1963	7	15		Zhejiang Prov., Mt. Siming
D. hikonensis				00945882	1958	11	3		Zhejiang Prov., Hangzhou city
D. hikonensis				00945892	1958	11	12		Jiangxi Prov., Yu Shan city, Mt. Huai Yu
D. kobayashii				01118804	1978	9	27	Li Jian-Xiu	Shangdong Prov., Mt. Kunyu
D . saxifraga				01118813	1981	8	21	Wang Jiang-zhong	Liaoning Prov., Fengcheng city, Mt. Fenghuang
D. varia				01362356	2005	12	16	Wuzhishan Fern Survey	Hainan Prov., Mt. Zhafu
D. varia				01923101	1990	7	6	Shi Lei	Chongqing city, Nanchuan
Korea									
D. bissetiana				01362500	2005	8	18	Zhang Xian-Chun	Cheju-do
D. erythrovaria	01104374				2010	6	29	S. Matsumoto	Cheju-do, Sop sum Island
D. hikonensis	01104349				2010	6	29	S. Matsumoto	Cheju-do, Sop sum Island
D. kobayashii				01362342	2005	2	26	X. C. Zhang	Hong dao
Taiwan									
D. varia		449120			2014	6	3	K. Hori	Taiwan, Taipei city, Urai

General Discussion

In this thesis, I have attempted to clarify the origin of the apogamous species of the *Dryopteris varia* complex on the basis of the "hybridization cycle hypothesis" by Lin *et al*. (1992). Lin *et al*. (1992) discovered unequal meiosis in the triploid apogamous sporophytes of *D. pacifica* (now *D. hikonensis* according to my new classification system), which produces diploid apogamous gametophytes and diploid apogamous sporophytes. The hybridization cycle hypothesis, which was proposed based on the finding of unequal meiosis, is the most effective way to explain the origin of morphological and genetic variations in a triploid apogamous fern complex. Later, Yamamoto (2013) discovered that a triploid apogamous species, *D. erythrosora*, can produce triploid apogamous hybrids through hybridization with a related diploid sexual species, *D. caudipinna*, under experimental conditions of their artificial crossing experiments. However, it is unclear how hybridization cycles contribute to the genetic diversification of a triploid apogamous fern complex in nature. This thesis is the first study that elucidated the hybrid origins of each species of a triploid apogamous fern complex considering the hybridization cycle hypothesis.

In Chapter 1, I clarified the reticulate evolution of the *Dryopteris varia* complex through hybridization between triploid apogamous species and diploid sexual species using a nuclear single copy gene, *PgiC*, as the nuclear genetic marker. The *PgiC* sequences of each diploid sexual species of the *D. varia* complex were distinguished and named as follows: *D. varia*, A; *D. saxifraga*, B; *D. protobissetiana*, C; *D. caudipinna*(+*D. koidzumiana*), D; *D.*

chinensis, E; *D. insularis*, M. Moreover, each apogamous species of the complex had two or three of the *PgiC* sequences in those of the diploid species as follows: *D. pacifica* (α), A+C; *D. pacifica* (β), A+B+C; *D. pacifica* (γ), A+C+D; *D. bissetiana*, B+C; *D. sacrosancta*, A+C+E; *D. kobayashii*, B+C+E; *D. insularis* var. *chichisimensis*, A+C+M. Thus, some of the triploid apogamous species had three *PgiC* sequences, suggesting that recurrent reticulation should have occurred through hybridization cycles.

Dryopteris protobissetiana, one of the diploid sexual species of the *D. varia* complex listed above, was a new species, which I fortunately discovered in Yakushima Island. Thousands of specimens of the *D. varia* complex have been collected from the entire area of Japan. However, only a few specimens of *D. protobissetiana* have been collected from the narrow areas of the western and southern parts of Yakushima Island. It was difficult to find them from the large number of specimens because each species of the complex demonstrates continuous morphological variation. *Dryopteris protobissetiana* is considered to be endemic to Yakushima Island because I was unable to find a specimen of this species even among those collected from the nearby Islands of Yakushima, such as the Amami Islands, Kuchinoerabu Island, Tanegashima Island, the Tokara Islands as well as from China, Korea, or Taiwan. The genome of *D. protobissetiana* (Type C) was widely shared by most members of the triploid apogamous species of the *D. varia* complex in contrast to the geographical distribution area of *D. protobissetiana*, which is narrower than that of the other members of the complex. It is likely that *D. protobissetiana* was widely distributed in Japan

earlier but that its distribution range shrank due to competiton and/or repeated hybridization with related diploid sexual and triploid apogamous species of the *D. varia* complex.

In Chapter 1, it is important to note that I discovered some members of the *Dryopteris varia* complex with the nuclear *PgiC* sequences of *D. caudipinna* and *D. chinensis*, which are not members of the complex but which belong to other section or subgenus of *Dryopteris*. Furthermore, this finding helped to solve the issues regarding the origin of large morphological variations in the *D. varia* complex. The contributions of these two species to the diversification of the *D. varia* complex have never been conceived because clear differences in morphological characteristics are observed between them. *Dryopteris caudipinna* is a diploid sexual species mainly distributed in the Izu Islands, the Oki Islands, and Tsuhima Island and rarely distributed in mainland Japan. *Dryopteris chinensis*, from which only triploid apogamous cytotype was reported, is distributed in Japan and surrounding countries. I found that the *PgiC* sequence of this species is shared by the triploid apogamous members of the *D. varia* complex: *D. kobayashii* and *D. sacrosancta*. Later, I fortunately discovered the diploid sexual cytotype of *D. chinensis* from Miyazaki Prefecture, Japan by checking thousands of specimens in the herbaria (MAK, TNS). The diploid sexual cytotype of *D. chinensis* was not found probably because its distribution area is much narrower than that of the triploid apogamous cytotype.

Dryopteris caudipinna and *D. chinensis* have never been included as

members of the *D. varia* complex because of the large differences in their morphological characteristics from the complex. However, in a molecular phylogenetic tree based on the nucleotide sequences of the plastid *rbcL* gene (Figure 1-2, 2–10), these two species seemed to be relatively closely related to the *D. varia* complex in the entire genus of *Dryopteris*. This means that morphological characteristics do not necessarily reflect phylogenetic relationships. Therefore, for elucidating the origin of triploid apogamous fern species, it is indispensable to collect geographically and phylogenetically wide samples and examine their reproductive mode and ploidy level. I collected such samples and conducted all of the analyses in this study.

In Chapter 2, I analyzed five nuclear markers—*AK1*, *Esterase*, *GapCp*, *G6pdh*, and *PgiC*—for the same plant materials as in Chapter 1 and additional materials of the *Dryopteris varia* complex to clarify whether chromosome recombination by recurrent reticulation through hybridization cycles occurs. As a result, the genotypes of each sample, including triploid apogamous individuals, were the same among the five nuclear loci. Recurrent reticulations accompanying chromosomal recombination seem to occur only a few times in the *D. varia* complex because the genotypes (allele combinations) were the same among the five nuclear loci used in this study as nuclear genetic markers. The genotypes (allele combinations) of each sample of the apogamous species of the complex were the same among the five nuclear loci: apogamous cytotype of *D. varia*, A; diploid apogamous *D. pacifica* (α), AC; triploid apogamous *D. pacifica* (α), AAC, ACC, or A/C (A/C means either AAC or ACC); *D.*

pacifica (β), ABC; *D. pacifica* (γ), ACD; *D. bissetiana*, BCC or B/C; *D. sacrosancta*, ACE; *D. kobayashii*, BCE; *D. insularis* var. *insularis* (diploid apogamous), M (MM); *D. insularis* var. *chichisimensis*, ACM. The individuals which had allele of one nuclear marker had the genotypes "AAC" did not have the genotypes "ACC." Similarly, the individuals which had allele of one nuclear marker had the genotypes "BCC" did not have the genotypes "BBC." However, for "BBC", I could not distinguish from "BCC" because there was not enough genetic variation in *D. saxifraga* (B). If more nuclear markers were analyzed, I may have been able to distinguish these genotypes and discuss whether there are chromosomal recombinations. Furthermore, in this study, I used only five nuclear markers, which are inadequate to uncover whether chromosomal recombination occurred in the whole genome because the base chromosome number of *Dryopteris* is 41 (Takamiya 1996). In the future, I would like to analyze nuclear markers covering loci on each of the 41 chromosomes.

In Chapter 3, I revised the classification of the *Dryopteris varia* complex based on the genome constitutions estimated by the five nuclear markers. Specifically, I revised *D. pacifica* (Nakai) Tagawa because the results in Chapter 2 clearly revealed that this apogamous species contains three species with different genome constitutions. The three types, α, β, and γ, of *D. pacifica* should be considered as distinct species. Moreover, the name *D. pacifica* (Nakai) Tagawa is an illegitimate name of *D. pacifica* Christ, which was published 13 years earlier than the former. I attributed the α type to *D. hikonensis* (H. Ito) Nakaike. I described the β type as *D. subhikonensis*

K.Hori et N.Murak and the γ type as *D. erythrovaria* K.Hori et N.Murak (Figure 3-1). The taxonomical problems of the apogamous fern complex have seldom been well resolved. Specifically, former Japanese pteridologists have considered that a clear classification is impossible for the apogamous species of the *D. varia* complex (Tagawa 1959, Iwatsuki 1995). This thesis is the first study that could revise the classification of the apogamous fern complex containing several triploid apogamous species on the basis of a clear standard: their genome constitutions.

This thesis is one of the best case studies on the evolution of the apogamous fern complex. Fern taxonomists should pay attention to several points when they discuss the evolutionary histories of the apogamous fern complex. Searches for diploid sexual species that are sources producing diversities of triploid apogamous species are indispensable. Even if DNA analyses using many nuclear genetic markers are performed without analyzing materials of diploid sexual species that contributed to the formation of the apogamous fern complex, it is impossible to identify the genomes contained in each apogamous species. However, it is difficult to completely collect all diploid sexual members as materials. Recent studies on the reticulate evolution of ferns had the problem of "missing diploid" (Dyer *et al*. 2012, Jaruwattanaphan *et al*. 2013, Sessa *et al*. 2012). Further, in the present study, it was most important to find the diploid sexual *Dryopteris protobissetiana* in Yakushima Island and the diploid sexual type of *D. chinensis* from Miyazaki Prefecture, as previously noted. Without analyzing them, I could not have resolved the complicated reticulate

evolution in the *D. varia* complex (Figure 3-1).

Fern taxonomists who wish to solve problems in the apogamous fern complex may first have to survey diploid sexual species or cytotypes, but diploid sexual species are often distributed in restricted areas like in the case of *Dryopteris protobissetiana*, *D. caudipinna*, and the diploid sexual cytotype of *D. chinensis* in this study. Therefore, the sampling of plant materials from wide geographical areas is needed. Many specimens of ferns have been collected from all over the world and deposited in herbaria. Therefore, the distributions of diploid sexual species or cytotypes can be estimated by using these specimens. Counting spore numbers per sporangium in herbarium specimens is quite effective for discovering the localities of diploid sexual species, though 64 spores per sporangium do not necessarily indicate diploid sexual species because there are many polyploid sexual species in ferns (Takamiya 1996). To solve this problem, ploidy analysis and counting chromosomes using living stocks are necessary. Therefore, fern taxonomists have to conduct both methods to solve the taxonomical problems of the apogamous fern complex.

Once plant samples of almost all diploid sexual species are obtained, fern taxonomists have to develop nuclear DNA markers to distinguish their genomes. The plastid *rbcL* gene is the most frequently used DNA marker to distinguish biological species (Yatabe *et al*. 2009, Yamada *et al*. 2016). However, this gene is maternally inherited and is thus useful only for determining maternal parental species. *GapCp* and *PgiC* genes are the widely used biparentally inherited nuclear DNA markers of ferns (Dyer *et*

al. 2012, Grusz *et al*. 2009, Ishikawa *et al.* 2002, Jaruwattanaphan *et al*. 2013, Sessa *et al*. 2012). However, the reported PCR primers for these genes are often mismatched to various taxa of ferns. It is becoming easier to design new PCR primers because the databases of the transcriptome in ferns are also being enriched. In this study, I was able to search for effective nuclear genes to resolve the genome constitutions of the members of the apogamous fern complex using such databases. Presently, enzyme genes, which have been used for allozyme electrophoretic analyses, are most effective in estimating the genome constitutions of apogamous ferns. PCR–SSCP analysis using acrylamide gels is a useful method to distinguish several alleles in each individual. However, in the future, analyses using dozens or hundreds of nuclear markers will be easily conducted through cost reductions in high-throughput sequencing.

It is still debatable whether fern taxonomists should use several nuclear markers because the allele constitutions of the five nuclear loci were concordant in the *Dryopteris varia* complex. If apogamous ferns do not cause chromosomal recombinations through unequal meiosis, there will be no problem in estimating genome constitutions and discussing the evolution of the apogamous fern complex using only one biparental nuclear marker. However, if some apogamous ferns cause chromosomal recombinations through unequal meiosis, pteridologists must use several nuclear DNA markers to resolve reticulate evolution of the apogamous fern complex. To answer this question, similar studies to this study using several unlinked nuclear genetic markers of several phylogenetically distant apogamous fern

complexes should be conducted.

If chromosomal recombinations does not occur in several groups of apogamous ferns, they might have some mechanisms which retains their original genome constitutions derived from diploid sexual progenitors. This phenomenon is hard to explain only by natural selection. If such hybridization cycles as shown in Figure 2-1 reoccur, the resultant apogamous species can display huge amounts of interclonal genetic variation. In the case of $x = 41$, chromosome constitutions can display a maximum of $3^{41} = 3.6472996e+19$ patterns in a triploid apogamous species as mentioned in Chapter 2. This means that offspring with the original genome constitution are not likely to be produced by chance. Therefore, there might be some mechanism particular to triploid apogamous species using in unequal meiosis to select chromosomes derived from a particular diploid sexual species. For example, only red chromosomes are selected when reduced spores are produced. Alternatively, triploid apogamous species having three different genomes originating from three diploid sexual species (e.g. *Dryopteris sacrosancta* was shown to have genomes from *D. varia*, *D. protobissetiana*, and *D. chinensis* in this study) might have some mechanism to stop unequal meiosis. If such a mechanism is uncovered in future studies, our understanding of apogamous ferns will be further deepened.

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Summary in Japanese

複数の核遺伝マーカーを用いたイタチシダ類（オシダ科）の無配生殖種における網状進化の解明（英文）

シダ植物には、二次的に有性生殖をやめて無配生殖と呼ばれる無性生殖を行っているものが少なくない。無配生殖とは、前葉体が胞子体を形成するとき受精をせずに次世代の胞子体を形成する生殖様式のことである。無配生殖種の子孫は親と遺伝的に同一（クローン）となり、形態や遺伝的多様性は少ないと考えられる。しかし、実際は無配生殖種にも幅広い形態変異や遺伝的多様性がみられることが多い。その理由を説明するための仮説として、三倍体無配生殖種が稀に不等減数分裂を起こして二倍体の精子を生じ（父親）、それが別の二倍体有性生殖種（母親）の一倍体の卵細胞と受精・交雑することが考えられている (Lin et al. 1995, Yamamoto 2012)。無配生殖種には大きな遺伝的多様性が見られるので、遠縁な二倍体有性生殖種も含めて交雑している可能性が考えられる。

そこで本研究では、無配生殖種を多く含み、形態的・遺伝的にも非常に多様なイタチシダ類を材料に用いることにした。日本では、ナンカイイタチシダ *Dryopteris varia*、イワイタチシダ *D. saxifraga*、モトイタチシダ *Dryopteris protobissetiana*、オオイタチシダ *Dryopteris pacifica*、ヤマイタチシダ *D. bissetiana*、リョウトウイタチシダ *D. kobayashii*、ヒメイタチシダ *D. sacrosancta* の 7 種がこの類として認識されている。これらのうち、2 倍体有性生殖型が知られているのはナンカイイタチシダ、イワイタチシダ、モトイタチシダだけである。これらの種における系統関係を、母性遺伝する葉緑体 *rbcL* 遺伝子と両性遺伝する核シングルコピー領域の 1 つである *PgiC* (phosphoglucose isomerase）遺伝子の塩基配列情報を用いて調べた。その結果、イタチシダ類の核 *PgiC* 遺伝子には A, B, C, D, E,の 5 タイプがあることが分かり、それぞれナンカイイタチシダ、イワイタチシダ、モトイタチシダ、ハチジョウベニシダ、ミサキカグマのものに対応した。さらに、イタチシダ類の無配生殖種の核 *PgiC* 遺伝子の型は、ヤマイタチシダ(BC)、オオイタチシダ(AC, ABC, ACD)、リョウ

トウイタチシダ(BCE)、ヒメイタチシダ(ACE)のように複数のタイプの *PgiC* 遺伝子を合わせもっていたことから、これらは複数の有性生殖種と無配生殖種の交雑によって起源したものであることが分かった。また、ハチジョウベニシダとミサキカグマの核 *PgiC* 遺伝子(D, E)がイタチシダ類に含まれていることも明らかになり、遠縁な種が交雑を起こしたことが強く示唆された。さらに、3 種類の核 *PgiC* 遺伝子をもつイタチシダ類の無配生殖種があることから、2 種類の核ゲノムをもった無配生殖種が別の 2 倍体有性生殖種と交雑を起こすことにより、新たな遺伝子型の 3 倍体無配生殖種が生じたと考えられる。

ところが、シダの無配生殖種群のほとんどは、胞子形成時に減数分裂を生じ、イタチシダ類もその例外ではない。交雑と減数分裂を繰り返せば、遺伝子座位ごとにゲノムの組み合わせが異なっている可能性がある。そこで次に、複数の核シングルコピー遺伝子座位を用いてゲノム構成を推定することにした。*AK1*、*Eseterase*、*GapCp*、*G6pdh* の 4 遺伝子座における DNA 塩基配列の解析を行った結果、ゲノム構成は *PgiC* 遺伝子と一致した。二倍体有性生殖種のモトイタチシダ *D. protobissetiana* では少なくとも *AK1* と *Esterase* が連鎖していないことも明らかになった。このことは、イタチシダ類の無配生殖種は、世代を重ねても基本的にはそのゲノム構成が固定していること、すなわち有性生殖種との交雑は少数回しか起きていないことを強く示唆している。

最後に、この結果をもとにイタチシダ類の分類学的再検討・新種記載を行った。従来、オオイタチシダに充てられていた *D. pacifica* という学名は、Christ によってサモア産の別種に与えられていたので非合法名である。オオイタチシダには AC, ABC, ACD の 3 種類のゲノム構成をもつ個体があることが分かったので、これらをそれぞれ、オオイタチシダ *D. hikonensis*, *D. subhikonensis*（新種）, *D. erythrovaria*（新種）として整理した。