Allelic Variation of the *Athyrium christensenianum* Complex (Athyriaceae)

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Summary *Athyrium christensenianum* has been considered a fern hybrid of diploid sexual *A. crenuloserrulatum* and tetraploid sexual *A. decurrentialatum*. Based on plastid (*rbcL*) and nuclear (*AKI*) DNA phylogeny, this study solved relationships between *A. crenuloserrulatum* (allele A), *A. decurrentialatum* (B) and *A. opacum* (C). Relationships of the complex suggested *A. christensenianum* had at least five allele constitution: α , AABB (tetraploid sexual); β , AAB (triploid sterile); γ , ABB (triploid sterile); δ , ABAB (tetraploid sterile); ϵ , ABC (triploid sterile). In addition, this study expected the existence of undetected tetraploid sexual species which is originated from hybrid between ancestral diploid sexual *A. decurrentialatum* and diploid sexual *A. opacum*.

Keywords *AKI*, Athyriaceae, Fern, Hybrid, *rbcL*, *Athyrium christensenianum*.

Apogamous reproduction is a type of asexual reproduction not unusual in ferns; approximately 3% of all fern species (Liu *et al.* 2012) and 13% of Japanese fern species for which information regarding reproductive modes is available reportedly exhibit apogamous reproduction (Takamiya 1996). Although several apogamous fern species do not require sexual reproduction to complete their life cycles, they exhibit extensive morphological and genetic variation and often form species complexes with a continuous morphological variation. Various studies have reported reticulate relationships between apogamous and sexual species in apogamous fern complexes. Previous studies reported reticulate relationships occur between apogamous and sexual fern species in the genus *Asplenium* (Aspleniaceae, Dyer *et al.* 2012), *Hymenasplenium* (Aspleniaceae, Watano and Iwatsuki, 1988), *Diplazium* (Athyriaceae, Hori and Murakami 2019a), *Dryopteris* (Dryopteridaceae, Lin *et al.* 1995, Ebihara *et al.* 2012, Hori *et al.* 2014, 2018a–c), *Cheilanthes* (Pteridaceae, Grusz *et al.* 2009) and *Pteris* (Pteridaceae, Walker 1962, Suzuki and Iwatsuki 1990, Chao *et al.* 2012, Jaruwattanaphan *et al.* 2013).

Few studies have suggested cases of triploid apogamous species originating from hybridization between diploid sexual species and tetraploid sexual species. In the genus *Athyrium*, *A. christensenianum* (Koidz.) Seriz, a triploid species, is considered a hybrid of diploid sexual *A. crenuloserrulatum* Makino and tetraploid sexual *A. decurrentialatum* (Hook.) Copel. (Kurita 1964, Hirabayashi 1970, Park and Kato 2003). Usually, sexual ferns produce 64 haploid spores per sporangium by meiosis after four mitotic divisions of one spore

mother cell (Manton 1950). Park and Kato (2003) found *A. christensenianum* produce young individuals from a parent in a nursery although previous studies report these triploid species produce only irregular-shaped spores (Kurita 1964, Hirabayashi 1970). Park and Kato (2003) reported *A. christensenianum* produce four types of sporogenesis depend on individuals: (1) most normal spores of 16 spores per sporangium; (2) most normal and a few abortive spores of about 64 spores; (3) most abortive and several normal spores of about 64 spores and (4) all abortive spores. They also considered triploid *A. christensenianum* has apogamous reproduction because the spores of some individuals produced sporophytes without forming archegonium and antheridium on gametophytes, artificial cultivation on agar medium. The mechanism of producing apogamous spores of *A. christensenianum* is not still resolved. Later, Hori and Murakami (2019b) found tetraploid sexual cytotype of *A. christensenianum* which produce 64 spores per sporangium. Recently, Hori (2019) reported complicated relationship in the *A. christensenianum* complex. He reported tetraploid sexual *A. christensenianum* had one allele of *A. crenuloserrulatum* and *A. decurrentialatum*, each in biparental inherited nuclear DNA marker of *AKI* gene. Otherwise, he found triploid *A. christensenianum* had two alleles of *A. crenuloserrulatum* and one allele of *A. decurrentialatum*. Therefore, he suggested two hypotheses: (1) First, tetraploid sexual *A. christensenianum* originates from the hybridization of diploid sexual *A. crenuloserrulatum* and an ancestral or extinct diploid *A. decurrentialatum*; (2) Triploid apogamous *A. christensenianum* originates from the hybridization of diploid sexual *A. crenuloserrulatum* and tetraploid sexual *A. christensenianum*. Furthermore, this study reports more allelic variation in the *A. christensenianum* complex ad-

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ditionally including *A. opacum*, using ploidy and, plastid and nuclear DNA analyses.

Materials and methods

Fifteen samples of *A. christensenianum*, seven *A. crenulatoserrulatum* and eight *A. decurrentialatum*, one *A. decurrentialatum* var. *pilosellum* (tetraploid sexual, Kurita 1964) and one *A. opacum* (diploid sexual, Kurita 1964; tetraploid sexual, Kurita 1964, Hirabayashi 1970) were collected for this study (Appendix 1). In the molecular analysis, *A. melanolepis*, four species of the genus *Deparia* and two or three *Diplazium* were used as outgroups. Living plants and voucher specimens were maintained in the herbaria of the Kochi Prefectural Makino Botanical Garden (MBK) or herbaria of Tokyo Metropolitan University (MAK). Voucher information about these samples is listed in Appendix 1. These materials included an individual of tetraploid sexual *A. christensenianum* (Hori 2974) (Hori and Murakami 2019a) and triploid apogamous *A. christensenianum* (Hori 2980) (Hori 2019). We checked shape of spores in the materials as well as possible. The sample was estimated to be sexually reproduced if the spore number in a sporangium was 64 (Manton 1950), whereas it was estimated to be sterile if the spores had various size or irregular shapes, or sporangium are shrunk.

The methods of ploidy analyses followed those of Hori *et al.* (2014). To determine the ploidy level, the DNA content (2C value) of each nucleus isolated from fresh pinnae was measured once for each sample by flow cytometry using a Cyflow Ploidy Analyzer PA-II (Partec, Munster, Germany) and a Cystain UV Precise P kit (Partec). Approximately 100 mm² of each pinna was torn into several pieces and finely chopped with a razor blade in 0.25 mL of nucleus isolation buffer from the kit. Then, 0.8 mL of staining solution from the kit was added to the chopped tissues. The crushed tissue in buffers was filtered through a 30- μ m nylon mesh (Partec) before measuring on the analyzer. Approximately 25 mm² of fresh leaf tissue of *Nicotiana tabacum* L. (2C value=10.04 pg, Johnston *et al.* 1999) was used as an internal standard. This study analyzed parts of samples which I could obtain living individuals or fresh leaves.

For molecular analysis, total DNA was extracted from silica gel-dried leaves using cetyltrimethylammonium bromide solution, as described by Doyle and Doyle (1990). Plastid *rbcL* was used as the maternally inherited cpDNA marker (Gastony and Yatskievych, 1992, Hori *et al.* 2018a), and *AKI* was used as the biparentally inherited nrDNA marker (Hori *et al.* 2018a). PCR-SSCP was used to determine allelic variations at the nuclear locus for each individual, according to the method described by Hori *et al.* (2019). Further, electrophoresis was performed using gels containing 2% glycerol at 15°C for 16 h at 300 V for *AKI*, followed by silver stain-

Fig. 1. Several patterns of bands in PCR-SSCP analysis of nuclear *AKI* gene: sample 1 (*A. christensenianum* β , Hori 2977), A2A3B3; 2 (γ , Hori 2981), A3B4B6; 3 (β , Hori 3254), A4A5B3; 4 (β , Hori 3256), A3B4; 5 (ϵ , Hori 3266), A2B8C1; 6 (ϵ , Hori 3267), A2B8C1; 7 (δ , Hori 3274), A4B3B5B6.

ing. Sequencing of the bands separated on the SSCP gels was performed by drying polyacrylamide gel following silver staining by sandwiching the gel between Kent paper and a cellophane sheet on an acrylic backplate at 55°C for 4 h. For DNA extraction, a piece of the DNA band was peeled from the dried gel using a cutter knife and then incubated in 50 μ L TE buffer (10 mM Tris-HCl and 1 mM EDTA; pH 8.0) at 4°C overnight. The obtained supernatant was used as a template for further PCR amplification using the same primer set that was used for the original PCR amplification. The PCR products were purified using ExoSAP-IT (USB, Ohio, USA) or Illustra ExoStar 1-Step (GE Healthcare, Wisconsin, USA) and then used as templates for direct sequencing. For sequencing, the reaction mixtures were prepared using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) and subsequently analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems). All plant samples were classified based on their PCR-SSCP banding patterns, and the genomic constitution of each pattern was identified by determining the nucleotide sequence of each DNA band separated on the SSCP gel (Fig. 1).

For phylogenetic analysis, only one sequence representing each allele of the nuclear gene locus (*i.e.*, *AKI*) and each haplotype of cpDNA (*i.e.*, *rbcL*) was used in the datasets. Sequences of all genes were aligned using the MUSCLE program (Edgar 2004) and analyzed using different methods, including Bayesian inference (BI) analysis using MrBayes 3.2.6 (Ronquist *et al.* 2012) and maximum parsimony (MP) analysis using MEGA 7.0 software (Kumar *et al.* 2016). In BI analysis, the best fitting model of sequence evolution for each DNA region was selected using jModelTest 2.1.10 (Darriba *et al.* 2012). The *rbcL* was constructed using the SYM+I+G model, *AKI* tree was constructed using the HKY+I model. Four chains of Markov chain Monte Carlo (MCMC) were run simultaneously and sampled every

100 generations for a total of 10 million generations. Tracer 1.7.1 (Rambaut *et al.* 2018) was used to examine the posterior distribution of all parameters and their associated statistics, including estimated sample sizes. The first 25000 of the sample trees from each run were discarded as a burn-in period. The MP tree was obtained using the subtree pruning-regrafting algorithm (Swafford *et al.* 1996) at the search level 1, at which the initial trees were obtained by random addition of sequences (10 replicates). The bootstrap method with 1000 replications was employed to estimate the confidence level of the monophyletic groups. Indels were treated as missing characters in the BI and MP analyses. Therefore, they were not distinguished in the molecular trees.

Results

A. crenulatoserrulatum, *A. decurrentialatum*, *A. decurrentialatum* var. *pilosellum* had 64 regular shaped spores per sporangium. In *A. christensenianum* (Type α), 64 regular shapes spores per sporangium, and in *A. christensenianum* (β – ϵ), only irregular-shaped sterile spores per shrunken sporangium were observed. This study could not obtain any spores of *A. opacum*. The DNA contents of the materials in this study are as follows (Table 1): *A. decurrentialatum*, 28.2 ± 1.5 pg ($N=6$); *A. decurrentialatum* var. *pilosellum*, 29.6 pg ($N=1$); *A. christensenianum* (α), 26.2 ± 0.6 pg ($N=4$); *A. christensenianum* (β), 19.0 ± 0.5 pg ($N=4$); *A. christensenianum* (γ), 20.5 pg ($N=1$); *A. christensenianum* (δ), 26.7 ± 0.8 pg ($N=2$); and *A. christensenianum* (ϵ), 21.1 ± 1.2 pg ($N=3$). The ploidy level of *A. decurrentialatum*, *A. decurrentialatum* var. *pilosellum*, *A. christensenianum* (δ) and *A. christensenianum* (α) were estimated to be tetraploid because their DNA contents were similar to one individual which was estimated to be tetraploid sexual by chromosome counts (Hori 1974, Hori and Murakami 2019b). The samples of *A. christensenianum* (β , γ , ϵ) were estimated to be triploid because their DNA contents were similar to one individual of *A. christensenianum* (β) which was estimated to be triploid by chromosome counts (Hori 1980, Hori 2019). Materials of *A. crenulatoserrulatum* and *A. opacum* were not available in this

study.

In most samples of the nuclear *AK1* marker, several alleles were detected by SSCP. In total, 27 distinct sequences were identified at the *AK1* loci. After editing, the data matrix for phylogenetic analyses included 573 characters, of which 122 (21%) were polymorphic and 78 (14%) were parsimoniously informative. The MP trees according to the sequences of *AK1* with Bayesian posterior probabilities (PP) and bootstrap percentages (BPs) of the MP analyses are partially shown in Fig. 2. The consistency index (CI) and retention index (RI) were 0.86 and 0.93, respectively. In the molecular tree of nuclear *AK1*, the sequences obtained from *A. crenulatoserrulatum*, *A. decurrentialatum* and *A. opacum* could be distinguished (Type A=*A. crenulatoserrulatum*, Type B=*A. decurrentialatum* and Type C=*A. opacum*) as belonging to the monophyletic groups. Type A had low supports of PP and BP, and type C had only

Fig. 2. MP tree based on sequence variation of gene *AK1* (CI=0.86, RI=0.93, Appendix 2) with PP (>0.90) and BPs (>50) of MP analyses on each branch. This study recognized three types of allele (A=corresponded to *A. crenulatoserrulatum*; B, *A. decurrentialatum*; C, *A. opacum*).

Table 1. Variation of the allelic constitution in the *Athyrium christensenianum* complex with DNA contents (2C), ploidy level and form of spores.

Species	DNA content (pg)	Ploidy	Ploidy (references)	Spores	Nuclear <i>AK1</i>	Plastid <i>rbcL</i>
<i>A. christensenianum</i> (β)	19.0 ± 0.5 , $N=4$	3x	3x (Kurita 1964, Hirabayashi 1970,	irregular	AAB	pA
<i>A. christensenianum</i> (γ)	20.5 , $N=1$	3x	Park and Kato 2003; it is not clear	irregular	ABB	pB
<i>A. christensenianum</i> (ϵ)	21.1 ± 1.2 , $N=3$	3x	which type did they use)	irregular	ABC	pB
<i>A. christensenianum</i> (α)	26.2 ± 0.6 , $N=4$	4x	Hori and Murakami (2019b)	regular	AABB	pA
<i>A. christensenianum</i> (δ)	26.7 ± 0.8 , $N=2$	4x		irregular	ABBB	pA or pB
<i>A. crenulatoserrulatum</i>	N/A	N/A	2x (Kurita 1964)	regular	AA	pA
<i>A. decurrentialatum</i>	28.2 ± 1.5 , $N=6$	4x	4x (Kurita 1964)	regular	BBBB	pB
<i>A. decurrentialatum</i> var. <i>pilosellum</i>	29.6 , $N=1$	4x	4x (Kurita 1964)	regular	BBBB	pB
<i>A. opacum</i>	N/A	N/A	2x (Kurita 1964)	N/A	CC or CCCC	pC
			4x (Kurita 1964, Hirabayashi 1970)

Fig. 3. MP tree based on sequence variation of gene *rbcL* (CI=0.69, RI=0.76, Appendix 2) with PP (>0.90) and BPs (>50) of MP analyses on each branch. This study recognized three types of allele (pA=corresponded to *A. crenatoserrulatum*; pB, *A. decurrentialatum*; pC, *A. opacum*).

one allele. The allele constitution was different between five types of *A. christensenianum* (Table 1, Appendix 1): α , AAB; β , AAB; γ , ABB; δ , ABBB and ϵ , ABC. The allele constitution of one sample of sterile triploid was not clear because it had only one allele A and one B. This sample might have two same alleles A and one B because morphological characteristics were very similar to β .

Two types of plastid *rbcL* sequences (haplotypes pA, pB and pC) were recognized from the materials except for the outgroups. After editing, the data matrix for phylogenetic analyses included 1,205 characters, of which 115 (9%) characters were polymorphic and 61 (5%) characters were parsimoniously informative. The BI and MP analyses resulted in the creation of phylogenetic trees with similar topologies. The MP tree is shown in Fig. 3. The consistency index (CI) and retention index (RI) were 0.69 and 0.73, respectively. *A. crenatoserrulatum*, *A. decurrentialatum*, *A. opacum*, *A. christensenianum* (α), (β), (γ), (δ), and (ϵ) had haplotype pA, pB, pC, pA, pA, pB, pB and pB, respectively (Table 1).

Discussion

The relationships in the *A. christensenianum* complex was shown in Fig. 4. This study suggests complicated hypotheses about hybridization between different sexual species or cytotypes: (1) First, tetraploid sexual *A. christensenianum* (α) originates from the hybridization of diploid sexual *A. crenatoserrulatum* (maternal) and ancestral or extinct diploid *A. decurrentialatum*; (2) Triploid sterile *A. christensenianum* (β) from the hybridization of diploid sexual *A. crenatoserrulatum* and tetraploid sexual *A. christensenianum* (α) (maternal species is not clear because the both of parents had the same haplotype of *rbcL* 'pA'); (3) triploid sterile *A. christensenianum* (γ) originates from the hybridization of diploid sexual *A. crenatoserrulatum* and tetraploid sexual *A. decurrentialatum* (maternal); (4) Tetraploid sterile *A. christensenianum* (δ) originates from the hybridization of tetraploid sexual *A. decurrentialatum* (biparental) and tetraploid sexual *A. christensenianum* (α) (biparental);

Fig. 4. Hybrid relationships in the *Athyrium christensenianum* complex based on the allele of nuclear *AKI* (A–C) and plastid *rbcL* haplotype (pA–pC). solid line, paternal inheritance; solid arrows, maternal inheritance; dashed line, hypothesized paternal inheritance; dashed arrow, hypothesized maternal inheritance; ellipse, spores regular; gray ellipse, sterile; dashed ellipse, hypothesized allelic types.

(5) Undetected tetraploid sexual species originates from the hybridization of diploid sexual *A. opacum* and ancestral or extinct diploid *A. decurrentialatum* (maternal); (6) triploid sterile *A. christensenianum* (ϵ) originates from the hybridization of diploid sexual *A. crenatoserrulatum* and the undetected tetraploid sexual species (maternal). It is difficult to identify *A. christensenianum* (ϵ) because the morphological characteristic is very similar to *A. christensenianum* (β).

In final, this study showed complicated relationships between diploid sexual, triploid sterile and tetraploid sexual species or cytotypes in the *A. christensenianum* complex by using plastid DNA *rbcL* and nuclear DNA *AKI*. This study found at least five entities of *A. christensenianum*, also expecting the existence of undetected diploid and tetraploid sexual cytotype and species. It needs to conduct taxonomical study of the *A. christensenianum* complex to distinguish these five entities in future studies.

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Appendix 1

Voucher specimens examined in this study.

Any allelic types of nuclear gene *AK1* that were identified by sequencing are in boldface. Otherwise, the allelic types of nuclear gene *AK1* were deduced from comparisons of band positions in SSCP gels. Data are in the order: Species name—locality; voucher; DNA contents (pg), ploidy level, shape of spore; allele of nuclear DNA *AK1*, the haplotype of plastid *rbcl* DNA.

***Athyrium christensenianum* (α)—Japan, Kochi pref.**, Takaoka county, Ochi town; *Hori 2974* (2n=160, Hori and Murakami

2019b, MBK); 26.6, 4x, regular; **A4B3**, A. *ibid.*, *Hori 2975* (MBK); 25.6, 4x, regular; A4B3; A. *ibid.*, *Hori 2976* (MBK); 26.6, 4x, regular; A4B3; A. **Fukuoka pref.**, Maebara town. Mt. Hagane, (cultivated in Koishikawa Botanical Garden, Tokyo University); *Hori 3268* (MBK); 25.6, 4x, regular; A4B3; A.

A. christensenianum (β)—Japan, Tokyo-to., Ome city, Koshizawa; *Hori 2977* (MBK); 18.9, 3x, sterile; **A2A3B3**, A. *ibid.*, *Hori 2980* (2n=120, Hori in press., MBK); 19.6, 3x, sterile; A2A3B3, A. **Kanagawa pref.**, Hakone town, Hatajuku; *Hori 3256* (MBK); 18.9, 3x, sterile; A4B3, A. **Osaka pref.**, Chihaya-Akasaka village, Mt. Kongou; *Hori3254* (MBK); 18.4, 3x, sterile; **A4A5B3**,

A.

- A. christensenianum* (γ)—Japan, Tokyo-to, Ome city, Koshizawa; *Hori* 2981 (MBK); 20.5, 3x, sterile; **A3B4B6**, B.
- A. christensenianum* (δ)—Japan, Kochi pref., Takaoka county, Ochi town; *Hori* 3278 (MBK); sterile; A4B3B5B6, B. **Fukuoka pref.**, Itoshima city, Shiraito; *Hori* 3273 (MBK); 26.2, 4x, sterile; A4B3B5B6, A. *ibid.*, *Hori* 3274 (MBK); 27.3, 4x, sterile; **A4B3B5B6**, B.
- A. christensenianum* (ϵ)—Japan, Akita pref., Yurihonjo city, Hachinosawa; *Hori* 3281 (MBK); 19.7, 3x, sterile; **A3B4C1**, B. **Fukuoka pref.**, Maebara town. Mt. Hagane, (cultivated in Koishikawa Botanical Garden, Tokyo University); *Hori* 3266 (MBK); 21.6, 3x, sterile; **A2B8C1**, B. *ibid.* (a young individual grew near *Hori* 3266), *Hori* 3267 (MBK); 21.7, 3x, sterile; **A2B8C1**, B.
- A. crenulatoserrulatum*—Japan, Akita pref., Yurihonjo city, Hachinosawa; *Hori* 3285 (MBK); regular; **A1A6**, A. **Tokyo-to**, Ome city, Mt. Mitake; *Hori* 2979 (MBK); **A1**, A; **Yamagata pref.**, Mamurogawa town, Nozoki, Nakanomata; *Hori* 3302 (MBK); regular: **A3A6**, A. *ibid.*, *Hori* 3303 (MBK); regular: **A1A7**, A. *ibid.*, *Hori* 3304 (MBK); regular: **A2A6**, A. *ibid.*, *Hori* 3305 (MBK); regular: **A6A8**, A. **Yamanashi pref.**, Hokuto city; *Hori* 3025 (MBK); **A1**, A.
- A. decurrentialatum*—Japan, Akita pref., Yurihonjo city, Hachinosawa; *Hori* 3282 (MBK); regular; **B5B6**, B. *ibid.*, *Hori* 3283 (MBK); 28.0, 4x, regular; B5B6, B. *ibid.*, Ishizawakyo; *Hori* 3287 (MBK); 27.3, 4x, regular; B4B7, B. *ibid.*, Ishizawakyo; *Hori* 3288 (MBK); 30.3, 4x, regular; B4B7, B. **Fukuoka pref.**, Tagawa county, Kawara town; *Hori* 2908 (MBK); regular; **B6B7**, B. **Kochi pref.**, Takaoka county, Ochi town; *Hori* 2973 (MBK); regular; **B1B4**, B. **Tokyo-to**, Hachioji city, Komagino; *Hori* 2986 (MBK); regular; **B1B2**, B. **Nagasaki pref.**, Isahaya city, Shiroyama park (cultivated in Koishikawa Botanical Garden, Tokyo University); *Hori* 3265 (MBK); 28.5, 4x, regular; **B4B7**, B.
- A. decurrentialatum* var. *pilosellum*—Japan, Fukuoka pref.,

Itoshima city, Shiraito; *Hori* 3271 (MBK); regular; **B5B6**, B.

- Deparia japonica*—Japan, Kyoto Pref., Kyoto city, Sakyo; *Hori* 3031 (MBK).
- D. pterorachis*—Japan, Hokkaido Pref., Ebetsu city; *Hori* 3055 (MBK).
- D. unifurcata*—Japan, Kochi Pref., Agawa county, Niyodogawa town; *Hori* 3021 (MBK).
- D. viridifrons*—Japan, Kochi Pref., Takaoka county, Ochi town; *Hori* 2971 (MBK).
- Diplazium chinense*—Japan, Kochi pref., Agawa county, Niyodogawa town; *Hori* 3023 (MBK).
- Di. wichurae*—Japan, Kanagawa pref., Zushi city, Jinnmuji; *Hori* 1763 (MAK).

Appendix 2

DNA data accession numbers of the obtained nucleotide sequences in this study.

<AKI>

A1, LC421516; A2, LC421517; A3, LC421518; A4, LC421519; A5, MN267423; A6, MN267425; A7, MN267426; A8, MN267427; B1, LC421512; B2, LC421513; B3, LC421514; B4, LC421515; B5, MN267428; B6, MN267429; B7, MN267430; B8, MN267424; C, MN267421; *Athyrium melanolepis*, MN267422; *Deparia japonica*, LC431726; *D. pterorachis*, LC421959; *D. unifurcata*, LC421961; *D. viridifrons*, LC421960; *Diplazium chinense*, LC421520; *D. esculentum*, LC468186; *D. wichurae*, LC468187, LC468188.

<rbcl>

pA, LC421509; pB, LC421510; pC, LC495480; *Athyrium melanolepis*, LC495481; *Deparia japonica*, LC421957; *D. pterorachis*, LC421959; *D. unifurcata*, LC421955; *D. viridifrons*, LC421952; *Diplazium chinense*, LC421508; *D. wichurae*, LC421511.