

Deparia xnanakuraensis K.Hori (Athyriaceae), a new hybrid pteridophyte from Japan

Kiyotaka Hori¹

¹ *The Kochi prefectural Makino Botanical Garden, Kochi, Japan*

Corresponding author: Kiyotaka Hori (khorik@makino.or.jp)

Academic editor: Th. Almeida | Received 20 August 2020 | Accepted 6 October 2020 | Published 28 October 2020

Citation: Hori K (2020) *Deparia xnanakuraensis* K.Hori (Athyriaceae), a new hybrid pteridophyte from Japan. *PhytoKeys* 165: 69–84. <https://doi.org/10.3897/phytokeys.165.57837>

Abstract

I describe *Deparia xnanakuraensis* **hyb. nov.** and discuss differences in morphological characteristics between parental species *D. pterorachis* and *D. viridifrons* with chromosome counting, plastid, and nuclear DNA markers. The new hybrid is endemic to the eastern and northern parts of Japan. Based on the criteria of the International Union for Conservation of Nature and Natural Resources, this new species is here considered Data Deficient. The ploidy level is diploid sterile.

Keywords

Athyriaceae, *Deparia*, new hybrid, Japan

Introduction

The genus *Deparia* Hook. & Grev. is one of the largest groups in the Athyriaceae family. It contains 60–90 species mostly in East Asia, with some species distributed in Africa, western Indian Ocean, northeastern North America, the Hawaiian Islands, Australia, New Zealand, and South Pacific Islands (Kato 1984; Rothfels et al. 2012; He et al. 2013; Kuo et al. 2016, 2018; PPG I 2016; Moran et al. 2019).

The genus is characterized by hair-like scales and disconnected grooves between rachises and costae (Kato 1973, 1977, 1984; Rothfels et al. 2012; Sundue and Rothfels 2014; Kuo et al. 2018). These two features have not been observed in the genera *Anisocampium* C.Presl, *Athyrium* Roth., *Diplazium* Sw., *Ephemeropteris* R.C.Moran & Sundue, and *Pseudathyrium* Newman but in some species in the Athyriaceae family

(Kato 1973; Rothfels et al. 2012; Moran et al. 2019). In addition, narrowly U-shaped rachis grooves are also a unique character of the genus *Deparia* (Kuo et al. 2018). The basic chromosome number of *Deparia* is 40, contrary to *Diplazium* of 41 (Sano et al. 2000; Rothfels et al. 2012).

In Japan, several hybrids of the genus *Deparia* have been described: *D. ×birii* Fraser-Jenk. (Fraser-Jenkins 2008), *D. ×kiyozumiana* (Sa.Kurata) Y.Shimura (Shimura 1980), pentaploid sterile *D. lancea* (Thunb.) Fraser-Jenk. (Nakato and Mitui 1979), *D. ×lobatocrenata* (Tagawa) M.Kato (Kato 1984; Ebihara 2017), *D. ×musashiensis* (H.Ohba) Seriz. (Serizawa 1981), pentaploid sterile *D. petersenii* (Kunze) M.Kato (Shinohara et al. 2003), *D. ×togakushiensis* Otsuka & Fujiw. (Otsuka and Fujiwara 1999), and *D. ×tomitaroana* (Masam.) R.Sano (Sano et al. 2000). Furthermore, Ebihara (2017) mentioned several combinations of hybrids that are not still described.

The *Deparia okuboana* complex (Athyriaceae) is recently defined by Ebihara (2017) as consisting of *D. okuboana* (Makino) M.Kato (apogamous triploid; Hirabayashi 1970), *D. coreana* (Christ) M.Kato (sexual tetraploid, Nakato and Ebihara 2018), *D. henryi* (Baker) M.Kato (apogamous triploid, Nakato and Ebihara 2018), *D. viridifrons* (Makino) M.Kato (sexual diploid; Hirabayashi 1970), *D. unifurcata* (Baker) M.Kato (apogamous triploid; Hirabayashi 1970), *D. pterorachis* (Christ) M.Kato (sexual diploid; Hirabayashi 1970). There is continuous morphological variation between *D. coreana*, *D. henryi*, *D. okuboana*, and *D. unifurcata* (Ebihara 2017). Kuo et al. (2018) identified that these members belong to sect. *Dryoathyrium*. Hori (2018) reported there were reticulate relationships in the *D. okuboana* complex with sect. *Lunathyrium* (Kuo et al. 2018) based on plastid and nuclear DNA marker. In addition, Ebihara (2017) mentioned undescribed diploid sterile hybrid between *D. pterorachis* and *D. viridifrons* based on morphology and ploidy level. This study described this new hybrid of *D. pterorachis* and *D. viridifrons*, *Deparia ×nanakuraensis* K.Hori, based on morphological characteristics, chromosome number, plastid, and nuclear DNA marker.

Materials and methods

Plant materials, Chromosome count, and DNA extraction

In this study, *Deparia viridifrons*, *D. ×nanakuraensis*, and *D. pterorachis* were investigated in molecular DNA analysis. Other members of the *D. okuboana* complex (*D. coreana*, *D. henryi*, *D. okuboana*, *D. unifurcata*) and Japanese members of the sect. *Lunathyrium* (*D. pycnosora* var. *albosquamata* M.Kato, *D. pycnosora* (Christ) M.Kato var. *pycnosora*, *D. pycnosora* var. *mucilagina* M.Kato) were also used as materials. Voucher information for all samples is listed in Appendix I. All voucher specimens have been deposited in the Makino Herbarium of Tokyo Metropolitan University (MAK), and/or the Kochi Prefectural Makino Botanical Garden (MBK). The DNA sequences of *Athyrium melanolepis* Christ, *A. crenuloserrulatum* Makino, *A. opacum*

Copel., *Diplazium chinense* (Baker) C.Chr., *Di. esculentum* (Retz.) Sw., *Di. wichurae* (Mett.) Diels were used as outgroups, quoted from the Genbank database.

Additionally, specimens from the Collection Database and Materials of TNS (<http://db.kahaku.go.jp/webmuseum/>), PE (<http://pe.ibcas.ac.cn/en/>), TAIF (<http://taif.tfri.gov.tw/search.php>), and from the JSTOR Global Plants (<https://plants.jstor.org/>) as well as from the Global Biodiversity Information Facility (GBIF: <https://www.gbif.org>) database were checked.

For the conservation assessment, the area of occupancy (AOO) and extent of occurrence (EOO) were estimated using GeoCAT (Bachman et al. 2011), default settings for grid size were applied. In addition, mitotic chromosomes from *D. xnanakuraensis* were counted.

To observe mitotic chromosomes, root tips were collected in the field, and pre-treated with 0.004 M 8-hydroxyquinoline for 6 h at approximately 17–20 °C. After fixation in ethanol and acetic acid (3:1) for 15–30 min, the root tips were hydrolyzed in 1 N HCl at 60 °C for 1–3 min and then squashed in 2% aceto-orcein solution. The chromosomes were observed under a microscope (Leica DM2500) and then photographed by using a digital camera (Leica MC170 HD).

For the molecular analyses, total DNA was extracted from silica-dried leaves using cetyltrimethylammonium bromide solution, according to Doyle and Doyle (1990).

Plastid and nuclear DNA sequencing

trnL-F was used as the maternally-inherited (Gastony and Yatskievych 1992; Kuo et al. 2018) plastid DNA marker (F: 5'-ATTTGAACTGGTGACACGAG-3' and FernL 1 Ir1: 5'-GGYAATCCTGAGCAAATC-3'; Taberlet et al. 1991; Li et al. 2009). *AK1* (AK4F: 5'-GATGAAGCCATCAAGAAACCA-3' and AKR2: 5'-ATGGATCCAGC-GACCAGTAA-3'; Hori and Murakami 2019) was used as a biparentally-inherited nuclear marker for polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis, which was used to determine allelic variation in each individual (Hori and Murakami 2019).

PCR amplification was performed using PrimeSTAR Max DNA Polymerase (Takara, Kyoto, Japan). PCR entailed an initial denaturation step at 95 °C for 10 min, followed by 35 cycles of denaturation, annealing, and elongation steps at 98 °C for 10 s, 55 °C for 5 s, and 72 °C for 5 s, respectively, using a Model 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

Gel electrophoresis of *AK1* PCR products was performed using gels of 50% MDE gel solution (Lonza) containing 2% glycerol at 15 °C for 16 h at 300 V, followed by silver staining. For sequencing of the bands separated on the gels, the polyacrylamide gel was dried after silver staining by sandwiching the gel between Kent paper and a cellophane sheet on an acrylic backplate at 55 °C for 4 h. To extract the DNA, a piece of the DNA band was peeled from the dried gel using a cutter knife and incubated in 50 µL of Tris-EDTA buffer (10-mM Tris-HCl and 1-mM EDTA, pH 8.0) at 4 °C

overnight. The supernatant solution was used as a template for further PCR amplification with the same primer set employed for initial PCR amplification.

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

Molecular analysis

The accession numbers of DNA sequences in the datasets were shown in Appendix I. The sequences were aligned using MUSCLE (Edgar 2004) and assessed with Bayesian inference (BI) analysis using MrBayes 3.2.6 (Ronquist et al. 2012), maximum parsimony (MP), and maximum likelihood (ML) analysis using the MEGA X software (Kumar et al. 2018). Indels were treated as missing characters in all analyses. In the BI analysis, the best-fit model (*trnL-F*: HKY+I model; *AKI*: HKY model) of sequence evolution for each DNA region was selected using jModelTest 2.1.10 (Darriba et al. 2012). Four Markov chain Monte Carlo chains were run simultaneously and sampled every 100 generations for 1 million generations in total. 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 2,500 sample trees from each run were discarded as burn-in periods. The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm (Nei and Kumar 2000) at search level 3, at which the initial trees were obtained by the random addition of sequences (100 replicates). The confidence level of the monophyletic groups was estimated with 1,000 MP bootstrap pseudo-replicates. In ML analysis, the best-fitting model of sequence evolution for each marker was selected using MEGA; Tamura 3-parameter + I model was used for *trnL-F* and HKY model for *AKI*. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach and then selecting the topology with superior log likelihood value. The bootstrap method with 1,000 replications was employed to estimate the confidence levels of monophyletic groups in MP and ML analysis.

Results

Chromosome count

Mitotic metaphase chromosome number observed in an individual of *D. xnanakuraensis* (Hori 3391) was $2n = 80$ (Figure 1). This individual had shrunken sporangium with no spores. The basic chromosome numbers of the genus *Deparia* is $x=40$ (Sano et al. 2000; Rothfels et al. 2012), and suitably, this sample was found to be a sterile diploid.



Figure 1. Photograph and sketch of mitotic metaphase chromosomes ($2n = 80$) of *D. × nanakuraensis* (Hori 3391).

Plastid and nuclear DNA phylogenetic trees

We sequenced 653–746 bp of the *trnL-F* intergenic spacer from different specimens. The aligned *trnL-F* matrix was 765 bp, of which 121 characters (15%) were parsimony-informative. For the *AK1* intron, we sequenced 338–590 bp of the intron for each specimen, yielding a 604 bp aligned matrix, of which 74 characters (12%) were parsimony-informative.

The ML trees according to the sequences of *trnL-F* ($\ln L = -2309.05$) and *AK1* ($\ln L = -1616.59$) with bootstrap percentages (BPs), Bayesian posterior probabilities (PP) were shown in Figures 2, 3, respectively. In the *trnL-F* phylogeny, the haplotype of *D. pterorachis* and *D. viridifrons* composed different clades with *D. coreana*, *D. henryi*, and *D. okuboana* which were supported by BP (>70) and PP (>0.90) values. In the *AK1* phylogeny, the two clades containing *D. pterorachis* and *D. viridifrons* were supported by BP, but *D. viridifrons* was not supported by PP value. *Deparia* × *nanakuraensis* had the same haplotype of *D. pterorachis* and *D. viridifrons* in both *trnL-F* and *AK1* phylogenies. Other members of the *D. okuboana* complex (*D. coreana*, *D. henryi*, *D. okuboana*, *D. unifurcata*) shared the same alleles with *D. viridifrons* partly (Hori 2018), but the combination of alleles was different from *D. ×nanakuraensis*. Japanese members of the sect. *Lunathyrium* (*D. pycnosora* var. *albosquamata*, *D. pycnosora* var. *pycnosora*, *D. pycnosora* var. *mucilaginata*) did not share any alleles with *D. ×nanakuraensis*. Therefore, *D. ×nanakuraensis* can be of origin hybrid from *D. pterorachis* and *D. viridifrons*.

Taxonomic treatment

Deparia* × *nanakuraensis* K.Hori, *hyb. nov.

urn:lsid:ipni.org:names:77212571-1

Figure 4

Type. JAPAN. Honshu: Akita prefecture, Noshiro city, Futatsui town, Nanakura-shrine, 40°12'9.48"N, 140°15'29.82"E, alt. 23 m, deciduous forest containing *Acer miyabei*



Figure 2. The ML tree based on the sequence variation of the gene *trnL-F* ($\ln L = -2309.05$) with PP (>0.90) and BP (>70) of ML/MP/BI analyses on each branch. The sequences with asterisks were quoted from Genbank.

Maxim., *Aesculus turbinata* Blume, *Cercidiphyllum japonicum* Siebold & Zucc., *Cryptomeria japonica* (Thunb. ex L.f.) D.Don, *Dryopteris monticola* (Makino) C.Chr., and *Pachysandra terminalis* Siebold & Zucc., on soil, 7 Jul 2020, K. Hori 3391 (holotype: MAK467056; isotype: MBK).

Description. *Terrestrial, summer green fern. Rhizomes* creeping, occasionally branched, with buds, stramineous, 15–25 × 4–7 cm, closely set with roots and persis-

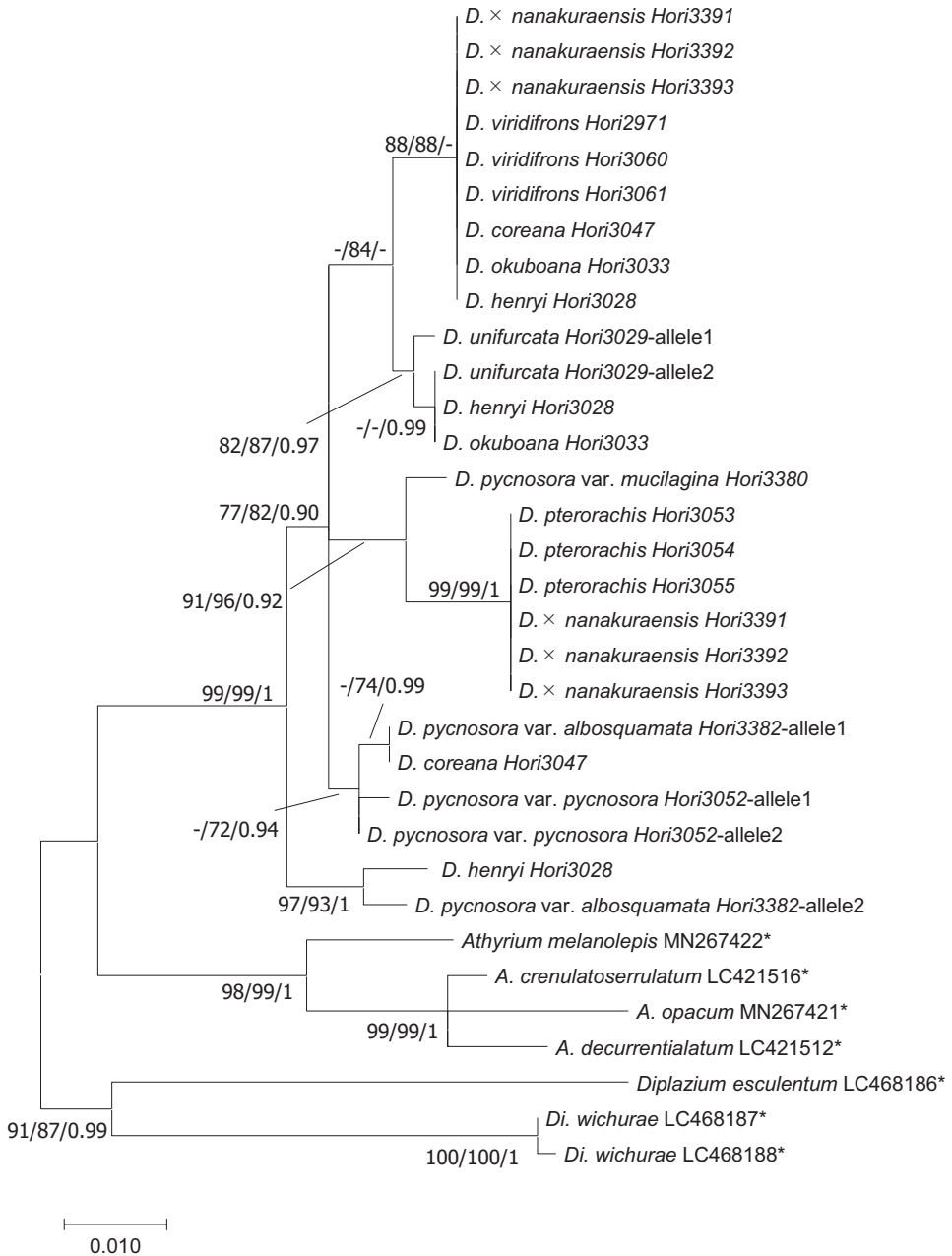


Figure 3. The ML tree based on the sequence variation of the gene *AKI* ($\ln L = -1616.59$) with PP (>0.90) and BP (>70) of ML/MP/BI analyses on each branch. The sequences with asterisks were quoted from Genbank.

tent, densely clothed by old stipe bases, glabrous; *fronds* 4–6 per rhizome; *stipes* whitish green, 30–40 × 0.8–1.5 cm, sparsely clothed with stramineous scales at the base (1–1.5 × 0.5–1 cm), ovate; *blades* yellowish green adaxially, 3-pinnate-pinnatifid at the

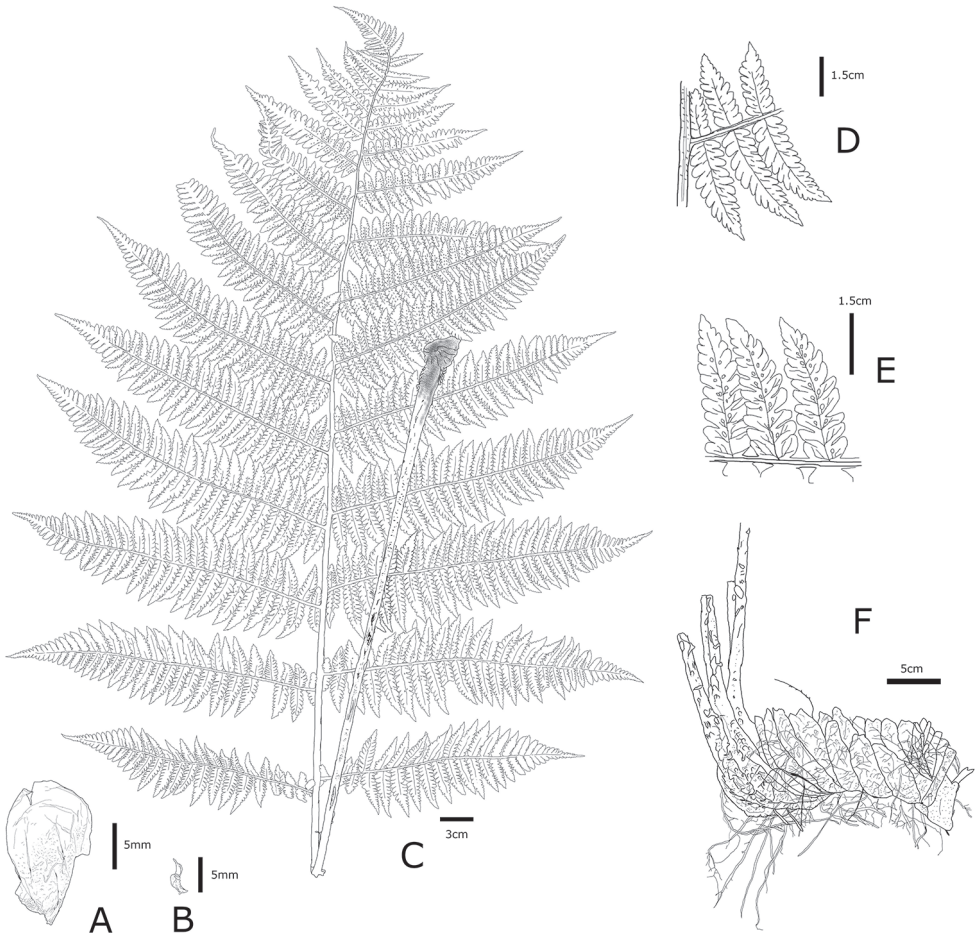


Figure 4. *D. xnanakuraensis* K.Hori **A** lower stipe scale **B** upper stipe scale **C** abaxial surface of frond and stipe **D** detail of adaxial pinnule **E** detail of abaxial pinnule, and **F** rhizome and base of stipes. **A–F** from the holotype (MAK467056) (illustration by K. Hori).

base, in the middle to upper section, 2-pinnate at the apex, 40–70 × 30–40 cm, deltoid; *rachises* whitish green, glabrous, sparsely clothed with stramineous scales (2–5 × 1–2 mm) and black hairs adaxially; *pinnae* 10–15 pairs, ascending, lanceolate, shrunk-en at base, alternate, petiolated (2–5 mm), sessile near the apex, lowest pinnae slightly reduced, second lowest pair usually the largest, 25–30 × 4–8 cm; *pinnules*, alternate on the basal and middle sections of the blade, 20–30 pairs on the basal and middle sections of the blade, 15–20 pairs on the apex of the blade, reduced distally, lanceolate, deeply serrated, vein-free, close to or reaching to the margin, 10–15 pairs in the middle lobe; *sori* brown, tending to appear on the abaxial surface of the middle or upper part of blades, oblong- to J-shaped, 1.5–3 mm long, on the apex or middle of veinlets, 5–10 per ultimate segment, persistent; *indusium* entire to serrated on margin, *sporangium* shrunken, spores absent.

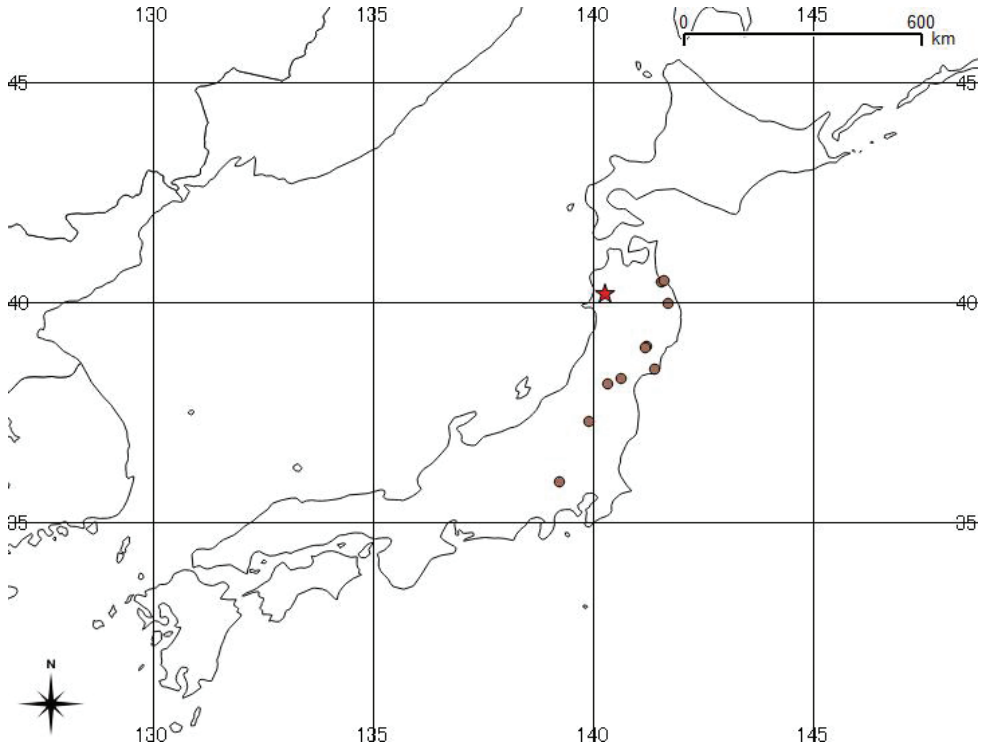


Figure 5. Map showing the known distribution of *D. xnanakuraensis* in Japan. Red star indicates type locality, other circles indicate examined specimens.

Etymology. The name derives from Nanakura-shrine, Futasui town, Noshiro City, Akita prefecture, northeast Japan, where *Deparia xnanakuraensis* was first found.

Specimens examined. Japan. Honshu: Akita pref., Noshiro city, Futatsui town, Nanakura-shrine, 40°12'9.48"N, 140°15'29.82"E, alt. 23 m, 7 Jul 2020, *K. Hori* 3392, *loc. cit.*, *K. Hori* 3393, *loc. cit.*, *K. Hori* 3394, *loc. cit.*, 10 Jul 2012, *Y. Horii* 35548 (TNS 01167830), *loc. cit.*, *Y. Horii* 35549 (TNS 01167829); Aomori pref., Hachinohe city, Same town, Kamikoswa, alt. 100 m, 23 Aug 1975, coll. *M. Neichi* (TNS 1170337, image!); *loc. cit.*, Kitsunetai, alt. 30m, 9 Jul 2005, coll. *M. Neichi* (TNS 01183638, image!); Iwate pref., Iwaizumi town, Atsuka, Matsugasawa, alt. 350 m, 18 Jul 1981, coll. *M. Neichi* (TNS 01161869, image!); *loc. cit.*, Ichinoseki city, Higashiyama cho, Nagasaka, Nagahira, alt. 180 m, 22 Aug 1987, coll. *M. Suzuki* (TNS 932028, image!); *loc. cit.*, Maikawa, Ohira, alt. 120 m, 22 Sep 1986, coll. *M. Suzuki* (TNS 9320284image!); Miyagi pref., Ishinomaki city, Mano, Uchihara, alt. 70 m, 25 May 1990, coll. *K. Shogo* (TNS01184195, image!); *loc. cit.*, Sendai city, Akiu town, Baba, alt. 200 m, 15 Oct 1983, coll. *K. Shogo* (TNS01184194, image!); Yamagata pref., Kamiyama city, Takano, alt. 250 m, 5 Jun 1983, coll. *N. Sakawa* (TNS01161877, image!); Fukushima pref., Minamiaizu county, Shimosato town, Yunokami, alt. 500 m, 8 Sep 1972, coll. *T. Waku* (TNS01161873, image!); Saitama pref., Hannnou city, Kasasugitouge, alt. 500 m, 21



Figure 6. Wild plant of *D. xnanakuraensis* in type locality.



Figure 7. Juvenile of *D. xnanakuraensis*.



Figure 8. Indefinite growth through bud (red arrow) on rhizome of *D. xnanakuraensis*.

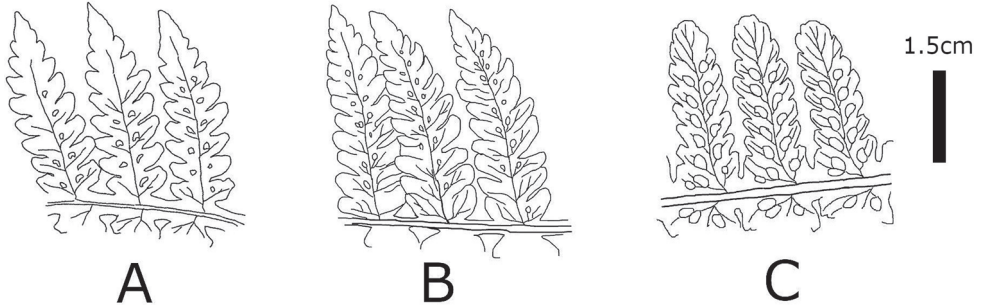


Figure 9. Abaxial surface of pinnule and sori of **A** *D. viridifrons* **B** *D. xnanakuraensis*, and **C** *D. pterorachis* (illustration by K. Hori).

Aug 1984, coll. *T. Iwata* (TNS01140142, image!); *loc. cit.*, 14 Sep 1980, coll. *Y. Kobayashi* (MBK0233005); *loc. cit.*, 14 June 1981, coll. *Y. Kobayashi* (MBK0232983).

Distribution and ecology. *Deparia* × *nanakuraensis* is known from the eastern and northern part of Honshu in Japan (Figure 5). It was observed to grow on soil under deciduous forest (Figure 6) or planted coniferous forest containing *Cryptomeria japonica*. This hybrid is endemic to Japan. In the type locality, this hybrid comprised a population of over 30 individuals with juveniles (Figure 7) although parents of *D. viridifrons*

and *D. pterorachis* were both absent, and sporangium had no spores. However, it is expected that *Deparia* × *nanakuraensis* can reproduce young individuals from buds on its rhizome (Figure 8).

Conservation status. IUCN Red List Category. Based on estimates from GeoCAT, the EOO of *D.* × *nanakuraensis* was 46,321 km². The known AOO of *D.* × *nanakuraensis* was 44 km². The localities correspond to less than 20 points, but I could not check the population size on each locality. Therefore, available information is inadequate to support the assessment of its extinction risk. According to the IUCN (2012) criteria, the category of Data Deficient (DD) is appropriate.

Discussion

Deparia × *nanakuraensis* presents almost intermediate morphologies between *D. viridifrons* and *D. pterorachis* species. *Deparia viridifrons* is characterized by having deltoid-ovate or ovate-lanceolate fronds, reniform to U-shaped sori, pinnules with costal wing, rounded serration of pinnules, and acute apex of pinnules. In contrast, *D. pterorachis* has oblong fronds, oblong to J-shaped sori, pinnules truncated to costa; truncate serration of pinnules, and obtuse apex of pinnules (He et al. 2013; Ebihara 2017). *Deparia* × *nanakuraensis* has deltoid fronds, oblong to J-shaped sori, pinnules with narrow costal wing, rather rounded serration of pinnules, and a rather acute apex of pinnules (Figure 9, Table 1).

Kuo et al. (2018) classified *D. viridifrons* and *D. pterorachis* as the members of sect. *Dryothyrium* because lateral pinnules are not auricled, and these are closely related in plastid DNA phylogeny (Kuo et al. 2018). Therefore, *Deparia* × *nanakuraensis* is infra section hybrid in the sect. *Dryothyrium*.

The ploidy level of this hybrid is the same as its parents because *D. viridifrons* and *D. pterorachis* are both sexual diploid (Kurita 1963; Mitui 1966, 1968, 1970; Hirabayashi 1970). In addition, this can be the first report of a diploid sterile hybrid of the genus *Deparia* from Japan although several hybrids have been described (Ebihara 2017).

In conclusion, this study described *Deparia* × *nanakuraensis* based on morphology, cytology, and molecular DNA analysis. The morphological characteristics were intermediate between its parents *D. viridifrons* and *D. pterorachis*. This hybrid can produce young individuals from buds on its rhizome. Based on the criteria of the International Union for Conservation of Nature and Natural Resources, this new species is here

Table 1. Morphological comparison among *D. nanakuraensis* and related species.

Characteristics	Shape of frond	Shape of sori	Margin of indusium	Base of pinnule	Serration of pinnules	Apex of pinnules
<i>D. viridifrons</i>	deltoid-ovate or ovate-lanceolate	reniform to U-shaped	serrated	with costal wing	rounded	acute
<i>D. nanakuraensis</i>	deltoid	oblong to J-shaped	entire to serrated	with narrow costal wing	rather rounded	rather acute
<i>D. pterorachis</i>	oblong	oblong to J-shaped	entire	truncated to costa	truncate	obtuse

considered Data Deficient. This hybrid can be the first report of diploid sterile hybrid of the genus *Deparia* from Japan. In future studies, it is expected that more hybrids of the genus *Deparia* will be discovered and described from Japan.

Acknowledgments

I am grateful to Mr. Y. Horii for plant collections. This study was supported by a Grant-in-Aid for JSPS Fellows 18K14785 to K. H.

References

- Bachman S, Moat J, Hill A, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9(8): 1–772. <https://doi.org/10.1038/nmeth.2109>
- Doyle JA, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* (San Francisco, Calif.) 12(1): 13–15.
- Ebihara A (2017) The standard of ferns and lycophytes in Japan 2. Gakken Plus, Tokyo. [in Japanese]
- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Fraser-Jenkins RC (2008) *Deparia* × *birii*. In: Fraser-Jenkins RC (Ed.) Taxonomic Revision of Three Hundred Indian Subcontinental Pteridophytes With a Revised Census-List. Bishen Singh Mahendra Pal Singh, Dehradun, 249 pp.
- Gastony GJ, Yatskievych G (1992) Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. *American Journal of Botany* 79(6): 716–722. <https://doi.org/10.1002/j.1537-2197.1992.tb14613.x>
- He Z, Wang Z, Kato M (2013) *Deparia*. In: Wu ZY, Raven PH, Hong DY (Eds) *Flora of China* 2–3: (Pteridophytes). Science Press, Beijing & Missouri Botanical Garden, St. Louis, 443–447.
- Hirabayashi H (1970) Chromosome numbers in several species of Aspidiaceae (2). *Shokubutsu Kenkyu Zasshi* 45: 45–52.
- Hori K (2018) Hybrid origin of some species in the *Deparia okuboana* complex (Athryiaceae, Polypodiidae) verified with DNA analysis. *Hikobia* 17: 315–320.
- Hori K, Murakami N (2019) Origin of the *Diplazium hachijoense* complex (Athryiaceae). *PhytoKeys* 124: 57–76. <https://doi.org/10.3897/phytokeys.124.35242>
- IUCN (2012) Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0. Gland, Switzerland and Cambridge, UK.

- Kato M (1973) Taxonomical evaluation of the articulated hairs found in the Athyriaceae. *Acta Phytotaxonomica et Geobotanica* 25: 119–126.
- Kato M (1977) Classification of Athyrium and allied genera of Japan. *The Botanical Magazine* 90(1): 23–40. <https://doi.org/10.1007/BF02489467>
- Kato M (1984) A taxonomic study of the athyroid fern genus *Deparia* with main reference to the Pacific species. *Journal of the Faculty of Science Section III* 13: 371–430.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGAX: Molecular evolutionary genetics analysis across Computing Platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Kuo LY, Ebihara A, Shinohara W, Rouhan G, Wood KR, Wang CN, Chiou WL (2016) Historical biogeography of the fern genus *Deparia* (Athyriaceae) and its relation with polyploidy. *Molecular Phylogenetics and Evolution* 104: 123–134. <https://doi.org/10.1016/j.ympev.2016.08.004> PubMed
- Kuo LY, Ebihara A, Hsu TC, Rouhan G, Huang YM, Wang CN, Chiou WL, Kato M (2018) Infrageneric revision of the fern genus *Deparia* (Athyriaceae, Aspleniineae, Polypodiales). *Systematic Botany* 43(3): 645–655. <https://doi.org/10.1600/036364418X697364>
- Kurita S (1963) Cytotaxonomical studies on some leptosporangiate ferns. *Journal of the College of Arts and Sciences. Chiba University* 4: 43–52.
- Li FW, Tan BC, Buchbender V, Moran RC, Rouhan G, Wang CN, Quandt D (2009) Identifying a mysterious aquatic fern gametophyte. *Plant Systematics and Evolution* 281(1): 77–86. <https://doi.org/10.1007/s00606-009-0188-2>
- Mitui K (1966) Chromosome studies on Japanese ferns (2). *Shokubutsu Kenkyu Zasshi* 41: 60–64.
- Mitui K (1968) Chromosomes and speciation in fern. *Science Reports of the Tokyo Kyoiku Daigaku. Section B* 13: 285–333.
- Mitui K (1970) Chromosomes studies on Japanese ferns (4). *Shokubutsu Kenkyu Zasshi* 45: 84–90.
- Moran RC, Hanks JG, Sundue M (2019) Phylogenetic relationships of Neotropical lady ferns (Athyriaceae), with a description of *Ephemeropteris*, gen. nov. *Taxon* 68(3): 425–441. <https://doi.org/10.1002/tax.12063>
- Nakato N, Ebihara A (2018) Chromosome Numbers of Eleven Ferns in Japan. (Athyriaceae, Dryopteridaceae and Tectariaceae). *Bulletin of National Museum of Nature and Science, Series B* 44: 23–30.
- Nakato N, Mitui K (1979) Intraspecific polyploidy in *Diplazium subsinuatum* (Wall.) Tagawa. *Journal of Japanese Botany* 54: 129–136.
- Nei M, Kumar S (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
- Otsuka K, Fujiwara M (1999) A new hybrid of the genus *Deparia* (Woodsiaceae) from Nagano prefecture, Japan. *The Journal of Phytogeography and Taxonomy* 47: 107–110. [in Japanese]
- PPG I (2016) A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54(6): 563–603. <https://doi.org/10.1111/jse.12229>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67(5): 901–904. <https://doi.org/10.1093/sysbio/syy032>

- Ronquist F, Teslenko M, Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rothfels CJ, Sundue MA, Kuo L-Y, Larsson A, Kato M, Schuettpeiz E, Pryer KM (2012) A revised family-level classification for eupolypod II ferns (Polypodiidae: Polypodiales). *Taxon* 61(3): 515–533. <https://doi.org/10.1002/tax.613003>
- Sano R, Takamiya M, Kurita S, Ito M, Hasebe M (2000) *Diplazium subsinuatum* and *Di. tomitaroanum* should be moved to *Deparia* according to molecular, morphological, and cytological characters. *Journal of Plant Research* 113(2): 157–163. <https://doi.org/10.1007/PL00013922>
- Serizawa S (1981) Notes of Japanese ferns (2). *The Journal of Phytogeography and Taxonomy* 29: 22–25. [in Japanese]
- Shimura Y (1980) Notes of hybrid fern (2). *The Journal of Phytogeography and Taxonomy* 28: 1–42. [in Japanese]
- Shinohara W, Takamiya M, Murakami N (2003) Taxonomic study of Japanese *Deparia peterse-nii* (Woodsiaceae) based on cytological and molecular information. *Acta Phytotaxonomica et Geobotanica* 54: 137–148.
- Sundue MA, Rothfels CJ (2014) Stasis and convergence characterize morphological evolution in eupolypod II ferns. *Annals of Botany* 113(1): 35–54. <https://doi.org/10.1093/aob/mct247>
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17(5): 1105–1109. <https://doi.org/10.1007/BF00037152>

Appendix I

Voucher specimens for DNA analysis in this study. Data are in the order: Species name –locality voucher (Herbarium); haplotype of plastid *trnL-F*; allele of nuclear *AK1*.

***Deparia xnanakuraensis* K.Hori**– JAPAN. Akita pref., Noshiro city, Futatsui town, Nanakura-shrine, 23m alt., 40°12'9.48"N, 140°15'29.82"E, 7 Jul 2020, *K. Hori* 3391 (MAK, MBK); MT898446 (*trnL-F*); MT887301, MT887307 (*AK1*). *ibid.*, *K. Hori* 3392 (MAK, MBK); MT898447 (*trnL-F*); MT887302, MT887308 (*AK1*). *ibid.*, *K. Hori* 3393 (MAK, MBK); MT898448 (*trnL-F*); MT887303, MT887309 (*AK1*).

***D. pterorachis* (Christ) M.Kato**– JAPAN. Hokkaido Pref., Sapporo city, Minami-ku, Jouzanneki, 530m alt., 42°55'36.8"N, 141°10'6.1"E, July 30 2018, *K. Hori* 3053 (MBK); MT898441 (*trnL-F*); MT887299 (*AK1*). *ibid.*, Ebetsu city, Nopporo nature park, July 30 2018, *K. Hori* 3054 (MBK); MT898442 (*trnL-F*); MT887300 (*AK1*). *ibid.*, *K. Hori* 3055 (MBK); MT898443 (*trnL-F*); LC421964 (*AK1*, Hori 2018).

***D. viridifrons* (Makino) M.Kato**– JAPAN. Kochi pref., Takaoka county, Ochi town, Mt. Yokogura, May 30 2018, *K. Hori* 2971 (MBK); LC421960 (*trnL-F*, Hori and Murakami 2019); LC468191 (*AK1*, Hori 2018). *ibid.*, Oct 17 2018, *K. Hori*

3060 (MAK); MT898444 (*trnL-F*); MT887305 (*AKI*). *ibid.*, Oct 17 2018, *K. Hori 3061* (MAK); MT898445 (*trnL-F*); MT887306 (*AKI*).

***D. coreana* (Christ) M.Kato**– JAPAN. Aomori Pref., Kamikita county, Shichinohe town, Jul 26 2018, *Hori 3047* (MBK); MW051518 (*trnL-F*); MW051522, MW051523 (*AKI*).

***D. henryi* (Baker) M.Kato**– JAPAN. Kyoto Pref., Kyoto City, Jul 14 2018, *Hori 3028* (MBK); MW051514 (*trnL-F*); MW051527, MW0515278, MW051529 (*AKI*).

***D. okuboana* (Makino) M.Kato**– JAPAN. Kyoto pref., Kyoto city, Jul 14 2018, *Hori 3033* (MBK); MW051515 (*trnL-F*); MW051530, MW051531 (*AKI*).

***D. pycnosora* (Christ) M.Kato var. *albosquamata* M.Kato**– JAPAN. Nagano Pref., Nagano city, Togakushi shrine, Okusha, Jul 9 2020, *K. Hori 3382* (MAK); MW051519 (*trnL-F*); MW051520, MW051521 (*AKI*).

***D. pycnosora* (Christ) M. Kato var. *mucilagina* M.Kato**– JAPAN. Nagano Pref., Nagano city, Togakushi shrine, Okusha, Jul 9 2020, *K. Hori 3380* (MAK); MW051516 (*trnL-F*); MW051526 (*AKI*).

***D. pycnosora* (Christ) M. Kato var. *pycnosora* M.Kato**– JAPAN. Aomori Pref., Kamikita county, Touhoku town, Otsutomo, Jul 26 2018, *K. Hori 3052* (MAK); MW051517 (*trnL-F*); MW051524, MW051525 (*AKI*).

***D. unifurcata* (Baker) M.Kato**– JAPAN. Kyoto Pref., Kyoto city, Jul 14 2018, *K. Hori 3029* (MBK); LC468192 (*trnL-F*, Hori and Murakami 2019); LC421961, LC421962 (*AKI*, Hori 2018).