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NOTES ON GEOGRAPHIC DISTRIBUTION

New records of ferns and lycophytes from Rishiri and Rebun Islands, with an updated list of species from the region

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Abstract. We update the checklist of ferns and lycophytes from Rishiri and Rebun Islands, Hokkaido, northern Japan. Based on a combination of extensive field surveys and thorough review of previous literature and herbarium records, we compile a robust and revised checklist. The updated list includes 78 taxa (67 ferns and 11 lycophytes), incorporating three newly recorded taxa on the two islands: *Botrychium multifidum* var. *multifidum*, *Dryopteris monticola*, and *Thelypteris pozoi* subsp. *mollissima*. Our findings highlight previously overlooked species diversity of ferns and lycophytes in the region.

Key words. DNA barcoding, East Asia, Hokkaido, rbcL

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INTRODUCTION

The Japanese Archipelago, stretching approximately 3,000 km from north to south, is characterized by latitudinally distinct climatic zones harboring unique flora in each region. From over a century of surveying efforts and understanding this unique flora, 5,016 species of angiosperms, 46 species of gymnosperms, 736 species of ferns, and 1,766 species of bryophytes have been recorded to the present day (Kato and Ebihara 2011; Ebihara et al. 2023).

Among the Japanese flora, the intensity of sampling of ferns has been particularly high; Ebihara and collaborators collected presence–absence data accompanied by voucher records in a 10-km grid across the entire Japanese Archipelago (Ebihara 2016, 2017). These tremendous efforts by both researchers and amateur enthusiasts has resulted in the Japanese Archipelago as one of the most thoroughly studied regions for fern flora in the world today (Ebihara and Nitta 2019).

However, there is still room for exploration, especially on remote islands where sampling is scarce. The recent surveys of those islands have led to numerous discoveries. For example, new records for Japan of *Hymenophyllum digitatum* (Sw.) Fosberg and *H. pilosissimum* C. Chr. from the Yaeyama Islands, Okinawa Prefecture, have been reported (Ebihara and Nakasone 2022; Naiki et al. 2023). Similarly, *Hymenasplenium murakami-hatanakae* Nakaike was documented as a new northern limit from Izu Oshima Island, the Izu Islands, Tokyo Prefecture (Yoneoka et al. 2024b). Additionally, a new species, *Dryopteris protobissetiana* K.Hori et N.Murak. was described from Yakushima Island, Kagoshima Pref. (Hori et al. 2015), and *Ctenitis microlepigera* (Nakai) Ching was rediscovered from Hahajima Island, Ogasawara Islands, Tokyo Pref. (Mizunashi et al. 2011). Those discoveries are important in unraveling the evolutionary history (Hori et al. 2014) and determining ecosystem management of and conservation strategies for ferns (Ministry of the Environment 2020; Yamamoto et al. 2023). Thus, those islands lacking sufficient surveys require comprehensive investigations to further understand their fern flora.

Rishiri and Rebun Islands are located at the northern tip of the Japanese Archipelago and east of Primorsky Krai, Russia. They are among a few islands in the Archipelago with a subarctic climate as defined in the Köppen classification (Köppen 1936; Kasai and Kimura 2013) and have a unique floristic composition.

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With a particular focus on ferns, at least four major surveys have been conducted on each island since 1934 and documented at least 61 species (52 species of ferns and 9 species of lycophytes) from this region (Takewaki 1934; Miyamoto 2012). However, the fern flora has not been updated for these islands in over 10 years. Considering ongoing climate change (Parmesan 2006) and long-distance dispersal of ferns (Geiger et al. 2007; Perrie and Brownsey 2007), northward expansions of species distribution and successions from the surrounding geographical areas are expected. Thus, the fern flora of the Rishiri and Rebun Islands needs another exploration to deepen our understanding of fern flora and its association with surrounding regions.

In this study, we conducted a comprehensive survey of the Rishiri and Rebun Islands to reexamine the fern flora there. Based on collected specimens and recorded occurrence data, we present an updated checklist of ferns and lycophytes from both islands here.

METHODS

Sporophytes of ferns and lycophytes were collected across nine different sites: Kutsugata, Minami-hama Wetland, Mount Rishiri (Oshidomari Route), Otadomari-numa, and Sakae-hama on Rishiri Island on 10–13 September 2023, and Kitousu River, Momoiwa, Rebun Forest Road, and Tonnai River on Rebun Island on 13–16 September 2023 (Figures 1, 2). At each sampling site, all the encountered species were collected as voucher specimens and longitude, latitude, altitude, and vegetation around the collection sites were re-

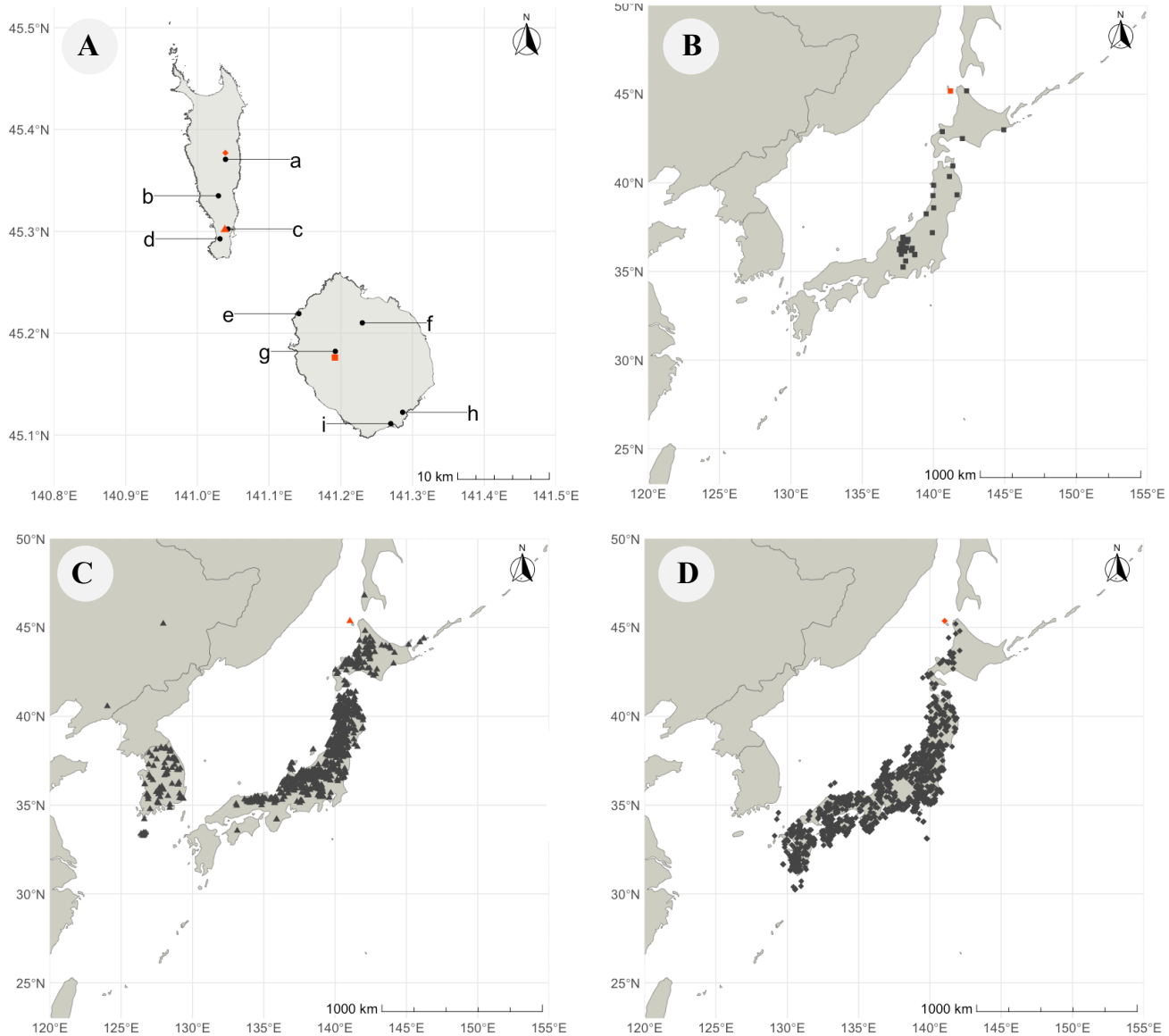


Figure 1. Maps of collection sites and distribution area of the three ferns newly recorded in this study. Black points, collection sites in this study. Red points, new occurrences discovered in this study. **A.** Nine collection sites established within Rishiri and Rebun Islands. a, Kitousu River. b, Rebun Forest Road. c, Tonnai River. d, Momoiwa. e, Sakae-hama. f, Mt Rishiri (Oshidomari Route). g, Kutsugata. h, Otadomari-numa. i, Minami-hama Wetland. **B.** *Botrychium multifidum* var. *multifidum*, filled squares. **C.** *Dryopteris monticola*, filled triangles. **D.** *Thelypteris pozoi* subsp. *mollissima*, filled diamonds.

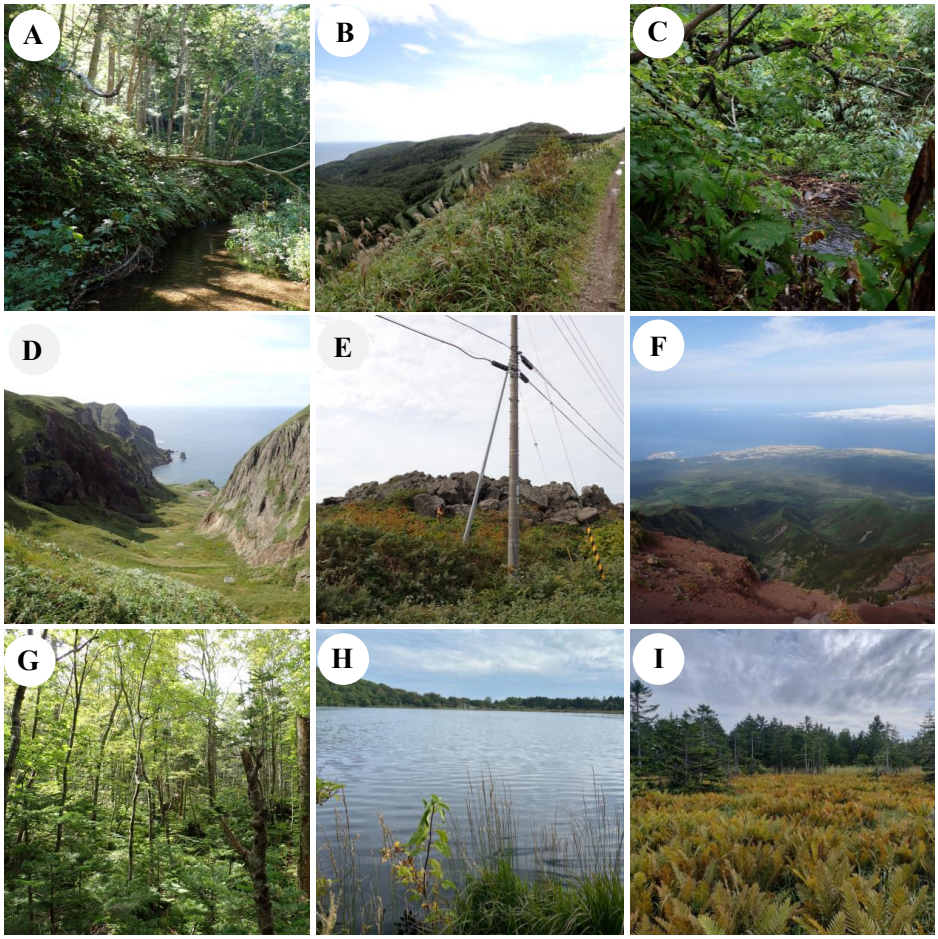


Figure 2. Landscapes of the collection sites. **A.** Kitousu River. **B.** Rebun Forest Road. **C.** Tonnai River. **D.** Momoiwa. **E.** Sakae-hama. **F.** Mt Rishiri (Oshidomari Route). **G.** Kutsugata. **H.** Otadomari-numa. **I.** Minami-hama Wetland.

corded for each collection point. The sample collections were conducted alongside the trails and streams to minimize habitat disturbance. For species identification, collected specimens were morphologically examined following the morphological descriptions provided by Ebihara (2016, 2017). For the taxonomic treatment, we consulted FernGreenList v. 2.0 (Ebihara et al. 2023). All the specimens were deposited in the Makino Herbarium of Tokyo Metropolitan University (MAK).

DNA barcoding analysis was performed for species challenging to identify based on morphological characteristics or expected to be novel entries. Total DNA was extracted from silica-gel-dried leaf tissue using a modified CTAB method (Doyle and Doyle 1987). The ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (rbcL) from the chloroplast genome was selectively amplified and sequenced based on Yoneoka et al. (2024a). Resulting sequences were compared against the GenBank library using the Basic Local Alignment Search Tool (BLAST), and the sequence in the library with the highest percent identity was recorded for each specimen.

To assess the details of species occurrence records, we referenced previous checklists from previous surveys and relevant literature (Tatewaki 1934, 1941; Nakamura 1968; Serizawa 1970; Umezawa 1997; Niwa et al. 2001; Sato et al. 2004; Miyamoto 2012; Ebihara 2016, 2017). In addition, we used occurrence data from the Global Biodiversity Information Facility (GBIF, last accessed 24 July 2025).

RESULTS

In the present study, 405 voucher specimens were collected from a total of nine locations of Rishiri and Rebun Islands, and 50 taxa (42 ferns and 8 lycophytes) were recognized through morphological examination (Table 1; Supplemental Data). Specifically, 43 taxa (37 ferns and 6 lycophytes) were found in Rishiri Island, and 30 taxa (23 ferns and 7 lycophytes) were collected in Rebun Island. Compared with the species list from previous studies (Miyamoto 2012; Ebihara 2016, 2017), three taxa were newly recorded from these islands: *Botrychium multifidum* (S.G.Gmel.) Rupr. var. *multifidum* from Rishiri Island; *Thelypteris pozoi* (Lag.) C.V.Morton subsp. *mollissima* (Fisch. ex Kunze) C.V.Morton, and *Dryopteris monticola* (Makino) C.Chr. from Rebun Island. Notably, our records of *T. pozoi* subsp. *mollissima* extend the northern distribution limit of this species (Ebihara 2016; GBIF last accessed 24 July 2025), highlighting the importance of the repeated updates

Table 1. Checklist of ferns and lycophytes on Rishiri and Rebun Islands: ○ = present; △ = record exists, but the specific island (Rishiri or Rebun Island) cannot be determined; ● = new recorded taxa; * = voucher specimen collected during this field survey; blank = absent.

No.	Family	Taxon	Rishiri	Rebun
1	Lycopodiaceae	*Huperzia miyoshiana* (Makino) Ching	○*	○*
2		*Huperzia serrata* (Thunb.) Trevis.	○*	○*
3		*Huperzia selago* (L.) Bernh. ex Schrank & Mart.	○*	○
4		*Lycopodium alpinum* L.		○*
5		*Lycopodium annotinum* L.	○	○
6		*Lycopodium clavatum* L. var. *asiaticum* Ching	○*	○*
7		*Lycopodium clavatum* L. var. *nipponicum* Nakai	○*	○*
8		*Lycopodium complanatum* L.	○	○*
9		*Lycopodium obscurum* L.	○*	○*
10	Selaginellaceae	*Selaginella helvetica* (L.) Spring	○	○
11		*Selaginella sibirica* (Milde) Hieron.	○	○
12	Equisetaceae	*Equisetum arvense* L.	○*	○
13		*Equisetum fluviatile* L.	○	○
14		*Equisetum hyemale* L. subsp. *hyemale*	○*	○*
15		*Equisetum palustre* L.		○
16	Ophioglossaceae	*Ophioglossum thermale* Kom.	△	△
17		*Ophioglossum vulgatum* L.	○	○
18		*Botrychium lanceolatum* (S.G.Gmel.) Ångstr.	○	
19		*Botrychium lunaria* (L.) Sw.	○*	○
20		*Botrychium multifidum* (S.G.Gmel.) Rupr. var. *multifidum*	●*	
21		*Botrychium multifidum* (S.G.Gmel.) Rupr. var. *robustum* (Rupr. ex Milde) C.Chr.	○*	○*
22		*Botrychium strictum* Underw.	○	
23		*Botrychium virginianum* (L.) Sw.	○	
24	Osmudaceae	*Osmundastrum cinnamomeum* (L.) C.Presl var. *fokiense* (Copel.) Tagawa	○*	○
25		*Osmunda japonica* Thunb.	○	
26	Hymenophyllaceae	*Hymenophyllum wrightii* Bosch	○*	○*
27	Pteridaceae	*Adiantum pedatum* L.	○	○*
28		*Cryptogramma crispa* (L.) R.Br.	○	
29		*Coniogramme intermedia* Hieron.	○*	○*
30	Dennstaedtiaceae	*Pteridium aquilinum* (L.) Kuhn subsp. *japonicum* (Nakai) A.Löve & S.Löve	○*	○
31		*Sitobolium wilfordii* (T.Moore) L.A.Triana & Sundue	○	△
32	Cystopteridaceae	*Cystopteris fragilis* (L.) Bernh.		○
33		*Gymnocarpium dryopteris* (L.) Newman	○*	○
34		*Gymnocarpium robertianum* (Hoffm.) Newman	○	
35	Aspleniaceae	*Asplenium incisum* Thunb.	○*	○*
36		*Asplenium scolopendrium* L.	○	○*
37	Woodsiaceae	*Woodsia ilvensis* (L.) R.Br.	○*	
38		*Woodsia polystichoides* D.C.Eaton	○*	○*
39		*Woodsia subcordata* Turcz.	○*	
40	Onocleaceae	*Onoclea orientalis* (Hook.) Hook.	○*	○*
41		*Onoclea sensibilis* L. var. interrupta Maxim.	○*	○
42		*Onoclea struthiopteris* (L.) Hoffm.	○*	○*
43	Blechnaceae	*Spicantopsis niponica* (Kunze) Nakai		○
44	Athyriaceae	*Athyrium brevifrons* Nakai ex Tagawa	○*	○*
45		*Athyrium deltoidofrons* Makino	○*	
46		*Athyrium melanolepis* (Franch. & Sav.) Christ	○*	
47		*Athyrium pinetorum* Tagawa	○	
48		*Athyrium rupestre* Kodama	○*	
49		*Athyrium spinulosum* (Maxim.) Milde		○
50		*Athyrium vidalii* (Franch. & Sav.) Nakai	○*	○*
51		*Athyrium yokoscense* (Franch. & Sav.) Christ	○*	○
52		*Athyrium* x *multifidum* Rosenst.	○	
53		*Deparia pterorachis* (Christ) M.Kato	○	○*
54		*Deparia pycnosora* (Christ) M.Kato var. *albosquamata* M.Kato	○*	
55		*Deparia pycnosora* (Christ) M.Kato var. *mucilagina* M.Kato	○*	○*
56		*Deparia pycnosora* (Christ) M.Kato var. *pycnosora*	○*	○*

No.	Family	Taxon	Rishiri	Rebun
57	Thelypteridaceae	*Phegopteris connectilis* (Michx.) Watt	○*	○*
58		*Thelypteris nipponica* (Franch. & Sav.) Ching	○*	
69		*Thelypteris palustris* Schott	○	△
60		*Thelypteris pozoi* (Lag.) C.V.Morton subsp. *mollissima* (Fisch. & Kunze) C.V.Morton		●*
61		*Thelypteris quelpaertensis* (Christ) Ching	○*	
62	Dryopteridaceae	*Arachniodes borealis* Seriz.	○*	○*
63		*Arachniodes mutica* (Franch. & Sav.) Ohwi	○	
64		*Dryopteris amurensis* (Milde) Christ	○	○
65		*Dryopteris coreanomontana* Nakai	○	
66		*Dryopteris crassirhizoma* Nakai	○*	○*
67		*Dryopteris expansa* (C.Presl) Fraser-Jenk. & Jermy	○*	○*
68		*Dryopteris fragrans* (L.) Schott	○*	
69		*Dryopteris monticola* (Makino) C.Chr.		●*
70		*Dryopteris tokyoensis* (Makino) C.Chr.	○	
71		*Polystichum braunii* (Spenn.) Fee	○*	○*
72		*Polystichum microchlamys* (Christ) Matsum. var. *azumiense* Seriz.	○*	
73		*Polystichum microchlamys* (Christ) Matsum. var. *microchlamys*	○	○
74		*Polystichum ovatopaleaceum* (Kodama) Sa.Kurata var. *coraiense* (Christ ex H.Lev.) Sa.Kurata	○	
75		*Polystichum tripteron* (Kunze) C.Presl	○*	○*
76	Polypodiaceae	*Lepisorus ussuriensis* (Regel & Maack) Ching var. *distans* (Makino) Tagawa	○	△
77		*Polypodium fauriei* Christ	○	△
78		*Polypodium sibiricum* Sipliv.	○*	○

of the fern flora of islands.

Integration of our findings with existing records (Miyamoto 2012; Ebihara 2016, 2017) revealed that the newly recorded taxa in this study constitute 3.8% of the total species richness of the Rishiri and Rebun Islands combined (78 taxa: 67 fern and 11 lycophyte taxa; Table 1).

Botrychium multifidum* (S.G.Gmel.) Rupr. var. *multifidum

New record. JAPAN — Hokkaido Pref. • Rishiri Island, Kutsugata; 45°10′56″N, 141°11′30″E; 406 m alt.; 11.IX.2023; Sosuke Ishii leg.; GenBank: LC885327; S. Ishii 23091110, MAK 480471.

Identification. The morphology of this taxon closely resembles that of *Botrychium multifidum* var. *robustum* (Rupr. ex Milde) C.Chr., which also occurs on Rishiri Island. However, *B. multifidum* var. *multifidum* can be distinguished by its thickly herbaceous, glabrous or glabrescent common stalk, ovate lobes with rounded tips, and finely serrated and flat leaf margins (Figure 3A, B). In contrast, *B. multifidum* var. *robustum* has a thick common stalk covered with numerous long white hairs, bluntly pointed lobe tips, and leaf margins that are coarsely crenate to bluntly serrate and curled (Ebihara 2016; personal observation by Ishii). A preliminary phylogenetic analysis revealed that these diploid varieties did not form a monophyletic group but instead exhibited a nested pattern with haplotype sharing. This pattern was also observed in a recent study focusing on hexaploids, suggesting the occurrence of incomplete lineage sorting or chloroplast capture (Ishii et al. 2025). Although rbcL sequences can aid in distinguishing between diploid and polyploid lineages, their resolution is usually insufficient to delimit species or lower taxa. In this study, the collected specimens were identified as *B. multifidum* var. *multifidum* based solely on morphological characteristics.

Notes. This taxon was recorded for the first time on Rishiri Island. The habitat was an open patch in *Betula ermanii* Cham. dominated forests. Co-habiting species consisted of *Botrychium multifidum* var. *robustum*, *Carex sachalinensis* F.Schmidt var. *iwakiana* Ohwi, and several unidentified species of Poaceae.

***Dryopteris monticola* (Makino) C.Chr.**

New record. JAPAN — Hokkaido Pref. • Rebun Island, Tonnai River; 45°18′06″N, 141°02′18″E; 68 m alt.; 14.IX.2023; Katsuhiro Yoneoka and Sosuke Ishii leg.; GenBank: LC885329; K. Yoneoka 3922, MAK 480475.

Identification. This taxon is morphologically distinct from other four *Dryopteris* species occurring on Rebun Island, *D. amurensis* (Milde) Christ, *D. coreanomontana* Nakai, *D. crassirhizoma* Nakai, and *D. expansa*, by its broadly lanceolate fronds, glossy scales with a dark brown center and pale brown margins, and a short-creeping rhizomes (Figure 3C–E; Ebihara 2017). The rbcL sequence of our voucher specimen was completely identical (1,205/1,205 bp) to that of *D. monticola* (AB575154) from Japan.

Notes. This taxon was recorded for the first time on Rebun Island. The habitat was the slope of adjacent

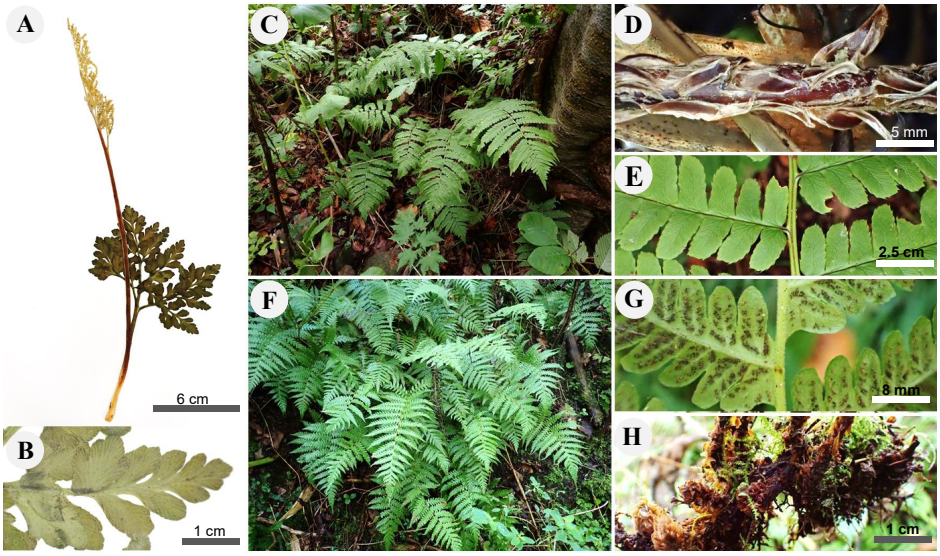


Figure 3. Newly recorded taxa collected in this study. **A.** Collected individual of *Botrychium multifidum* var. *multifidum* (MAK480471) from Kutsugata, Rishiri Island. **B.** Pinna of *B. multifidum* var. *multifidum*. **C.** Collected population of *Dryopteris monticola* (MAK 480475) along the Tonnai River. **D.** Scales at the base of the stipe of *D. monticola*. **E.** Basal pinnae of *D. monticola*. **F.** Collected population of *Thelypteris pozoi* subsp. *mollissima* (MAK480474) along the Kitousu River. **G.** Basal pinnae showing exposed sori of *T. pozoi* subsp. *mollissima*. **H.** Long-creeping rhizome of *T. pozoi* subsp. *mollissima*.

to small stream in a forest composed of *Acer pictum* Thunb., *Betula ermanii*, and *Urtica platyphylla* Wedd. Co-habiting species consisted of *Dryopteris crassirhizoma*, *Onoclea orientalis* (Hook.) Hook., *Polystichum braunii* (Spenn.) Fée, and *P. tripteron* (Kunze) C.Presl. Only one population composed of three individuals was found in this study.

***Thelypteris pozoi* (Lag.) C.V.Morton subsp. *mollissima* (Fisch. ex Kunze) C.V.Morton**

New records. JAPAN — Hokkaido Pref. • Rebun Island: Kitousu River; 45°22′16″N, 141°02′21″E; 90 m alt.; 15.IX.2023; Katsuhiro Yoneoka leg.; GenBank: LC885328; Yoneoka 3946, MAK 480474.

Identification. This taxon is morphologically distinct from *T. palustris*, another species of *Thelypteris* that occur on Rebun Island by its linear sori without indusia (Fig. 3F–H; Ebihara 2017). Supportively, the rbcL sequence of our voucher was completely identical (1,205/1,205 bp) to *T. pozoi* subsp. *mollissima* (AB575006) from Japan.

Notes. This taxon was recorded for the first time on Rebun Island. Also, this record expanded the northern distribution limit of this taxon by 20 km beyond Wakkanai, Hokkaido, Japan (GBIF, last accessed 24 July 2025). The habitat was the wet steam-side creek bank adjacent to a river in a dark forest consists of *Abies sachalinensis* (F.Schmidt) Mast., *Betula ermanii*, *Viburnum wrightii* Miq. var. *wrightii*, and *Solidago virgaurea* L. subsp. *asiatica* (Nakai ex H.Hara) Kitam. ex H.Hara. Co-habiting species consisted of *Coniogramme intermedia* Hieron., *Deparia pterorachis* (Christ) M.Kato, *Polystichum braunii*, and *P. tripteron*. Additionally, the population was large, covering approximately 5 × 20 m of the creek bank.

DISCUSSION

Based on our field surveys and literature reviews, we successfully reconstructed an inventory of 78 taxa (67 ferns and 11 lycophytes). The new checklist includes three newly recorded taxa from this region, one of which is an extension of the northern distribution limit of the respective taxon. These results may simply be due to some of the newly recorded taxa in this study being overlooked from morphologically similar taxa in previous studies (e.g. *Botrychium multifidum* var. *multifidum* possibly overlooked from *Botrychium multifidum* var. *robustum*). However, we also cannot deny the possibility that the recent settlement from surrounding geographical regions (including the lower latitudes) contributed to newly recorded taxa. Thus, future phylogeographic approaches to taxa found on Rishiri and Rebun Islands and surrounding regions may explain the origin of newly recorded fern taxa in the present study.

Despite the close physical proximity of 20 km between Rishiri and Rebun Islands, our updated checklist highlights notable differences between them, particularly in the significantly higher diversity of fern flora on Rishiri Island compared to Rebun Island. Among 78 taxa contained in our updated checklist, Rishiri Island comprises 70 taxa, and Rebun Island comprises 50 taxa, showing that the species richness of Rishiri Island is 40% higher than that of Rebun Island (Table 1). In addition, while 23 taxa were unique to Rishiri Island, only seven taxa are unique to Rebun Island (29.5% and 0.9% among total species richness, respectively) (Table 1). This exceptionally high diversity on Rishiri Island compared to Rebun Island may associated with the high heterogeneity of environments present on Rishiri Island. Rebun Island is topologically dominated by the hilly

terrain with relatively uniform environmental conditions, with the highest peak at 490 m; on the other hand, on Rishiri Island Mount Rishiri reaching 1,721 m in elevation, producing high environmental heterogeneity along the elevational and oriental gradient (Haruki et al. 2004). Such environmental differences reflect the number of vegetation types on each island (Haruki et al. 2004), and the analogy likely applies to fern flora. Moreover, the topographic complexity of the mountain provides various microclimatic niche spaces harboring high species diversity (Hampe and Petit 2005). Supportively, some of the fern taxa unique to Rishiri Island, such as *Athyrium rupestre* Kodama, *A. pinetorum* Tagawa, *Cryptogramma crispa* (L.) R.Br., *Cystopteris fragilis* (L.) Bernh., and *Woodsia ilvensis* (L.) R.Br., are restricted to mountainous habitats, highlighting the importance of these habitats in shaping the fern flora of Rishiri Island. Further understanding of both environmental characteristics and ecological requirements of fern taxa would allow us to predict the dynamics of fern flora in the island system with surrounding areas.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: KY, RK, SI. Data curation: KY, RK, TO. Formal analysis: KY, TK. Funding acquisition: KY, SI, NM. Investigation: KY, RK. Methodology: KY. Supervision: NM. Visualization: KY, RK. Writing – original draft: KY, RK, SI. Writing – review and editing: KY, RK, SI.

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Data availability

All data that support the findings of this study are available in the main text and in the supplemental data.
Supplemental file. List of all specimens collected in this study.

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