

A Reconsideration of Taxonomic Treatments of *Festuca ovina* and Allies (Poaceae) in Japan based on Analyses of Morphology, Ploidy Level and Phylogeny

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Due to broad distribution and great morphological diversity, *Festuca ovina* and allies, have long been controversial with respect to their taxonomic treatments. Here, their taxonomic treatments are reconsidered using individuals broadly distributed in Japan, based on analyses of morphology (including leaf anatomy), ploidy level, and molecular phylogeny. Molecular analyses based on 70 chloroplast coding sequences (CDS) (52,164 bp in total) from 16 samples distributed from Hokkaido to Kinki District, Honshu, resulted in two robust clades with 100% bootstrap support in the ML tree: one consisting of individuals with sparse leaves, and the other consisting of individuals with dense leaves. Morphologically, the individuals in the latter clade clearly differ from those in the former clade in that at least one nest (none in the former) of old concentric sheaths envelopes four or more new leaf fascicles. As they sometimes grow sympatrically, individuals in the former clade are considered to be *F. ruprechtii* (lectotypified in this study) and those in the latter clade as *F. ovina* s.s. The analyses showed that the two species include both diploids and tetraploids and that *F. probatovae* and *F. chiisanensis* are synonyms of *F. ruprechtii* and *F. ovina*, respectively.

Keywords: diploid, *Festuca chiisanensis*, *Festuca ovina*, *Festuca probatovae*, *Festuca ruprechtii*, lectotypification, morphology, phylogeny, taxonomy, tetraploid

Festuca L. represents one of the most complex genera of Poaceae (Loureiro *et al.* 2007), consisting of 743 species distributed worldwide (POWO 2025). Among them, *F. ovina* L. is widely distributed in the northern hemisphere, and from Hokkaido to Yakushima Island, Kyushu, in Japan, where it grows in dry grasslands and rocky sites from the seashore to high mountains. While *F. ovina* is characterized by involute filiform leaves and membranaceous rounded auricles (Osada 1993), it is highly diverse in height, awn length, inflorescence type and leaf density. As a result, many infraspecific taxa (even separate species) have been proposed (Fig. 1).

However, taxonomic treatments of *F. ovina* and its allies have been long controversial (Fig. 1). For example, Ohwi (1941) recognized seven

varieties under *F. ovina*, while Alexeev (1988) recognized four subspecies under *F. ovina* in addition to *F. chiisanensis* (Ohwi) E. B. Alexeev and *F. probatovae* E. B. Alexeev. Osada (1993) recognized two subspecies and five varieties under *F. ovina*, reducing *F. chiisanensis* to a variety of *F. ovina*, ignoring *F. probatovae*. Koba (2020) reduced all of the infraspecific taxa, as well as allied species, to *F. ovina* without recognizing them at any rank (Fig. 1). These treatments have never been evaluated in light of molecular evidence.

Cytologically, *F. ovina* includes diploids to octoploids worldwide (Qiu *et al.* 2020). For Japanese individuals, Tateoka (unpubl.; for the summary, see Tateoka 1985) studied chromosomes of ca. 700 samples from ca. 50 localities and showed



that only diploids and tetraploids existed in Japan, with one exception of a triploid. However, it has been uncertain how ploidy levels occur in species and are associated with infraspecific taxa.

The purpose of this study was to reconsider the taxonomy of *Festuca ovina* and its allies in Japan based on molecular phylogenetic analysis using chloroplast coding sequences (CDS), morphology including leaf anatomy, and ploidy levels determined with flow cytometry.

Materials and Methods

Materials

The sources of materials used in this study are listed in Table 1. Sixteen samples of *Festuca ovina sensu* Koba (2020) were collected from the localities mapped in Fig. 2. Two samples of *F. rubra* L. and one sample of *Vulpia octoflora* Rydb. were used as outgroups. Voucher specimens are preserved in the Herbarium of Kyoto University (KYO).

Phylogenetic analysis

Total DNA was extracted from silica gel dried leaves using the column extraction method with the EconoSpin column IIa (Funakoshi Co., Ltd., Tokyo, Japan). Library preparation was performed using the xGen™ DNA Library Prep EZ UNI Kit with xGen Stubby Adapter and UDI Primer Pairs (IDT, Iowa, USA). Sequencing was conducted by shotgun sequencing on a DNB-SEQ-G400 (BGI, Shenzhen, China) platform. Adapter sequences were removed from the obtained reads using fastp v.0.23.2 (Chen *et al.* 2018). Short reads were mapped to the complete chloroplast genome sequences of *F. ovina* (GenBank accession number MN309824.1; Qiu *et al.* 2019) using the ‘Map to reference’ function in Geneious Prime 2020.1.2. (Geneious, Boston, MA, USA). Coding sequences (70 regions) were recovered using the ‘transfer annotations’ function in Geneious Prime. These CDS sequences were concatenated and aligned using MAFFT v.7.450 (Katoh & Standley 2013). A maximum likelihood (ML) phylogenetic tree was constructed

with RAxML-NG v.1.2.2 (Kozlov *et al.* 2019). Model selection was conducted with ModelTest-NG v.0.1.7 (Darriba *et al.* 2020), resulting in the selection of the TVM+I model. One thousand bootstrap replicates were performed. The phylogenetic tree was displayed using FigTree v.1.4.4 (Rambaut & Drummond 2018). Raw sequence data of short reads were deposited in the DNA Data Bank of Japan (DDBJ) (accession number PRJDB35494).

Observation/measurements of morphological characters

Fourteen morphological characters (including anatomical ones) were observed/measured (Table 2).

In *Festuca ovina* and its allies, two to five new basal leaves are fasciculate, and a further one to several of the fascicles are enveloped by a nest of old concentric sheaths. We counted the number of the fascicles enveloped by a nest of old concentric sheaths, when at least one of the fascicles was fertile (yielding a culm). Additionally, we calculated frequencies of the nests of old concentric sheaths that envelope four or more fascicles to those enveloping three or less fascicles within an individual, when at least one of the fascicles was fertile. The measured portions of plant height, inflorescence length, first lemma length and awn length are indicated in Fig. 3A, B.

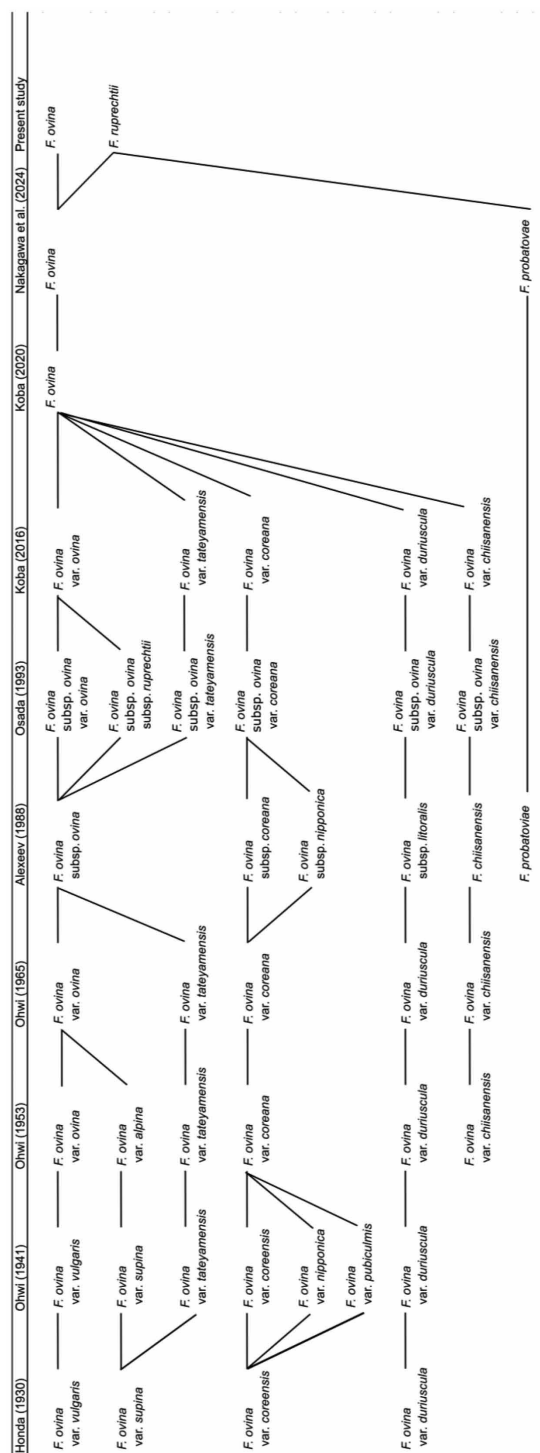
For the anatomical characteristics of the basal leaves, the middle part of the leaves of herbarium specimens were excised, stored in 3% aqueous ammonia solution for ca. 12 hours, and then washed with water. Cross sections of the leaves were made by hand using a razor blade and observed/measured with a light microscope. The measured portions of the anatomical characters are indicated in Fig. 3C. As for the latter three characters, five random measurements were made for each specimen, or if fewer than five were available, all available parts were measured.

Estimation of ploidy levels

Ploidy levels of the sixteen samples were estimated by flow cytometry (Fig. 4) (Doležel *et al.* 2007). Approximately 4 cm of silica gel dried

TABLE 1. Sources of materials of *Festuca ovina sensu* Koba (2020) (O1–O16) and an outgroup (R1, R2, V1) used in this study. Sample codes defined in this table were used throughout the study.

Sample Code	Alexeev (1988)	Osada (1993)	Japanese names	Present study	Locality	Altitude	Habitat	Voucher (KYO)
O1	<i>F. probatovae</i>	<i>F. ovina</i> var. <i>tateyamensis</i>	Saharin-ushinoke-gusa	<i>F. ruprechtii</i>	Hokkaido: Mt. Ashibetsu	1,579 m	Sunny, on rocks	<i>Kobayashi 1770</i>
O2	<i>F. ovina</i> subsp. <i>litardis</i>	<i>F. ovina</i> var. <i>duriuscula</i>	Korai-ushinoke-gusa	<i>F. ovina</i>	Hokkaido: Mt. Hakkenzan, Sapporo	396 m	Semi-shaded, steep slope with sandy soil.	<i>Kobayashi 1784</i>
O3	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> subsp. <i>ruprechtii</i>	Miyama-ushinoke-gusa	<i>F. ruprechtii</i>	Pref. Gunma: Mt. Shibutsu	2,102 m	Sunny, on serpentine rocks.	<i>Kobayashi 1763</i>
O4	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> var. <i>tateyamensis</i>	Takane-ushinoke-gusa	<i>F. ruprechtii</i>	Pref. Yamaguchi: Mt. Kitadake	3,026 m	Sunny, in rocky crevice.	<i>Kobayashi 1915</i>
O5	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> subsp. <i>ruprechtii</i>	Miyama-ushinoke-gusa	<i>F. ovina</i>	Pref. Yamaguchi: Mt. Anodake	3,189 m	Sunny, in gravelly sites.	<i>Kobayashi 1923</i>
O6	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> subsp. <i>ruprechtii</i>	Miyama-ushinoke-gusa	<i>F. ruprechtii</i>	Pref. Nagano: Mt. Amakazari	1,963 m	Sunny, in gravelly sites.	<i>Kobayashi 1864</i>
O7	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> subsp. <i>ruprechtii</i>	Miyama-ushinoke-gusa	<i>F. ruprechtii</i>	Pref. Nagano: Mt. Shirouma	2,932 m	Sunny, in rocky crevice.	<i>Kobayashi 1849</i>
O8	<i>F. probatovae</i>	<i>F. ovina</i> var. <i>tateyamensis</i>	Saharin-ushinoke-gusa	<i>F. ruprechtii</i>	Pref. Nagano: Mt. Shirouma-Yari	2,903 m	Sunny, in rocky crevice.	<i>Kobayashi 1847</i>
O9	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> var. <i>ovina</i>	Ushinoke-gusa	<i>F. ovina</i>	Pref. Nagano: Mt. Yatsugatake	1,509 m	Sunny, <i>Larix</i> plantation margin.	<i>Kobayashi 1595</i>
O10	<i>F. probatovae</i>	<i>F. ovina</i> var. <i>tateyamensis</i>	Saharin-ushinoke-gusa	<i>F. ruprechtii</i>	Pref. Nagano: Mt. Kisokoma	2,956 m	Sunny, in rocky crevice.	<i>Kobayashi 1690</i>
O11	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> subsp. <i>ruprechtii</i>	Miyama-ushinoke-gusa	<i>F. ovina</i>	Pref. Nagano: Mt. Kisokoma	2,956 m	Sunny, in rocky crevice.	<i>Kobayashi 1689</i>
O12	<i>F. ovina</i> subsp. <i>ovina</i>	<i>F. ovina</i> var. <i>ovina</i>	Ushinoke-gusa	<i>F. ruprechtii</i>	Pref. Ishikawa: Mt. Hakusan	2,196 m	Sunny, in gravelly sites.	<i>Kobayashi 1675</i>
O13	<i>F. ovina</i> subsp. <i>litardis</i>	<i>F. ovina</i> var. <i>duriuscula</i>	Korai-ushinoke-gusa	<i>F. ovina</i>	Pref. Ishikawa: Mt. Hakusan	6 m	Sunny, on tuff.	<i>Kobayashi 1589</i>
O14	<i>F. ovina</i> subsp. <i>nipponica</i>	<i>F. ovina</i> var. <i>coreana</i>	Ao-ushinoke-gusa	<i>F. ovina</i>	Pref. Kyoto: Ohara-Momoi, Sakyo	647 m	Sunny, on sandstone cliff.	<i>Kobayashi 1881</i>
O15	<i>F. ovina</i> subsp. <i>nipponica</i>	<i>F. ovina</i> var. <i>coreana</i>	Ao-ushinoke-gusa	<i>F. ovina</i>	Pref. Kyoto: Kameoka	121 m	Sunny, on sandstone cliff.	<i>Kobayashi 1506</i>
O16	<i>F. chisanensis</i>	<i>F. ovina</i> var. <i>chisanensis</i>	Chuisan-ushinoke-gusa	<i>F. ovina</i>	Pref. Nara: Mt. Inamura	1,685 m	Sunny, on rocks.	<i>Kobayashi 1557</i>
R1	<i>F. rubra</i> subsp. <i>multiflora</i>	<i>F. rubra</i> var. <i>pacifica</i>	Hirohano-o-ushinoke-gusa	<i>F. rubra</i>	Pref. Niigata: Itoigawa	2 m	Sunny, sandy ground.	<i>Kobayashi 1866</i>
R2	<i>F. rubra</i> subsp. <i>rubra</i>	<i>F. rubra</i> var. <i>rubra</i>	O-ushinoke-gusa	<i>F. rubra</i>	Pref. Nagano: Mt. Shiomi	3,012 m	Sunny, in gravelly sites.	<i>Kobayashi 1906</i>
V1	—	<i>Vulpia octiflora</i>	Murasaki-Naginata-gaya	<i>V. octiflora</i>	Pref. Kyoto: Takano, Sakyo	62 m	Sunny, in garden.	<i>Kobayashi 1457</i>

FIG. 1. Comparison of classifications of *Festuca ovina* and allies in Japan in previous and present studies.

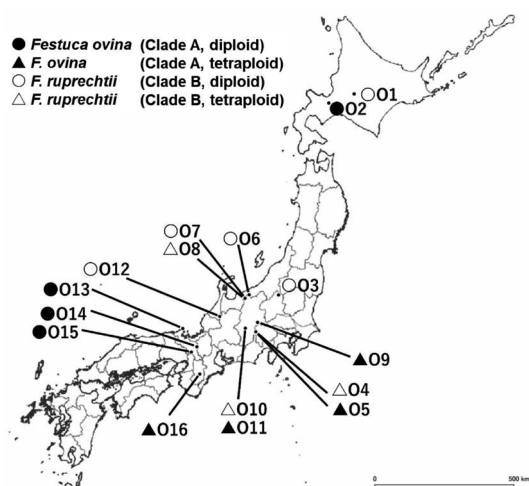


FIG. 2. Distribution of specimens of *Festuca ovina* sensu Koba (2020) used in this study. Sample codes correspond to those in Table 1. Map is from the Geospatial Information Authority of Japan (2013).

leaves were excised, and then mixed with the nuclei extraction buffer of Cystain UV Precise P (Sysmex Partec, Gorlitz, Germany). The nuclei were separated from the leaves by chopping with a razor blade and stained with DAPI included in the buffer Cystain UV Precise P (Sysmex Partec, Gorlitz, Germany). The stained nuclei were counted using CyFlow® PA (Sysmex Partec, Gorlitz, Germany). *Pisum sativum* L. was used as an internal reference standard. To evaluate significant differences, we performed a Mann-Whitney U test, which is a non-parametric test between two groups. The level of significance was set at $P < 0.05$. The analyses were run in R (R Core Team 2024).

Results

Phylogenetic analysis

Seventy chloroplast CDS of *Festuca ovina* in Japan were concatenated and aligned, resulting in a length of 52,164 bp after alignment. The ML tree based on the 52,164 bp (Fig. 5) consisted of two robust clades with 100% bootstrap support, i.e. Clade A (O2, O5, O9, O11, O13, O14, O15, O16) and Clade B (O1, O3, O4, O6, O7, O8, O10,

O12). The Clade A further comprised two subclades: one (100% BS) of four diploid samples (O2, O13, O14, O15) and one tetraploid sample from Mts. Ohmine, Pref. Nara (O16), and the other ($< 50\%$ BS) of three tetraploid samples from the southern area of Japan Alps and the adjacent mountains, Pref. Nagano (O5, O9, O11). In Clade B, a diploid sample from Mt. Shibutsu, Pref. Gunma (O3), diverged as the first branch (99% BS), followed successively by a tetraploid sample from Mt. Kitadake, Pref. Yamanashi (O4) (100% BS), two tetraploid samples from the North and Central Japan Alps, Pref. Nagano (O8, O10) ($< 50\%$ BS), a diploid sample from Mt. Ashibetsu, Hokkaido (O1) (100% BS), and three diploid samples from high mountains of the Chubu District facing the Japan Sea (O6, O7, O12).

Observation/measurements of morphological characters

The results of morphological observation and measurements are shown in Table 2. The number of new leaf fascicles (including at least one fertile fascicle) enveloped by each nest of old concentric sheaths ranged from one to seven. In the samples of Clade A (O2, O5, O9, O11, O13, O14, O15, O16), it was usually 4 to 7 (Fig. 6A). However, it was 2 for a few fascicles of O5 or 3 for some fascicles of O2, O5 and O15. More than 1/3 of the nests of old concentric sheaths per individual enveloped four or more new leaf fascicles (including fertile one). In comparison, in the samples of Clade B (O1, O3, O4, O6, O7, O8, O10, O12), the number of new leaf fascicles (including fertile one) enveloped by each nest of old concentric sheaths was always 1 to 3 (Fig. 6B).

Plant height ranged from 12 to 55 cm. The samples of Clade A were generally taller (15–55 cm tall) than those of Clade B (12–28 cm tall). The length of the basal leaves ranged from 3 to 36 cm. Basal leaves of Clade A were generally longer (3–36 cm long) than those of Clade B (3–17 cm long). The inflorescences ranged from 2.1 to 9.3 cm long. Inflorescences of Clade A were generally longer (2.9–9.3 cm long) than those of Clade B (2.1–5 cm long). The samples of Clade A always had panicles, but those of Clade B some-

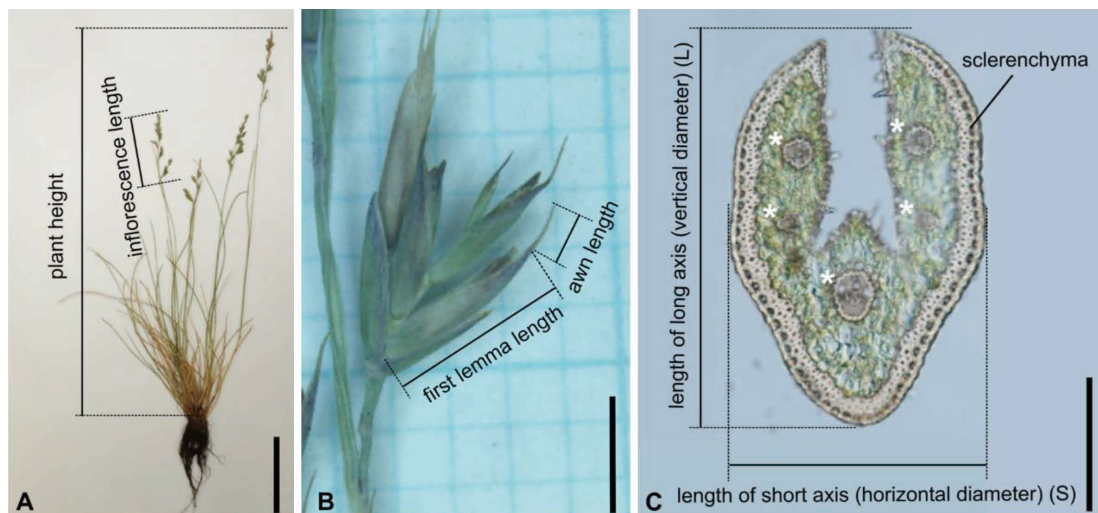


FIG. 3. Plant parts measured in this study. **A**, habit, bar = 5 cm. **B**, spikelet, bar = 2 mm. **C**, cross section of basal leaves, bar = 200 μ m. white asterisks (*) indicate vascular bundles (Kobayashi 1675, KYO).

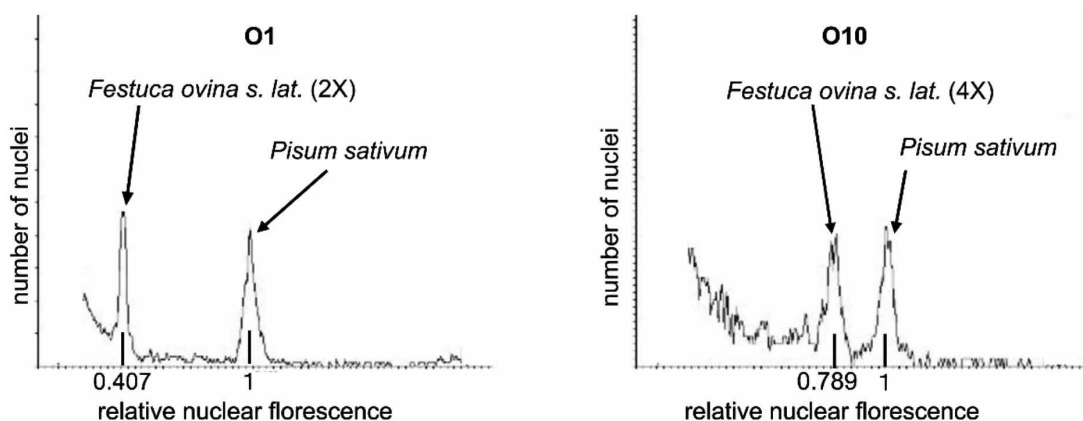


FIG. 4. Histogram examples of relative nuclear fluorescence obtained from flow cytometry analysis of *Festuca ovina sensu* Koba (2020) (O1, O10) and *Pisum sativum* (control). Sample codes correspond to those of Table 1.

times had panicles and sometimes racemes. Thus, the plants of Clade A were primarily larger than those of Clade B.

Although the culm surface was used as a diagnostic characteristic among varieties of *Festuca ovina* by Ohwi (1965), both scabrous and smooth surfaces were found in the samples of both clades. Thus, the culm surface turned out to be of no diagnostic value, at least between Clades A and B.

Alexeev (1988) considered sclerenchyma of the basal leaves to be important for species recognition of *F. ovina* and allies. In this study, three patterns of leaf sclerenchyma in cross section

were found in *F. ovina*; continuous sclerenchyma, indistinctly separated sclerenchyma in five bundles, and distinctly isolated sclerenchyma in seven bundles (Fig. 7). These three patterns were constant and stable among the individuals of the same population. However, the continuous pattern and distinct seven-bundle pattern were found in both Clades A and B, this characteristic was of no diagnostic value between the two clades. As for other characteristics of the basal leaves, there were no conspicuous differences between the two clades.

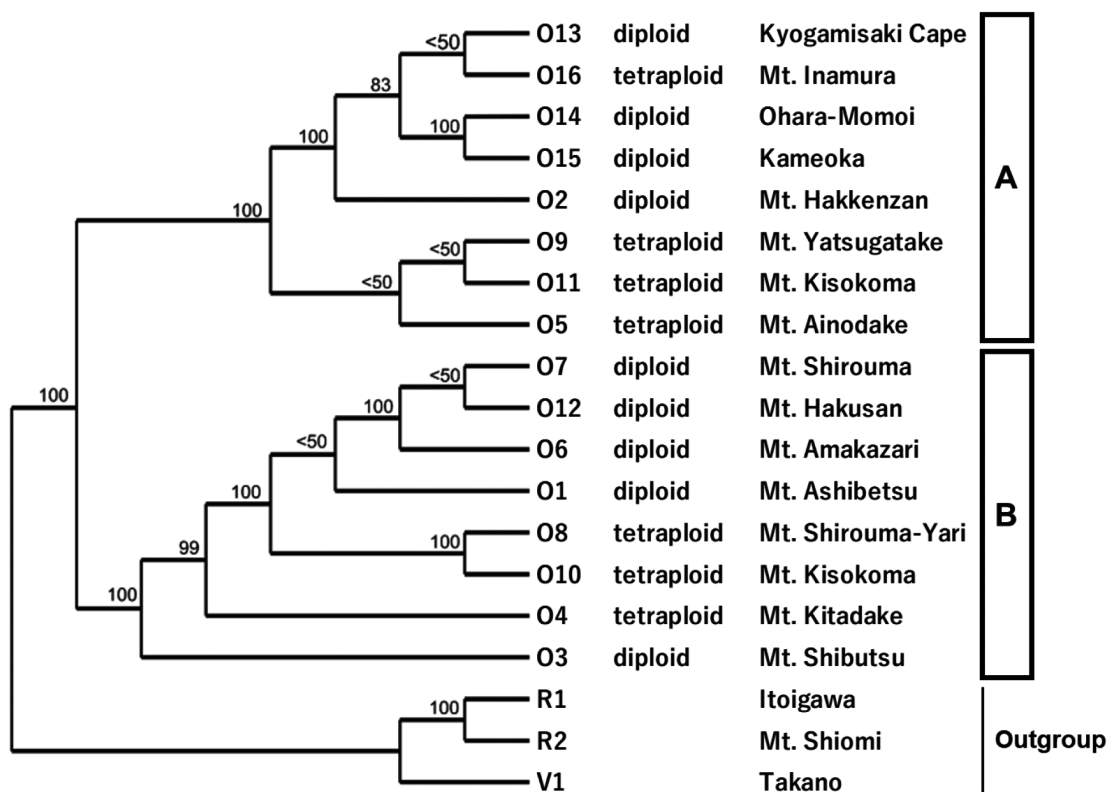


FIG. 5. Maximum likelihood tree of *Festuca ovina sensu* Koba (2020) (O1–O16) and its outgroups (R1, R2, V1) based on chloroplast CDS (70 loci, 52,164bp). Numbers above branches indicate bootstrap values (%). Sample codes correspond to those of Table 1.

Estimation of ploidy levels

The results of flow cytometry are shown in Table 2. As the relative nuclear fluorescence of diploids significantly differed from that of tetraploids (Fig. 4) (Mann–Whitney U test, $P < 0.05$), ploidy level was estimated based on relative nuclear fluorescence, *i.e.* diploid or tetraploid in this study, since Tateoka (1985) indicated that only diploids and tetraploids are in Japan.

Among diploids, relative nuclear fluorescence of Clade A (O2, O13, O14, O15) significantly differed from that of Clade B (O1, O3, O6, O7, O12) (Mann–Whitney U test, $P < 0.05$). The diploids of Clade A grow at lower elevation, *i.e.* 0–700 m, while those of Clade B grow at higher elevations, *i.e.* 1,500–3,000 m. Further, the diploids were taller (25–55 cm high) than the latter (12–25 cm high), and the basal leaves of the former were longer (9–36 cm long) than those of the latter (3–15 cm long).

Other noteworthy features was the fact that the abaxial leaf surface of non-coastal diploids of Clade A (O2, O14, O15) was scabrous in contrast to the smooth/slightly scabrous abaxial leaf surface of the other *F. ovina* in Japan.

Further, although Koba (2020) measured the length of the first lemma of *F. ovina* to be 3–4.5 mm long, tetraploids of Clade B (O4, O8, O10) had a longer first lemma (4.9–5.6 mm long, 4.0–4.7 mm long, 4.8–5.9 mm long, respectively) than in Koba's measurements.

Discussion

Taxonomy of Festuca ovina sensu Koba (2020) in Japan

Based on the ML tree (Fig. 5), *Festuca ovina sensu* Koba (2020) in Japan consists of two robust clades with 100% bootstrap support. One is basi-

TABLE 2. Results of morphological observation/measurements and flow cytometry analysis of *Festuca ovina sensu* Koba (2020) used in this study and the related lectotypes. Sample codes correspond to those of Table 1. For clades, see Fig. 5.

Sample code	Clade	Number* of new leaf fascicles that envelope four or more each nest of old concentric sheaths	Plant height (cm)	Culm surface	Length (cm)	Basal leaves				Inflorescence ramification	Inflorescence length (cm)	First lemma length (mm)	Awn length (mm)	Relative nuclear fluorescence ratio (Sample/Standard)	Ploidy level
						Length of long axis (vertical diameter) in cross section (L) (μm)	Length of short axis (horizontal diameter) in cross section (S) (μm)	Leaf shape in cross section (S/L)	Distribution of abaxial sclerenchyma in cross section						
O2	A	3–4	50	scabrous	9–19	609	335	0.55	continuous	panicle	4.6–5.2	3.7–4.1	0.71–1.23	0.47	2x
O5	A	2–4	37	smooth	3–10	630	359	0.57	continuous	panicle	2.9–3.6	3.2–3.8	0.58–1.33	0.77	4x
O9	A	4	100	scabrous	8–22	465	381	0.82	continuous	panicle	5.4–5.5	3.6–4.1	0.83–1.14	0.79	4x
O11	A	4–5	100	scabrous	3–9	649	448	0.69	continuous	panicle	3.3–5.0	3.5–3.7	0.71–0.83	0.78	4x
O13	A	6	100	smooth	13–20	829	481	0.58	continuous	panicle	4.8–5.4	4.0–4.5	0.22–0.31	0.52	2x
O14	A	4	100	scabrous	18–34	712	370	0.52	continuous	panicle	6.2–7.0	3.2–3.9	0–0.23	0.48	2x
O15	A	3–4	50	scabrous	11–36	624	387	0.62	continuous	panicle	7.8–9.3	3.5–4.0	0–0.12	0.49	2x
O16	A	5–7	100	scabrous	7–15	637	433	0.68	distinct 7 bundles	panicle	3.6–5.0	3.2–4.2	0.89–1.10	0.82	4x
Summary	A	2–7	37–100	smooth/scabrous	3–36	465–829	335–481	0.52–0.82	continuous/distinct 7 bundles	panicle	2.9–9.3	3.2–4.5	0–1.33	0.47–0.52/ 0.78–0.82	2x/4x
Lectotype of <i>F. ovina</i> (<i>Limnæi</i> 92-J, LINN)															
O1	B	1–3	0	smooth	5–13	430	301	0.70	distinct 7 bundles	raceme/panicle	2.1–2.6	3.5–3.6	0.63–1.30	0.41	2x
O3	B	3	0	smooth	3–10	453	308	0.68	continuous	raceme/panicle	2.1–2.5	2.8–3.1	0.18–0.60	0.42	2x
O4	B	1–2	0	smooth	8–15	965	579	0.60	indistinct 5 bundles	raceme/panicle	3.9–4.6	4.9–5.6	0–1.74	0.76	4x
O6	B	1	0	slightly scabrous	5–13	567	312	0.55	continuous	raceme	2.6–3.6	3.2–3.8	0.29–0.62	0.43	2x
O7	B	2	0	smooth	5–11	600	336	0.56	continuous	panicle	3.0–4.5	4.0	0.36–0.82	0.43	2x
O8	B	3	0	smooth	8–13	863	440	0.51	distinct 7 bundles	raceme	3.8–4.5	4.0–4.7	1.00–1.56	0.80	4x
O10	B	1–2	0	slightly scabrous	5–17	832	499	0.60	distinct 7 bundles	raceme/panicle	4.5–5.0	4.8–5.9	1.36–1.60	0.79	4x
O12	B	1–2	0	smooth	5–15	578	376	0.65	continuous	panicle	4.2–4.9	3.3–3.9	0.58–0.72	0.42	2x
Summary	B	1–3	0	smooth/scabrous	3–17	430–965	301–579	0.51–0.68	continuous/indistinct 5 bundles/distinct 7 bundles	raceme/panicle	2.1–5	2.8–5.9	0–1.74	0.41–0.43/ 0.76–0.80	2x/4x
Lectotype of <i>F. ruprechtii</i> (<i>Ruprechtii</i> s.n., G)															
-	-	3	0	-	2–8	-	-	-	continuous	raceme	2.5–3.5	ca. 4	-	-	-

*We counted this number only in case at least one of the new leaf fascicles yields a culm.

**We counted this percentage only in case at least one of the new leaf fascicles yields a culm.

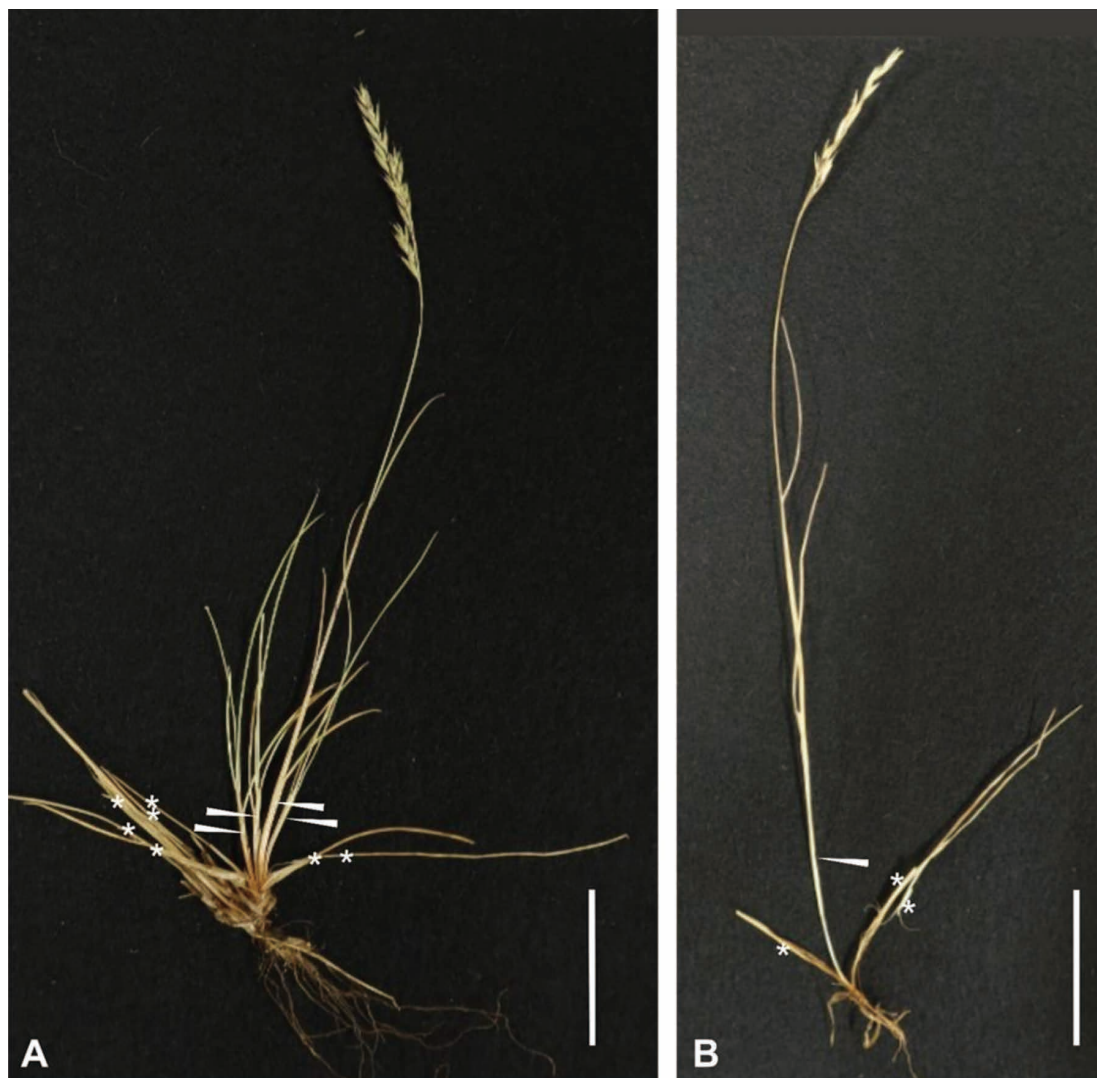


FIG. 6. Comparison between vertically divided portions of an individual. Both A and B were originally enveloped by a nest of old concentric sheaths, but the nest of old concentric sheaths was artificially opened to see inside. **A**, four new leaf fascicles were enveloped (*Kobayashi 1689*, KYO). **B**, one new leaf fascicle was enveloped (*Kobayashi 1915*, KYO). White arrowheads indicate new leaf fascicles. White asterisks (*) indicate parts of opened nest of old concentric sheaths. Bars = 3 cm.

cally distributed in the high mountains (above ca. 2,000 m elevation in Honshu and more than ca. 1,500 m elevation in Hokkaido) and has sparse leaves and racemes / smaller panicles (Fig. 6B); the other is widely distributed from seashores to high mountains (0–3,190 m elevation) with dense leaves and larger panicles (Fig. 6A) (Table 1, Fig. 2).

They were exactly distinguished from each other in number of new leaf fascicles enveloped

by a nest of old concentric sheaths (Table 2). In the former clade, all nests of old concentric sheaths envelop fewer than three (1–3) new leaf fascicles each, whether or not they are accompanied by culms, and no nest of old concentric sheaths envelops more than four new leaf fascicles. In comparison, more than 1/3 of the nests of old concentric sheaths within an individual always envelop more than four (4–7) new leaf fas-

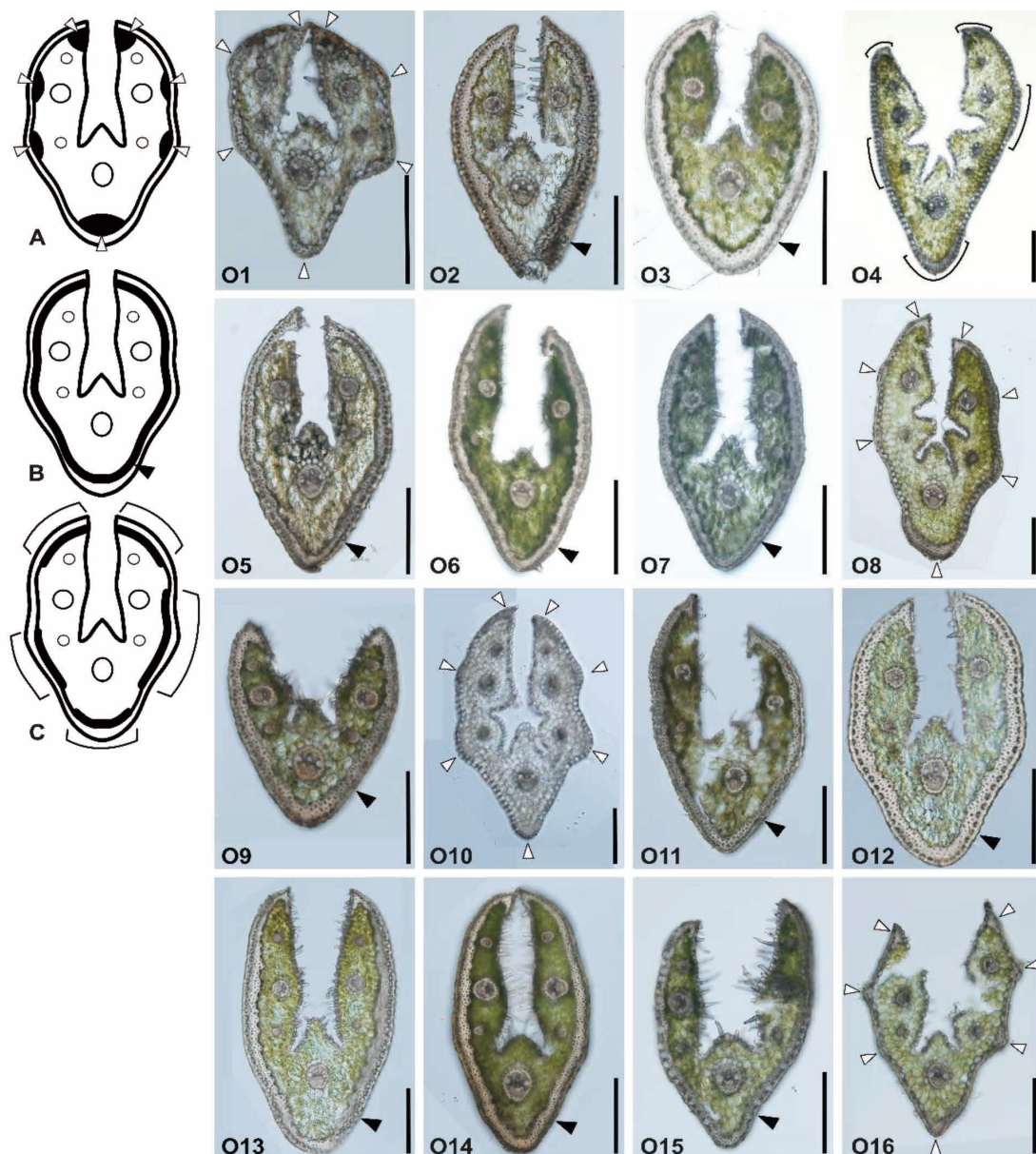


FIG. 7. Comparison of cross sections of basal leaves between samples of *Festuca ovina sensu* Koba (2020) (O1–O16) and schematic illustrations (A–C). Sample codes (O1–O16) correspond to those of Table 1. In A–C, black thickened parts indicate sclerenchyma, and open circles indicate vascular bundles. White arrowheads indicate distinct sclerenchyma bundles; black arrowheads indicate continuous sclerenchyma; arcs indicate indistinct sclerenchyma bundles. Bars = 200 μ m.

cicles, among which at least one new leaf fascicle is accompanied by a culm. The remaining less than 2/3 envelop fewer (2 or 3) new leaf fascicles in the latter clade.

The two types sometimes grow sympatrical-

ly. Around the summit area of Mt. Kisokoma, Pref. Nagano, they grow only ca. 10 m apart (O10 & O11 in Table 1 and Fig. 2) in the absence of any morphologically intermediate individuals (Fig. 8). Thus, we consider them distinct species. As

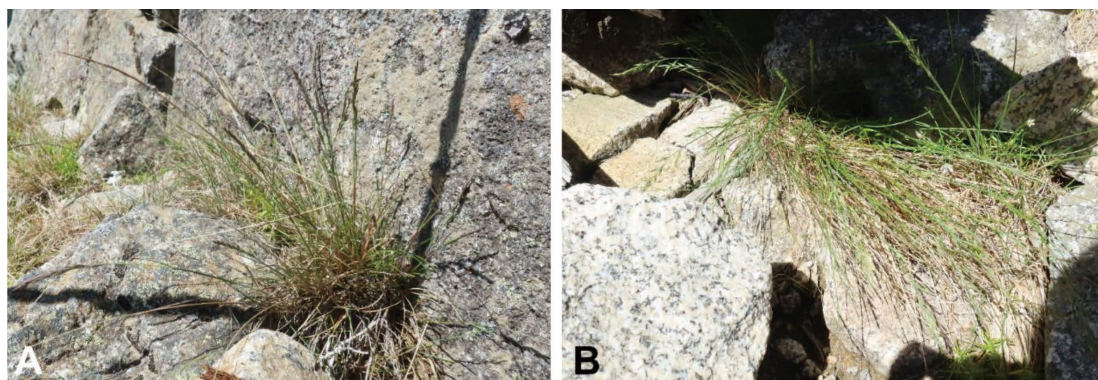


FIG. 8. Habitat of *Festuca ovina* (A, O11) and *F. ruprechtii* (B, O10). They grow only ca. 10 m apart at the summit area of Mt. Kisokoma, Pref. Nagano. Photographs were taken on 13 July 2024. Sample codes correspond to those of Table 1.

the lectotypes of *Festuca ruprechtii* (Boiss) V.I.Krecz. & Bobr. (*F. J. Ruprecht s.n.*, G) and *F. ovina* (Herb. Linnaei 92-I, LINN) were within the range of morphological variation revealed in clades B and A, respectively (Table 2), we recognize the former *F. ruprechtii* and the latter as *F. ovina*.

Alexeev (1988) distinguished *F. chiisanensis* and *F. probatovae* from *F. ovina* in having 5–7 distinctly separate bundles of sclerenchyma just below the abaxial leaf epidermis in cross section. *F. ovina* usually has continuous or rarely more or less disjunct sclerenchyma just below the abaxial leaf epidermis in cross section. Based on our molecular phylogenetic results (Fig. 5), however, *F. chiisanensis* (O16) is included in the clade of *F. ovina*; *F. probatovae* (O1, O8 & O10) is in the clade of *F. ruprechtii*. Additionally, nests of old concentric sheaths of *F. chiisanensis* (O16) envelop 5–7 new leaf fascicles, within the range of *F. ovina*, and those of *F. probatovae* (O1, O8 & O10) envelop 1–3 new leaf fascicles, within the range of variation of *F. ruprechtii* (Table 2). Thus, we reduce *F. chiisanensis* and *F. probatovae* to *F. ovina* and *F. ruprechtii*, respectively and consider the distribution of the abaxial sclerenchyma in cross section of the basal leaves to be of no diagnostic value at the species level.

Polyploidization of Festuca ovina sensu Koba (2020) in Japan

Festuca ovina and *F. ruprechtii* in Japan both

contain diploids and tetraploids (Table 2). Both tetraploids of each species consist of two different lineages (Fig. 5), indicating that tetraploidization occurred at least twice independently in both species. As diploids and tetraploids of *F. ovina* grow at lower (0–650 m) and higher elevations (1,500–3,190 m), respectively, it might be possible for two parallel tetraploidization events to have allowed *F. ovina* to grow in the new alpine environments.

The two tetraploid lineages of each species differ also morphologically. The two of *F. ovina* have continuous sclerenchyma (Fig. 7 O5, O9 & O11) and seven distinctly separated sclerenchyma (Fig. 7 O16) in cross sections of the leaves, respectively. In *F. ruprechtii*, both have separate (discontinuous) sclerenchyma in cross section of the leaves, one with seven distinctly separate sclerenchyma bundles (Fig. 7 O8, O10), the other with five indistinctly separate sclerenchyma bundles (Fig. 7 O4). Since individuals with intermediate morphology between the two tetraploid lineages of each species have never been found, the two tetraploid lineages of each species may not be hybrids based on our morphological observations (Table 2). This hypothesis is partly supported by the disjunct distribution between the two tetraploid lineages of *F. ovina*, i.e. one in the south-central part of the Chubu Mountain region, Pref. Nagano, and the other in Mts. Ohmine, Pref. Nara (Fig. 2). Further studies of the nuclear genome are needed to confirm this hypothesis.

Taxonomic treatment

Key to the species of *Festuca ovina sensu* Koba (2020) in Japan

- 1a. Leaves sparse; each nest of old concentric sheaths enveloping three or fewer new leaf fascicles; inflorescences racemes or panicles 2. *F. ruprechtii*
 1b. Leaves dense; at least one nest of old concentric sheaths within an individual enveloping four or more new leaf fascicles; inflorescences panicles 1. *F. ovina*

1. *Festuca ovina* L. in Sp. Pl.: 73 (1753). —Type: Sweden, Lapland, *Herb. Linnaei 92-1* (lecto- digital image LINN!, Jarvis *et al.* 1987).

F. duriuscula L. in Sp. Pl.: 74 (1753). —*F. ovina* L. var. *duriuscula* (L.) W. D. J. Koch in Syn. Pl. Germ. Helv.: 812 (1837). —Type: Europe, *A. van Royen 913-7-451* (lecto- L, *n.v.*, van der Meijden 2000).

F. ovina L. subsp. *ovina* var. *vulgaris* Wallr. subvar. *coreana* St. Yves in Bull. Soc. Bot. France 71: 33 (1924). —Type: KOREA, Port-Hamilton, *C. Willford 700* (holo- P, iso- digital image LE 01002326!, digital image K000913240!).

F. ovina L. var. *coreensis* Hack. ex Nakai in Saishu-to Kuan-to Shokubutsu Hokoku-sho [Fl. Quelpaert Isl.]: 19 (1914), nom. inval.; T. Mori in Enum. Pl. Corea 43 (1922), nom. inval.; Honda in J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 3(1): 45, 49 (1930). —Type: Quelpaert, Hongno, *Taquet 1866* (lecto- TI00418446!, **hic designatus**).

F. ovina L. var. *nipponica* Ohwi in Acta Phytotax. Geobot. 10: 111 (1941). —Type: JAPAN, Prov. Bungo, Tsurumi-sanmyaku, *Z. Tashiro s.n.* (holo- KYO00078576!).

F. ovina L. var. *pubiculmis* Ohwi in Acta Phytotax. Geobot. 10: 110 (1941). —Type: JAPAN, Prov. Settsu, Mt. Mayasan, *Z. Tashiro s.n.* (holo- KYO00078577!).

F. ovina L. var. *chiisanensis* Ohwi in Acta Phytotax. Geobot. 10: 111 (1941). —Type: KOREA, Keinan, Mt. Chiisan, *J. Ohwi 7136* (holo- KYO00076328!).

F. ovina L. var. *litoralis* Tzvelev in Novosti Sist. Vyssh. Rast. 9: 45 (1972). —Type: RUSSIA, Primorsky, *B. Kolesnikov 147* (holo- digital image LE01011356!).

Japanese name. Ushinoke-gusa.

Distribution. Circumboreal. Japan: Hokkaido to Kyushu (northward from Yakushima Isl.)

Habitat in Japan. Sunny, open cliffs and rocky sites, 0–3,190 m elevation.

Ploidy levels in Japan. diploid and tetraploid.

2. *Festuca ruprechtii* (Boiss.) V. I. Krecz. & Bobr. in Fl. URSS 2: 507 (1934). —*F. ovina* L. subsp. *laevis* Hack. var. *ruprechtii* Boiss. in Fl. Orient. 5: 619 (1884). —Type: Caucasus, *F. J. Ruprecht s.n.* (lecto- digital image G00785806!, **hic designatus**).

F. supina Schur in Enum. Pl. Transsilv.: 784 (1866). —Type: Carpathians, Mt. Ablas, *Schur s.n.* (lecto- LW, *n.v.*, Tzvelev 1976).

F. ovina L. var. *tateyamensis* Ohwi in Acta Phytotax. Geobot. 10: 111 (1941). —Type: JAPAN, Prov. Ecchu, Mt. Tateyama, *S. Sakai s.n.* (holo- KYO00078578!).

F. probatovae E. B. Alexeev in Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol. 87(5): 102 (1982), '*probatoviae*'. —Type: RUSSIA, Sakhalin, *N. Probatova 683* (holo- photo VLA10270!; iso- digital image LE01011338!, digital image MO2114916!, digital image MW0591402!)

Japanese name. Miyama-ushinoke-gusa.

Distribution. Circumboreal. Japan: Hokkaido to C. Honshu.

Habitat in Japan. Sunny, open cliffs and rocky sites, 1,500–3,030 m elevation.

Ploidy levels in Japan. diploid and tetraploid.

We express our cordial gratitude to Prof. Hidetoshi Nagamasu for comments on nomenclature. We are deeply grateful to Dr. Kotaro Shutoh for valuable information during our fieldworks. Our thanks are due to Prof. Emeritus Hiroshi Tobe and Dr. Koji Takayama for their insightful advices on analytical and experimental methods. Thanks are also due to the directors and staff members of the herbaria KYO and TI for allowing us to access their collection. This study was supported in part by the Joint Usage/Research Center of Osaka Metropolitan University, funded by the MEXT Promotion of Distinctive Joint Usage/Research Center Support Program (Grant Number JPMXP0723833155) and the Japan Society for the Promotion of Science (JSPS) KAKENHI grant-in-aid for scientific research (Grant Number JP21KK0132).

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Received May 14, 2025; accepted July 4, 2025