

Surviving at the Southern Edge: The Rear-Edge Population of *Viola brevistipulata* var. *acuminata* Persisting in Disturbance-Mediated Refugium

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The Ashiu area of Kyoto Prefecture is renowned for its role as a refugium for relict plants that migrated from northern regions in glacial periods. *Viola brevistipulata sensu lato* in Ashiu represents such a rear-edge population at low latitude, yet its biogeographic and conservation importance has been obscured due to taxonomic issues. This study aimed to clarify the taxonomy of the Ashiu population of *V. brevistipulata s.l.* through phylogenetic analysis of three varieties occurring in Honshu, Japan. The analysis confirmed the genetic distinctness of var. *acuminata* from a group including var. *brevistipulata* and var. *minor*, and clearly identified the Ashiu population as var. *acuminata*, which prefers a subalpine climate. Surveys of the distribution of var. *acuminata* populations in Ashiu revealed their persistence at low altitudes that correspond to upper warm temperate to lower cool temperate climate zones. The riverside rocky habitats that exclude competing plants and the unique life history characteristics of var. *acuminata* appear to allow these relict populations to survive in a climatically less suitable area.

Key words: molecular identification, relict population, southern distribution limit, violet

Ashiu Forest of Kyoto University, located in Nantan City, Kyoto Prefecture, is situated at an elevation of less than 1,000 meters. It hosts old-growth temperate forest vegetation. Despite its relatively low altitude, the forest is home to several species of plants with their main area of distribution in northern Japan. Species such as the sub-alpine *Avenella flexuosa* (L.) Drejer and *Trautvetteria caroliniensis* (Walter) Vail var. *japonica* (Siebold & Zucc.) T. Shimizu, along with cool-temperate species such as *Hemerocallis middendorffii* Trautv. & C. A. Mey. var. *esculenta* (Koidz.) Ohwi, *Carex podogyna* Franch. & Sav. and *Diphylleia grayi* F. Schmidt have been documented in Ashiu Forest at elevations within the upper warm-temperate and lower cool-tem-

perate zones (Yasuda & Nagamasu, 1995). Those species occur primarily on rocky substrates in steep terrain and along streams within the forest, suggesting that they may have survived in these specific locations by avoiding competition with other plants, which would have otherwise caused their extirpation under local climatic conditions (Yasuda & Nagamasu, 1995, Sakaguchi 2023, Murata *et al.*, 1995, Fukumoto, 2021). Recent studies have investigated the genetic consequences of the persistence of northern plant populations within the forest. For instance, long-term geographic isolation has led to distinct lineage differentiation in the rear-edge population of *H. middendorffii* var. *esculenta* (Masuda *et al.*, 2023). Similarly, microsatellite alleles in the Ashiu pop-



ulation of *Carex podogyna* have become completely fixed at the southwestern edge of its distribution (Nagasawa *et al.* 2022). These genetic findings indicate that the microrefugia of Ashiu have enabled plants from northern regions to survive, isolated from the main northern distributions.

The Yura River, originating in the Ashiu Forest, provides a meandering stream environment with riffles, pools and small waterfalls at elevations between 400 and 600 meters. In this stream zone, species such as *C. podogyna*, *Caltha palustris* L. var. *pygmaea* Makino, and *Viola brevistipulata* (Franch. & Sav.) W. Becker, which are considered to be relict populations, thrive (Fig. 1a, b). *Viola brevistipulata* s.l., the target species of this study, is a yellow-flowered perennial violet (Becker 1916, Franchet & Savatier 1875) belonging to sect. *Chamaemelianum* (Marcussen *et al.* 2022). The species is endemic to Japan and occurs primarily in the snowy regions of Hokkaido and the Sea of Japan side of Honshu. In Honshu, in addition to var. *brevistipulata*, other varieties include var. *acuminata* Nakai (Nakai 1928), which has lanceolate leaves and short internodes, var. *ciliata* M. Kikuchi, which is on the Pacific side in the Tohoku region (Kikuchi 1950, Nakai *et al.* 1996, Takeda 2017), var. *kishidae* (Nakai) F. Maek. & T. Hashim. (Maekawa & Hashimoto 1968) on windswept mountain slopes and ultramafic areas and the dwarf var. *minor* Nakai (Nakai 1933). In the Kinki region, *V. brevistipulata* s.l. is in northern Shiga and Kyoto Prefectures, but there are differing opinions regarding the taxonomy of these populations (Japan Violet Society 2020, Murata, 2004). Gen Murata, a plant taxonomist who compiled the flora of the Kinki region, reclassified plants on granite mountains in Shiga Prefecture previously considered to be var. *minor* as var. *brevistipulata*. He concluded that var. *brevistipulata* was the only variety in Kyoto and Shiga prefectures. Despite the herbarium of Kyoto University, where Murata worked, having voucher specimens of plants similar to var. *acuminata* collected in the Kinki region, he did not mention the occurrence of var. *acuminata* being in the region in his book, 'Flora of the Kinki Region' (Murata 2004). In fact, Murata himself an-

notated a specimen collected in Gifu Prefecture (*G. Murata 30029*, KYO) as "the plant is f. *acuminata* (Nakai) S. Watanabe", a synonym of var. *acuminata* (Watanabe 1961), considered it inseparable from var. *brevistipulata* at the rank of variety. In a monograph on violets in the Kinki region (Japan Violet Society 2020), var. *brevistipulata* and var. *acuminata* are treated as distinct and both varieties are documented to occur in both Kyoto and Shiga Prefectures.

A comparison of the literature on *V. brevistipulata* s.l. in the Kinki region highlights two main differences: (1) whether var. *acuminata* should be recognized as distinct from var. *brevistipulata*, and (2) if recognized, whether both taxa are in Kyoto Prefecture. In Kyoto, there is only one known locality for *V. brevistipulata* s.l., which is in the stream zone in Ashiu Forest. Clarifying whether the plants in the forest are the cool-temperate var. *brevistipulata* or the subalpine to cool-temperate var. *acuminata* (or both) is important for understanding the biogeography of relict plants from northern regions. Against this background, this study aimed to determine the taxonomy of the Ashiu population of *V. brevistipulata* s.l. through phylogenetic analysis and to re-evaluate the role of the special habitat as a climatic refugium for relict plants.

Materials & Methods

Phylogenetic analysis of V. brevistipulata s.l.

Our provisional surveys of specimens in the Kyoto University Museum (KYO) suggested that two morphological groups, corresponding to var. *brevistipulata* and var. *acuminata*, were in the Kinki region (Fig. 2a, b). Var. *brevistipulata* has round to broadly ovate lowermost stem leaves (leaf blade length to width ratio of 0.8–1.3) with a deeply cordate base, and the lowest leaf is always separated from the one above it (internode length > 0 mm) (Fig. 2b). Older individuals are characterized by crowded stems due to their short rhizomes. Such plants are known from Takashima City in northwestern Shiga Prefecture, and around Mt. Akasaka, Mt. Mikuni and Kuroko

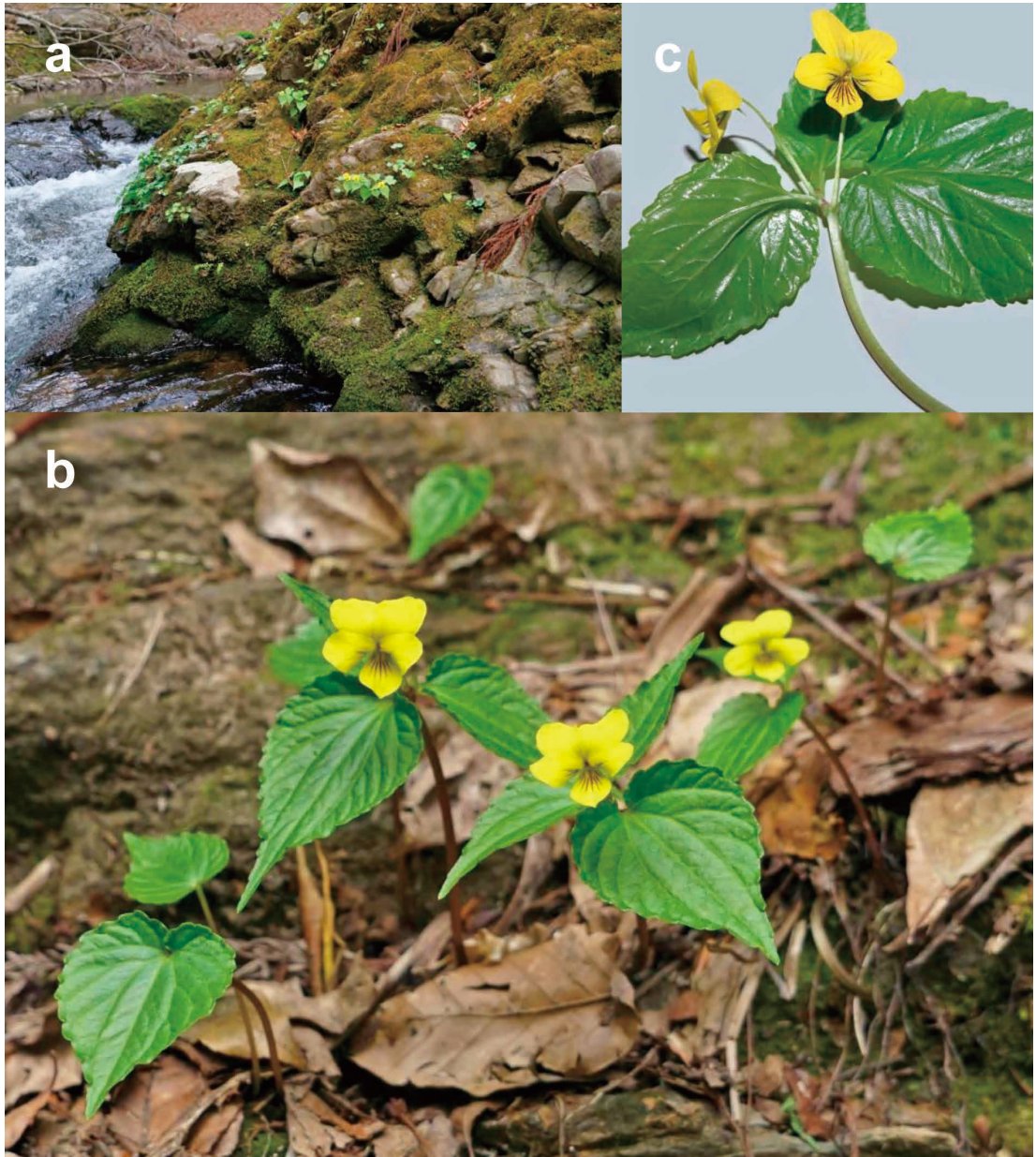


FIG. 1. **a**, Riverside habitat of *Viola brevistipulata* var. *acuminata* in Ashiu Forest. Photograph was taken on April 20, 2022. **b** & **c**, Habit of *V. brevistipulata* var. *acuminata* in Ashiu.

Pass. Var. *acuminata* type plants have lanceolate to ovate lowermost stem leaves (leaf blade length to width ratio of 1.3–2.4) with a shallowly cordate base (Fig. 2b). The internode between the lowermost stem leaf and the leaf just above is, in most cases, shorter than in var. *brevistipulata* (Fig. 2b), and most individuals have three leaves in a clus-

tered arrangement (Fig. 1c), consistent with the original description by Nakai (1933). However, in some large individuals, the lower stem leaves are attached at some distance from each other, as in var. *brevistipulata*. The rhizomes of var. *acuminata* in the Kinki region are longer, and the plants spread by vegetative reproduction (Fig. 1b). Var.

TABLE 1. *Viola brevistipulata* materials used for phylogenetic analysis.

Intraspecific taxon	Population code	Locality	Latitude	Longitude	Elevation (m)	Sample size	Vouchers representing the population
var. <i>acuminata</i>	SS547	Nishimurayama-gun, Yamagata	38°32'	139°59'	1,250	2	YAMA 27965
	SS1060	Kitaazumino-gun, Nagano	36°44'	137°47'	1,599	4	SHIN 146143
	SS1282	Azumino, Nagano	36°17'	137°43'	2,300	4	KPM 2342
	SS1141	Ohno-gun, Gifu	36°15'	136°50'	1,457	4	TNS 647208
	SS696	Ashiu, Nantan, Kyoto	35°19'	135°46'	559	4	KYO 26890
var. <i>brevistipulata</i>	SS298	Mogami-gun, Yamagata	38°57'	140°19'	271	3	YAMA 26363
	SS1022	Nagaoka, Niigata	37°12'	138°51'	171	3	KYO 25849
	SS1019	Takashima, Shiga	35°31'	136°01'	771	4	OSA 65624
var. <i>minor</i>	SS1033	Saihaku-gun, Tottori	35°22'	133°32'	1,297	3	TNS 746146
Total						31	

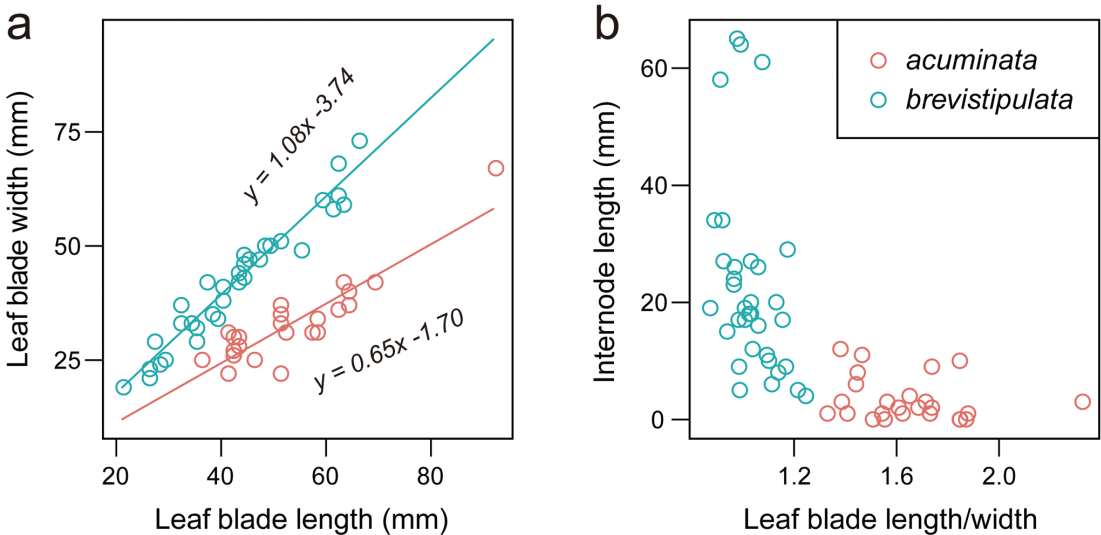


FIG. 2. **a**, Scatterplot of leaf blade width against width of two varieties of *Viola brevistipulata*. **b**, Scatterplot of internode length against leaf blade length/width ratio.

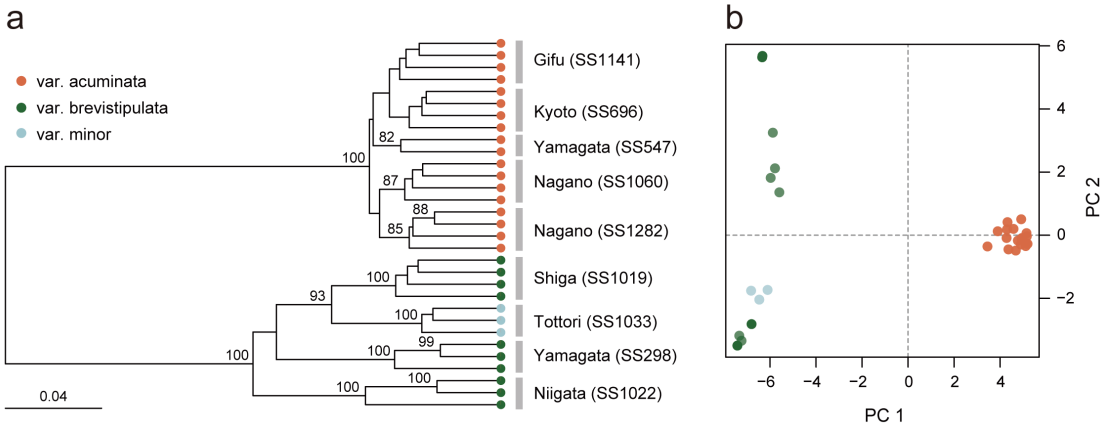


FIG. 3. **a**, Neighbor-joining tree of *Viola brevistipulata* s.l. samples in Honshu. Values shown at each node represent bootstrap support. **b**, Individual plot of principal component analysis scores on first two PCA axes.

acuminata is known from northeastern Shiga Prefecture (around Maibara City) (Murase 2007), Mt. Sanjo, Kutsuki Village (Onyu-dani and Furuya) in Takashima City (based on field surveys by the authors), and Nantan City (Ashiu) in Kyoto Prefecture.

It is not known if the two morphologically distinct groups in the Kinki region, are genetically distinct. Therefore, we conducted a genetic analysis, including populations from other areas of Honshu, to investigate this point. As shown in Table 1, three populations of var. *brevistipulata*, including one from Takashima, Shiga Prefecture, five populations of var. *acuminata*, including one from the Ashiu Forest, and one population of var. *minor* from Tottori Prefecture were included. In the Ashiu population, we collected four individuals from two distinct areas (two plants from each), focusing on internode length ranging from 0 mm to 8 mm, to represent the morphological variation observed in Ashiu Forest. Leaves collected in the field were immediately dried in silica gel and stored in the dark until DNA extraction. Approximately 1 cm² of leaf material was placed in tubes containing zirconia beads and pulverized using a TissueLyser II (QIAGEN, Germany). DNA was isolated from the leaf material using the cetyl trimethyl ammonium bromide (CTAB) method (Murray & Thompson 1980). The extracted DNA was dissolved in TE buffer and used for subsequent DNA library preparation. We employed a method of reduced representation sequencing of genomes, MIG-seq (Multiplexed ISSR Genotyping by Sequencing) (Suyama & Matsuki 2015) to obtain SNP (Single Nucleotide Polymorphism) markers. MIG-seq libraries were prepared following the protocol in Suyama & Matsuki (2015), with the exception that the annealing temperature in the first PCR was changed from 48°C to 38°C. The amplicons were purified and sequenced on an Illumina MiSeq Sequencer using the MiSeq Reagent Kit v.3 (150 cycles) (Illumina, San Diego, CA, USA). We skipped the sequencing of the first 17 bases of reads 1 and 2 (SSR primer regions and anchors) using the ‘DarkCycle’ technique. Low-quality reads and adapter sequences in the obtained reads were removed using Trim-

omatic 0.39 (Bolger *et al.* 2014). The data were then analyzed using UNEAK (Universal Network-Enabled Analysis Kit) (Lu *et al.* 2013). SNP markers were extracted using UNEAK, following the method described by Sakaguchi *et al.* (2022). Briefly, Tag counts were generated from fastq files with the FastqToTagCountPlugin, then merged with the MergeMultipleTagCountPlugin, with the minimum count of a tag that must be present defined as five. Tag pair was identified for SNP calling with UtagCountToTagPairPlugin. The error tolerance rate was specified as 0.03.

A neighbor-joining (NJ) tree was inferred based on the genetic polymorphism data (31 individuals genotyped at 313 SNPs) obtained from the UNEAK pipeline, using the R *Poppr* package (Kamvar *et al.* 2014). Clade support on the phylogenetic tree was evaluated based on 1,000 bootstrap data. In addition, a principal component analysis (PCA) of the genotypes was used to ordinate individuals on the major axis of genetic variations. The R *ade4* package (Jombart 2008) was used for the PCA analysis.

Distribution Survey of V. brevistipulata s.l. in Ashiu Forest

We surveyed the distribution of populations of *Viola brevistipulata s.l.* along the Yura River in Ashiu Forest, the only known locality of the species in Kyoto, to determine their spatial distribution and elevation. For the survey, we went upstream to the headwaters of the Yura River at an elevation of 370 m a.s.l. where the Ashiu Forest Research Station office is located. The geographic locations of the populations of *V. brevistipulata s.l.* and the number of plants were recorded during the flowering season of April 20–21, 2018. *Viola brevistipulata s.l.* in Ashiu exhibits a mixed reproductive strategy of vegetative growth through rhizomes and seed production through open or chasmogamous flowers. This reproduction mode results in a hierarchical distribution structure of the populations of *Viola brevistipulata s.l.* and typically consisted of a clump of ramets within a distance of 50 cm, including both flowering and non-flowering ramets, (defined as ‘individual’, here). The groups of such scattered clumps in

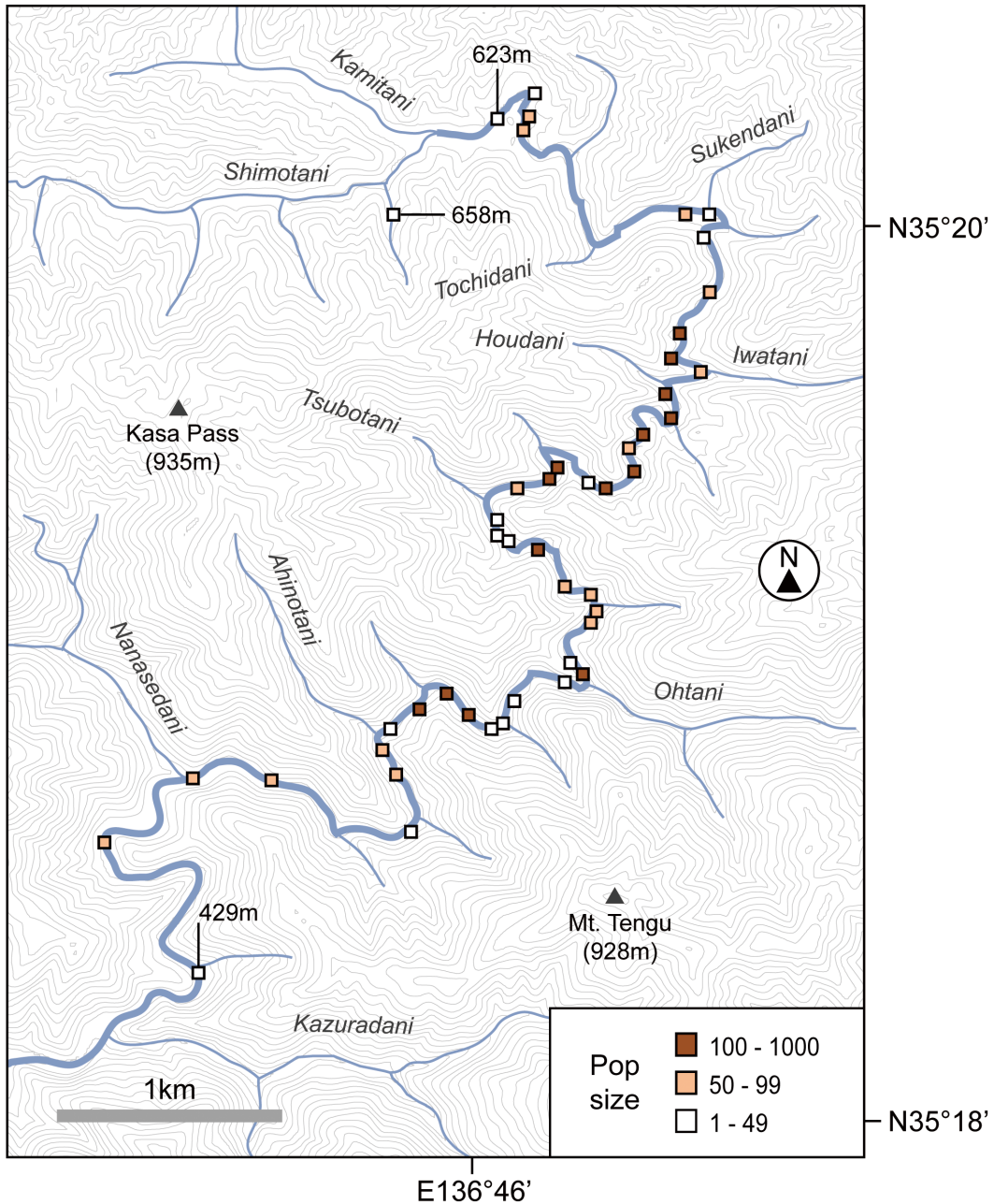


FIG. 4. Map showing spatial distribution of *Viola brevistipulata* var. *acuminata* along the Yura River in Ashiu Forest.

each location were defined as ‘populations.’ As an example of local distribution, a typical population is shown in the photograph (Fig. 1a) where a group of individuals each consisting of multiple ramets (< 10) formed a population on a riverside rock.

To explore the geographic and climatic context of the populations of *Viola brevistipulata* s.l. in Ashiu Forest (see Results), we compiled distribution records for a variety of *acuminata* across Honshu based on data from the Science Museum Net (<https://science-net.kahaku.go.jp/>) and the lo-

cality data recorded by the authors.

Results

Phylogenetic position of the Ashiu population of V. brevistipulata s.l.

The genetic analysis of the samples of *V. brevistipulata s.l.* using neighbor-joining tree and principal component analysis consistently revealed two major clusters among the 31 samples. The first cluster, comprising 18 samples, morphologically identified as var. *acuminata*, formed a distinct, well-supported monophyletic group in the NJ tree (Fig. 3a). Principal component analysis indicated that the samples of var. *acuminata* clustered together in the positive region of the first PCA axis, clearly separating them from the remaining samples of var. *brevistipulata* and var. *minor* (Fig. 3b). Varieties *brevistipulata* and *minor* together formed a single, strongly supported cluster in the NJ tree (bootstrap support = 100%).

Distribution patterns of V. brevistipulata s.l. in Ashiu Forest

The distribution patterns of *V. brevistipulata s.l.* along the Yura River in April 2018 are illustrated in Figure 4. Forty-six populations were observed along the main stream of the Yura River (from Kamitani to Kazura-dani), from altitudes ranging from 429 m near the Kazura-dani outflow to 623 m (Fig. 4). The mean number of individuals in a population was 237 (standard deviation: 340), with a maximum of 1,856 individuals and a minimum of 1 plant. Additionally, one population was in a tributary of the Shimotani watershed, a branch from the main stream, at an altitude of 658 m, which marked the highest distribution point in the forest. This section of the Yura River is 10.9 km long; 10,912 plants of *V. brevistipulata s.l.* were growing there. The distribution density was notably high in the section from Ahinodani at 480 m elevation to Iwatani at 600 m elevation where the Yura River meanders and forms a zone with widely exposed rocks on both sides (Fig. 4).

Figure 5a illustrates the distribution of var.

acuminata in Honshu. Our genetic analysis confirmed that Ashiu Forest in Kyoto Prefecture represents the southwestern limit of distribution of *V. brevistipulata s.l.* in Honshu, with the species typically occurring in the subalpine to cool temperate mountains south of Aomori Prefecture. In the Tohoku and Chubu regions, the distribution of var. *acuminata* generally increased in elevation with decreasing latitude. However, in Ashiu Forest, as its southern limit, var. *acuminata* was unexpectedly descends to the upper warm temperate zone (Fig. 5b).

Discussion

Taxonomic recognition of V. brevistipulata var. acuminata

Viola brevistipulata var. *acuminata* was originally described by Takenoshin Nakai in 1928 as a variety of *V. brevistipulata* (Nakai 1928), based on a type specimen from Mt. Yarigatake, Nagano Prefecture. Subsequently, Sadamoto Watanabe, in his re-examination of the taxonomy of *V. brevistipulata s.l.*, suggested that var. *acuminata* be considered “a form of *V. brevistipulata* with soft leaves” and treated it at the rank of forma (Watanabe 1961). Watanabe’s perspective likely stemmed from his observations of the morphology of var. *brevistipulata* and var. *acuminata* in southwestern Hokkaido, as indicated by the distribution map in his paper. In southwestern Hokkaido, plants corresponding to var. *brevistipulata* and var. *acuminata* occur, but the length of the stem between the lowest leaf and the leaf immediately above it (*i.e.*, first internode length) overlaps in distribution, making it challenging to differentiate them based on Nakai’s criteria (Igashiri 1996, Hosokawa 2004). Hence, Watanabe, who likely observed the morphological variation of *V. brevistipulata s.l.* in southwestern Hokkaido, may have regarded var. *acuminata* as merely a form of *V. brevistipulata*. Watanabe’s treatment of ‘var. *acuminata*’ as a forma was adopted in subsequent monographs of *Viola* in Japan (Igari 2016, Hashimoto 1967, Hama 2002) and may have influenced the treatment of *V. brevistipulata*

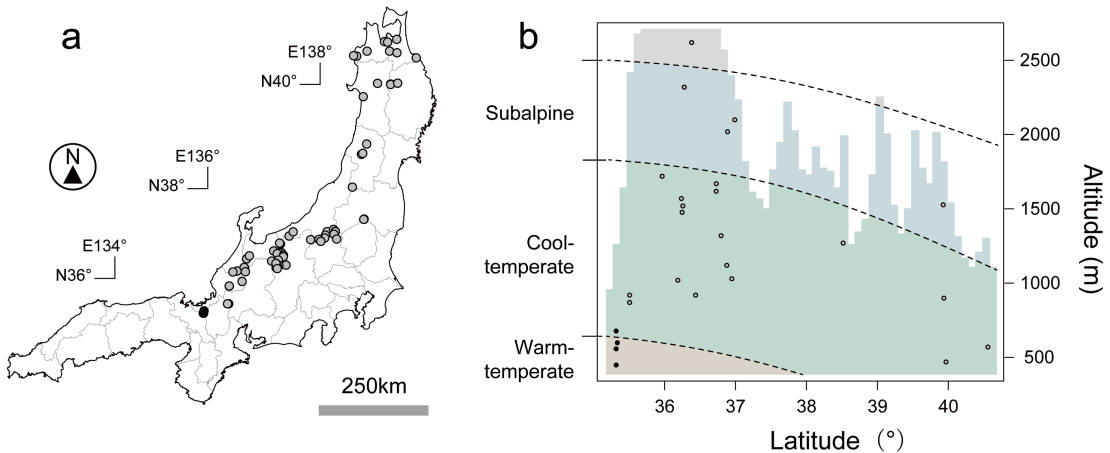


FIG. 5. **a**, Geographic distribution of *Viola brevistipulata* var. *acuminata* on Honshu Island, Japan. Black circles indicate populations in Ashiu and adjacent area. **b**, Elevational distribution of var. *acuminata* plotted against latitude. Climate zones are shown by different colors. Note that number of records used in panels **a** & **b** differs, as some voucher specimens lacked elevational information

s.l. in the Kinki region by Gen Murata.

In comparison, var. *acuminata* and var. *brevistipulata* in Honshu exhibit differentiation in terms of elevations, with the former tending to be at higher elevations (Igari 2016, Kudo 2010). A study comparing the var. *acuminata*-type population at high elevations on Mt. Gassan in Yamagata Prefecture (1,200–1,500 m above sea level) and the var. *brevistipulata*-type population at low elevation altitude (< 600 m a.s.l.) reported that the majority (83%) of individuals in the high-elevation population had a 0 mm-long first internode, and the leaf blade ratio tended to be higher than in the low elevation population. Although there was a certain degree of morphological differentiation between leaf blade shape, such as narrower blade in var. *acuminata* compared to the lowland var. *brevistipulata* type (Kudo 2010), the leaf blade length/width ratio of the var. *acuminata* type ranged from 0.5 to 1.0, overlapping with that of the var. *brevistipulata* type (0.65 to 1.25) (Kudo 2010). In more southerly regions, the morphological differentiation in leaf blade shape between var. *acuminata* and var. *brevistipulata* becomes clearer, and in the Kinki region, the two groups can be distinguished by the ratio index alone, as represented in Fig. 2. A comparison of morphological characteristics in different regions reveals that there is geographical variation in the

leaf shape in var. *acuminata*, with the two varieties being clearly distinct in the Chubu region (in the type locality of var. *acuminata*) and westwards, whereas in Hokkaido and eastern Japan, var. *acuminata* tends to exhibit a shape similar to var. *brevistipulata*.

In this study, the phylogenetic analysis demonstrated that var. *acuminata* and var. *brevistipulata*, despite their overlapping distributions, were distinctly clustered into separate groups (Fig. 3), suggesting that, at least in Honshu, they maintain genetic coherence, possibly due to some reproductive isolation barriers that reduce opportunities for gene exchange. Therefore, var. *acuminata* plausibly deserves recognition at a rank higher than variety. However, definitive conclusions regarding the taxonomy of var. *acuminata* must await an evaluation of the genetic variations of plants in Hokkaido, which were not included in our phylogenetic analysis. Our phylogenetic analysis showed that var. *minor* was embedded in the var. *brevistipulata* populations, suggesting that its taxonomic treatment may require further investigation. Future research to reassess its taxonomy should therefore encompass a comprehensive analysis of the phylogeny, morphology, and reproductive characteristics involving all intra-specific taxa of *Viola brevistipulata s.l.* not covered in this study.

Distribution of two varieties in Kinki region

Despite the presence of two morphological groups of *V. brevistipulata* s.l. in the Kinki region, there have been differing views on the classification of the populations of *V. brevistipulata* s.l. in Kyoto. Murata (2004) did not distinguish between var. *acuminata* and var. *brevistipulata*, considering the plants in Kyoto to be variety *brevistipulata*. In contrast, the Japan Violet Society (2020) accepted the presence of both var. *acuminata* and var. *brevistipulata* in Kyoto Prefecture without providing specific locality or voucher specimen information. Our phylogenetic analysis (Fig. 3) determined the plants of *V. brevistipulata* s.l. in Ashiu Forest to be var. *acuminata*, supporting the Japan Violet Society's (2020) assertion. However, despite our thorough survey of Ashiu Forest, the sole native habitat of *V. brevistipulata* s.l. in Kyoto Prefecture, all plants observed exhibited lanceolate leaf blades, indicating the exclusive distribution of var. *acuminata* in the area. Notably, the characteristic of 'leaves arranged as a whole', typically associated with var. *acuminata*, was not consistently observed in the Ashiu population, with some individuals displaying a first internode of 1.0 cm or more (Fig. 2). For example, specimens of var. *acuminata* in the Kyoto University Museum (KYO) included individuals with varying internode lengths, presumably collected from the same patch (e.g., *G. Koidzumi* s.n. May 1922, *S. Okamoto* s.n., May 1936). Those plants, if internode length were considered a defining feature of var. *acuminata*, might be classified as var. *brevistipulata*. Hence, it is possible that the Japan Violet Society's (2020) report of var. *brevistipulata* in Kyoto Prefecture was based on misidentification of var. *acuminata* with long internodes.

Survival of var. acuminata populations at low altitudes in the southern distribution limit

Based on the results of our phylogenetic analysis (Fig. 3), it is evident that the distribution of var. *acuminata* in Honshu extends from its northern limit in Aomori Prefecture to Ashiu Forest in Kyoto Prefecture at the southwestern end of its range (Fig. 5a, Appendix 1). In the

Tohoku and Chubu regions, var. *acuminata* is often in small clusters on stream slopes in *Fagus crenata* Blume and *Betula ermanii* Cham. forests, as well as at the edges of subalpine shrub and herbaceous vegetation, displaying a wide distribution across the cool temperate and subalpine zones in terms of range of elevation. The distribution of var. *acuminata* tends to increase in elevation at lower latitudes, with a distribution range of 1,000–2,000 m in the central mountains (Fig. 5b). However, in Ashiu Forest, var. *acuminata* is at elevations ranging from 429 m to 658 m, which is unusually low for the latitude of this area (Fig. 5b). The habitat is also unique, as it grows in the rocky terrain in the mountain stream belt, a characteristic not observed in other regions. The ability of var. *acuminata*, which typically thrives in cooler climates, to survive in the warm temperate zone of Kyoto Prefecture may be attributed to the challenging conditions in the riverside habitats that are frequently disturbed by floods and do not allow the invasion of competitive trees and tall herbs. This environment may have allowed relict plants such as var. *acuminata* to persist without being outcompeted (Sakaguchi 2023, Fukumoto 2021). However, even though these plants have not been eliminated by interspecific competition, they remain vulnerable to disturbance by water flowing in the stream zone. In the Yura River headwaters, populations of var. *acuminata* are not significantly affected by flood disturbance during the rainy season and subsequent typhoons, as they exhibit a life cycle as a spring plant. They flower from late April to early May and then go dormant before the onset of early summer. Such life history characteristics may also contribute to the maintenance of var. *acuminata* at low elevations at the southern limit of its distribution, where climatic conditions are typically less favorable.

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APPENDIX 1. Specimens of *Viola brevistipulata* var. *acuminata* in Kinki.

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- Kyoto Pref.**: Ashiu, Nantan, 20–25 May 1911, *G. Koidzumi s.n.* [*fr.*] (KYO!); same locality, 13 May *S. Okamoto s.n.* [*fl.*] (KYO!), 25 Apr. 1992, *S. Yasuda 201* [*fl.*] (KYO!), 20 Apr. 2022, *S. Sakaguchi, J. Yu, K. Masuda, S. Fukumoto SS220420A* [*fl.*] (KYO 00026890!), *S. Sakaguchi, J. Yu, K. Masuda, S. Fukumoto SS220420B* [*fl.*] (KYO 00026891!). —**Shiga Pref.**: Mt. Sanjo, Takashima, 27 May 1928 *C. Watanabe s.n.* (KYO!), Onyu-dani, Kutsuki-mura, Takashima 5 May 2023 *T. Fujimura SS230505* [KYO 00026998!], Ibuki-cho, Sakata, 11 May 1991 *G. Murata 70175* [*fl.*] (KYO!)
