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New Species of *Thalictrum* (Ranunculaceae) from Okinawa Island in Japan and Its Phylogenetic Implications

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Abstract

This study aims to compare *Thalictrum* (Ranunculaceae) plant species, tentatively identified as *T. minus,* from the Yambaru region of Okinawa Island, Japan, with closely related species using morphometric and molecular phylogenetic analyses. Morphological observations revealed that the unique plants from Okinawa Island had clavate filaments and thickened roots. These traits differed significantly from those of typical *T. minus*, which have filamentous filaments and slender roots. Molecular phylogenetic analyses revealed that the unique plant on Okinawa Island is sister to *T. urbaini*, endemic to Taiwan, and formed a separate clade from those containing *T. minus*. Based on current morphological comparisons and molecular phylogenetic analyses, the unique plant on Okinawa Island was concluded to be a previously undescribed species, newly named *Thalictrum yambaruense*. Furthermore, our molecular phylogenetic analyses of *Thalictrum* in Japan and Taiwan revealed that *T. tuberiferum* var. *yakusimense*, endemic to Yakushima Island, Japan, is phylogenetically distinct from *T. tuberiferum* var. *tuberiferum*. Therefore, we propose elevating the taxonomic status of *T. tuberiferum* var. *yakusimense* to the species level as *T. yakusimense*.

Key words: meadow rue, MIG-seq, Ryukyu Archipelago, Yakushima Island, Yambaru National Park

Introduction

The genus *Thalictrum* Linnaeus (1753: 545), commonly called meadow rue, includes approximately 120–200 species, primarily found in temperate regions worldwide (Park & Festerling 1997). In Japan, *Thalictrum* is divided into the following sections: *Tripterium* Candolle (1817: 169), *Physocarpum* Candolle (1817: 171), *Baicalensis* (Tamura) Emura (1972: 107), *Thalictrum*, *Purpurea* (Tamura) Emura (1972: 125), *Erythrandra* B.Boivin (1944: 391), and *Actaeifolia* (Tamura) Emura (1972: 126) (Kadota & Nishikawa 2006).

Taxonomic challenges in *Thalictrum* species largely stem from difficulties in classification based on morphological traits (Park & Festerling 1997). These challenges arise due to several factors, such as extensive morphological variability

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within species and reliance on subtle and often inconsistent traits, such as the persistence of sepals (Kadota 2016). Consequently, taxonomic interpretations of certain species vary among taxonomists. For example, *T. yakusimense* Koidzumi (1917: 91) is recognized as an independent species in some literature (e.g. Tamura, 1953), whereas in other sources it is treated as a variety of *T. tuberiferum* Maximowicz (1877: 227), namely, *T. tuberiferum* var. *yakusimense* (Koidz.) Emura (1972: 103) (e.g. Kadota, 2016). Similarly, *T. watanabei* Yatabe (1892: 307) is sometimes regarded as an independent species (Tamura, 1953) and, at other times, treated as a variety of *T. filamentosum* Maximowicz (1859: 13), that is, *T. filamentosum* var. *watanabei* (Yatabe) Kitam. in Kitamura & Murata (1962: 203) (Kitamura & Murata, 1961). Therefore, comprehensive phylogenetic analyses using genetic markers are crucial for the accurate classification of *Thalictrum* species.

One of the most complex taxonomic challenges in *Thalicturum* is *T. minus* Linnaeus (1753: 546), commonly referred to as lesser meadow-rue. This perennial herb is found in diverse habitats, including sunny meadows, forest margins, and rocky areas, ranging from subalpine regions to coastal zones throughout Eurasia (Kadota 2016). Five varieties of this species have been identified in Japan (Kadota & Nishikawa 2006). Its extensive morphological variation has led to ongoing taxonomic uncertainty.

Yokota *et al*. (1997) report a population of *T. minus* in the Yambaru region of northern Okinawa Island, Japan (Fig. 1). This population was found growing on cliff tops, where water drips down, and consisting of a small number of individuals (Yokota *et al*. 1997). This environment is an unusual habitat for *T. minus*. Additionally, the individuals in this population are significantly smaller than those in other regions (Yokota *et al*. 1997). These observations suggest that the taxonomic classification of this population warrants reconsideration.

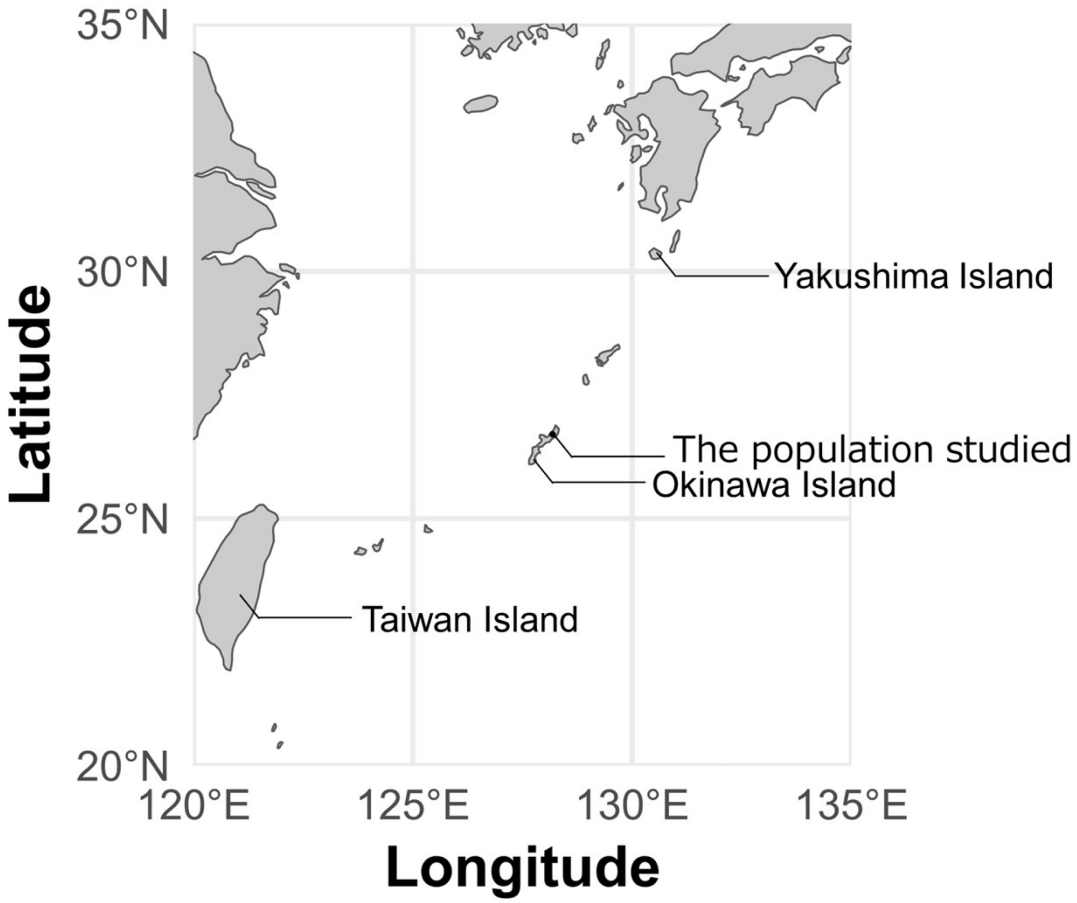

FIGURE 1. Map of Okinawa Island and its surrounding area, with a closed circle indicating the location of the studied population on Okinawa Island.

Our preliminary survey revealed that the unique plants in Yambaru exhibit thickened roots and clavate filaments, traits not typical of Sect. *Thalictrum* (including *T. minus*) but rather characteristic of Sect. *Physocarpum,* which includes *T. watanabei* and *T. urbaini* Hayata (1911: 25). Therefore, this study aims to clarify the taxonomic status of the unique plant on Okinawa Island by comparing it with other *Thalictrum* species in the Japanese Archipelago and surrounding

regions. We specifically focus on the phylogenetic relationships between the focal species and its congeners in Taiwan, an area adjacent to Okinawa Island.

This study aims to clarify the taxonomic uncertainties surrounding *Thalictrum* species on Okinawa Island. Resolving these uncertainties is crucial for understanding the evolutionary relationships within the genus and for guiding conservation strategies for populations with potential ecological or phylogenetic significance. This study seeks to evaluate whether the unique traits of the Yambaru population indicate an unrecognized lineage or reflect adaptive divergence within *T. minus*. Through this work, we aim to refine the taxonomic framework of *Thalictrum* in Okinawa and the surrounding region and contribute to a broader understanding of its evolutionary diversity in the region.

Material and methods

Sample collection for morphological observation

We conducted comparative observations of the floral and root morphologies by visually inspecting the unique plant on Okinawa Island and typical *T. minus* populations collected from natural populations outside Okinawa Prefecture, as well as specimens deposited in the Tohoku University Herbarium (TUS). Additionally, we compared the morphology of the plant with that of *T. watanabei* and *T. urbaini*, which shared similar morphological traits with the unique Okinawa Island plant based on our preliminary observations. Detailed morphological characters of the unique Okinawa plants and *T. urbaini* were carefully documented using high-resolution digital photographs (Figs. 2–3), providing visual evidence for our observations.

For related species that could not be collected in the field, comparative analyses were based on literature, specifically Kadota (2016) and Wang (2018). Ecological photographs and specimen images were referenced from the Plant Science Data Center (https://www.plantplus.cn/) and the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/).

Sample collections for molecular phylogenetics

We collected seven individuals from the unique plant population on Okinawa Island for molecular phylogenetic analyses. We also sampled additional *Thalictrum* species from Japan and Taiwan. Mature leaf samples from each individual were collected, dried with silica gel, and stored at a temperature of around 25°C until DNA extraction. Voucher specimens were deposited in the herbarium at the Botanical Gardens, Tohoku University (Appendix 1).

DNA extraction and Sanger sequencings of chloroplast DNA (cpDNA) and nucleotide ribosomal DNA (nrDNA)

Total DNA was extracted from dried leaves using a modified CTAB method (Doyle & Doyle 1987). An intron of *rpl16* in chloroplast DNA (cpDNA) was amplified through PCR with primers rpL16F71 (GCT ATG CTT AGT GTG TGA CTC GTT G) and rpL16R1516 (CCC TTC ATT CTT CCT CTA TGT TG) (Shaw & Small 2005). Nuclear ribosomal DNA (nrDNA) was also amplified using PCR using primers ITS5 (GGA AGT AAA AGT CGT AAC AAG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (White *et al*. 1990). All PCR amplification reactions were conducted in 10-μL volumes, containing 7.9 μL double-distilled water, 0.1 μL Taq polymerase (Takara Bio, Kusatsu, Japan), 1.0 μL 10 × PCR buffer (15 mM $MgCl_2$), 0.2 μL $MgCl_2$ (25 mM), 0.2 μL dNTPs (10 mM), 0.15 μM of each primer (10 μM), and 0.3 μL extracted DNA solution. Thermal cycling for both amplifications was initiated at 95°C for 4 min, followed by 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 30 s. The reaction concluded with a final extension at 72°C for 5 min. The PCR products were purified with ExoSAP-IT Express (Thermo Fisher Scientific, Waltham, MA, USA) and diluted to 100 μL using deionized water. Cycle sequencing was conducted in 10μL volumes, consisting of 7.7 μL purified PCR products, 0.2 μM of each primer, 1.9 μL of 5 × sequencing buffer, and 0.2 μL of BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). Thermal cycling began at 96°C for 1 min, followed by 30 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min, concluding with a final extension at 60°C for 1 min. Sanger sequencing was performed using an ABI Prism 3100 or 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) following the instructions of the manufacturer.

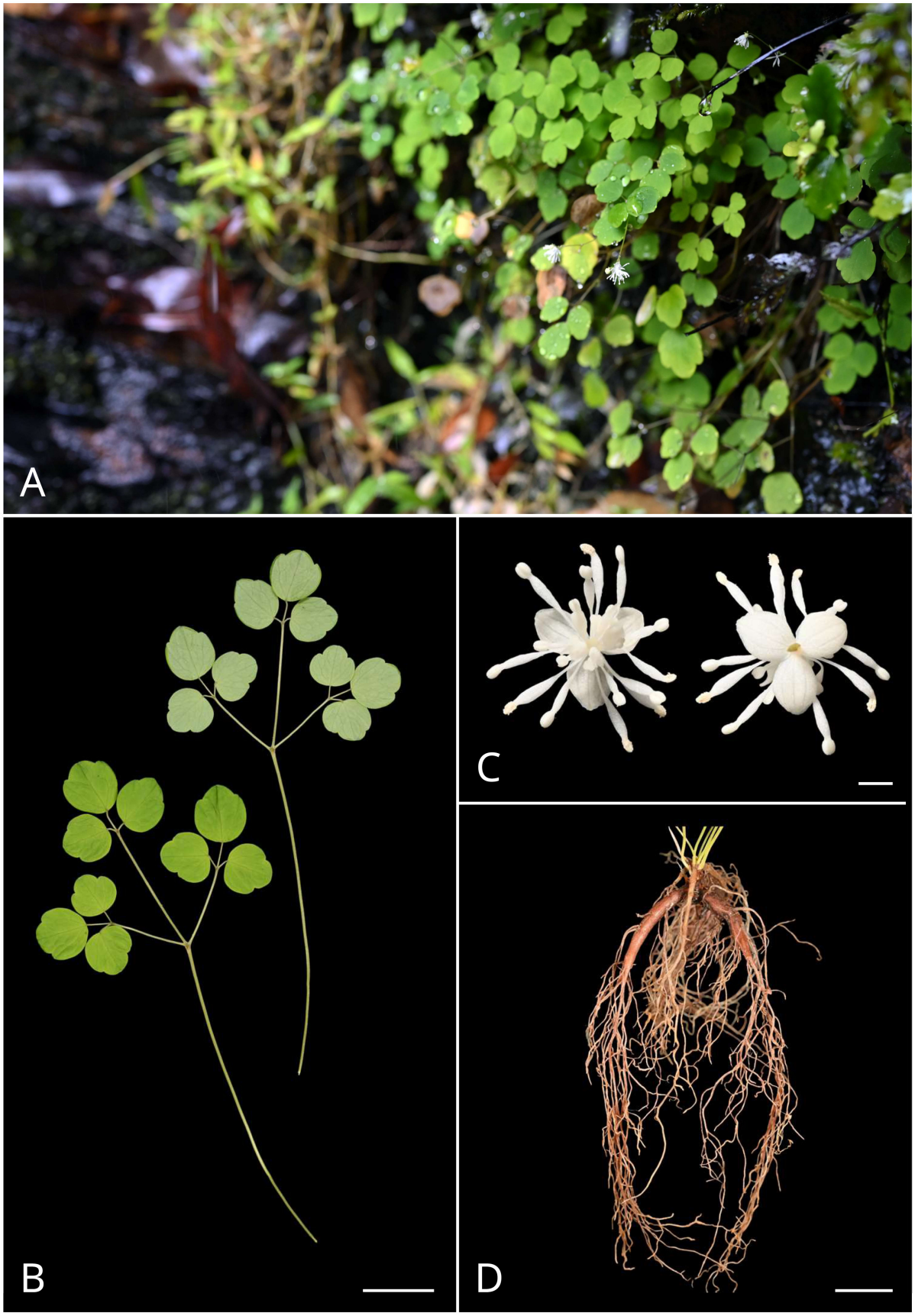

FIGURE 2. *Thalictrum yambaruense nov.* from Okinawa Island. A, Habit; B, Leaves; C, Flowers; D, Tuberous roots. B & D: scale bar = 1 cm; C: scale bar = 1 mm. [A. Wild individuals on Okinawa Island. B–D from *Takuro Ito 7857*. All photos from Takuro Ito].

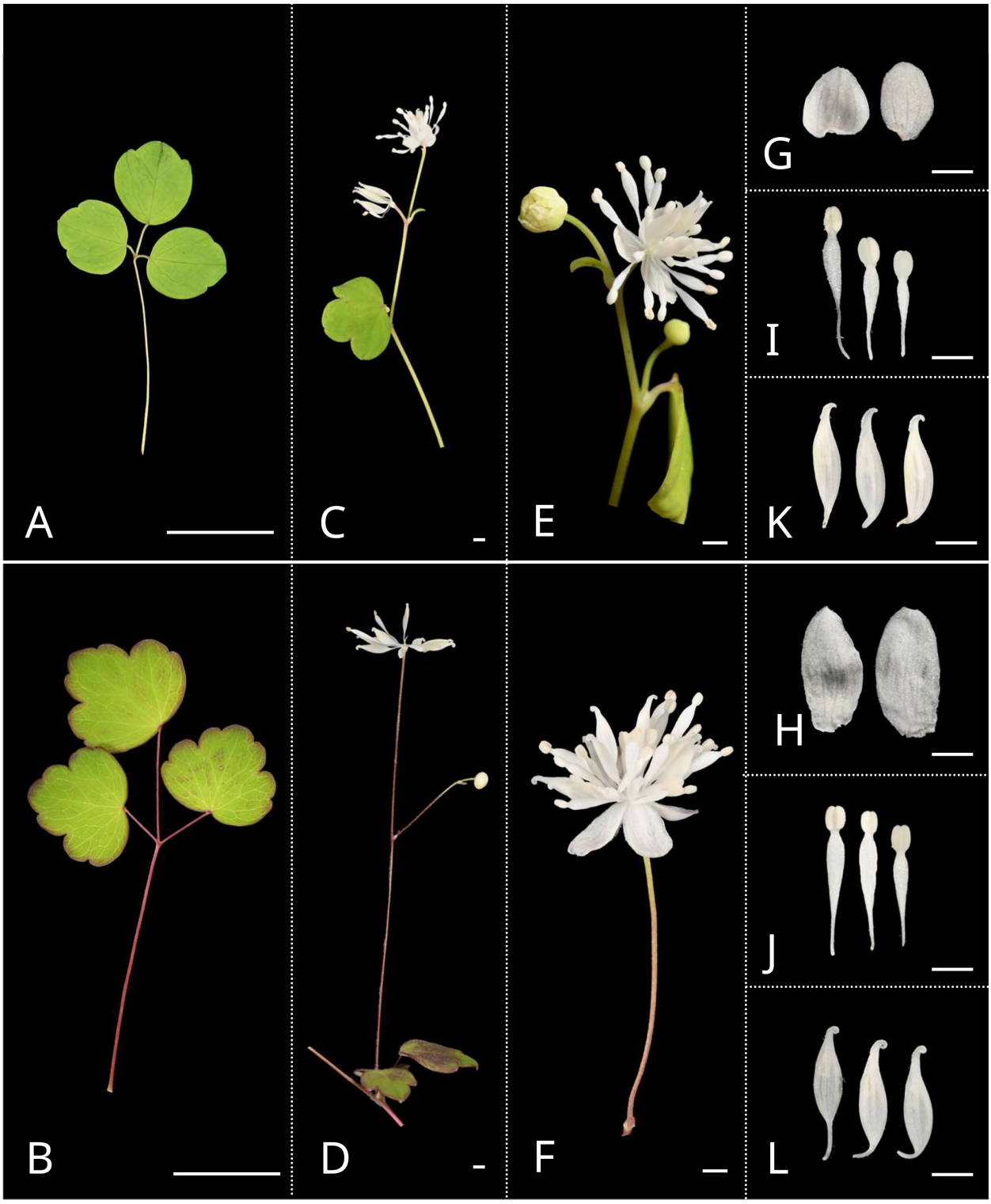

FIGURE 3. Morphological comparisons between *T. yambaruense nov.* from Okinawa Island (A, C, E, G, I, & K) and *T. urbaini* (B, D, F, H, J, & L). A & B: Leaflets; C–F: Inflorescence; G & H: Sepals; I & J: Stamens; K & L: Carpels. A& B: scale bar = 1 cm; C–L: scale bar = 1 mm. [A, C, E, G, I & K from *Takuro Ito 7857*; B, D, F, H, J & L from *Takuro Ito 8947*. All photos from Takuro Ito].

High-throughput genomic analysis using the MIG-seq method

To analyze phylogenetic relationships more accurately, we obtained single nucleotide polymorphisms (SNPs) using Multiplexed ISSR Genotyping by sequencing (MIG-seq) with a next-generation sequencer (Suyama & Matsuki 2015). The species from Sect. *Physocarpum* in Japan and Taiwan were used in this analysis. MIG-seq libraries were

constructed following the protocols of Suyama and Matsuki (2015) and Suyama *et al*. (2022). In the first-round PCR (1st PCR), primers targeting SSR regions, developed by Suyama and Matsuki (2015), were used. The first PCR conditions included an initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 38°C for 1 min, and extension at 72°C for 1 min, with a final extension step at 72°C for 10 min. In the second-round PCR (2nd PCR), adapter and index sequences were added to the purified 1st PCR products. The second PCR was conducted for 12 cycles with the following conditions: 98°C for 10 s, 54°C for 15 s, and 68°C for 1 min. The second PCR products were purified, and fragments ranging from 300–800 bp were isolated using a BluePippin system (Sage Science, Beverly, MA, USA). The final DNA concentrations were measured using a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and a 4200 TapeStation (Agilent, Santa Clara, CA, USA).

The multiplexed library was sequenced on an Illumina MiSeq platform with a MiSeq Reagent Kit 3 (150 cycles; Illumina, San Diego, CA, USA) using the dark cycle option. Following the protocol outlined by Suyama and Matsuki (2015), the first 17 bases of Read 1 and the first three bases of Read 2 were excluded. This resulted in sequence reads of 80 bp (Read 1) and 94 bp (Read 2), respectively.

For quality control, the first 14 bases of Read 2 were trimmed using fastx_trimmer in the FASTX-Toolkit v.0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/). The resulting reads were processed with Trimmomatic v0.39 (Bolger *et al.* 2014) to remove adapter sequences. The sliding window size was set to four and reads with an average quality value < 15 were discarded. Additionally, the MINLEN parameter was used to exclude reads shorter than 74 bp. The filtered reads were aligned to the reference genome of *T. thalictroides* (L.) A.J.Eames & B.Boivin in Boivin (1957: 319) (GCA_013358455.1) using BWA-MEM2 v.2.2.1 (Vasimuddin *et al.* 2019). After obtaining SAM files, they were compressed to *.bam format. They were then sorted and indexed using SAMtools v.1.6 (Li *et al.* 2009). Sorted BAM files were used for SNP detection with Stacks 2.60 (Catchen *et al*. 2013; Rochette *et al*. 2019). The population program was set with the following parameters: a minimum percentage of individuals across populations required to process a locus (-R) at 0.4 and a maximum observed heterozygosity (-max-obs-het) at 0.6 to exclude the influence of paralogous regions. Furthermore, linkage sites in the genomic data were removed using PLINK v1.9 software (Chang *et al.,* 2015) with the parameters (-indep-pairwise 50 5 0.4).

Phylogenetic analyses

For the phylogenetic analysis of cpDNA and nrDNA, we used *Leptopyrum fumarioides* Reichenbach (1828: 192) (Ranunculaceae) as the outgroup taxon for *Thalictrum* populations from Japan, Taiwan, and China, as it has been identified as a sister taxon to *Thalictrum* (Wang *et al*. 2009, Soza *et al.* 2013, Michimoto *et al.* 2022). Sequence data for the *rpl16* intron and nrITS regions of *L. fumarioides* were retrieved from the DDBJ/ENA/NCBI database (accession no. JF742070 and JF742128, respectively).

All cpDNA and nrDNA sequences from this study, along with those of the outgroup, were aligned using MAFFT (Katoh & Standley 2013) and trimmed with trimAl version 1.2 (Capella-Gutiérrez *et al.* 2009). The base substitution model was determined using Modeltest-NG 0.1.6 (Darriba *et al.* 2020) based on the Bayesian Information Criterion (BIC). Maximum likelihood phylogenetic trees were generated under the selected model using RAxML-NG 0.9.0 (Kozlov *et al.* 2019), with 1,000 bootstrap replicates to assess the reliability of the trees. The ML tree was visualized using FigTree v1.4.3 (Rambaut 2017).

For species in Sect, *Physocarpum,* we reconstructed the maximum likelihood phylogenetic tree using SNP data obtained by MIG-seq. Outgroup taxa included *T. aquilegiifolium* Linnaeus (1753: 547) var. *sibiricum* Regel & Tiling (1859: 23) (Sect. *Tripterium*), *T. alpinum* Linnaeus (1753: 545) (Sect. *Thalictrum*), and *T. foetidum* Linnaeus (1753: 545) (Sect. *Thalictrum*). These taxa belong to a sister clade of the monophyletic group containing Sect. *Physocarpum*, making them suitable for rooting the tree (Soza *et al.* 2013, Ling *et al.* 2024). To ensure accuracy, we used the ascbias.py script (Martin 2018) to detect and remove invariant sites from the MIG-seq alignment dataset. Additionally, we applied Stamatakis bias correction simultaneously, based on the number of bases at each invariant site, using the same script. The base substitution model, as for cpDNA and nrDNA, was selected based on the Bayesian Information Criterion (BIC) using Modeltest-NG 0.1.6 (Darriba *et al.* 2020). Maximum likelihood phylogenetic trees were then estimated under this model using RAxML-NG 0.9.0 (Kozlov *et al.* 2019), with 1,000 bootstrap replicates to assess the tree reliability. The ML tree was visualized using FigTree v.1.4.3 (Rambaut 2017).

Results

Morphological distinctness of Thalictrum *species from Okinawa Island*

The unique plants on Okinawa Island exhibited morphological characteristics consistent with those of Sect. *Physocarpum*, including (1) clavate filaments (Fig. 2C) and (2) tuberous roots (Fig. 2D). These features contrast with the fibrous roots and filiform filaments commonly found in *T. minus* (Emura 1972, Kadota 2016). Morphologically, the unique plant resembles *T. watanabei* and *T. urbaini*. The leaflet margins and petioles of the unique plant are green (Fig. 3A), while those of *T. urbaini* are purple-brown (Fig. 3B). The pedicel of the unique plant is 5 mm or less (Fig. 3C), significantly shorter than those of *T. watanabei* (10–15 mm; Kadota 2016) and *T. urbaini* (approximately 10 mm; Fig. 3D) (Table 1). Additionally, the sepals of the unique plant differ in size from those of *T. urbaini.* The sepals of the unique plant measure approximately 2 × 1 mm (Fig. 3G), while those of *T. urbaini* are 3–4 × 1.5–2 mm (Fig. 3H) (Wang 2018). Furthermore, the carpels of the unique plant are straight or slightly bent (Fig. 3K), while those of *T. urbaini* are strongly bent (Fig. 3L) (Table 1).

TABLE 1. Comparison of morphological features of the Yambaru plant in Okinawa with *T. urbaini* and *T. watanabei.* Asterisks are indicated based on the literature: Kadota & Nishikawa (2006) *; Wang (2018) **.

	Yambaru plant (*Thalictrum yambaruense*)	*T. urbaini*	*T. watanabei*
Pedicel length	< 5 mm	ca. 10 mm	10–15 mm*
Sepal (length × width)	ca. 2 × 1 mm	3–4 × 1.5–2 mm**	2–3 × ca. 2mm*
Pistil form	straight or slightly bend	strongly bend	straight*
Color of leaf margins and petioles	Green	purple-brown	Green

Phylogenetic tree of Thalictrum based on the chloroplast rpl16 intron and internal transcribed spacer (ITS) region of nuclear ribosomal DNA

The cpDNA and nrDNA sequences from this study were deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers LC807741–LC807763, LC807816–LC807844, and LC823724–LC823729. The DDBJ has been in operation since 1983.

The nucleotide substitution model F81+G4, selected by Modeltest-NG 0.1.6 (Darriba *et al.* 2020), was used to construct the maximum likelihood phylogenetic tree for the chloroplast *rpl16* intron sequence data (723 bp). This analysis showed the following clades: Clade I (Bootstrap Value [BSV] = 72 and Clade II (BSV = 71) (Fig. 4A). Clade II exclusively contained the Yambaru plants and species of Sect. *Physocarpum*, while Clade I included other sections of *Thalictrum*. Within Clade I, Sect. *Thalictrum* was not monophyletic and formed a clade with Sect. *Erythrandra* and Sect. *Purpurea* (BSV = 70). The unique plants from Okinawa Island formed a monophyletic clade with other species of Sect. *Physocarpum* (Clade II), not with those of Sect. *Thalictrum*. In Clade II, *T. watanabei, T. microspermum* Ohwi (1936: 183)*,* and *T. toyamae* Hatusima & Ohwi (1943: 293) formed a clade (BSV = 64), while other species of Sect. *Physocarpum* formed another clade (BSV = 81). The unique plants formed a monophyletic clade (BSV = 80) and were sister to a clade containing Taiwanese species, *T. urbaini* and *T. rubescens* Ohwi (1933: 156) (BSV = 93).

The TrN+G4 nucleotide substitution model was used to construct the maximum likelihood phylogenetic tree based on the 412 bp ITS region sequences. Similar to the ML tree based on *rpl16* intron sequences, the following clades were identified: Clade I (BSV = 100) and Clade II (BSV = 61) (Fig. 4B). Clade II included the unique plants from Okinawa Island and species of Sect. *Physocarpum*, while Clade I contained species from other sections, consistent with the *rpl16* intron tree. In Clade I, *Thalictrum* species native to Taiwan exhibited basal divergence and Sect. *Thalictrum*, Sect. *Erythrandra*, Sect. *Tripterium*, and Sect. *Purpurea* formed a clade (BSV = 75). Sect. *Thalictrum* was not monophyletic. As in the *rpl16* intron tree, the unique plants did not group with Sect. *Thalictrum* species but instead were included in the clade with Sect. *Physocarpum* species (Clade II). Within Clade II, the unique plants did not form a monophyletic group, instead forming a subclade with *T. urbaini* var. *majus* Shimizu (1963: 27) (BSV = 81). *T. watanabei, T. microspermum,* and *T. toyamae* also formed a subclade (BSV = 83) similar to that of the *rpl16* intron tree.

Phylogenetic tree of Sect. Physocarpum based on MIG-seq SNPs

The sequence data obtained by MIG-seq in this study were deposited in the DDBJ under accession numbers DRR581707–DRR581751 (DDBJ has been operational since 1983).

After removing primer sequences and low-quality reads, 16,817,276 reads (343,210 ± 66,583 reads per sample) were retained from an initial 18,777,802 raw reads (375,556 ± 74,435 reads per sample). The phylogenetic analysis of the entire Sect. *Physocarpum* was based on 539 loci with a genotyping rate of 65.08% and 2,077 parsimoniously informative SNPs.

The TVMef+G4+ASC_LEWIS nucleotide substitution model was used to construct the maximum likelihood phylogenetic tree for Sect. *Physocarpum*. Consequently, two well-supported clades were identified (Clade I: BSV = 100, Clade II: BSV = 100) (Fig. 5).

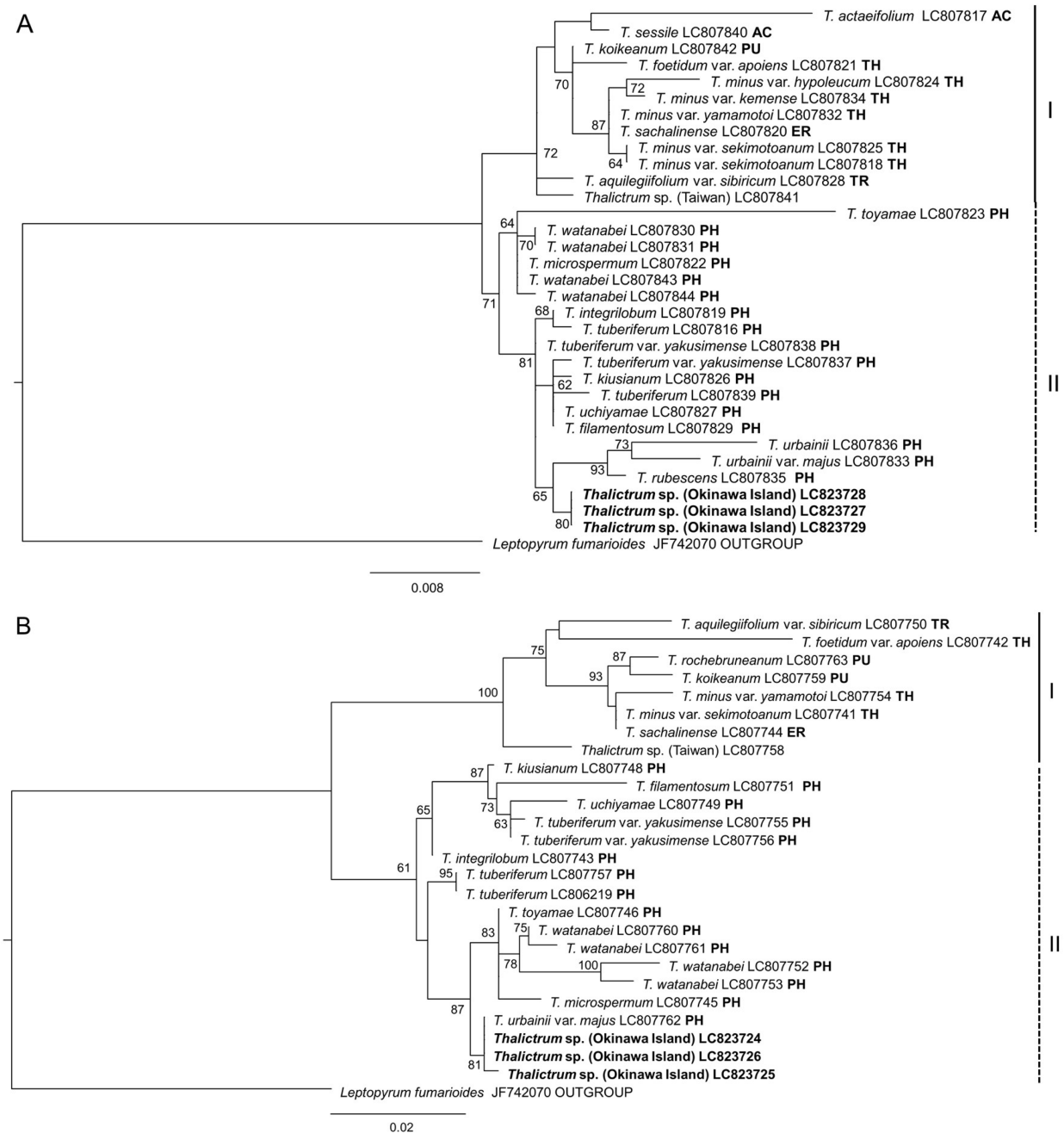

FIGURE 4. Phylogenetic relationships in the genus *Thalictrum* (Ranunculaceae) from Japan and Taiwan. **A**. ML tree based on the chloroplast *rpL16* intron region. **B**. ML tree based on the nuclear ITS. Likelihood bootstrap values > 60% are shown below the branches. **AC:** Sect. *Actaefolium*, **ER:** Sect. *Erythrandra*, **PH:** Sect. *Physocarpum*, **PU:** Sect. *Purpurea*, **TH:** Sect. *Thalictrum*, **TR:** Sect. *Tripterium*. Abbreviation: ITS, internal transcribed spacer region.

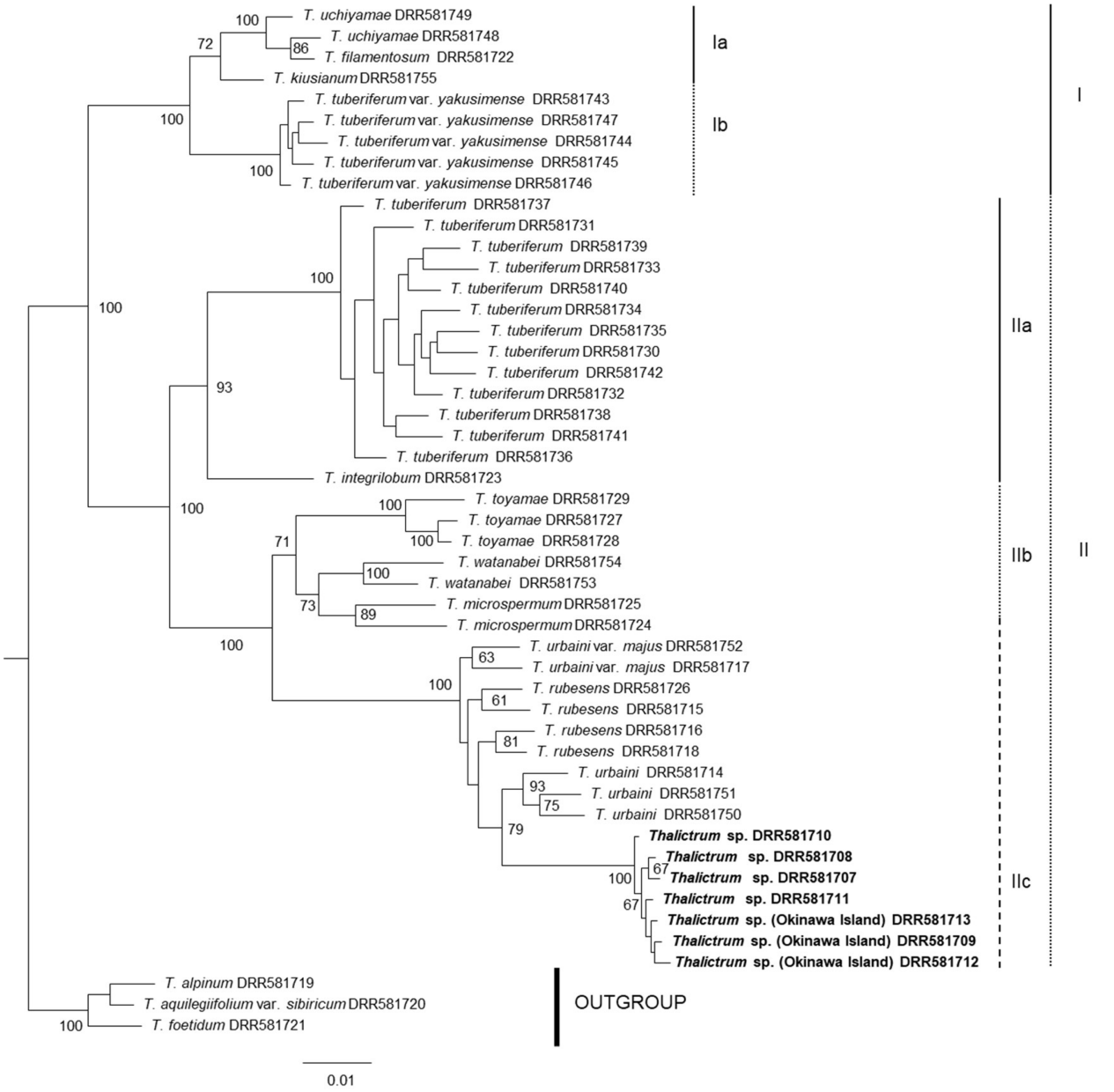

FIGURE 5. Phylogenetic tree of Sect. *Physocarpum* reconstructed from MIG-seq data based on 2077 SNPs (R = 0.4). Nodes with bootstrap values > 60% are not shown. Branch length represents the average number of substitutions per site.

In Clade I, a subclade (Clade Ia) comprised *T. uchiyamae* Nakai (1909: 15), *T. filamentosum*, and *T. kiusianum* Nakai (1928: 1) (BSV = 72), while another subclade (Clade Ib) included *T. tuberiferum* var. *yakusimense,* which was monophyletic (BSV = 100). In Clade Ia, *T. kiusianum* diverged first, followed by *T. uchiyamae,* which was paraphyletic and formed a clade with *T. filamentosum* (BSV = 100). In Clade Ib, *T. tuberiferum* var. *yakusimense* formed a monophyletic clade (BSV = 100). *T. tuberiferum* var. *yakusimense* and *T. tuberiferum* (sensu stricto) do not belong to the same clade (see below).

In Clade II, a basal divergence was observed in a clade consisting of *T. integrilobum* Maximowicz (1888: 477) and *T. tuberiferum* (sensu stricto) (Clade IIa) (BSV = 93). Clade IIb comprised *T. watanabei*, *T. microspermum*, and *T. toyamae* (BSV = 71). Clade IIc included Taiwanese *Thalictrum* species and the unique plant from Okinawa Island (BSV = 100). In Clade IIb, *T. toyamae* diverged basally, while *T. microspermum* and *T. watanabei* each formed monophyletic clades (BSV = 89, 100). In Clade IIc, *Thalictrum* species in Taiwan were paraphyletic, and the unique plants formed a monophyletic clade (BSV = 100). *T. urbaini* (sensu lato) was not monophyletic and grouped with *T. rubesens*.

Discussion

Taxonomic Reexamination of Thalictrum *on Okinawa Island*

In Japan, five varieties of the highly variable species *T. minus* are recognized (Kadota 2016). These varieties are found across a range of regions and habitats in Japan, from subalpine zones to coastal areas (Kadota 2016). However, the plants on Okinawa Island occupy a distinct environment: cliff faces beside a waterfall within natural forests, contrasting sharply with the typical habitats of *T. minus* (Yokota 1997, Kyushu Regional Forest Office 2000) (Fig. 2A).

Although *T. minus* is classified in Sect, *Thalictrum*, which is characterized by nearly sessile achenes and filiform filaments (Park & Festerling 1997), our morphological surveys identified distinct features in the unique plants on Okinawa Island, such as thick roots and clavate floral filaments, indicating they belong to Sect. *Physocarpum* (Figs. 2C & D). Phylogenetic analyses using nrITS and cpDNA sequences further supported this, placing the unique plants in a clade with species of Sect. *Physocarpum* is distinct from *T. minus* (Fig. 4).

The unique plants morphologically resemble *T. watanabei* and *T. urbaini*, both of which are members of Sect. *Physocarpum*. However, a key difference in pedicel length sets the unique plants apart. The pedicel of this unique plant is significantly shorter, measuring ≤ 5 mm, compared to 10–15 mm in *T. watanabei* and approximately 10 mm in *T. urbaini* (Table 1; Fig. 3B).

To further clarify the phylogenetic position of the unique plants, an additional phylogenetic analysis was conducted using SNPs derived from MIG-seq data for Sect. *Physocarpum* and the unique plants on Okinawa Island. The results showed that the unique plants are sisters to the *Thalictrum* species in Taiwan (Fig. 5). These multifaceted analyses revealed that the unique plants on Okinawa Island represent a distinct species, establishing their sister group relationship with certain congeners in Taiwan. Therefore, we describe it as a novel species, *Thalictrum yambaruense*.

Taxonomic status of Thalictrum tuberiferum *var.* yakusimense and its allies

Phylogenetic analyses using the MIG-seq method revealed that *T. tuberiferum* var. *yakusimense*, which is endemic to Yakushima Island, Japan (Fig. 1), formed a distinct genetic cluster, diverging from typical *T. tuberiferum* populations (Fig. 5). *T. tuberiferum* var. *yakusimense* belongs to a cluster that includes *T. uchiyamae*, *T. filamentosum*, and *T. kiusianum* (BSV = 100) (Fig. 5: Clade Ia).

The leaflets of *T. tuberiferum* (sensu stricto) morphologically range from narrowly ovate to widely ovate, or ovate-rhombic, measuring 1.5–8 cm long and 1–8 wide (Kadota & Nishikawa 2006). The achenes of *T. tuberiferum* (sensu stricto) are typically 4–5 mm long, and the filiform stipe measures 3–4 mm in length (Kadota & Nishikawa 2006, Kadota 2016). In contrast, *T. tuberiferum* var. *yakusimense* has suborbicular leaflets, 1–2 cm in length and width, with achenes measuring 3–4 mm in length and a stipe measuring 1–2 mm long (Kadota & Nishikawa 2006, Kadota 2016). Based on this strong morphological and genetic evidence, we propose elevating *T. tuberiferum* var. *yakusimense* to full species status as *T. yakusimense*.

Study Limitations

This study has a limitation that should be acknowledged. First, the sampling of *Thalictrum* species in Taiwan was limited, which may have influenced the observed phylogenetic relationships, such as the paraphyletic grouping of *T. urbaini* (sensu lato) with *T. rubesens*. A more extensive sampling across Taiwan's diverse habitats is necessary to comprehensively evaluate the taxonomic treatment of endemic species groups in the region. Future studies should incorporate increased sampling coverage to refine the taxonomic framework for *Thalictrum* species in Taiwan and adjacent regions.

Taxonomic treatment

Thalictrum yambaruense Michim., Yokota & S.Fujii, *sp. nov.*

***Diagnosis*:** Similar to *Thalictrum watanabei*, this species differs in having pedicels shorter than 5 mm (vs. 10–15 mm in *T. watanabei*). Sepals are approximately 2 × 1 mm (vs. 3–4 × 1.5–2 mm). Pistils are not bent or are slightly bent (vs. strongly bent (Table 1; Figs. 2 & 3).

***Holotype*:** JAPAN, Okinawa Prefecture: Okinawa Island, Kunigami-gun, Kunigami-son, Aha. 16 April 2022, A. Abe, M. Yokota, T. Ito, K. Michimoto, T. Nakasone, S. Higa & J. Shinjo 2095 (OCF).

***Description*:** Herbs, perennial, glabrous, up to 20 cm tall. Roots tuberous. Stems simple or few branched. Basal leaves 3–5; leaf blade 1- or 2-ternate; leaflets broadly ovate or orbicular, herbaceous, abaxially greenish white, 1–1.8 × 1–1.5 cm, apex 3-lobed; petiole 2–5cm. Inflorescences cymes with 2–15 flowers. Flowers 0.3–0.5 cm in diam.; pedicels less than 5 mm. Sepals 3–4, persistent, white, elliptic, ca. 2 × 1mm. Stamens white, 2–4mm; filament base filiform, apex oblanceolate, same as broad as anther. Carpels 5–10; style not bend or bend slightly, stigmas lanceolate. Achenes stipe ca. 0.5 mm, body fusiform, compressed, ca. 2 mm.

***Distribution and habitat*:** Known only from the type locality in the Yambaru region. The upper reaches of the river basin consist of well-preserved mountain streams in natural forest stands (Kyushu Regional Forest Office, 2000). The species grows on dripping cliff faces.

***Etymology*:** The specific epithet "yambaruense" refers to the type locality, the Yambaru region.

***Japanese common name*:** Yambaru-karamatsu (nov.).

***Paratypes*:** JAPAN, Okinawa Prefecture: Okinawa Island, Kunigami-gun, Kunigami-son, Aha. 16 April 2022, Takuro Ito 7857 (TUS). 29 August 1994, M. Yokota 10369 (RYU).

***Conservation status*:** *Thalictrum yambaruense* is known from a single population, exclusively located in the upper reaches of the Yambaru region in northern Okinawa Island (Yokota *et al.* 1997). It is classified as a critically endangered species (Category IA, CR), similar to *Thalictrum minus* (Okinawa Prefectural Department of Environmental Affairs 2018). The total number of mature plants or ramets is estimated to be fewer than 50, classifying it as a critically endangered species IA (CR) according to the IUCN Red List Categories and Criteria "D" (IUCN 2000). A water storage dam built in the lower reaches may have eradicated unknown populations downstream.

Key to the new *Thalictrum* species, with the related species of *T. filamentosum, T. tuberiferum,* and *T. watanabei*

1	crescent-shaped pistils; ellipse stigmas	
1a	opposite cauline leaf	*T. filamentosum*
1b	alternate cauline leaf	*T. tuberiferum*
2	spindle-shaped pistils; lanceolate stigmas	
2a	Pedicels 10–15 mm	*T. watanabei*
2b	Pedicels less than 5 mm	*T. yambaruense*

Thalictrum yakusimense Koidz. in Ic. Pl. Koisikavenses (Matsumura, ed.) 3: 91, t. 191 (1917). Holotype (designated by Koidzumi 1917): JAPAN, Kyushu, Yakushima Island (Ohsumi Province), no collection data, *Y. Yoshii s.n*. (TI!).

Thalictrum filamentosum Maxim. var. *yakusimense* (Koidz.) Tamura in Acta Pytotax. Geobot. 15: 85 (1953).

T. tuberiferum Maxim. var. *yakusimense* (Koidz.) Emura in J. Fac. Sci. Univ. Tokyo, sect. 3, Bot. 11(3): 103 (1972).

Note: Tamura (1953) classified *Thalictrum tuberiferum* and *T. yakusimense* as intraspecific taxa of *T. filamentosum.* Subsequently, Emura (1972) recognized *T. tuberiferum* as an independent species, separating it from *T. filamentosum* and reclassifying *T. tuberiferum* (sensu stricto) and *T. yakusimense* under *T. tuberiferum*. Phylogenetic analysis in this study showed that this taxon is genetically distinct from *T. tuberiferum* and *T. filamentosum*, leading us to classify it as *T. yakusimense*. To examine the morphology and validate the holotype of *T. tuberiferum* var. *yakusimense*, we studied specimens from Tohoku University (TUS), Kyoto University (KYO), and Tokyo University (TI). This specimen, previously unrecognized as the holotype, was validated and established as legitimate through our detailed examination.

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Author Contributions

KM, TI, and MM conceived and designed the study. KM, MY, AA, SF, DT, CCY, TI, and MM conducted the fieldwork and collected the data. KM and SK performed the laboratory analyses. KM and TI analyzed the data. KM wrote the initial draft of the manuscript. All authors read and approved the final manuscript.

Reference

Boivin, B. (1944) Contributions from the Gray Herbarium of Harvard University—No. clii. American Thalictra and their old world allies. *Rhodora* 46: 337–377.
https://doi.org/10.5962/p.336293

Boivin, B. (1957) Études Thalictrologiques III: Réduction du genre Anemonella Spach (Ranunculaceae). *Bulletin de la Société Royale de Botanique de Belgique* 89: 319–321. Available from: http://www.jstor.org/stable/20792255 (accessed 27 March 2025)

Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.
https://doi.org/10.1093/bioinformatics/btu170

Candolle, A. (1817) *Regni vegetabilis systema naturale, sive Ordines, genera et species plantarum secundum methodi naturalis normas digestarum et descriptarum*. Treuttel et Würtz, Paris, 564 pp.
https://doi.org/10.5962/bhl.title.59874

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25: 1972–1973.
https://doi.org/10.1093/bioinformatics/btp348

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A. & Cresko, W.A. (2013) Stacks: an analysis tool set for population genomics. *Molecular Ecology* 22: 3124–3140.
https://doi.org/10.1111/mec.12354

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M. & Lee, J.J. (2015) Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience* 4: s13742-015-0047–0048.
https://doi.org/10.1186/s13742-015-0047-8

Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B. & Flouri, T. (2020) ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37: 291–294.
https://doi.org/10.1093/molbev/msz189

DDBJ. (1983) DNA data of bank of Japan, National Institute of Genetics, Japan, Mishima. Available from: http://www.ddbj.nig.ac.jp/ (accessed 29 October 2024)

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Emura, K. (1972) Cytotaxonomical studies on the genus *Thalictrum*. *Journal of the Faculty of Science, University of Tokyo. Sect. 3, Botany* 11: 93–136.

Hatusima, S. & Ohwi, J. (1943) Two new plants from Mt. Kurokami, Prov. Hizen in Kiushiu. *Journal of Japanese Botany* 19: 293–294.

Hayata, B. (1911) *Icones plantarum formosanarum nec non et contributiones ad floram formosanam: or, Icones of the plants of Formosa, and materials for a flora of the island, based on a study of the collections of the Botanical survey of the Government of Formosa*. Bureau of Productive Industry, Government of Formosa, Taihoku, 265 pp.
https://doi.org/10.5962/bhl.title.18786

IUCN. (2020) The IUCN Red list of threatened species, version 2024.2. Available from: http://www.iucnredlist.org (accessed 4 December 2024)

Kadota, Y. & Nishikawa, T. (2006) Ranunculaceae. *In*: Iwatsuki, K., Boufford, D.E. & Ohba, H. (Eds.) *Flora of Japan*. Kodansha, Tokyo, pp. 258–341.

Kadota, Y. (2016) *Thalictrum*. *In*: Ohashi, H., Kadota, Y., Murata, J. & Yonekura, K. (Eds.) *Wild flowers of Japan*. Heibonsha, Tokyo, pp. 162–167. [In Japanese]

Katoh, K. & Standley, D.M. (2013) MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. https://doi.org/10.1093/molbev/mst010

Kitamura, S. & Murata, G. (1961) *Thalictrum* L. *In: Coloured illustrations of herbaceous plants of Japan II (Choripetalae)*. Hoikusha, Osaka, pp. 235–239. [In Japanese]

Kitamura, S. & Murata, G. (1962) New names and new conceptions adopted in our coloured illustrations of herbaceous plants of Japan II (Choripetalae). (in Japanese). *Acta Phytotaxonomica et Geobotanica* 20: 195–208. https://doi.org/10.18942/bunruichiri.KJ00002992778

Koidzumi, G. (1917) *Thalictrum yakusimense* Koidz. *In*: Matsumura, J. (Ed.) *Icones Plantarum Koisikavenses*. Maruzen-Kabushiki-Kaisha, Tokyo, pp. 91, t. 191.

Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019) RAxML-NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35: 4453–4455. https://doi.org/10.1093/bioinformatics/btz305

Kyushu Regional Forest Office. (2000) Report on the Basic Survey of Forest Environment in Northern Okinawa National Forests (Additional Survey). 13 pp. [In Japanese] Available from: https://www.rinya.maff.go.jp/j/kokuyu_rinya/kakusyu_siryo/pdf/00321_7_h11_all.pdf (accessed 9 May 2024)

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. & 1000 Genome Project Data Processing Subgroup. (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25: 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Ling, Y.-Y., Peng, H.-W., Lian, L., Erst, A.S., Xiang, K.-L. & Wang, W. (2024) Out of and in East Asia: Phylogeny, biogeography and diversification of *Thalictroideae* (Ranunculaceae) in the Northern Hemisphere. *Annals of Botany* 134 (7): 1251–1262. https://doi.org/10.1093/aob/mcae148

Linnaeus, C. (1753) *Species Plantarum 1*. Laurentii Salvii, Holmiae, 560 pp.

Martin, B.T. (2018) raxml_ascbias, GitHub, San Francisco (CA). Available from: https://github.com/btmartin721/raxml_ascbias/ (accessed 29 October 2024)

Maximowicz, C.J. (1859) *Primitiae florae Amurensis*. Buchdruckerei der kaiserlichen Akademie der Wissenschaften, St. Petersburg, 504 pp.

Maximowicz, C.J. (1877) Diagonoses plantarum novarum Japoniae et Mandshuriae. *Bulletin de l'Académie impériale des sciences de St.-Pétersbourg* 22: 209–264.

Maximowicz, C.J. (1888) Diagonoses plantarum novarum asiaticarum. VII. *Bulletin de l'Académie impériale des sciences de St.-Pétersbourg* 32: 477–629.

Michimoto, K., Ito, T. & Maki, M. (2022) The complete chloroplast genome sequence of *Thalictrum aquilegiifolium var. sibiricum* (Ranunculaceae). *Mitochondrial DNA Part B* 7: 1171–1173. https://doi.org/10.1080/23802359.2022.2088309

Nakai, T. (1909) Flora Koreana. *Journal of the College of Science, Imperial University of Tokyo* 26 (art. 1): 1–304.

Nakai, T. (1928) Notulæ ad plantas Japoniæ & Koreæ. XXXV. *The Botanical Magazine, Tokyo* 42: 451–479. https://doi.org/10.15281/jplantres1887.42.451

Nature Conservation Division, Department of Environmental Affairs, Okinawa Prefectural Government. (2018) Threatened Wildlife in Okinawa. 3rd ed. Japanese: (Fungi and Plants) -Red Data Okinawa-. Nature Conservation Division Department of Environmenta, Okinawa. Available from: https://www.pref.okinawa.jp/_res/projects/default_project/_page_/001/004/629/reddataokinawa.pdf (accessed 29 October 2024)

Ohwi, J. (1933) Symbolae ad Floram Asrae Orientalis, 9. *Acta Phytotaxonomica et Geobotanica* 2: 149–170.

Ohwi, J. (1936) Synbolae ad floram Asiae orientalis 14. *Acta Phytotaxonomica et Geobotanica* 5: 179–188.

Park, M.M. & Festerling, Jr. D. (1997) *Thalictrum Linnaeus*. *In*: Flora of North America editorial committee (Ed.) *Flora of North America: North of Mexico; Magnoliophyta: Magnoliidae and Hamamelidae*. Vol. 3. Oxford University Press, New York, Oxford, pp. 258–259.

Rambaut, A. (2017) Figtree-version 1.4.3, a graphical viewer of phylogenetic trees. Computer program distributed by the author. Available from: http://tree.bio.ed.ac.uk/software/figtree (accessed 11 December 2024)

Regel, E. & Tiling, H. (1859) Florula Ajanensis. *Nouveaux mémoires de la Société impériale des naturalistes de Moscou* 11: 1–140.

Reichenbach, H.G.L. (1828) *Conspectus Regni Vegetabilis per gradus naturales evoluti*. Apud C. Cnobloch, Lipsiae, 294 pp. https://doi.org/10.5962/bhl.title.127418

Rochette, N.C., Rivera-Colón, A.G. & Catchen, J.M. (2019) Stacks 2: Analytical methods for paired-end sequencing improve RADseq-

based population genomics. *Molecular Ecology* 28: 4737–4754.
https://doi.org/10.1111/mec.15253

Shaw, J. & Small, R.L. (2005) Chloroplast DNA phylogeny and phylogeography of the North American plums (*Prunus* subgenus *Prunus* section *Prunocerasus*, Rosaceae). *American Journal of Botany* 92: 2011–2030.
https://doi.org/10.3732/ajb.92.12.2011

Shimizu, T. (1963) Studies on the limestone flora of Japan and Taiwan part II. *The Faculty of Textile Science and Technology, Shinshu University, Ser. A* 12: 1–88. [https://soar-ir.repo.nii.ac.jp/record/13654/files/Textile_A12-01.pdf]

Soza, V.L., Haworth, K.L. & Di Stilio, V.S. (2013) Timing and consequences of recurrent polyploidy in meadow-rues (*Thalictrum*, Ranunculaceae). *Molecular Biology and Evolution* 30: 1940–1954.
https://doi.org/10.1093/molbev/mst101

Suyama, Y., Hirota, S.K., Matsuo, A., Tsunamoto, Y., Mitsuyuki, C., Shimura, A. & Okano, K. (2022) Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. *Ecological Research* 37: 171–181.
https://doi.org/10.1111/1440-1703.12270

Suyama, Y. & Matsuki, Y. (2015) MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Scientific Reports* 5: 16963.
https://doi.org/10.1038/srep16963

Tamura, M. (1953) Thalictra of Japan, Kuriles, Saghalin, and Corea. *Acta Phytotaxonomica et Geobotanica* 15: 80–88. [In Japanese]

Vasimuddin, Md., Misra, S., Li, H. & Aluru, S. (2019) Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. *In: 2019 IEEE International Parallel and Distributed Processing Symposium.* IEEE, pp. 314–324.
https://doi.org/10.1109/IPDPS.2019.00041

Wang, W., Lu, A.M., Ren, Y., Endress, M.E. & Chen, Z.D. (2009) Phylogeny and classification of Ranunculales: Evidence from four molecular loci and morphological data. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 81–110.
https://doi.org/10.1016/j.ppees.2009.01.001

Wang, W.T. (2018) *Thalictrum (Ranunculaceae) in China*. Peking University Press, Peking, 376 pp. [In Chinese]

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) 38 - Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols*. Academic Press, San Diego, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yatabe, R. (1892) New or little known plants of Japan. *The Botanical Magazine, Tokyo* 6: 177–180.
https://doi.org/10.15281/jplantres1887.6.177

Yokota, M., Hiraiwa, A., Kanemoto, T. & Komesu, K. (1997) Note of the distribution of plants in the Ryukyus 15. *The Biological Magazine Okinawa* 35: 55–64. [In Japanese]

Appendix 1: Taxa and GenBank accession numbers for specimens studied.

Species: location collected, collector, and voucher specimen number: ITS ([1]), rpL16 ([2]), and MIG-seq data ([3]).
***Thalictrum actaeifolium*.** JAPAN: Honshu, Shiga, Inukami-gun. Taga-cho, S.*Fujii 19177*, LC807817[2]
Thalictrum alpinum. JAPAN: Honshu, Nagano, Kitaazumi-gun, Hakuba-mura, *T. Ito 8161*, DRR581719[3]
Thalictrum aquilegiifolium var. sibiricum. JAPAN: Honshu, Miyagi, Igu-gun, Marumori-machi. *K. Michimoto 1*, LC807750[1], LC807828[2], DRR581720[3]
Thalictrum filamentosum. JAPAN: Nagasaki, Hirado-shi, Mt. Shijiki. *K. Michimoto 22062001*, LC807751[1], LC807829[2], DRR581722[3]
Thalictrum foetidum* var. *apoiense. JAPAN: Nagasaki, Hirado-shi, Mt. Shijiki. *K. Mihicmoto 21090501*, LC807742[1], LC807821[2], DRR581721[3]
Thalictrum integrilobum. JAPAN: Hokkaido, Samani-gun, Samani-cho. *K. Michimoto 21090503*, LC807743[1], LC807819[2], DRR581723[3]
Thalictrum kiusianum. cultivar, *K.Michimoto s.n.*, LC807748[1], LC807826[2], DRR581755[3]
Thalictrum koikeanum. JAPAN: Honshu, Hiroshima, Shobara-shi. *K. Michimoto NS2*, LC807759[1], LC807842[2]
Thalictrum microspermum. JAPAN: Kyushu, Miyazaki, Hyuga-shi. *T. Hoson THH33*, LC807745[1], LC807822[2], DRR581724[3]; JAPAN: Shikoku, Tokushima, Naka-gun, Naka-cho. *K. Michimoto 220509*, DRR581725[3]
Thalictrum minus* var. *hypoleucum. Japan: Honshu, Tokyo, Izu Islands, Tokyo, Nii-jima Isl. *T. Ito 7320*, LC807824[2]
Thalictrum minus* var. *kemense. JAPAN: Honshu, Nagano, Kitaazumi-gun, Hakuba-mura. *T. Ito 8156*, LC807834[2]
Thalictrum minus* var. *sekimotoanum. JAPAN: Honshu, Aomori, Sannohe-gun, Hashikami-cho. *K. Michimoto 42*, LC807741[1], LC807818[2]; JAPAN: Honshu, Tochigi, Tochigi-shi. *K. Michimoto 21092601*, LC807825[2]
Thalictrum minus* var. *yamamotoi. JAPAN: Kyushu, Miyazaki, Higashiusuki-gun, Shiiba-son, Mt. Shiraiwa. *T. Hoson THH30*, LC807754[1], LC807832[2]
Thalictrum rochebruneanum. JAPAN: Honshu, Nagano, Nagano-shi. *T. Ito s.n.,* LC807763[1]
Thalictrum rubesens. TAIWAN: Ilan, Datong Hsiang. *T. Ito 1490*, DRR581715[3]; TAIWAN, Chiayi, Alishan Hsiang. *T. Ito 1930*, DRR581716[3]; TAIWAN: Chiayi, Alishan Hsiang. T. Ito 4339, DRR581718[3]; TAIWAN: Nantou, Jenai Hsiang. *T. Ito 8912*, LC807835[2], DRR581726[3]
Thalictrum sachalinense. JAPAN: Hokkaido, Kushiro-shi. *K. Michimoto 21090104*, LC807744[1], LC807820[2]
Thalictrum sessile. TAIWAN: Nantou, Jenai Hsiang. *T. Ito 6193*, LC807840[2]
Thalictrum toyamae. JAPAN: Kyushu, Saga, Imari-shi. *M. Maki 23013*, LC807746[1], LC807823[2], DRR581727[3]; JAPAN: Kyushu, Saga, Takeo-shi. *M. Maki 23014*, DRR581728[3]; JAPAN: Kyushu, Nagasaki, Saikai-shi. *M. Maki 23015*, DRR581729[3]
Thalictrum tuberiferum. JAPAN: Honshu, Nagano, Ina-shi. *S.Fujii s.n.*, DRR581730[3]; JAPAN: Honshu, Niigata, Sado-shi, Sado Isl. D. *Takahashi s.n.*, DRR581731[3]; JAPAN: Honshu, Miyagi, Natori-shi. *K. Michimoto 11*, DRR581732[3]; JAPAN: Shikoku, Ehime, Saijo-shi. *D. Takahashi 202106212132*, LC807757[1], LC807839[2], DRR581733[3]; JAPAN: Honshu, Miyagi, Shiroishi-shi. *K. Michimoto 21091405*, LC806219[1], LC807816[2], DRR581734[3]; JAPAN: Honshu, Yamagata, Yonezawa-shi. *K. Michimoto NS3*, DRR581735[3]; JAPAN: Honshu, Iwate, Oshu-shi. *T. Ito 7562*, DRR581736[3]; JAPAN: Honshu, Saitama, Chichibu-gun, Ogano-machi. *K. Michimoto 21092401*, DRR581737[3]; JAPAN: Honshu, Nagano, Nagano-shi. *T. Ito 8153*, DRR581738[3]; JAPAN: Honshu, Hiroshima, Yamagata-gun, Akiota-cho. *K. Michimoto 220622*, DRR581739[3]; JAPAN: Honshu: Nagano, Kitaazumi-gun, Hakuba-mura. *T. Ito 8163*, DRR581740[3]; JAPAN: Kyushu, Oita, Bungo-Ono-shi. *T. Hoson THH26*, DRR581741[3]; JAPAN: Honshu, Akita, Senboku-gun, Misato-cho. *T. Ito 1235*, DRR581742[3]
Thalictrum tuberiferum* var. *yakusimense. JAPAN: Kyushu, Kagoshima, Yakushima Isl. *T. Ito s.n.*, LC807755[1], LC807837[2], DRR581743[3]; JAPAN: Kyushu, Kagoshima, Yakushima Isl. *T. Ito s.n.*, DRR581744[3]; JAPAN: Kyushu, Kagoshima, Yakushima Isl. *T. Ito s.n.*, LC807756[1], LC807838[2], DRR581745[3]; JAPAN: Kyushu, Kagoshima, Yakushima Isl. *T. Ito 183KM.*, DRR581746[3]; JAPAN, Kyushu, Kagoshima, Yakushima Isl. *T. Ito s.n.*, DRR581747[3]
Thalictrum uchiyamae. JAPAN: Kyushu, Nagasaki, Hirado-shi, Mt. Shijiki-san. *K. Michimoto 2022061801*, DRR581748[3]; JAPAN: Kyushu, Nagasaki, Hirado-shi, Mt. Shijiki-san. *K. Michimoto 202206102*, LC807749[1], LC807827[2], DRR581749[3]
Thalictrum urbaini. TAIWAN: New Taipei City, Pingxi District. *T. Ito 8939*, DRR581750[3]; TAIWAN: Ilan, Datong Hsiang. *T. Ito 1441*, DRR581714[3]; TAIWAN: Taipei City, Beitou District. *T. Ito 8947*, LC807836[2], DRR581751[3]
Thalictrum urbaini* var. *majus. TAIWAN: Taipei City, Beitou District. *T. Ito 3300*, DRR581717[3]; TAIWAN: Taipei City, Beitou District. *T. Ito 3303*, LC807762[1], LC807833[2], DRR581752[3]

Thalictrum watanabei. JAPAN: Kyushu, Miyazaki, Mt. Wanitsuka. *T. Ito s.n.*, LC807760[1], LC807761[1], LC807843[2], LC807844[2], DRR581753[3]; JAPAN: Shikoku, Kagawa, Mt. Ryuo. *K. Mihicmoto 23062002,* LC807752[1], LC807753[1], LC807830[2], LC807831[2], DRR581754[3]

***Thalictrum* sp.** TAIWAN: Nantou, Jenai Hsiang. T. Ito, 1344, LC807758[1], LC807841[2]

Thalictrum yambaruense. JAPAN: Central Ryukyu, Okinawa, Okinawa Isl. *T. Ito 7857*, LC823724[1], LC823725[1], LC823726[1], LC823727[2], LC823728[2], LC823729[2], DRR581707[3], DRR581708[3], DRR581709[3], DRR581710[3], DRR581711[3], DRR581712[3].