Botanica Pacifica. 2023. 12(2):32–41 (This article belongs to the Special Issue dedicated to the memory of Alexey Shipunov)
DOI: 10.17581/bp.2023.12s09

Endemic *Trollius* from southeast Sakhalin: revealing another taxonomic confusion in *Trollius* L. taxonomy – the “*T. miyabei* case”

Michael M. Serebryanyi* & Alina V. Fedorova

Michael M. Serebryanyi*
e-mail: misha@florin.ru

Alina V. Fedorova
e-mail: alina_77777@mail.ru

N.V. Tsitsin Main Botanical Garden RAS, Moscow, Russia

* corresponding author

Manuscript received: 09.06.2023
Review completed: 02.10.2023
Accepted for publication: 05.10.2023
Published online: 07.10.2023

ABSTRACT

Taxonomic history of *Trollius miyabei* Sipliv. is presented together with a review of status and usage of that name in botanical and popular-scientific literature. The name *T. miyabei* Sipliv. should be treated as a synonym of *T. riederianus* Fisch. et C.A. Mey. The wrong attribution of that name – for *T. altaicus* C.A. Mey. subsp. *sachalinensis* Kadota – is analyzed in details. A molecular genetic research together with a morphological study prove that subspecies to deserve a species status; the necessary new combination – *T. sachalinensis* (Kadota) Serebryanyi – is proposed, detailed morphological description is provided. *Trollius altaicus* subsp. *pulcher* (Makino) Kadota is not present in the Sakhalin flora: previous identifications appeared wrong and should be attributed as *T. sachalinensis*. Taxonomic affinity of the species is reviewed; distribution of *T. sachalinensis* in southeast Sakhalin is revised, corrected and mapped. Thus, three species of the genus *Trollius* occur in Sakhalin: *T. riederianus*, *T. chinensis* and *T. sachalinensis* (Kadota) Serebryanyi.

Keywords: *Trollius* taxonomy, southeast Sakhalin, *Trollius miyabei*, *Trollius altaicus* subsp. *sachalinensis*, *Trollius sachalinensis*, new combination

РЕЗЮМЕ

Серебряный М.М., Федорова А.В. Эндемичная купальница юго-восточного Сахалина: прояснение еще одной проблемы в таксономии рода *Trollius* L. – статуса и атрибуции названия *T. miyabei*. В статье приведена таксономическая история названия *Trollius miyabei* Sipliv. и подтвержден единственно верный его статус – как синонима *T. riederianus* Fisch. et C.A. Mey. Широко распространенная ошибочная атрибуция этого названия как *T. altaicus* C.A. Mey. subsp. *sachalinensis* Kadota детально рассмотрена и проанализирована. Молекулярно-генетическое исследование группы видов рода *Trollius* вкупе с анализом морфологических признаков родства *T. altaicus* показывают необходимость пересмотра статуса этого таксона как самостоятельного вида; предложена необходимая новая комбинация – *T. sachalinensis* (Kadota) Serebryanyi, приводится детальное морфологическое описание этого вида. Установлено, что ранее указывавшийся для флоры Сахалина *T. altaicus* subsp. *pulcher* (Makino) Kadota – результат ошибочных определений *T. sachalinensis*. Обсуждено родство *T. sachalinensis*; уточнен и скорректирован ареал этого вида. В настоящее время на острове Сахалин произрастают три вида *Trollius*: *T. riederianus*, *T. chinensis* Bunge и *T. sachalinensis* (Kadota) Serebryanyi.

Ключевые слова: систематика *Trollius*, юго-восток Сахалина, *Trollius miyabei*, *Trollius altaicus* subsp. *sachalinensis*, *Trollius sachalinensis*, новая комбинация

The taxonomic history of the genus *Trollius* L. (Ranunculaceae: *Adonideae*) is full of invalid names, misidentifications and misattributions, which is not a rare occurrence in plant taxonomy. However, some of these errors and wrong taxonomic decisions persist for a very long time and lead to unfortunate and confusing consequences, migrating from one publication to another. Some of them were analyzed/solved in our previous publications (Serebryanyi 2019, Serebryanyi & Fedorova 2023). The Russian Far East is one of the most affected areas in this respect: several names are now considered illegitimate or have been moved into synonymy – for example, the currently accepted broad concept of *Trollius riederianus* Fisch. & C.A. Mey. (Kadota 2016) results in about 10 synonyms of this species; the combination *T. macropetalus* (Regel) F. Schmidt is considered invalidly published and should therefore be withdrawn from usage (Serebryanyi 2019). One major confusion remains to be overcome – the attribution and status of the name *Trollius miyabe* Sipliv., to which the present paper is devoted. This issue cannot be fully resolved without assessing the taxonomic status of *T. altaicus* C.A. Mey. subsp. *sachalinensis* Kadota, an insufficiently known taxon of *Trollius* endemic to southeast Sakhalin, so we had to accept the challenge.

Taxonomy and taxonomic history

The history of the name *Trollius miyabei* Sipliv. is relatively short – it was published by V.N. Siplivinsky (1972) as a replacement for *T. schipczinskii* Miyabe, Acta Phytotax. Geobot. 13:14 (1943), nom. invalid. (no Latin diagnosis provided, types not properly cited/not assigned, list of synonyms included different taxa). Siplivinsky composed the Latin diagnosis, assigned the holotype (Ins. Sachalin, jugum Sudzujensis prope Jushnosachalinsk, declivitas montium Czechovii (Sudzue-dake), 800 m, ad rivum montanum, 22 VII 1948, M. Popov (LE 01038891!) [О. Сахалин, Судзуйский хр., возле Южно-Сахалинска, склон горы Чехова (Sudzue-dake), 800 м выс., по горному ручью 22 VII 1948, М.Г. Попов (LE 01038891!)]) and six paratypes, added notes on the species' affinity ("Affintas" section) and removed Miyabe's list of synonyms, leaving only one synonym: *T. schipczinskii*, p.p. It should be noted that Siplivinsky cited Exs. Gerb. Fl. SSSR No. 3862 immediately after the name *T. schipczinskii*, p.p. (op. cit., p. 176), which is misleading because the specimens (exsicattae) erroneously identified by A.I. Tolmachev as *T. japonicus* Miq. represent a different species, now known as *T. altaicus* C.A. Mey. subsp. *sachalinensis* Kadota (see discussion below). Moreover, these specimens were selected as paratypes of the latter taxon by Y. Kadota (1991).

The Affinitas section reads as follows: "A *T. membranostyli* Hulten caulibus ramosis, omnis partibus duplo majoribus et rostellis folliculorum apteris bene differt" (op. cit., p. 176). In brief, the plants of *T. miyabe* are easily distinguishable from *T. membranostylis*, being twice as large in all parts, having branching stems and not keeled beaks of follicle. It is important to note that the color of the pistils (styles and stigmas) is described by both authors (Miyabe 1943, Siplivinsky 1972) as green/yellowish green. A well-preserved holotype contains flowers and developing (not mature) follicles with beaks scarcely keeled along abaxial sutures; red coloration of pistils in the specimen is absent (Fig. 1).

A. Doroszewska (1974) included *T. schipczinsky* in her revision of the genus *Trollius* (apparently omitting the publication of Siplivinsky (1972)). Besides discussing the opinions by Japanese authors on the group of species, including *T. japonicus* Miq., *T. membranostrylis*, *T. riederianus* and *T. schipczinskii* she noted that the beak shape can be variable even in a single individual (op. cit., p. 132).

Y. Kadota (2016) substantiated the broad concept of *T. riederianus* Fisch. et C.A. Mey., including plants 20–100 cm tall, often with branched stems, flowers solitary or 2–3 in a loose cyme, 3–6 cm in diameter, parabolic to saucer-shaped, of 5–14 patent sepals. Carpels 7–40; styles 0.9–2.5 mm long, erect, thick, angled or sometimes keeled. Petals fleshy, (4)6–13 mm long, shorter than – to as long as – or sometimes slightly longer than stamens (it is worth mentioning that stamens fall down first during the late phase of flowering period, while petals remain intact). Follicles erect or suberect, ca. 10 mm long, keeled along the abaxial sutures; beaks thick, hamately curved (that character applies only to mature follicles).

It is obvious and logical that both *T. membranostylis* and *T. miyabei* plunged into the synonymy of *T. riederianus*, along with other Far East species (*T. kurilensis* Sipliv., *T. japonicus* Miq.) which was done by Kadota (2016). This could (and should) have been a natural conclusion of the "*Trollius miyabei* case", but, unfortunately, it is only the beginning.

The name '*Trollius miyabei*' appears again in a checklist of vascular plants of Sakhalin Island (Barkalov & Taran 2004). It is not possible to judge what was meant by this name (as this publication is a common checklist with geographical attribution). The next important evidence is represented by the photographs 154a (by A.V. Petuchov) and 154b (by A.V. Kordjukov) in Atlas plantarum vascularium vicinitatis civitatis Austro-Sachalinski (Petuchov et al. 2010), which show a completely different plant under the name *Trollius miyabei* – *Trollius altaicus* C.A. Mey. subsp. *sachalinensis* Kadota, published 13 years earlier (Kadota 1991). Since then, this misidentification/misapplication has spread in scientific and popular science literature up to the review of the genus *Trollius* in the Russian Far East (Luferov et al. 2018), where a photograph of *Trollius altaicus* subsp. *sachalinensis* reappeared under the name *Trollius miyabei* (this time by V.Y. Barkalov: op. cit. p. 114, fig. 1G).

Trollius altaicus subsp. *sachalinensis* belongs to a small group of taxa within the genus, having (dark) red to black-purple/violet pistils/styles and stigmas: *T. altaicus* subsp. *altaicus*, *T. altaicus* subsp. pulcher (Makino) Kadota and inter-species hybrids *T. asiaticus* L. × *T. altaicus*, *T. dschungaricus* Regel × *T. altaicus* (Fig. 2). The nature of this phenomenon and pigment(s) responsible for the character are still not determined and deserve further study. It is worth mentioning that another inter-species hybrid involving *T. altaicus* – *T. ilmensis* Sipliv. (*T. altaicus* × *T. europaeus*) – does not have that syndrome: the pistils in that taxon are entirely green.

Trollius europaeus L., *T. altaicus* and *T. dschungaricus* comprise a group of species within the genus, diagnosed by the petals falling down first in the late phase of flowering period, while stamens remain intact (as we pointed out above, in *T. riederianus* it is vice versa). This syndrome, together with some other morphological characters allow us to distinguish a natural infrageneric group, provisionally having the status of a section close to the section *Trollius* Dorosz. (but excluding *T. ranunculinus* (Sm.) Stearn). A new/adequate infrageneric classification of the genus *Trollius* is urgently needed, and we are currently working on a separate publication on this issue.

The taxonomic statuses of *T. altaicus* subsp. *sachalinensis* and *T. altaicus* subsp. *pulcher* have not yet been definitively decided: both taxa are morphologically distinct and easily recognizable. The decision of Kadota to assign subspecies rank to these taxa was partly based on geographical considerations derived from the revision by Doroszewska (1974): the distribution range of *T. altaicus* presented in the monograph and redrawn by Kadota (op.cit., p. 55, fig. 5) covering a significant part of the Russian Far East is completely inadequate. In fact, non-hybrid populations of *T. altaicus* are not recorded east of the Eastern Sayan, and hybrid populations involving the species – east of the Khamar-Daban Range. Thus, considering these three taxa as vicariants is not a substantial approach, and the disjunction between the ranges of *T. altaicus* s. str. and two Far East Pacific taxa – *T. altaicus* subsp. *sachalinensis* and *T. altaicus* subsp. *pulcher* (narrow endemics of Sakhalin and Rishiri islands, respectively) – becomes a very serious geographical phenomenon requiring explanation. For this reason, in course of ongoing revision of the genus *Trollius* by the first author, the status of *T. altaicus* subsp. *sachalinensis* remained doubtful and required a separate in-depth study.

Molecular genetic study

We decided to include specimens of *Trollius altaicus* subsp. *sachalinensis* in our ongoing molecular genetic study of the genus *Trollius* to reveal phylogenetic relationships of the allied taxa (no molecular data on this taxon had been obtained earlier). Specimens were collected by Ms. Tatiana Konovalova in 2022 at two localities within the Susunai

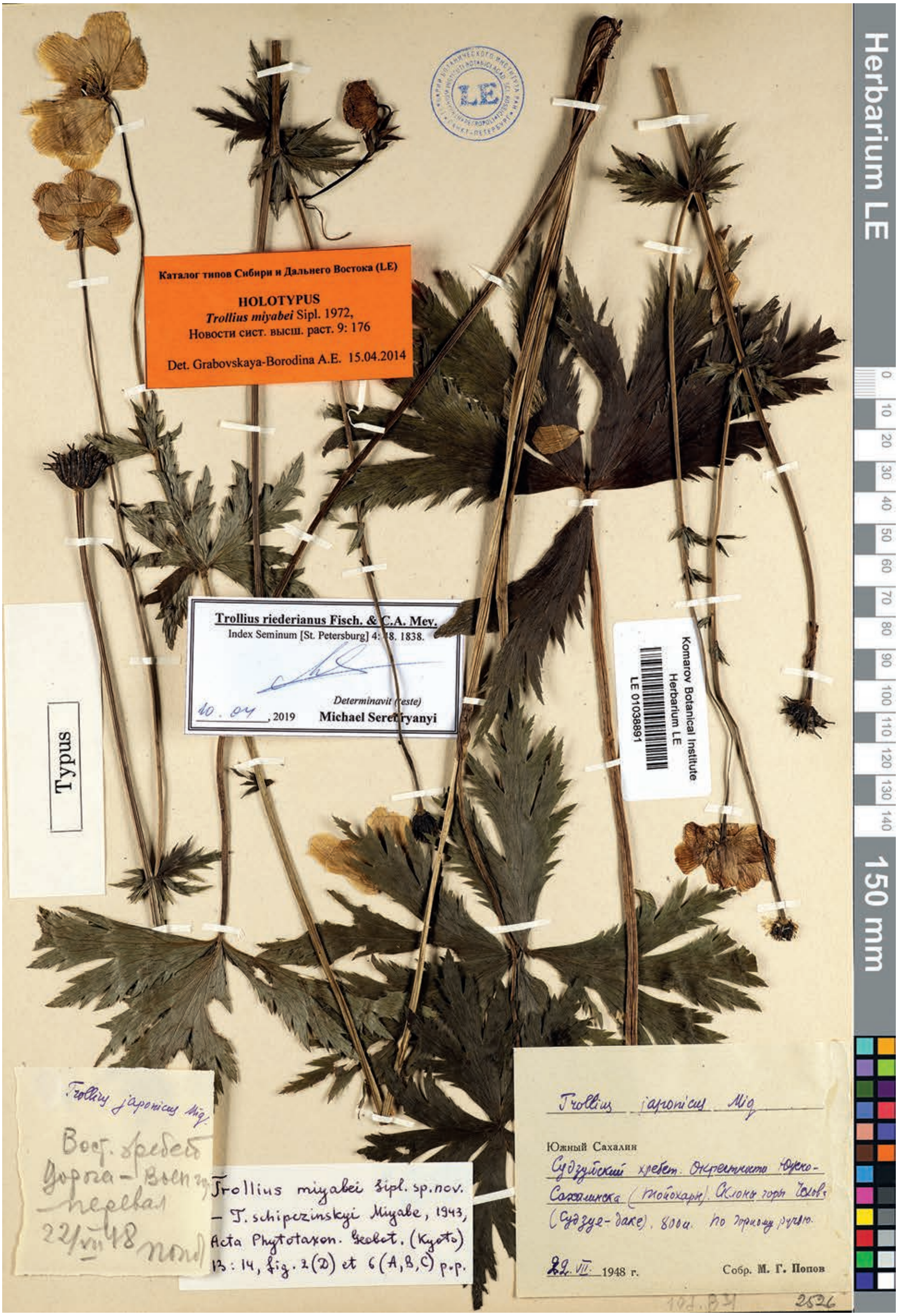

Figure 1 The holotype of *Trollius miyabei* Sipliv. (LE 01038891)

Table 1. Morphological characters, differentiating *Trollius altaicus* and *T. sachalinensis*.

Morphological character	*Trollius altaicus* s.str.	*Trollius sachalinensis*
Basal leaf blade texture & color	Thin to thin coriaceous; upper surface bright- to dark-green; lower surface much paler, showing contrast colour pattern in sicco	Thin; light- to bright-green above, slightly paler and papillate below, showing no contrast in sicco
Basal leaf venation	Veins impressed in the upper surface of leaf blade, prominent but not raised/elevated on the lower surface, not or moderately dense	Veins impressed in the upper surface of leaf blade, prominently raised and elevated on the lower surface, extremely dense
Teeth of leaf blade margin (incision)	Lobes in basal leaves with broadly-triangular to more or less ovate teeth, extremely shortly apiculate/pointed at the top – to obtuse at the top	Lobes in basal leaves shallowly pinnately incised with longish lanceolate-oblique laciniae, those more or less shortly apiculate at the top in vivo, acuminate at the top in sicco, never obtuse
Petals	Membranous, flat to slightly convex, round to obtuse at the apices, 7–16 mm long; nectary pits elliptic to pyriform , slightly inflated on the abaxial side	Fleshy, clearly convex , round to subtruncate at the apices, 4–6.5 mm long; nectary pits circular to elliptic/pyriform, slightly inflated on the abaxial side
Pistils' pigmentation/colour	Entire pistils dark red – to dark purple; less often ovaries reddish-purple, styles and stigmas dark purple	Ovaries light green, styles and stigmas reddish-purple; colour getting more intense bottom-up (style/upper zone of the style – stigma)
Follicles	(7)11–25, erect, 11–17 mm long	(6)7–11, diverging from each other, 9–10 mm long
Beak of the follicle	Shortly bent outwards at the base, then sharply hooked inwards, thick and firm, up to 4 mm long	Distinctly bent outwards (up to 90º angle to the follicle axis), up to 3 mm long

Range. The selection studied included also the specimens of three species from section *Trollius* – *T. europaeus* (the type species of the genus and section), *T. altaicus* s. str. and *T. riederianus* (the most common species in the Russian Far East, also quite common on Sakhalin). *Trollius chinensis* Bunge, representing section *Longipetala* Dorosz. (characterized by long, flat and thin petals much larger than the stamens; the latter fall first in the late flowering phase, while the petals remain intact), with a vast distribution range, also occurring in Sakhalin, was used as the outgroup. Unfortunately, we have not yet been able to obtain specimens of *T. altaicus* subsp. *pulcher*. We will make every effort to obtain the specimens necessary to assess the taxonomic status of *T. altaicus* subsp. *pulcher*. We are fully aware that the results presented below are preliminary and a more representative analysis will be conducted in the near future.

Due to the low variability of genome regions traditionally used in molecular genetic studies of the genus *Trollius*, it is necessary to continue screening of its chloroplast genome to identify the most informative and variable regions (genes and intergenic spacers). To date, we have tested two regions of the nuclear genome (ITS and ETS) and 7 regions of chloroplast DNA (*mat*K, *trn*L-*trn*F, *pet*A-*psb*J, *psb*B-*psb*H, *psb*A-*trn*H, *ndh*C-*trn*V, *rpo*B-*trn*C) for 11 species and subspecies of *Trollius* (*T. lilacinus* Bunge, *T. komarovii* Pachom., *T. chartosepalus* Schipcz., *T. ranunculoides* Hemsl.., *T. farreri* Stapf, *T. altaicus*, *T. chinensis*, *T. riederianus*, *T. altaicus* susbsp. *sachalinensis*, *T. europaeus*, *T. sibiricus* Schipcz.).

Comparative analysis of the nucleotide sequences we obtained showed that the following IGS, *psb*A-*trn*H and *ndh*C-*trn*V, can be successfully used to study groups of closely related species within the genus *Trollius* (Serebryanyi & Fedorova 2023). At the same time, ETS, *psb*A-*trn*H and *rpo*B- *trn*C proved to be the most variable when comparing distant species/species groups.

In the present study, in addition to the *psb*A-*trn*H, which is one of the most frequently used and informative regions for most taxonomic groups of land plants (Dong et al. 2012, Pang et al. 2012, Korotkova et al. 2011, Shaw et al. 2007), we obtained the nucleotide sequences of the *ndh*C-*trn*V region for all samples included in the analysis. The choice of this IGS was due to its successful use in our previous studies of *Rosa* L., *Eversmannia* Bunge, and other genera (Schanzer et al. 2020, Fedorova et al. 2010, Fedorova 2012, Stepanova 2023).

MATERIAL AND METHODS

Taxon sampling

A total of 19 specimens representing 5 species of *Trollius*: *T. altaicus* (6), *T. chinensis* (3), *T. riederianus* (7) *T. altaicus* subsp. *sachalinensis* (1), *T. europaeus* (2), were analyzed in the present study. All specimens were obtained

Figure 2 Dark red/purple pigmentation of pistils in *Trollius* L. A: *Trollius altaicus* C.A. Mey. – Kazakhstan, Alabel' passover (Kolbintsev & Komarov 2016–2019); 2.06.2016; photo by V. Kolbintsev, used with permission. B: *Trollius altaicus* C.A. Mey – Kazakhstan, urochische Ul'ken-Kaindy (Kolbintsev 2014–2022); 30.05.2003; photo by Arie Peterse, used with permission. C: *Trollius asiaticus* L. × *T. altaicus* – Altai Territory, Kosh-Agach District, NW of Chuysk Depression, Mt. Sukor; 28.06.2021; photo by Natalia Shchegoleva, used with permission. D: *Trollius dschungaricus* Regel × *T. altaicus* – West Mongolia, Bayan-Ulgii province, Sagsai sum; 2479 m; photo by Gyu Young Chung, used with permission

from herbarium specimens in two herbaria (MHA, TK). *T. chinensis* specimens from the Tubota River basin, Tunka depression, Republic of Buryatia, collected and logged *in situ*, were selected as outgroups (Serebryanyi 2019). A detailed list of the samples used is presented in Appendix 1.

Molecular methods

Total DNA was extracted from herbarium specimens of *Trollius* using the CTAB procedure (Doyle & Doyle 1987) according to the manufacturer's protocol. Four markers were used for molecular phylogenetic study: nuclear ribosomal internal transcribed spacers (ITS 1, 2), external transcribed spacer (ETS) and two plastid intergenic spacers (IGS) – *trn*V–*ndh*C and *psb*A–*trn*H. The nuclear regions were amplified using the primers: NNC–18S10 (AGGAGAAGTCGTAACAA), C26A (GTTTCTTTTCC TCCGCT) for ITS and ETS1f (CTGTGGCGTCGCAT GAGTGG), 18S-R (AGACAAGCATATGACTACTGG CAGG) for ETS. For the amplification and subsequent sequencing of chloroplast loci *trn*H–*psb*A we used: *trn*H (CGCGCATGGTGGATTCACAATCC) and *psb*A (GTTA TGCATGAACGTAATGCTC); *trn*V–*ndh*C: ndhC (ATTA GAAATGYCCARAAAATATCAT) and trnV(UAC)x2 (GTCTACGGTTCGARTCCGTA) (Shaw et al. 2005, Wen & Zimmer 1996, Starr & Harris 2003). Primers used for PCR were synthesized and purified in PAAG by Syntol Ltd. (Moscow, Russia). PCR reaction mixture (20 μL) contained 1.5–2 ng of DNA, 5 pmol of each primer, 4 μL of PCR Master MIX, containing a hotstart SmarTaq DNA polymerase (Dialat LTD, Russia) and 13 μL deionized water. PCR reaction was performed on a MJ Research PTC220 DNA Engine Dyad Thermal Cycler (BioRad Laboratories, United States) under the following conditions. For ITS1, 2: 94°C – 3 min; 94°C – 20 s, 58°C – 30 s, 72°C – 40s (34 cycles); 72°C – 3 min. For ETS: 95°C – 2 min; 95°C – 45 s, 58°C – 1 min, and 72°C – 2 min (35 cycles); 72°C – 5 min. For *psb*A–*trn*H: 94°C – 3 min; 94°C – 30 s, 52,5°C – 30 s, 72°C – 90 s (40 cycles); 72°C – 7 min and for *trn*V–*ndh*C: 95°C–180 s; 95°C–60 s, 57°C–40 s, 60°C–80 s (35 cycles); 57°C–40 s, 60°C–80 s (2 cycles). A control, containing all the components except genomic DNA, was included in reactions to prove that no contamination occurred. PCR products were separated on 1 % agarose gel in 0.5 × TBE (pH = 8.3) buffer containing ethidium bromide and purified by precipitation in 0.125M/l ammonium acetate solution in 70 % ethanol. Cleaned products were directly sequenced in both directions with the same primers as for PCR, using the ABI PRISM© BigDye™ Terminator v. 3. kit (Applied Biosystems) at the Syntol Ltd. (Moscow, Russia). All sequences were deposited to GenBank (https://www.ncbi.nlm.nih.gov/), accession numbers of the sequences are presented in Appendix 2.

Sequence alignment and phylogenetic analyses

DNA sequences were first aligned by ClustalW in BioEdit (Hall 1999), followed by manual adjustments. Positions of indels were treated as missing data for all specimens. The alignments of the nuclear and plastid sequences data were analyzed as the concatenated matrix. Since the nucleotide sequence data for ITS 1, 2 proved to be extremely low informative, we excluded the data from the final analysis. The final dataset contained 19 accessions for all regions. The Maximum Likelihood (ML) analysis (Felsenstein 1981) was conducted with RAxML ver. 8.2.12 using raxmlGUI version 2.0.7 with GTR plus GAMMA model (Stamatakis 2014, Kozlov et al. 2019, Edler et al. 2021). ML bootstrap values were based on 1000 fast replicates. We visualized the resulting tree in FigTree v.1.4.3 (Rambaut 2009) and finally prepared the figure using the Inkscape v.1.1.2 (b8e25be833, 2022-02-05; https://inkscape.org). Also we used the Neighbour Net method implemented in SplitsTree v.4.16.2 (Huson & Bryant 2006) to visualize affinities between samples. The Neighbour Net split network was built based on nr ETS and cp *trn*V-*ndh*C, *trn*H-*psb*A datasets with 1,000 bootstrap iterations.

RESULTS AND DISCUSSION

Molecular phylogeny

The total number of characters in the final concatenated alignment was 1,803 bp; 84 sites were variable, 33 were parsimony informative. ML analysis of the concatenated dataset resulted in a tree with lnL= 2866.310217 (Fig. 3). The ETS region was the least informative in this case: its length was 436 bp and it was characterized by 3 substitutions (377 position: T/A for all *T. chinensis* specimens; 373 position: T/C for TRD6; 178 position: G/A for two *T. riederianus* specimens from the Kuril Islands TRD4 and TRD11) and one indel (A/ – in the 10th position of the alignment for all specimens except *T. europaeus*). Both cp regions showed a sufficiently high polymorphism: 80 sites were variable, 31 were parsimony informative. When the tree was rooted on *T. chinensis* (Fig. 3), two clades were formed: the first one (bootstrap support 64) was constituted by two clades of the *T. europaeus* specimens (bootstrap support 100) and the specimens of *T. riederianus* (bootstrap support 97). Moreover, within the *T. riederianus* clade, the samples are clearly separated by geographic origin: specimens from the Kuril Islands (bootstrap support 94) and specimens from the Magadan Region (bootstrap support 78). The second clade contains specimens of *T. altaicus*, *T. altaicus* subsp. *sachalinensis* and two specimens of *T. riederianus* from the Altai Territory. Within this clade, the following division is observed: 4 specimens of *T. altaicus* (TAL5, 6, 9, 10) form their own clade (bootstrap support 90), while their sister clade is composed of two specimens of *T. riederianus* (TRD2 and TRD6) from the Amur Region and Khabarovsk Territory; the specimen of *T. altaicus* subsp. *sachalinensis* is nested within it. A separate clade (bootstrap support 82) is formed by TAL4 and TAL11 from the Altai Territory. Neighbour Net split network (Fig. 4) reveals the same divergence as in RAxML. All specimens are divided into six network clades: *T. altaicus*, *T. chinensis*, *T. riederianus*, *T. europaeus* and *T. sachalinensis*. Specimens of *T. altaicus* and *T. chinensis* have extremely low bootstrap supports and are located closer to the center of the network. *T. sachalinensis* (bootstrap support 100) is the furthest away from the center of the network, indicating a large amount of variation in its nucleotide sequences. The *T. riederianus* specimens split into two well-supported clades (99 and 98), which are located in opposite sides from the center of the network.

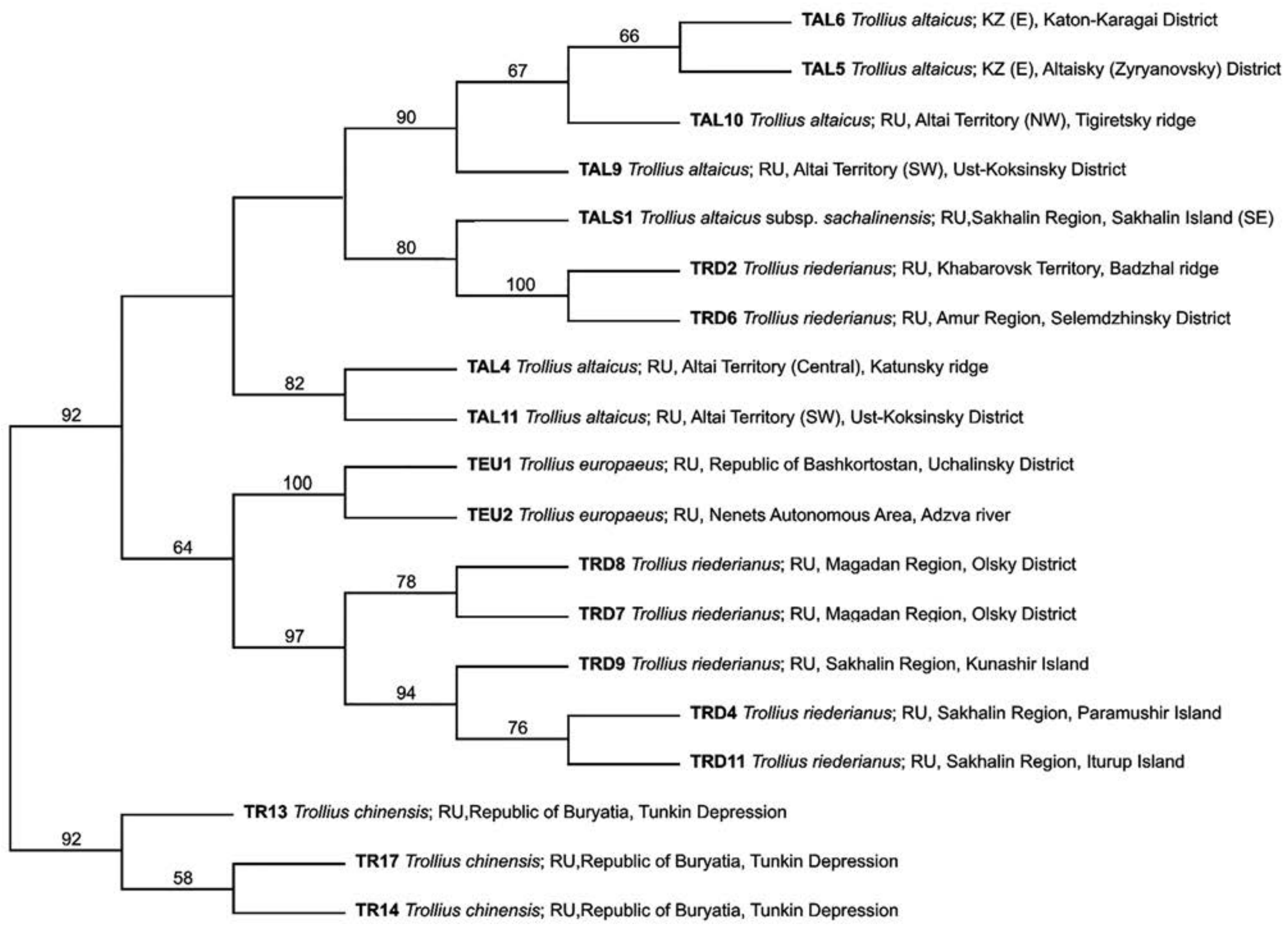

Figure 3 The best tree (lnL= 2866.310217) recovered from the ML analysis (RAxML with GTR + GAMMA) of the concatenated nr and cp data. Numbers above branches indicate ML Bootstrap support values that are equal to or more than 50 %. Countries are encoded in alpha-2 (ISO 3166) format

Two main results of the molecular genetic study are of primary importance: the position of *T. altaicus* subsp. *sachalinensis* and splitting of *T. riederianus* specimens into two well-supported clades: the "Mainland" (TRD2 & TRD6) and "Islands" (the rest of the specimens).

Trollius riederianus splitting in two clades

Evidently, the phenomenon requires additional in-depth study. The following hypotheses can be evaluated:

– differences in genome as a result of a long isolation of mainland and islands populations;

– possible hybridogenesis of mainland populations where the analyzed samples were collected;

– attribution of the mainland specimens to the doubtful species *T. uniflorus* Sipliv. which taxonomic position and status are not completely clarified.

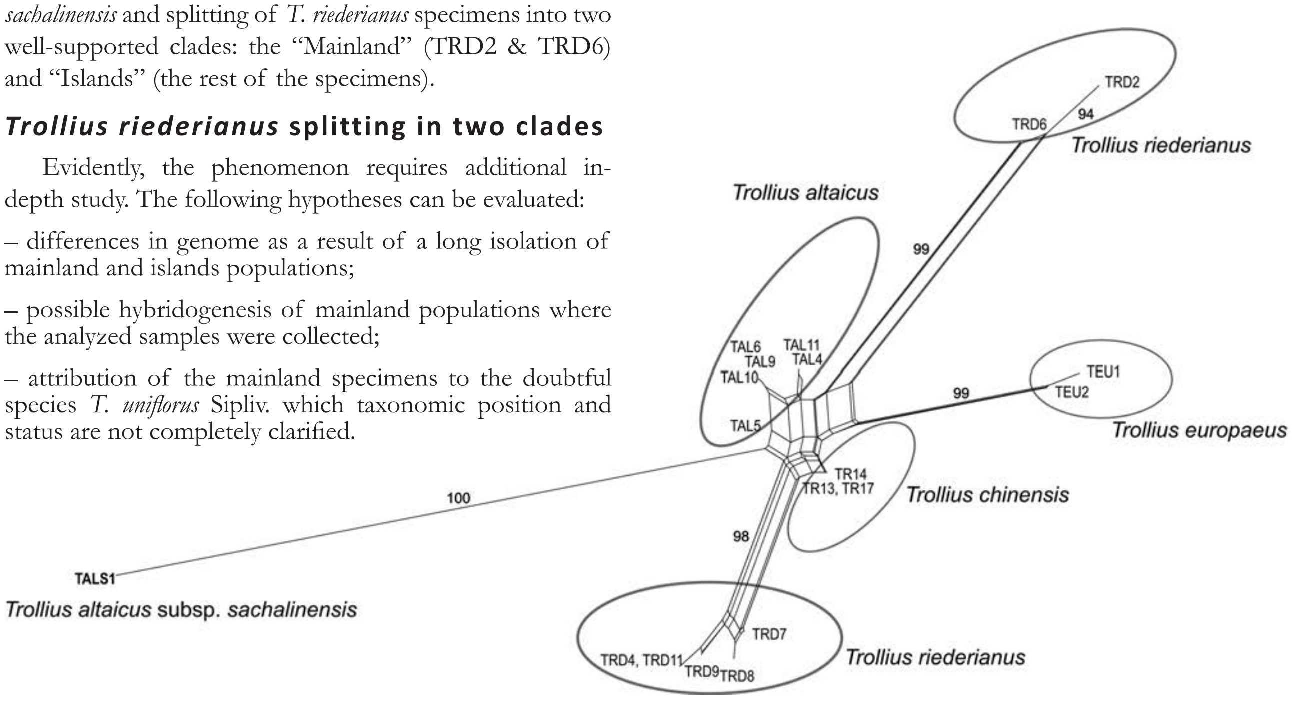

Figure 4 Splitgraph of analyzed samples originated from the nr ETS and cp *trn*V–*ndh*C, *trn*H–*psb*A dataset. The bootstrap values appeared from 1,000 iterations of the bootstrap analysis in Splitstree 4

Taxonomic status of *Trollius altaicus* subsp. *sachalinensis*

It became obvious that the subspecies rank does not correspond to the status of the Southeast Sakhalin endemic *Trollius*, and not only from the molecular-genetic point of view: practically all parts of plants of this taxon are strikingly different from *T. altaicus*: basal and cauline leaves (texture, veining, dissection, color), pigmentation/color of pistils, follicles/beak, etc. (Table 1). In the light of the results obtained, a new combination inevitably emerged, which is following:

Trollius sachalinensis (Kadota) Serebryanyi, **stat. et comb. nov.** (≡ *Trollius altaicus* subsp. *sachalinensis* Kadota, Mem. Natl. Sci. Mus. (Tokyo) 24:57 (1991). *Trollius pulcher* Makino f. *grandiflorus* Miyabe, in sched. *Trollius pulcher* auct. non Makino: Miyabe, Acta Phytotax. Geobot. (Kyoto) 1 3:8, fig 2 (B), 5(B) (1943), pro parte; Siplivinsky, Nov. Syst. Pl. Vasc. 9: 176 (1972); Czerepanov, Add. Corr. Fl. URSS 497 (1973), pro parte; Pt. Vasc. URSS 428 (1983), pro parte; Doroszewska, Monogr. Bot. (Warszawa) 41:125, fig. 15, 3 (1974); Voroschilov, Opred. Rast. Soviet. Daln. Vostoka 261 (1982), pro parte. *Trollius japonicus* auct. non Miq.: Schipczinsky, Fl. URSS 7: 50 (1937), pro parte.

Type: S Sakhalin, Mt. Ninushi, July 1, 1908, T. Miyake s. n. (**holotype:** TNS 380857!; **isotypes**: TNS 380856!, 380859!).

Description. Stem 35–55(60) cm tall, more or less lucid in sicco, smooth, striate, not twisted in the upper part/peduncle. Leaf blades reniform in outline, basal petiolate, 12–15 cm in diameter, light- to bright-green above, slightly paler and papillate below, cauline leaves (smaller and reduced) significantly paler than the basal ones; lobes in basal leaves shallowly pinnately incised with longish lanceolate-oblique laciniae, the latter more or less shortly apiculate at the top (apicules whitish in vivo), acuminate at the top in sicco, forming extremely acute angle with the segment's margin; veins deeply impressed in the upper surface of leaf blade, prominently raised and elevated on the lower surface. Upper/lower surfaces of leaf blades do not show contrast color pattern in herbarium specimens. Flowers 1–3, subglobose, 4–5 cm in diameter. Sepals 11–13, imbricate, broadly obovate to elliptic, 15–20 mm long, minutely dentate along the proximal margin, slightly sinuate at the top. Petals fleshy, 4–7 mm long and always shorter than the stamens; blades spathulate to broadly linear, round-truncate at the apices; nectary pits elliptic to pyriform, slightly inflated and glabrous at the abaxial side; claws 0.5–1 mm long, thickened. Anthers 2–3 mm long. Pistils 7–11; ovaries elongate, green; styles straight to slightly curved outwards, reddish purple; stigmata reddish purple. Fruit up to 18 mm in diameter, 10 mm tall; follicles (6)7–11, diverging from each other, 9–10 mm long, beaks up to 3 mm long, distinctly bent outwards (up to 90º angle to the follicle axis). [Fruit details are published for the first time.] Flowers in June–July, fruits in July. Fig. 5.

Distribution: the Susunai and Tonino-Aniva ranges, southeast Sakhalin endemic (Fig. 6).

Representative specimens studied: Insula Sachalin. In viciniis opp. Dolinsk. In herbosis elatis apud rivum in «Cholodnaya dolina». 22.06.1953. A. Gizha, L. Motorina A. Czernjaeva s.n. (Gerb. Fl. SSSR No. 3862) [О-в Сахалин, окр. г. Долинска. В зарослях крупнотравья у речки в Холодной долине. 22.06.1953. А. Гижа, Л. Моторина, А. Черняева s.n. (Герб. Флоры СССР № 3862)] – LE!, TK!); Russia, Sakhalin Region, Aniva District, the Susunai Range, small plateau 1.5 km S of Mt. Bykov (ca. 8 km S of Bereznyaki settl.). In small meadow plots in-between *Pinus pumila* (Pall.) Regel thickets. Ca. 650 m alt. 47.113367ºN 142.872350ºE; 16.07.2022. T. Konovalova s.n. (MHA0341835!); Russia, Sakhalin Region, Yuzhno-Sakhalinsk vicinity, the Susunai Range, SW

Figure 5 *Trollius sachalinensis* (Kadota) Serebryanyi in natural community. Sakhalin, Yuzhno-Sakhalinsk vicinity, the Susunai Range, SW macroslope of the Chekhov's Peak. In small meadow plots in communities with *Betula ermanii* Cham. in tree stratum. Ca. 600 m alt. 4.07.2022. Photo by T. Konovalova, used with permission

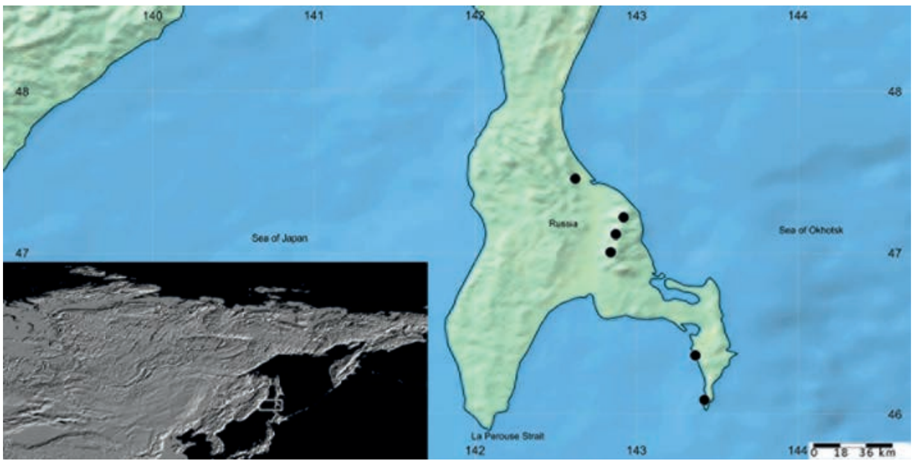

Figure 6 Distribution of *Trollius sachalinensis* in southeast Sakhalin (created with Simplemappr, Shorthouse 2010)

macroslope of the Chekhov's Peak. In small meadow plots in communities with *Betula ermanii* Cham. in tree stratum. Ca. 600 m alt. 46.999324ºN 142.837116ºE. 4.07.2022. T. Konovalova s.n. (MHA!); [USSR] Sakhalin, Mt. Omanbetsu, July 15, 1908, T. Miyake s. n. (SAPT); [USSR] Sakhalin, Cape Naka-Shiretoko, Mt. Juzosan, July 11, 1908, T. Miyake s.n. (SAPT, TNS!).

Attribution of obsolete Sakhalin toponyms is an extremely difficult problem, requiring complex and non-formalizable procedures: even a correct transcription from Japanese to English is not at all sufficient, since toponyms in Japanese are essentially the result of translations from Ainu to Japanese. Numerous errors (typos, letter transpositions, etc.) were made during the Ainu-Japanese-Russian translations/transliterations, sometimes followed by brand new Russian toponyms from the Soviet period. For example, on the 1:200000 topographic map of Sakhalin (1944), the type locality of *T. sachalinensis*, Mt. Ninushi, is designated as Inunusi-dake [Инунуси-дакэ] (Susunai Range, elevation 772 m); Mt. Juzosan (Juzozan) – an obsolete toponym of Kruzenshtern Peak (the highest point of Cape Tonino-Aniva – 670 m above sea level) – is designated as Siretoko-yama [Сирэтоко-яма] on the same map. At present it is practically impossible to indicate the exact location of Mt. Omanbetsu, so we could place it only tentatively. We took into account the data of Takahashi et al. (2004) for several toponyms, but the attribution of Mt. Ninushi there is accompanied by a question mark without any further explanation.

CONCLUSIONS

The name *Trollius miyabei* Sipliv. should be treated as one of the synonyms of *T. riederianus* Fisch. et C.A. Mey. Its misattribution as *Trollius sachalinensis* (Kadota) Serebryanyi should be considered a bad practice and discontinued. Three species of the genus *Trollius* occur in Sakhalin: *T. riederianus*, *T. chinensis* and *T. sachalinensis* (Kadota) Serebryanyi. All previous records of *T. pulcher* Makino (*T. altaicus* subsp. *pulcher*) in Sakhalin, including those of Siplivinsky (1972), have been shown to be misidentifications of *T. sachalinensis*. There is no reliable information on spontaneous interspecific hybrids in Sakhalin yet, but this does not mean that they do not occur there, as contact zones between populations of *T. riederianus* and *T. sachalinensis* exist in the Susunai and Tonino-Aniva Ranges.

ACKNOWLEDGEMENTS

The first author is deeply and sincerely grateful to late Dr. Alexei Shipunov for long-term fruitful collaboration, particularly during his stay in Japan. Alexei supported the *Trollius* revision project with unfeigned eagerness and enthusiasm.

Keepers, curators and staff members of the following Herbaria are greatly acknowledged for assistance: MHA, TNS, LE, TK. Drs. Alexander Ebel and Natalia Shchegoleva (Tomsk State University) are acknowledged for the material of *Trollius altaicus* for the molecular-genetic part of the research. The authors are deeply grateful to Tatiana Konovalova for collecting the herbarium specimens of *Trollius sachalinensis* in SE Sakhalin and sharing the respective field notes.

Dr. Nobuyuki Tanaka (TNS) is greatly acknowledged for providing hi-resolution scans of *T. altaicus* subsp. *sachalinensis* type material and his kind assistance in discussing the obsolete Sakhalin toponyms. The authors are sincerely grateful to Dr. Lyudmila Zinchuk (Chief of the Department of Cartography of Russian State Library) and Marina Legczenko (LE) for their valuable help in identification/attribution of the obsolete Sakhalin toponyms.

We are grateful to the authors of the excellent photos of *Trollius* species and inter-species hybrids, kindly permitted us to use them in the present paper – Vladimir Kolbintsev, Arie Peterse, Natalia Shchegoleva, Gyu Young Chung.

We acknowledge the support from the Tsitsin Main Botanical Garden state assignment no. 122042700002-6. We also thank the Ministry of Higher Education and Science of the Russian Federation for support and the Center of Collective Use "Herbarium MBG RAS" (grant 075-15-2021-678)

LITERATURE CITED

Barkalov, V.Y. & A.A. Taran 2004. A checklist of vascular plants of the Sakhalin Island. In: *Flora and Fauna of Sakhalin Island (Materials of International Sakhalin Island Project)* (S.Yu. Storozhenko, ed.), pp. 39–66, Dal'nauka, Vladivostok (in Russian with English abstract). [Баркалов В.Ю., Таран А.А. 2004. Список видов сосудистых растений острова Сахалин // Растительный и животный мир острова Сахалин (Материалы Международного сахалинского проекта) / под ред. С.Ю. Стороженко. Владивосток: Дальнаука. С. 39–66].

Dong, W., J. Liu, J. Yu, L. Wang & S. Zhou 2012. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. *PLoS ONE* 7(4):e35071.

Doroczewska, A. 1974. *The genus Trollius L. A taxonomical study*. Panstwowe wydawnictwo naukowe, Warszawa, 184 pp.

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

Edler, D., J. Klein, A. Antonelli & D. Silvestro 2021. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12:373–377.

Fedorova, A.V., I.A. Schanzer & A.A. Kagalo 2010. Local differentiation and hybridization in populations of wild roses in W Ukraine. *Wulfenia* 17: 99–115.

Fedorova, A.V., I.A. Schanzer & I.G. Meshchersky 2012. Hybridization between *Rosa rubiginosa* L. and *R. villosa* L. in "Belogoriye" State Reserve ("Stenki lzgoriya" Area) and the nature of *R. oskolensis* Buzunova et Grigorj. *Byulleten' Glavnogo Botanicheskogo Sada* 198(4):33–40 (in Russian). [Федорова А.В., Шанцер И.А., Мещерский И.Г. 2012. Гибридизация между *Rosa rubiginosa* L. и *R.villosa* L. в условиях заповедника «Белогорье» (участок «Стенки Изгорья») и природа *R. oskolensis* Buzunova et Grigorj. // Бюллетень Главного ботанического сада. Т. 198, № 4. С. 33–40].

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17:368–376.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.

Huson, D.H. & D. Bryant 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23(2):254–267.

Kadota, Y. 1991. Taxonomic status of *Trollius* (Ranunculaceae) from Rishiri Island, Hokkaido, Northern Japan. *Memoirs of the National Science Museum* 24:49–60.

Kadota, Y. 2016. A revision of the genus *Trollius* (Ranunculaceae) in Japan. *Journal of Japanese Botany* 91:178–200.

Kolbintsev, V. & E. Komarov 2016–2019. Alabel' passover – geographic point: photos of landscapes and habitats. In: *Plantarium. Plants and lichens of Russia and neighboring countries: open online galleries and plant identification guide*. URL: https://www.plantarium.ru/lang/en/page/landscapes/point/8741.html Last accessed 01.06.2023.

Kolbintsev, V. 2014—2022. Urochische Ul'ken-Kaindy – geographic point: photos of landscapes and habitats. In: *Plantarium. Plants and lichens of Russia and neighboring countries: open online galleries and plant identification guide*. URL: https://www.plantarium.ru/lang/en/page/landscapes/point/2887.html Last accessed 01.06.2023.

Kozlov, A.M., D. Darriba, T. Flouri, B. Morel & A. Stamatakis 2019. RAxML-NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35:4453–4455.

Luferov, A.N., A.S. Erst, D.N. Luferov, A.I. Shmakov & W. Wang 2018. The genus *Trollius* (Ranunculaceae) in the Russian Far East. *Turczaninowia* 21(2):110–116.

RKKA 1944. *Map of the Sakhalin Island, scale 1:200000* (in Russian). [Карта РККА острова Сахалин, М 1:200000]. Available online: http://www.etomesto.ru/map-sakhalin_rkka-2km/# Last accessed 01.06.2023.

Miyabe, K. 1943. On the species of *Trollius* in Japan. *Acta Phytotaxonomica et Geobotanica* 13:1–16.

Pang, X., C. Liu, L. Shi, R. Liu, D. Liang, H. Li, S.S. Cherny & S. Chen 2012. Utility of the *trn*H–*psb*A intergenic spacer region and its combinations as plant DNA barcodes: A meta-analysis. *PLoS ONE* 7(11):e48833.

Petuchov, A.V., A.V. Kordjukov & L.N. Baranczuk-Czervonnyj 2010. *Atlas of vascular plants of the vicinity of Yuzhno-Sakhalinsk. 2nd edition, revised and supplemented*. Eikon, Yuzhno-Sakhalinsk, 220 pp. (in Russian). [Петухов А.В., Кордюков А.В., Баранчук-Червонный Л.Н. 2010. Атлас сосудистых растений окрестностей Южно-Сахалинска. 2-е издание, исправленное и дополненное. Южно-Сахалинск: Эйкон. 220 с.]

Rambaut, A. 2006. *FigTree v.1.4.3*. Available from: http://tree.bio.ed.ac.uk/software/figtree/ Last accessed 16.09.2020.

Schanzer, I.A., A.V. Fedorova, M.A. Galkina, E.A. Chubar, A.V. Rodionov & V.V. Kotseruba 2020. Is *Rosa* × *archipelagica* (Rosaceae, Rosoideae) really a spontaneous intersectional hybrid between *R. rugosa* and *R. maximowicziana*? Molecular data confirmation and evidence of paternal leakage. *Phytotaxa* 428(2):93–103.

Schipczinsky, N.V. 1924. On the geographical distribution of species of the genus *Trollius* and their genetic relationship. *Izvestiya Glavnogo botanicheskogo sada RSFSR* 23:55–73 (in Russian with German abstract) [Шипчинский Н.В. 1924. О географическом распространении видов рода *Trollius* и о генетической их связи // Известия Главного ботанического сада РСФСР. Т. 23. С. 55–73].

Schipczinsky, N.V. 1937. The genus *Trollius* L. In: *Flora of the URSS, vol. 7* (V.L. Komarov, ed.), pp. 42–53, AN SSSR, Moscow, Leningrad (in Russian). [Шипчинский Н.В. 1937. Род Купальница – *Trollius* L. // Флора СССР. / под ред. В.Л. Комарова. М.; Л.: Изд-во АН СССР. Т. 7. С. 42–53].

Serebryanyi, M.M. 2019. Towards a taxonomic revision of the genus *Trollius* (Ranunculaceae) in the Asian part of Russia. I. *Trollius chinensis*: taxonomic and geographical reconsiderations. *Novosti sistematiki vysshih rastenii* 50: 101–114 (in Russian with English abstract) [Серебряный М.М. 2019. К таксономической ревизии рода *Trollius* (Ranunculaceae) в Азиатской части России. I. *Trollius chinensis*: критический анализ таксономии и географии // Новости систематики высших растений. Т. 50. С. 101–114].

Serebryanyi, M. & A. Fedorova 2023. On the species of *Trollius* L., often allocated to the genus *Hegemone* Bunge ex Ledeb. (Ranunculaceae). *Byulleten' Moskovskogo obshchestva ispytatelei prirody. Otdel biologicheskii* 128(5) (in print) (in Russian with English abstract) [Серебряный М., Федорова А. 2023. О видах *Trollius* L., нередко выделяемых в род *Hegemone* Bunge ex Ledeb. (Ranunculaceae) // Бюл. МОИП. Отд. Биол. Т. 128, вып. 5. (в печати)].

Shaw, J., E.B. Lickey, J.T. Beck, S.B. Farmer, W. Liu, J. Miller, K.C. Siripun, C.T. Winder, E.E. Schilling & R.L. Small 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92:142–166.

Shaw, J., E.B. Lickey, E.E. Schilling & R.L. Small 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94(3):275–288.

Shorthouse, D.P. 2010. *SimpleMappr, an online tool to produce publication-quality point maps*. Retrieved from https://www.simplemappr.net. Last accessed 30.04.2022.

Siplivinsky, V.N. 1972. The genus *Trollius* L. in northern and eastern Asia. *Novosti sistematiki vysshih rastenii* 9:163–182 (in Russian with Latin diagnoses) [Сипливинский В.Н. 1972. Род *Trollius* L. на севере и востоке Азии // Новости систематики высших растений. Т. 9. С.163–182].

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.

Starr, J.R., S.A. Harris & D.A. Simpson 2003. Potential of the 5' and 3' ends of the intergenic spacer (IGS) of *r*DNA in the Cyperaceae: new sequences for lower-level phylogenies in sedges with an example from Uncinia Pers. *International Journal of Plant Sciences* 164(2):213–227.

Stepanova, N.Yu., A.V. Fedorova & I.A. Schanzer 2023. On the genetic structure of *Eversmannia subspinosa* populations in Russia. *Turczaninowia* 26(1):83–94 (in Russian). [Степанова Н.Ю., Федорова А.В., Шанцер И.А. 2023. О генетической структуре популяций *Eversmannia subspinosa* в России // Turczaninowia. Т. 26, № 1. С. 83–94].

Takahashi, H, T. Fukuda & A. Taran 2004. Locality and collector names for the plant specimens collected in Sakhalin and deposited in Japanese herbaria // Bunrui 4(2):153–176 (in Japanese).

Voroschilov, V.N. 1982. *The guide of plants of the Soviet Far East*. Nauka, Moscow, 672 pp. (in Russian) [Ворошилов В.Н. 1982. Определитель растений советского Дальнего Востока. М.: Наука. 672 с.].

Wen, J. & E. Zimmer 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* 6:167–177.

Appendix 1. The list of specimens analyzed in course of molecular genetic study

Specimen code	Label	Herbarium code or specimen barcode, if present
Trollius altaicus subsp. sachalinensis		
TALS1	Russia, Sakhalin Region, Aniva District, the Susunai Range, small plateau 1.5 km S of Mt. Bykov (ca. 8 km S of Bereznyaki settl.). In small meadow plots in-between *Pinus pumila* (Pall.) Regel thickets. 47.113367ºN 142.872350ºE; 16.07.2022. T. Konovalova s.n.	MHA0341836
Trollius europaeus		
TEU1	Republic of Bashkortostan, Uchalinsky District, ca. 4 km SE Akhunovo settl. in a damp birch-aspen forest near a stream. 7.06.1990. T. Konovalova, N. Shevyryova s.n.	MHA0122708
TEU2	NE of European Russia, Nenets Autonomous Area. Middle course of Adz'va River. 67°06'N 60°36'E, 100 m alt. 30.08.2009. M. Ignatov, N. Stepanova, O. Ivanov, D. Donskov NS-09-1032	MHA0122663
Trollius altaicus		
TAL4	Montain Altai, Katun' Ridge, river Akkem source. Grassy tundra between the moraine lines of Rodzevich & Friendship Glaciers. H=2200 m. 9 VI 1974. N. Revyakina s.n.	TK
TAL5	East Kazakhstan, Zyryanovsky District. Holzun Ridge, riber Beryozovka upstream, opp. Khamira, alpine meadow. 10.08.85. A. Ryovushkin, S. Vydrina, S. Pul'kina, A. Rakitin, N. Sergeyeva s.n.	TK
TAL6	[Kazakhstan] Semipalatinsk Province, Katon-Karagaj. Mt. Sarym-Sakty. Alpine formations. 28.06.1920. Prof. V. Sapozhnikov expedition s.n.	TK
TAL7	Montain Altai, Terektinsky Ridge, river Tyungur upstream, alpine meadow. 29 VII 1983. A. Ryovushkin, S. Vydrina, N. Gordeyeva, V. Maltsev s.n.	TK
TAL8	Mountain Altai, Ongudaisky District, Mt. Sarlyk, small nival meadow. 13.7.1986. A. Ryovushkin, S. Vydrina, A. Rakitin, N. Sergeyeva, S. Pshevorskaya, S. Pul'kina s.n.	TK
TAL9	Altai, Ust'-Koksinsky aimak. Left bank of Krosnoyarka river. Meadow. VI. 1957. G. Perminova, A. Votinova s.n.	TK
TAL10	Altai, Tigeretsky Ridge. River Korgon source. Alpine small meadows on the valley bottom. 29.06.1961. V.N. Siplivinsky, L.I. Lazutskaya s.n.	TK
TAL11	Altai, Ust'-Koksinsky District, river Multa valley, the right bank of the 3rd SW cirque, 2280 m alt, swamped plot. 10.VII.1970. Sholomovaskaya, Dubrovina s.n.	TK
Trollius riederianus		
TRD2	Khabarovsk Territory, Badzhal Ridge, river Badzhal sources, 1600–1700 m alt, below the snow patches. 23 VII 1984. Slotgauer 10104.	MHA0306114
TRD4	Kuril Isnalds, Paramushir Isl., Severo-Kurilsk vicinity, on nival small meadows along the ocean terrace slope, widespread, mass. 31 VIII 1979. V.Yu. Barkalov s.n.	MHA0306130
TRD6	Amur Region, Selemdzhinsky District, Kovrizhka river valley. 1200 m alt. 2 VIII 1983. Gotvansky s.n.	MHA0306080
TRD7	Magadan Region, Ol'sky District, Oksa, damp meadow near the stream. 7 VI 1971. A. Khokhrjakov s.n.	MHA0306096
TRD8	Magadan Region, Ol'sky District, Atargan settl., along the road to Chistoe Lake, in *Duschekia* thickets. 11.6.1973. M. Mazurenko, S. Ershova, A. Khokhrjakov s.n.	MHA0306090
TRD9	Kunashir Isl., river Serebryanka bank, *Salix* thickets with tallgrass. 25 VII 1974. L. Alexeyeva, Ye. Tsvetkova 5762.	MHA0306060
TRD11	Iturup Isl., SE Kasatka, Lake Sredneye bank. 30 VIII 1965. Ye. Egorova, L. Kolchanova 3191.	MHA0306067
Trollius chinensis		
TR13, TR14, TR17	study plot 2019-Tubota_main (3.4 × 7.5 m 2 ; 51.872618 102.802318; h = 1039 m a. s. l.). 21 VII 2019 M. Serebryanyi, K. Savov	Original data (Serebryanyi 2019)

Appendix 2. Newly obtained GenBank accession numbers of the analyzed *Trollius* specimens.

Specimens	Specimen code	ITS1, 2	ETS	*trn*H–*psb*A	*trn*V–*ndh*C
Trollius chinensis	TR13	OQ848201	OQ869239	OQ869228	OQ869217
Trollius chinensis	TR14	OQ848202	OQ869240	OQ869229	OQ869218
Trollius chinensis	TR17	OQ848203	OQ869241	OQ869230	OQ869219
Trollius riederianus	TRD2	OQ848204	OQ869242	OQ869231	OQ869220
Trollius riederianus	TRD4	OQ848205	OQ869243	OQ869232	OQ869221
Trollius riederianus	TRD6	OQ848206	OQ869244	OQ869233	OQ869222
Trollius riederianus	TRD7	OQ848207	OQ869245	OQ869234	OQ869223
Trollius riederianus	TRD8	OQ848208	OQ869246	OQ869235	OQ869224
Trollius riederianus	TRD9	OQ848209	OQ869247	OQ869236	OQ869225
Trollius riederianus	TRD11	OQ848210	OQ869248	OQ869237	OQ869226
Trollius altaicus subsp. *sachalinensis*	TALS1	OQ848211	OQ869249	OQ869238	OQ869227
Trollius altaicus	TAL4	—	OQ938649	OQ938641	OQ938657
Trollius altaicus	TAL5	—	OQ938650	OQ938642	OQ938658
Trollius altaicus	TAL6	—	OQ938651	OQ938643	OQ938659
Trollius altaicus	TAL9	—	OQ938652	OQ938644	OQ938660
Trollius altaicus	TAL10	—	OQ938653	OQ938645	OQ938661
Trollius altaicus	TAL11	—	OQ938654	OQ938646	OQ938662
Trollius europaeus	TEU1	—	OQ938655	OQ938647	OQ938663
Trollius europaeus	TEU2	—	OQ938656	OQ938648	OQ938664