

Heracleum sosnowskyi or Heracleum mantegazzianum? DNA-based identification of invasive hogweeds (Apiaceae) in two key regions of the species' invasion history in the territory of the former Soviet Union

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Abstract

Heracleum mantegazzianum Sommier & Levier and *Heracleum sosnowskyi* Manden. are two species that belong to the giant invasive hogweed complex. *H. mantegazzianum* is predominantly found in Western European countries, while *H. sosnowskyi* is invasive in the European part of Russia and Eastern European countries. The taxonomy of the *Heracleum* genus is quite complex, and identifying these species requires extensive expertise. Surprisingly, although *H. mantegazzianum* and *H. sosnowskyi* are considered separate species, their morphological and ecological-physiological properties, as well as their ontogeny and population structure, exhibit remarkable similarities, making them ecological twins. The intentional introduction of this invasive species was initially conducted in the cities of Kirovsk city (Murmansk region, Russia) and Syktyvkar city (Komi Republic, Russia). Plant materials sourced from these two regions were subsequently distributed to all regions encompassing the modern hogweed invasion range across the former USSR countries. The objective of this study was to test the hypothesis that the plants initially introduced in Kirovsk and Syktyvkar actually belong to *H. mantegazzianum*. To accomplish this, herbarium material was collected, and DNA barcoding was performed on 16 samples of giant invasive hogweed from the vicinity of the cities of Kirovsk and Syktyvkar, as well as on 30 *H. mantegazzianum* samples collected within its native range in the Western Caucasus. The results of morphological identification combined with DNA barcoding demonstrate that *H. mantegazzianum* and the plants growing in Kirovsk and Syktyvkar belong to the same species – *H. mantegazzianum*, rather than *H. sosnowskyi* as previously believed.

Introduction

The invasive species *Heracleum mantegazzianum* Sommier & Levier (Sommier and Levier 1895) and *Heracleum sosnowskyi* Manden. (Mandenova 1944) are the prominent representatives of the “giant hogweeds” complex (Jahodová et al. 2007a; Nielsen et al. 2008). Their natural range is limited to the Caucasus. Both species were included by I. Mandenova in the section Pubescentia (Mandenova 1950). The size of these herb species (up to 4–5 m) is their most distinctive trait. One can see a nearly sharp border between the invasive ranges of these species (Fig. 1) on the European continent. The common explanation of this distribution pattern is the contrast in the invasion history of these two species. *H. mantegazzianum* was introduced in Western Europe during the 19th century as an ornamental plant, while *H. sosnowskyi* was introduced as a crop plant in the Soviet Union during the latter half of the 20th century (Pyšek et al. 2007).

Another hypothesis put forward to account for the resemblance in the invasive range boundaries of *H. mantegazzianum* and *H. sosnowskyi*, as well as their alignment with political borders in Europe, is the variability in the regional use of their names. This variation might have arisen from established traditions that emerged following the first descriptions of these invasive species occurrences in different European regions. It is generally recognized that determination of the boundaries of Pubescentia species is challenging (Mandenova 1950; Jahodová et al. 2007a; Pimenov and Ostroumova 2012; Ebel et al. 2018; Vladimirov et al. 2019). The *Heracleum* genus has a complex evolutionary history, including hybridization,

which has resulted in significant variations in morphology. *H. mantegazzianum* and *H. sosnowskyi* species have many identical traits and can be considered as ecological twins (Pyšek et al. 2007; Dalke et al. 2015). This makes the distinction of these two species by morphological characters very difficult. It is important to take into account that when introducing plants of this genus, people did not always carry out the identification and documentation of them (Kudinov et al. 1980; Skupchenko 1989; Pyšek et al. 2007). According to some authors (Pyšek et al. 2007), it would be extremely difficult for introducers to collect mericarps of only one species in the common growth area for both *H. mantegazzianum* and *H. sosnowskyi* (Fig. 1). Ebel et al. (2018) noted that "...the use of the name *Heracleum sosnowskyi* for wildings of the giant *Heracleum* species is rather tentative".

The first samples of plants, under the name *Heracleum sosnowskyi*, were brought from the Caucasus region (near Nalchik city) to the Polar-Alpine Botanical Garden-Institute in Kirovsk (Murmansk region, Russia) in 1947. The purpose of this transfer was to intentionally introduce it as a silage crop (Ozerova and Krivosheina 2018). In 1952, the seeds (mericarps) of this plant were transferred to the Institute of Biology of the Komi Branch of the USSR Academy of Sciences in Syktyvkar (Komi Republic, Russia). At this institute, extensive work began to develop agricultural technology for the cultivation of *Heracleum sosnowskyi*, as well as breeding a new variety of this species. The Institute of Biology in Syktyvkar was recognized as the all-Union center for the introduction of new types of silage plants into cultivation. From 1962 to 1966, 5500 kg of seeds from these new silage plants were sent from Syktyvkar to nearly 1500 farms throughout the former Soviet Union (Moiseev 1967). Mainly located in the European part (Fig. 1). Thus, the primary plant populations of the invasive giant *Heracleum* species, known as *Heracleum sosnowskyi*, which were created in Kirovsk and Syktyvkar, became the main source of seeds for deliberate introduction in the vast territory of the former USSR countries, especially in its European part. The species identification of giant invasive *Heracleum* species specimens collected near Kirovsk and Syktyvkar can be applied to a significant portion of their invasive range.

The predominant characters in the identification keys that differentiate between *H. mantegazzianum* and *H. sosnowskyi* pertain to the morphology of their leaves (Mandenova 1950; Tsvelev 2000; Pimenov and Ostroumova 2012; Vladimirov et al. 2019) and provide a succinct description of the leaf morphology distinguishing these two species: first order leaf-lobes of leaves are pinnately-divided (*H. mantegazzianum*) or the leaf-lobes are pinnately-lobed (*H. sosnowskyi*). It is noteworthy that Ida Mandenova, the first to formally describe Sosnovsky's hogweed species, cautioned against relying on leaf shape as a reliable identification feature for *Heracleum* species due to significant variability. According to I. Mandenova (1950), the most straightforward distinguishing characteristic between these two species is their height: up to 150 cm for *H. sosnowskyi* and up to 300 cm for *H. mantegazzianum*. However, the majority of *H. sosnowskyi* and *H. mantegazzianum* plants described within their invasive range have heights ranging from 250–350 cm up to 500 cm (Pyšek et al. 2007; Dalke et al. 2015). Developing identification keys that can effectively distinguish between *H. sosnowskyi* and *H. mantegazzianum* is challenging due to the scarcity and suboptimal quality of herbarium samples available for study. Collecting herbarium material that adequately represents their morphological diversity is a daunting task due to the size of these species.

Because there are no consistent morphological features that distinguish between these two species, the identification issue can be resolved by employing appropriate DNA markers and methods of DNA barcoding (Hebert et al. 2003; Kress and Erickson 2007). The barcoding of representatives of the plant kingdom requires the use of *rbcL* and *matK* genes, as well as intergenic spacer *trnH-psbA* of chloroplast DNA (cpDNA) and sequence ITS1-5.8s-ITS2 of the ribosomal nuclear DNA cluster (nrDNA) (Shneyer and Rodionov 2019; Shekhovtsov et al. 2019; Shadrin 2021). The ITS region of nrDNA has been employed for the most detailed account, until now, of the molecular phylogenetic relationships and the origin and migration of representatives of the Apiaceae family (Downie et al. 2001, 2010; Banasiak et al. 2013). To identify a number of subtaxa of the Apiaceae family, an attempt was made to use the cpDNA *trnH-psbA* intergenic spacer region (Degtjareva et al. 2012), as well as a combination of ITS and *trnH-psbA* sequences (Logacheva et al. 2008; Yu et al. 2011; Liu et al. 2014; Valuyskikh and Shadrin 2021). Logacheva et al. (2008) declared that the high conservation of the intergenic spacer *psbA-trnH* poses a limitation on the identification of representatives of the *Heracleum* genus and related taxa.

An effective marker for phylogenetic studies of the tribe Tordylieae, which comprises the *Heracleum* genus, is the nrDNA ETS (external transcribed spacer) sequence, especially in combination with the ITS sequence (Logacheva et al. 2010). In addition to the markers listed above, for the molecular phylogenetic analysis of representatives of the *Heracleum* genus in the Chinese flora, noncoding cpDNA sequences were used: *rps16* intron, *trnQ-rps16*, *rps16-trnK* and *rp32-trnL* (Yu et al. 2011). The discrepancy between the phylogenies of the *Heracleum* genus based on the comparison of nrDNA and cpDNA sequences was also noted there, which, according to the authors, is an unprecedented case in the molecular phylogenetic system of the Apiaceae system (Yu et al. 2011).

We believe that it is crucial to identify the giant invasive plants of the genus *Heracleum*, which have a significant distribution range across Eastern Europe and the European part of Russia. Although the observed taxonomic differences between the two species may appear insignificant, their incorrect identification can have severe consequences for invasive plant management. Consequently, it is important to avoid such errors from both a scientific and managerial point of view. The name *H. sosnowskyi* is referenced widely in official and unofficial eradication guides, as well as in regulatory and legal acts at the regional and municipal levels of Russia, Belarus, Latvia, Lithuania, and Estonia. Our purpose was to tackle this issue by comparing the DNA barcode markers of plants that exhibit distinct *H. mantegazzianum* characteristics obtained from their natural habitat area (Western Caucasus) with the invasive giant *Heracleum* plants from two locations where the introduction of "*Heracleum sosnowskyi*" as a crop plant began (Murmansk region and Komi Republic).

Our goal was to address this issue by comparing the DNA barcode markers of plants displaying distinct *H. mantegazzianum* characteristics obtained from their natural habitat in the Western Caucasus with invasive giant *Heracleum* plants from two locations in European East Russia: the Murmansk region and the Komi Republic. These locations served as sources for the distribution of these species throughout the extensive territory currently affected by their invasive range.

Materials and methods

Sampling

A list of all 114 accessions of *Heracleum* and its allies considered in this investigation, including place of origin, voucher information and GenBank reference numbers, is presented in Online Resource 1 and Online Resource 2.

All samples of giant hogweed complex plants were collected in the European part of Russia (Fig. 2, Fig. 3). The samples were collected within the natural range of *H. mantegazzianum* in the Western Caucasus (30 samples), as well as in the areas where *H. sosnowskyi* plants were first introduced as an agricultural crop: the Murmansk Region and Komi Republic (16 samples). The four samples of *Heracleum sibiricum* L. = *Heracleum sphondylium* subsp. *sibiricum* (L.) Simonk., were collected for use as a sister group. The samples were taken from reproductive plants in the flowering or initial fruiting phase. Samples dedicated for genetic analysis were put into paper bags. Herbarium specimens were obtained from most of the samples: a small segment of the inflorescence and the middle leaf were cut into several parts according to the size of the herbarium sheet. Each plant was photographed from several angles, and the height and diameter at the base were measured.

A priori identification of samples

Samples of *H. sibiricum* L. were identified using the key published in «Flora of the Northeast of the European Part of the USSR» (Tolmatchev 1977). The samples of the giant hogweed complex were identified with four identification keys (Mandenova 1950; Tsvelev 2000; Pimenov and Ostroumova 2012; Vladimirov et al. 2019). When using each of these keys, we received cases of ambiguous identification, when we could not attribute the sample clearly to *H. mantegazzianum* or *H. sosnowskyi*. Later in the work, we reported the results of identification obtained using the key published in the monograph by Pimenov and Ostroumova (2012). These authors recognize two species – *H. sosnowskyi* and *H. mantegazzianum*, but they consider *H. mantegazzianum* Sommier & Levier along with *Heracleum persicum* Desf. as synonymous with *Heracleum wilhelmsii* Fisch. & Avé-Lall. At the same time, they admit that, "The name *H. mantegazzianum* is much more commonly used in literature than the name *H. wilhelmsii*, as the plant with beautiful foliage is widely cultivated under the name *H. mantegazzianum* in botanical gardens in Europe and America, and has become invasive in many areas. Therefore, it would be worth considering conserving the name *H. mantegazzianum* instead of using *H. wilhelmsii* and *H. persicum*" (p. 334).

The identification was mainly based on the shape of the segments and leaf lobes of the samples. The use of the shape of the stylopodium, size of the stiles, and color of the anthers as characters was challenging due to difficulties in correlating the verbal description of their morphology with the observed state of the characters. Furthermore, the state of these characteristics at times failed to correspond to any of the descriptions in the species diagnosis. In some cases, not only quantitative but also qualitative

properties were found to be somewhere between the typical attributes for *H. sosnowskyi* and *H. mantegazzianum*. Each giant hogweed specimen was assigned to either *H. sosnowskyi* or *H. mantegazzianum* based on the count of characters that matched the species description. Samples with an equal number of characters assigned to each species or with a predominance of an intermediary character state were labeled hybrids of *H. sosnowskyi* × *H. mantegazzianum* (see Online Resource 1).

DNA Extraction, Amplification, Sequencing

Total DNA was extracted from dried leaves using the DNA-Extran-3 kit (Sintol, Russia) according to the manufacturer's manual. Polymerase chain reaction (PCR) of the fragments was carried out in 50 µl of a mixture containing 10 µl of ScreenMix (Eurogen, Russia), 10 µl of each primer (0.3 µM) (Eurogen, Russia), 18 µl of ddH₂O (Paneco, Russia) and 2 µl of DNA matrix (1–100 ng). Primers for the amplification of DNA sections used in the analysis and the conditions for carrying out the polymerase chain reaction are presented in Table 1.

Table 1
Primers and conditions used for the polymerase chain reaction

Marker	Primer	Sequence positions of forward / reverse primers (5'-3')	Author	PCR conditions
rbcL	SL_For/ SL_Rev	ATGTCACCACAAACAGAGACTAAAGC/ GTAAAATCAAGTCCACCCRCG	(Kress et al. 2009)	95 °C – 1 min; 95 °C – 30 c, 50 °C – 30 c, 72 °C – 45 c, 35 cycles; 72 °C – 5 min.
matK	KIM 3F/ KIM 3R	CGTACAGTACTTTTGTGTTTACGAG/ ACCCAGTCCATCTGGAAATCTTGGTTC	(Kress et al. 2009)	95 °C – 1 min; 95 °C – 30 c, 60 °C – 30 c, 72 °C – 45 c, 35 cycles; 72 °C – 5 min.
trnL	trnL5/ trnL7	CATTACAAATGCGATGCTCT/ TCTACCGATTTTCGCCATATC	(Taberlet et al. 1991)	95 °C – 1 min; 95 °C – 30 c, 55 °C – 30 c, 72 °C – 45 c, 35 cycles; 72 °C – 5 min.
trnH- psbA	trnH2/ psbAF	CGCGCATGGTGGATTACAAATCC/ GTTATGCATGAACGTAATGCTC	(Tate and Simpson 2003)	95 °C – 1 min; 95 °C – 30 c, 60 °C – 30 c, 72 °C – 45 c, 40 cycles; 72 °C – 5 min.

Marker	Primer	Sequence positions of forward / reverse primers (5'-3')	Author	PCR conditions
ITS	ITS-4/ ITS-5	TCCTCCGCTTATTGATATGC/ GGAAGTAAAAGTCGTAACAAGG	(Baldwin et al. 1995)	95 °C – 1 min; 95 °C – 30 c, 55 °C – 30 c, 72 °C – 45 c, 35 cycles; 72 °C – 5 min.
ETS	18S-ETS/ Umb-ETS	ACTTACACATGCATGGCTTAATCT/ GCGCATGAGTGGTGAWTKGTA	(Logacheva et al. 2010)	95 °C – 1 min; 95 °C – 30 c, 60 °C – 30 c, 72 °C – 45 c, 35 cycles; 72 °C – 5 min.
rps16 intron	rps16-F/ rps16-R	ATAGACGGCTCATTG GGA/ CGT GCG ACT TGA AGG	(Wang et al. 2008)	95 °C – 3 min; 94 °C – 1 min, 52 °C – 1 min, 72 °C – 1,5 min,
trnQ- rps16	trnQ/ rps16-1R	CCCGCTATTCGGAGGTTCTGA/ ATCGTGTCTTCAAGTCGCA	(Calviño and Downie 2007)	30 cycles; 72 °C – 10 min.
rps16- trnK	3exon-1/ trnK	TTCCTTGAAAAGGGCGCTCA/ TACTCTACCGTTGAGTTA	(Calviño and Downie 2007)	
rpl32- trnL	rpl32-F/ trnL	CAGTTCCAAAAAACGTACTTC/ CTGCTTCCTAAGAGCAGCGT	(Timme et al. 2007)	

The PCR products were separated in a 1.5% agarose gel based on tris-acetate-ethylene-diamine-tetraacetic acid (TAE). The PCR products were purified using the ColGen DNA purification kit (Sintol, Russia) according to the manufacturer's manual. The sequencing products were separated on a NANOFOR 05 DNA sequencer (Sintol, Russia). DNA isolation, PCR, and sequencing were performed using the equipment of the “Molecular Biology” Center for Collective Use, the Institute of Biology, Federal Research Center of the Komi Research Center, Ural Branch of the Russian Academy of Sciences, Syktyvkar.

Data analysis

Multiple alignment of nucleotide sequences was performed using the ClustalW algorithm in MegaX (Thompson et al. 1994; Kumar et al. 2008). Phylogenetic trees were built in MegaX using the maximum likelihood method (Maximum Likelihood, ML). When constructing molecular phylogenetic trees, we utilized the nucleotide sequences obtained by our team (see Online Resource 2), as well as data from other authors available in the GenBank (<https://www.ncbi.nlm.nih.gov>) and BOLD Systems (<http://www.boldsystems.org>) databases. *Azilia eryngioides* (Pau) Hedge & Lamond was used as an outgroup in the reconstruction of phylogenetic trees. The analyses were conducted for individual sequences and combined data.

Results

The molecular phylogenetic analysis included 50 samples of plants of the *Heracleum* genus (see Online Resources 1 and 2). If no differences were found in the given DNA marker between samples of both species (*H. sosnowskyi* and *H. mantegazzianum*) in a small sample subset, the marker was not sequenced in any additional samples. Overall, a total of 224 sequences were generated for nine markers (Table 2).

Table 2
Characteristics of DNA marker sequences of *Heracleum mantegazzianum*, *H. sosnowskyi* and *H. sosnowskyi* × *H. mantegazzianum*

DNA marker	Sequence length (bp)	Alignment positions	Number of constant positions	Number of variable characters	Number of parsimony-informative positions	Percentage of variable characters
<i>rbcL</i>	544	544	544	0	0	0.0
<i>matK</i>	719	719	718	1	1	0.1
<i>psbA-trnH</i>	188–189	189	177	11	2	5.8
<i>rps16</i> intron	755–757	757	752	3	2	0.4
<i>trnQ-rps16</i>	1217–1219	1221	1210	8	1	0.1
<i>rps16-trnK</i>	668–671	673	667	1	0	0.8
<i>rp132-trnL</i>	955–968	978	970	7	5	0.7
ITS	602	602	600	2	0	0.3
ETS	382–385	385	369	16	12	4.2
Combined (ITS + ETS)	984–987	987	969	18	12	1.8

After analyzing the *rbcL* and *matK* genes, as well as the *psbA-trnH* intergenic spacer in the cpDNA of plants belonging to the *Heracleum* genus, which were available in GenBank and BOLD Systems, we concluded that these sequences are highly conserved. Consequently, these genetic markers are not suitable for DNA barcoding of plants in this particular group. However, we obtained the sequences of the *rbcL*, and *matK* genes, and the *psbA-trnH* cpDNA intergenic spacer for the plant samples we identified as *H. sosnowskyi* and *H. mantegazzianum* (Table 2). The reason behind this is that the information available on these particular species was previously confined to a single sequence of *rbcL* and *matK* for *H. mantegazzianum* only, and a *psbA-trnH* sequence solely for *H. sosnowskyi*.

After sequencing the *rbcL* and *matK* DNA markers that were isolated from our 27 samples and combining them with data obtained for other species in the *Heracleum* genus (refer to NCBI, BOLD Systems), we obtained the expected results. We found that there is a remarkably low level of divergence both within the group of giant hogweeds and for all *Heracleum* genera. The degree of pairwise genetic differences between the compared samples ranged from 0 to 1.1% and from 0 to 1.5% for the *rbcL* and *matK* genes, respectively. Among the plant samples a priori identified by us as *H. sosnowskyi* and *H.*

mantegazzianum, the degree of pairwise genetic differences was 0%. Thus, it was confirmed that these markers are not applicable for the identification of plants of the *Heracleum* genus.

Logacheva et al. (2008) demonstrated several variations within the *psbA-trnH* sequence, two of which were found in our DNA samples at positions 60 to 76 bp (Fig. 4). The first variant is TTGTTCTATCA, and the second variant is TGATAGAACAA, and both of them are repeated twice in the sequences we obtained. We obtained a total of 41 *psbA-trnH* sequences from *H. sosnowskyi* and *H. mantegazzianum* (Table 2). These variants of the *psbA-trnH* sequence occur regardless of species, including *H. sosnowskyi* and *H. mantegazzianum*, as well as other species included in the analysis. Logacheva et al. (2008) explained that such variability is caused by a combination of two independent evolutionary events, namely inversion and duplication, which occurred independently of each other on several occasions. Our analysis of the pairwise genetic variability between the samples of *H. mantegazzianum* and *H. sosnowskyi* resulted in 5.3%. We found no correlation between this type of variability and the species names of the samples in our analysis of both variability variants. We also discovered that another polymorphic site at position 30 (G/T) in the study sample did not exhibit any correlation with the species.

Next, 10 plant samples were studied to assess the variability of the sequences of the *rps16* intron and three noncoding cpDNA intergenic spacers – *trnQ-rps16*, *rps16-K* and *rp132-trnL*. Previously, these markers were used to study molecular phylogenetic relationships in the genus *Heracleum* (Yu et al. 2011) and to identify *H. mantegazzianum* (Barcoding Facility for Organisms and Tissues of Policy Concern 2019). Sequence alignment showed that the *rps16* intron and intergene spacer *trnQ-rps16* have a less pronounced level of variability than intergenic spacers *rps16-trnK* and *rp132-trnL* (Table 2). The polymorphism of the *rps16* intron fragments and the *trnQ-rps16* intergenic spacer is mainly explained by single nucleotide substitutions. The variability of the *rps16-trnK* intergenic spacer was determined by single nucleotide insertions and deletions. In the *Heracleum* samples under study, the *rp132-trnL* intergenic spacer included three indels 12, 10 and 6 bp long, with a total alignment length of 978 bp. The same polymorphism of the *rp132-trnL* marker has been previously reported in other Magnoliophyta species (Shaw et al. 2007; Miller et al. 2009).

Nevertheless, the revealed variability for the *rps16* intron sequences and cpDNA intergenic spacers *trnQ-rps16*, *rps16-trnK* and *rp132-trnL* of the sample series under study did not allow us to allocate the samples based on their reference to *H. mantegazzianum* or *H. sosnowskyi* (Fig. 5).

The nrDNA ITS sequence analysis revealed resolution in the topology of the phylogenetic tree for most species of the genus *Heracleum* (Fig. 5). The pairwise genetic distance between different species ranged from 0 to 2.1%. Meanwhile, for the group of samples including according to our data, *H. mantegazzianum*, *H. sosnowskyi*, *H. sosnowskyi* × *H. mantegazzianum* a very low level of variability in the ITS sequence may be noted. The pairwise genetic distance ranged from 0 to 0.3%. A value of 0.3% was associated with a hybrid peak (R) in the 5.8s region in three samples of *H. sosnowskyi* collected in the North Caucasus (NCBI: OQ222183, OQ222184, OQ222185).

The samples assigned by us to *H. mantegazzianum*, *H. sosnowskyi* and *H. sosnowskyi* × *H. mantegazzianum* with a high bootstrap value (99) grouped into a separate clade differing from the rest of the species of the genus (Fig. 6). After overall alignment of the sequences of different species of the *Heracleum* genus at position 125 for ITS1, a unique substitution (A/G) typical for just the mentioned plant samples was found. The sequences we obtained for *H. sibiricum* were found to be identical to the GenBank data for this species, as shown in the molecular phylogenetic tree (Fig. 6).

Another genetic marker that was used as a potential DNA barcode to distinguish between the analyzed samples was the nrDNA ETS sequence. A revision of genetic databases (NCBI 2023; BOLD Systems 2023) showed that data on ETS are not available for *H. sosnowskyi*, and for *H. mantegazzianum* are presented with regard to one sample. We obtained ETS sequences for 46 plant samples that we identified as *H. mantegazzianum*, *H. sosnowskyi* and *H. sosnowskyi* × *H. mantegazzianum*. A total of 81 ETS sequences for 32 species of the genus *Heracleum* were included in the analysis (Fig. 6). The ratio of variable sites to the total alignment length within the analyzed group (*H. mantegazzianum*, *H. sosnowskyi* and *H. sosnowskyi* × *H. mantegazzianum*) was 4.2% (Table 2).

Based on the comparison of *H. sosnowskyi* plant sequences with the only available *H. mantegazzianum* sample in genetic databases, we found the use of the ETS sequence for species distinction upon involving more *H. mantegazzianum* samples as promising. The qualitative analysis of the ETS sequences of *H. mantegazzianum* from the North Caucasus revealed that they are more similar to plants growing in northeast Russia than to the *H. mantegazzianum* sample presented in the genetic database (NCBI: FJ807509) (Fig. 7). The average pairwise genetic distance within the sample group under analysis ranged from 0.0 to 0.5%. The average pairwise genetic distance between other compared *Heracleum* species ranged from 3.0 to 22.7%. A qualitative analysis of sequence variability showed no correlation between the identified changes and the species affiliation of the *H. sosnowskyi* and *H. mantegazzianum* samples.

We identified four substitutions at positions 34 (T/C), 186 (A/C, A/G), 259 (C/A) and 357 (A/G) attributable only to 46 samples of the plants classified by us as *Heracleum mantegazzianum*, *H. sosnowskyi*, and *H. sosnowskyi* × *H. mantegazzianum*, including a sample from the GenBank database (NCBI:FJ807509). Upon analyzing the alignment of the combined sequences (ITS + ETS = 984 bp), we observed a consistent general topology in the phylogenies derived from the ITS and ETS sequences separately.

Discussion

Using nine DNA markers, we examined the distinctions between *H. mantegazzianum* specimens collected in the Western Caucasus and giant *Heracleum* specimens gathered from their initial introduction sites located in Northern Russia. Among these markers, seven were chloroplast DNA fragments, namely, *rbcL*, *matK*, *psbA-trnH*, *rps16* intron, *trnQ-rps16*, *rps16-trnK*, and *rp132-trnL*, and two were from nuclear DNA, namely, ITS and ETS.

The markers *rbcL* and *matK* DNA exhibited a minimal level of polymorphism in the studied *Heracleum* samples, rendering them unsuitable for distinguishing not only species within the giant hogweed complex but also some other *Heracleum* species.

The markers *psbA-trnH*, *rps16* intron, *trnQ-rps16*, *rps16-K*, and *rp132-trnL* showed varying degrees of polymorphism, but their variability was insufficient for discriminating between *H. mantegazzianum* specimens collected within the natural range and giant hogweed complex specimens collected in the invasive part of the range.

Our results are in good agreement with previously obtained data on the use of chloroplast DNA fragments for reconstructing the phylogeny and identifying species of the Apiaceae family. The markers *rbcL* and *matK* are impractical for distinguishing between species in the Apiaceae family. However, markers such as *psbA-trnH*, *rps16* intron, *trnQ-rps16*, *rps16-K*, and *rp132-trnL* exhibit varying levels of intraspecific and interspecific variability for different species in this family (Logacheva et al. 2008; Liu et al. 2014).

The nuclear markers ITS and ETS demonstrated a significantly greater ability to differentiate between species of the Apiaceae family. However, the evolution of these sequences does not always correspond well with the evolution of morphological traits of the species (Logacheva et al. 2010; Yu et al. 2011; Liu et al. 2014). Our results indicate that the use of these markers, either individually or in combination, enables the reliable separation of samples of giant invasive hogweed collected from the sites of primary introduction in northern Russia and within the boundaries of the natural range of *H. mantegazzianum* from other species in this genus. To clarify, both *H. mantegazzianum* and invasive hogweeds collected within the invasive range of Northern European Russia belong to the same species. All plant samples from the 'giant *Heracleum* complex' collected by us in Northern European Russia a priori identified by us as *H. mantegazzianum*, *H. sosnowskyi*, and *H. sosnowskyi* × *H. mantegazzianum* should be identified as *H. mantegazzianum*.

As mentioned in the introduction, the populations of giant invasive hogweed from the Murmansk region and the Komi Republic were used as the foundation for planting these plants in most of their current invasive habitats. According to our research findings, we can confidently state that the invasive range of *H. mantegazzianum* includes not only Western Europe but nearly the entire European continent.

These results lead us to two possible conclusions. First, *H. mantegazzianum* hogweed, but not *H. sosnowskyi*, may have been introduced to the North of Russia (Murmansk region and the Komi Republic) in the mid-20th century. Alternatively, *H. sosnowskyi* hogweed could be considered a synonym for *H. mantegazzianum*. We currently do not have adequate data to support the preceding statement. For this reason, it is necessary to gather samples for herbarization and sequencing from the areas where type specimens of *H. sosnowskyi* were previously collected; this includes the vicinity of Lelovani village, located in the Adigeni Municipality of the Samtskhe-Javakheti region in Georgia. We also need to collect samples from other locations detailed in Ida Mandenova's monograph (1950) where *H. sosnowskyi* samples were obtained.

Our results appear to contradict a comprehensive study on the genetic diversity of the giant hogweed complex by Jahodová et al. (Jahodová et al. 2007b). In their study, AFLP-markers were effectively used to determine genetic distances between samples of *H. sosnowskyi*, *H. mantegazzianum*, and *H. persicum* collected from both the invasive and endemic ranges. However, our results demonstrate that although specimens identified as *H. sosnowskyi* and *H. mantegazzianum* typically form distinct clusters on the AFLP-derived dendrogram, they actually belong to the same higher-level clade. Within this clade, specimens of *H. sosnowskyi* and *H. mantegazzianum* are separated with a bootstrap support of less than 50%. It is also worth noting that the AFLP method allows for the acquisition of a larger number of loci for analysis and, typically, enables the identification of a greater number of homogeneous groups within the studied plant species compared to the use of ISSR and SSR (microsatellite) markers (Nybohm 2004).

Population genetic studies of *H. mantegazzianum* using SSR markers in the UK (Walker et al. 2003) and Finland (Niinikoski and Korpelainen 2015) revealed the presence of distinct groups within this species. In Great Britain, these differences were associated with the boundaries between river catchments. The genetic variances between the two clusters of the eight populations of *H. mantegazzianum* studied in Finland were attributed to their independent introduction to that country's territory. At the same time, microsatellite markers did not reveal any differences between *H. persicum* Desf. ex Fisch. and *H. rechingeri* Manden. (Daemi-Saeidabad et al. 2020). This indicates that distinguishing species within the Pubescentia section of the *Heracleum* genus solely based on morphological characteristics is controversial.

If samples of *H. mantegazzianum* plants and giant invasive *Heracleum* plants collected in the areas of the primary introduction of giant *Heracleum* species in northern European Russia show inconsistent differences in morphological characters, similar ecological traits and do not differ in DNA markers, it would be appropriate to recognize them simply as a single polymorphic species. It should be acknowledged that there have been no studies on crossbreeding *H. sosnowskyi* and *H. mantegazzianum*, as it is impossible to obtain pure lines of both species for such an experiment. Perhaps in the mid-20th century, populations of *H. sosnowskyi* and *H. mantegazzianum* from various regions of the Greater Caucasus could still be easily distinguished. However, with the initiation of intensive introduction efforts, the gene pools of both species have intermingled, resulting in the inability to identify *H. sosnowskyi* in its pure form within the invasive range. To verify the validity of isolating *H. sosnowskyi* as a distinct species, it is essential to compare the DNA markers of *H. mantegazzianum* with those of plant material collected from the *locus classicus* of *H. sosnowskyi*.

The data obtained from the study revealed that the name *H. sosnowskyi* is being used in the eastern part of the extensive invasive range of giant hogweed, only in accordance with established tradition. The correct name for most giant invasive *Heracleum* species in European Russia is *H. mantegazzianum*.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dmitry Shadrin, Dalke Igor, Zakhozhiy Ilya, Shilnikov Dmitry, Kozhin Mikhail, Chadin Ivan. The first draft of the manuscript was written by Dmitry Shadrin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

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Figures

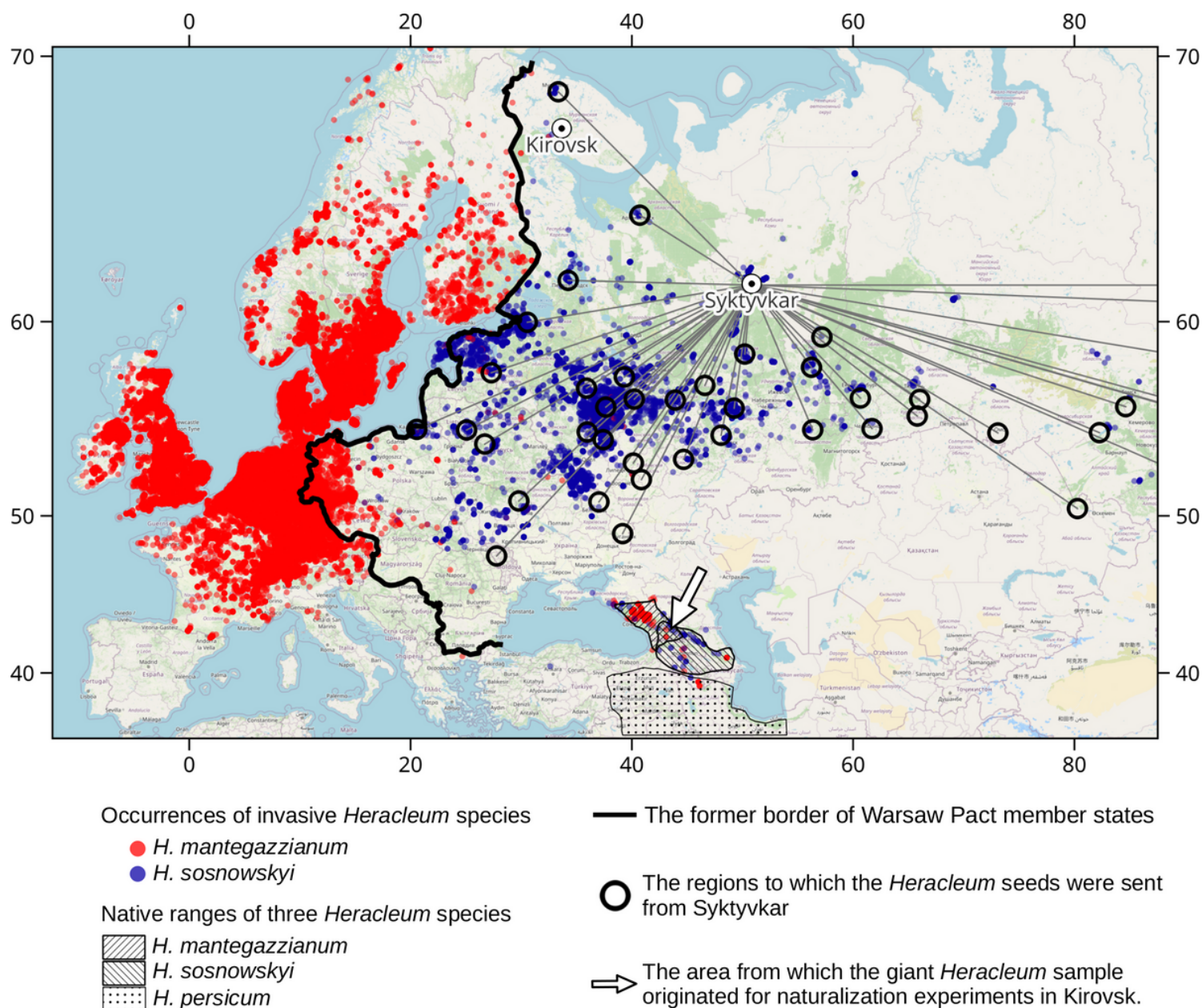


Figure 1

Modern distribution of invasive *Heracleum* species (*H. mantegazzianum*, *H. sosnowskyi*) on the European continent (GBIF.Org User 2023) and the regions to which the seeds of *Heracleum sosnowskyi* were dispatched from Syktyvkar as of 1967 (Moiseev 1967).

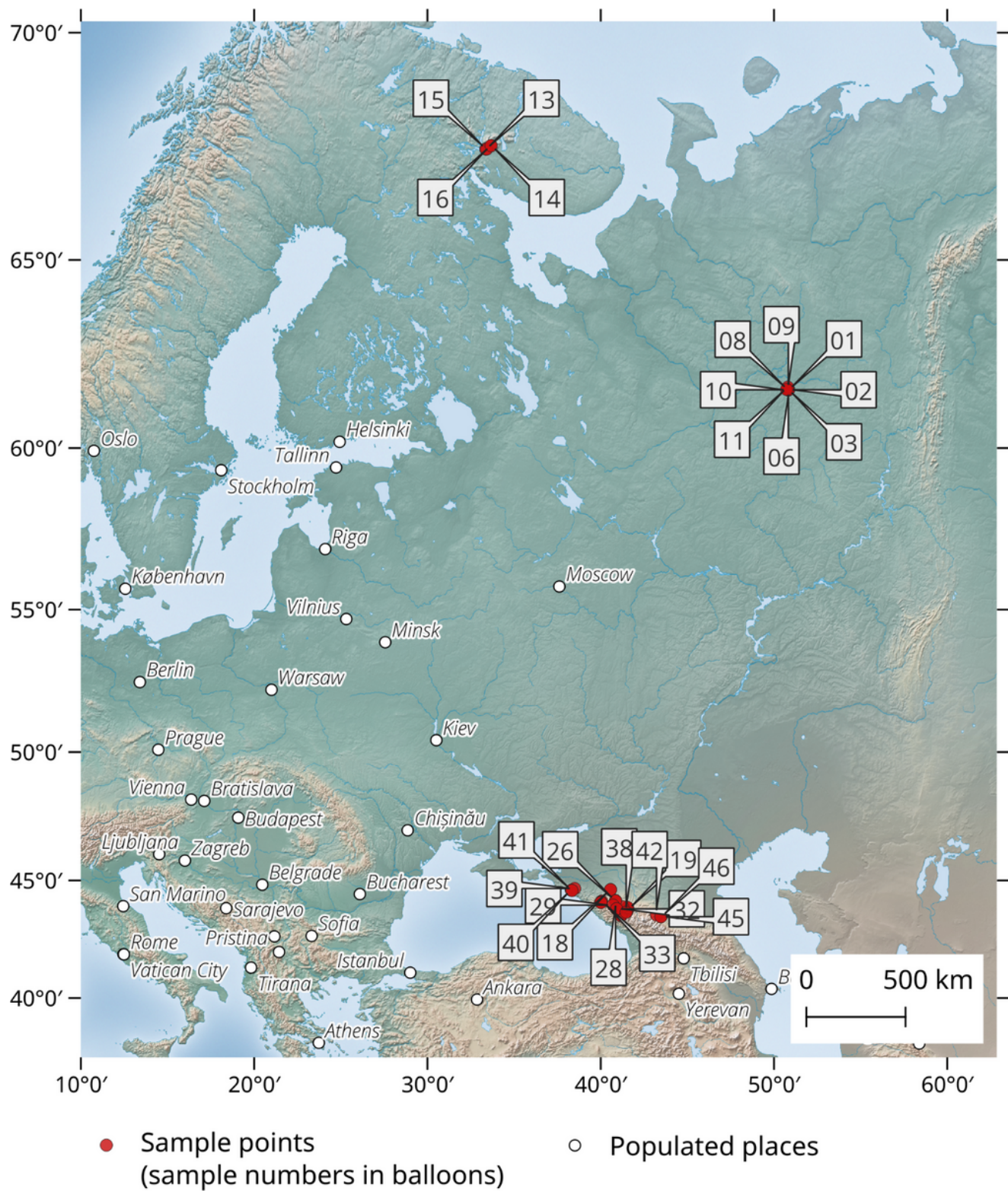


Figure 2

Heracleum sample collection sites within invasive and natural ranges.

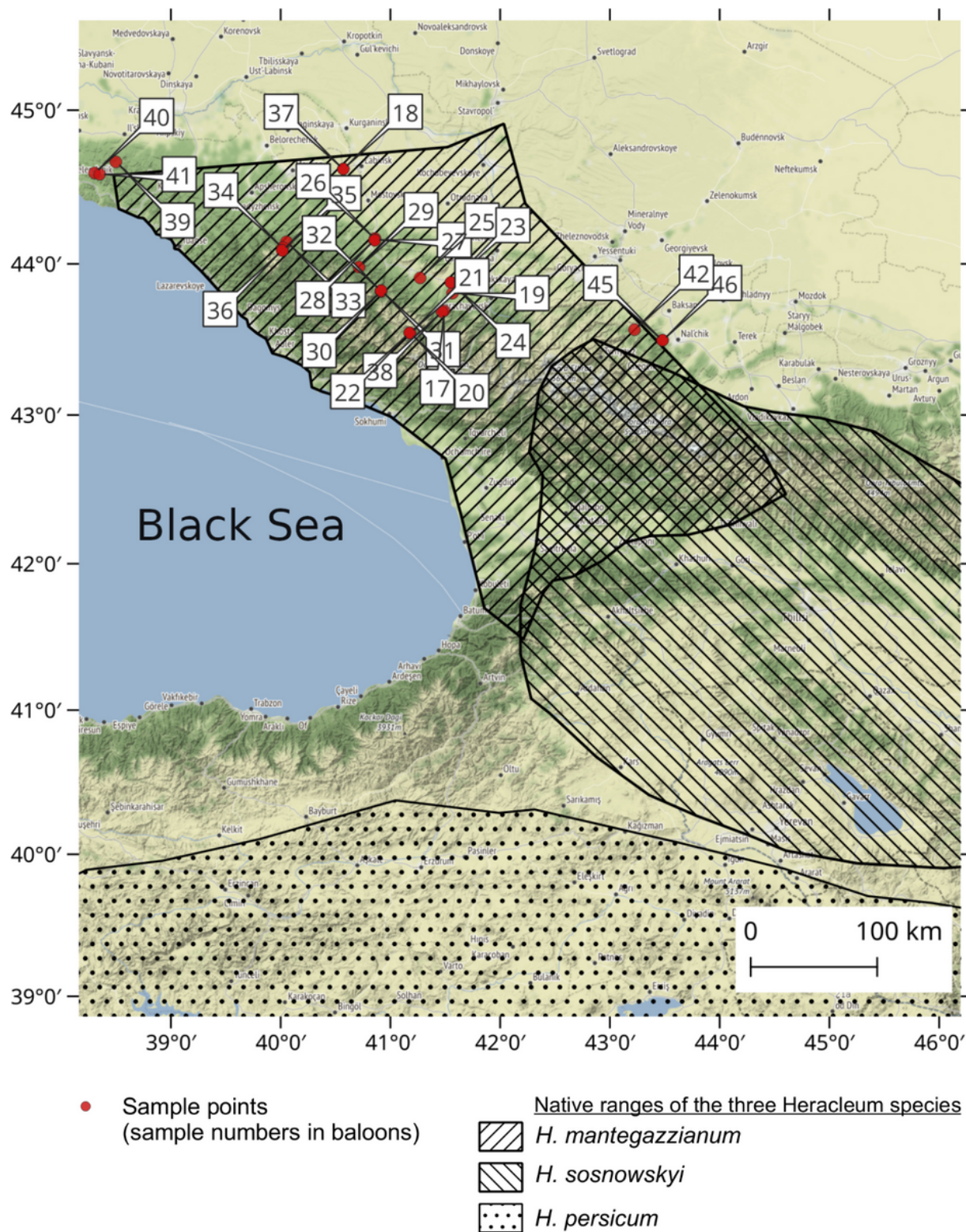


Figure 3

Natural range boundaries for *Heracleum mantegazzianum*, *H. sosnowskyi*, *H. persicum* and sample collection sites. The range boundaries are provided according to (Jahodová et al. 2007a).

			3666667777
			0023581346
Heracleum	cf. sosnowskyi	OQ274556	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OQ274557	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OQ274558	TTCTTTTCTT
Heracleum	cf. sosnowskyi	OQ274559	TTCTTTTCTT
Heracleum	mantegazzianum	OL762530	TTCTTTTCTT
Heracleum	mantegazzianum	OL762531	GAAGAAAAGA
Heracleum	mantegazzianum	OL762532	GAAGAAAAGA
Heracleum	mantegazzianum	OL762533	TTCTTTTCTT
Heracleum	mantegazzianum	OL762534	TTCTTTTCTT
Heracleum	mantegazzianum	OL762535	TTCTTTTCTT
Heracleum	mantegazzianum	OL762536	GTCTTTTCTT
Heracleum	mantegazzianum	OL762537	GAAGAAAAGA
Heracleum	mantegazzianum	OL762538	GAAGAAAAGA
Heracleum	mantegazzianum	OL762539	GAAGAAAAGA
Heracleum	mantegazzianum	OL762540	GAAGAAAAGA
Heracleum	mantegazzianum	OL762541	GAAGAAAAGA
Heracleum	mantegazzianum	OL762542	GAAGAAAAGA
Heracleum	mantegazzianum	OL762543	TTCTTTTCTT
Heracleum	mantegazzianum	OL762544	GAAGAAAAGA
Heracleum	mantegazzianum	OL762545	GTCTTTTCTT
Heracleum	mantegazzianum	OL762546	TTCTTTTCTT
Heracleum	mantegazzianum	OL762547	TTCTTTTCTT
Heracleum	mantegazzianum	OL762548	TTCTTTTCTT
Heracleum	mantegazzianum	OL762549	TTCTTTTCTT
Heracleum	mantegazzianum	OL762550	TAAGAAAAGA
Heracleum	mantegazzianum	OL762551	TTCTTTTCTT
Heracleum	mantegazzianum	OL762552	TTCTTTTCTT
Heracleum	mantegazzianum	OL762553	GTCTTTTCTT
Heracleum	cf. sosnowskyi	OQ274554	GTCTTTTCTT
Heracleum	cf. sosnowskyi	OQ274555	TTCTTTTCTT
Heracleum	cf. mantegazzianum	OL762500	GAAGAAAAGA
Heracleum	cf. mantegazzianum	OL762501	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OL762502	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OL762503	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OL762498	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OL762499	GAAGAAAAGA
Heracleum	cf. mantegazzianum	OL762493	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OL762494	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OL762495	GAAGAAAAGA
Heracleum	cf. sosnowskyi	OL762496	GAAGAAAAGA
Heracleum	cf. mantegazzianum	OL762497	GAAGAAAAGA
Heracleum	sosnowskyi	DQ996584	TAAGAAAAGA
Heracleum	transcaucasicum	DQ869374	GAAGAAAAGA
Heracleum	sphondylium subsp. sibiricum	EU594934	GTCTTTTCTT
Heracleum	sphondylium subsp. sibiricum	OL762504	TTCTTTTCTT
Heracleum	sphondylium subsp. sibiricum	OL762505	TTCTTTTCTT
Heracleum	sphondylium subsp. sibiricum	OL762506	TTCTTTTCTT
Heracleum	sphondylium subsp. sibiricum	OL762507	TTCTTTTCTT
Heracleum	persicum	EU594939	GTCTTTTCTT

Figure 4

The variable sites from an alignment of *trnH-psbA* cpDNA sequences.

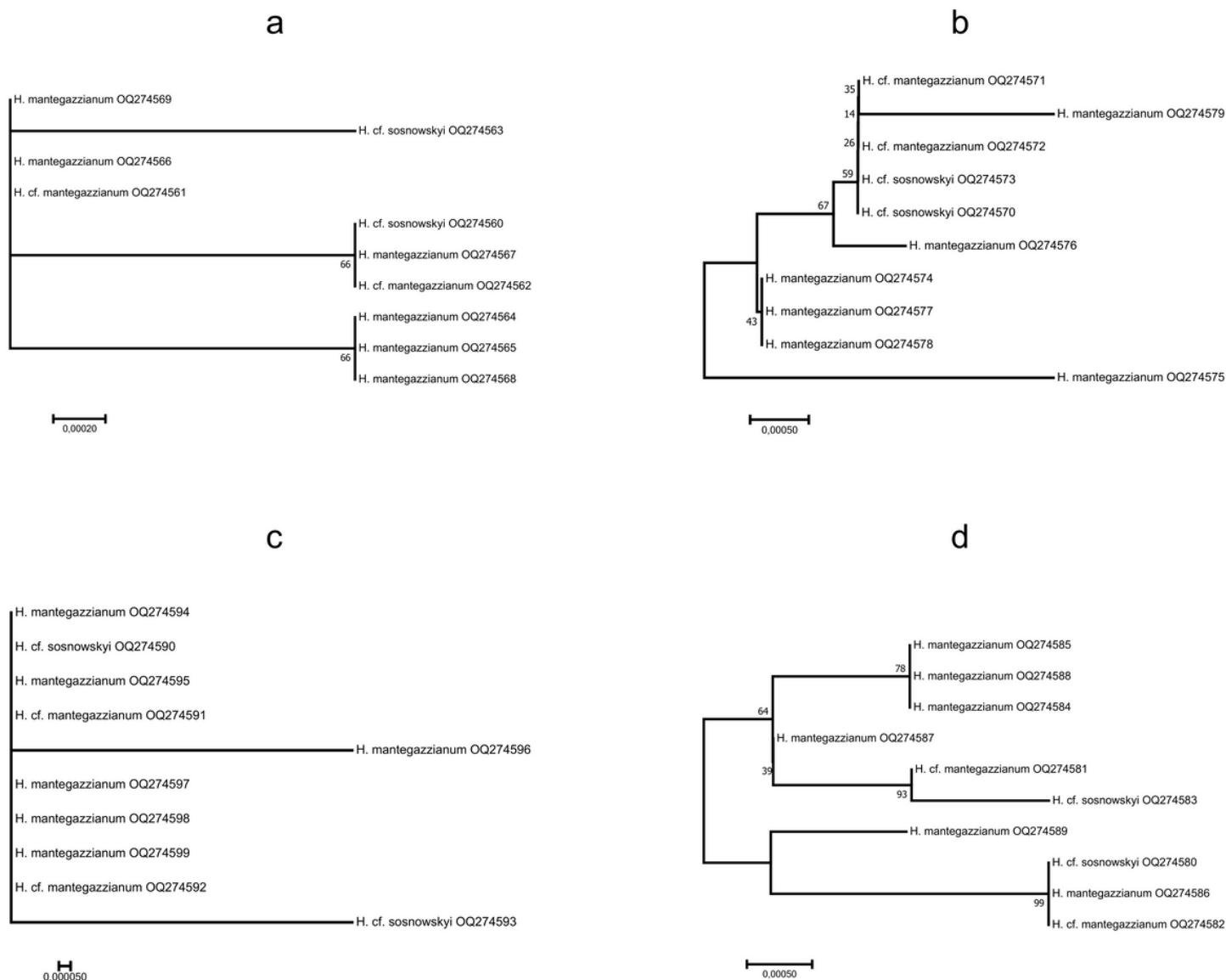


Figure 5

Molecular phylogenetic trees constructed based on sequence comparison of 10 plant samples of giant hogweed. Trees were constructed using the maximum likelihood method. The specimen GenBank accession numbers in parentheses follow the names. a) *rps16* intron , b) *trnQ-rps16*, c) *rps16-trnK*, d) *rp32-trnL*.

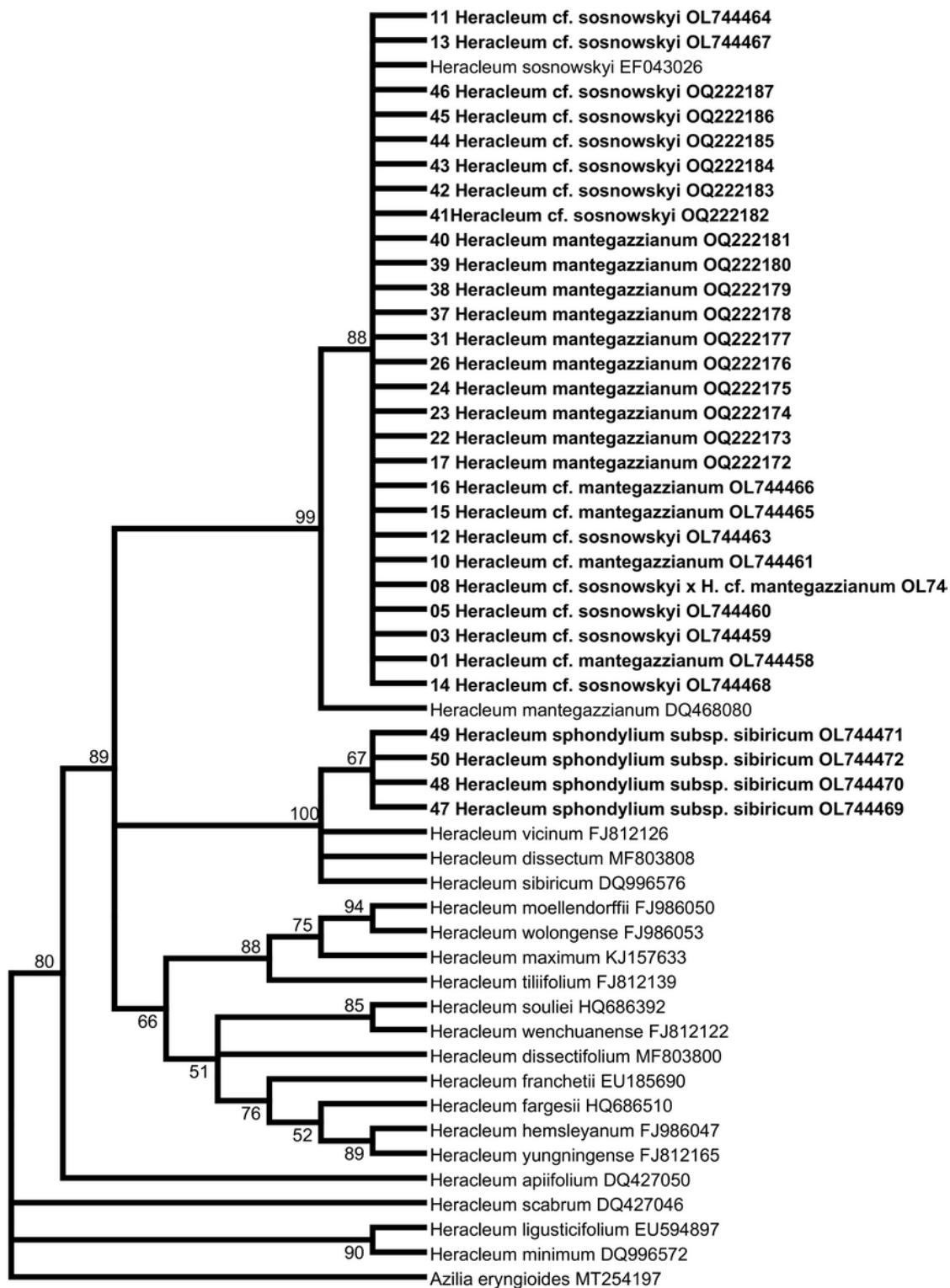


Figure 6

The optimal phylogenetic tree of the *Heracleum* species generated with maximum likelihood analysis for the ITS nrDNA. The specimen GenBank accession numbers in parentheses follow the names. Samples obtained by the authors are highlighted in bold.

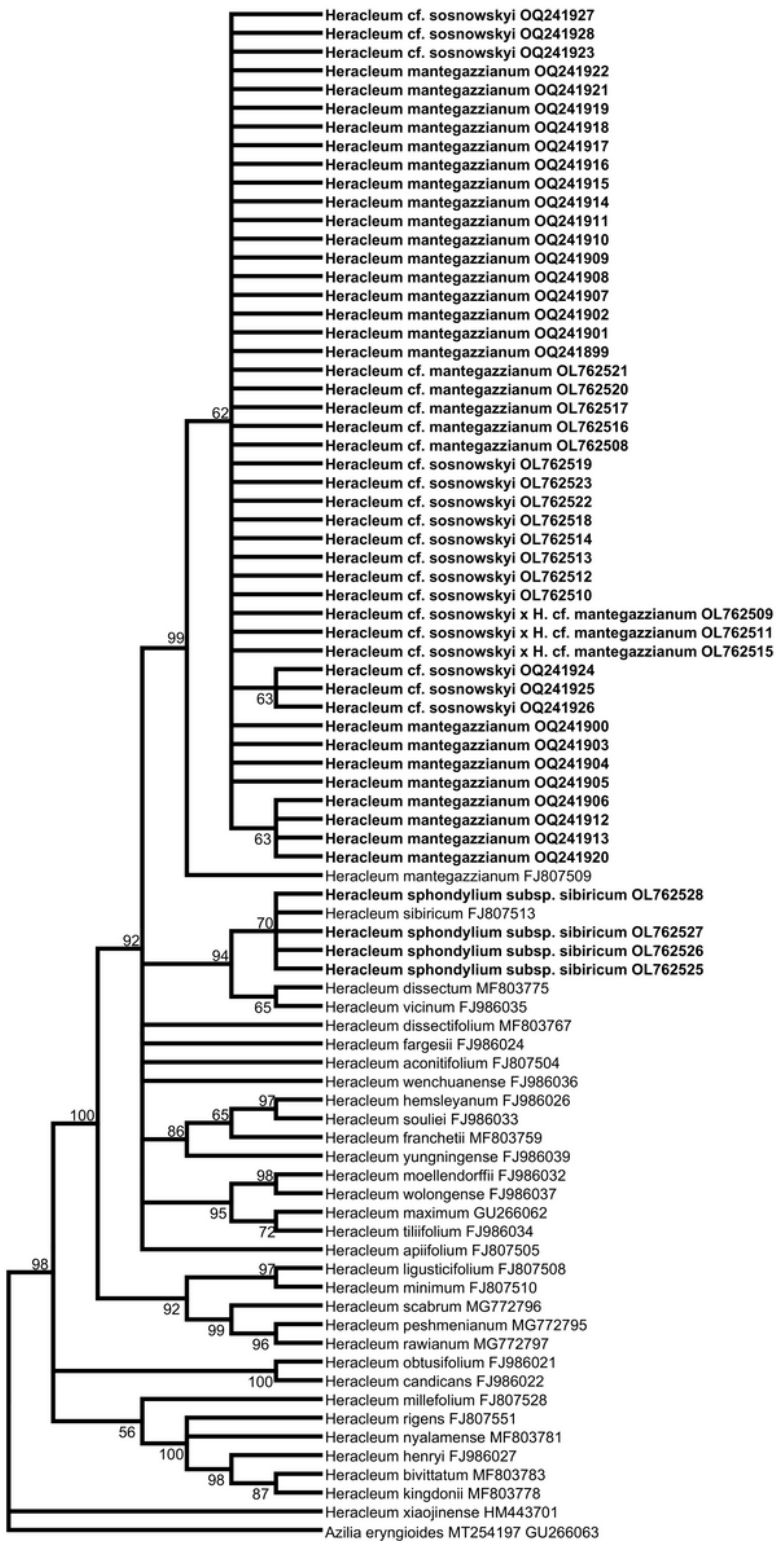


Figure 7

The optimal phylogenetic tree of the *Heracleum* species generated with maximum likelihood analysis for the ETS nrDNA data. The specimen GenBank accession numbers in parentheses follow the names. Samples obtained by the authors are highlighted in bold.

Supplementary Files

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