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ORIGINAL RESEARCH PAPER in RECENT DEVELOPMENTS IN TAXONOMY AND PHYLOGENY OF PLANTS

Taxonomic Evaluation of *Hedera crebrescens*: A Potentially Invasive Ivy in Central Europe

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Abstract

Although *Hedera helix* is the only native ivy distributed in Central Europe, other ivy species are cultivated in this region and have horticultural importance, including *H. hibernica*, *H. colchica*, and *H. azorica*. On the basis of morphological, cytological, and phenological studies, a newly identified diploid species of ivy, *H. crebrescens* Bényei-Himmer & Höhn, was recently described from Hungary. Due to its high reproductive capacity and vigorous growth, this species can be considered a potentially invasive plant that could readily supplant *H. helix* in its native habitats. To characterize the molecular taxonomic status of *H. crebrescens*, we conducted a molecular genetic analysis based on five chloroplast and one nuclear DNA regions. Our phylogenetic reconstruction supported the monophyly of *Hedera*, with a tree topology similar to that previously obtained based on phylogenetic cpDNA analyses. Mediterranean species of ivy were well separated from the remainder of the European species, as well as from Asian species. *Hedera crebrescens* represented a single independent haplotype within the Asian–European cluster, whereas *H. helix* proved to be polyphyletic. The detected species-specific haplotype and invariability among studied specimens obtained from different geographical locations, provide support for the taxonomical autonomy of *H. crebrescens*.

Keywords

Hedera crebrescens; invasive species; ivy; molecular study; phylogeny; taxonomy

1. Introduction

Currently, one of the most substantial threats to the terrestrial ecosystems is the rapid decline in biodiversity, which has become a particularly important focus of nature conservation initiatives. In this regard, invasive species are considered among the most prominent causes of biodiversity loss, second only to habitat destruction or transformation. Invaded ecosystems are typically damaged to varying degrees by non-native species via changes in species diversity and community structures. Non-native species that spread rapidly over extensive areas, and thereby threaten local biological diversity, are considered invasive species (Genovesi & Shine, 2004). In worst case scenarios, the mass distribution of new organisms can lead to local extinctions among native species (Vilà et al., 2007). By cultivating alien plants in residential and botanical gardens, humans unwittingly contribute to the spread of these plants. Cultivated taxa capable of self-propagation can readily disperse into natural environments, some of which are able to establish populations in different habitats. Breeding or hybridization in gardens can even alter the genetic make-up of species, and consequently, escaped specimens might have already undergone

adaptive changes. Adequate knowledge of the origin and distribution of alien plant species and the ecology of populations is crucial for the conservation of biodiversity (Bevill & Louda, 1999; Duncan & Young, 2000).

The genus *Hedera* is the single representative of the Araliaceae plant family in Europe. According to McAllister and Marshall (2017) and Valcárcel et al. (2017), the genus comprises 12 evergreen-vine species distributed across the Northern Hemisphere, from Macaronesia, Europe, and North Africa to South and East Asia. It is well known that *Hedera* species are difficult to identify, owing to the diverse morphological characters of their leaves, which can be affected by environmental conditions and differ according to ontogenic stage during individual development. Indeed, there can be significant differences in the appearance between the juvenile and adult shoots of an individual, and ontogenic changes are known to be reversible (Robbins, 1957, 1960; Rogler & Hackett, 1975).

The first attempts to classify *Hedera* were based on morphological traits of the vegetative organs, for which leaf morphology and the foliar trichomes were used as the main diagnostic characters in species delimitation. Subsequently, however, trichome morphology was shown to be not decisive trait for the delimitation of different taxa, as these features were considered evolutionary unstable (Green et al., 2011; Vargas et al., 1999). During the early 1980s, cytological analyses were introduced in taxonomical studies. For example, the separation of *H. hibernica* from *H. helix*, which were previously assumed to be taxonomically identical, was based on the different levels of ploidy (*H. hibernica* is a tetraploid, whereas *H. helix* is a diploid) in addition to differences in trichomes (Jacobsen, 1954; McAllister & Rutherford, 1990). *Hedera algeriensis* (2x) and *H. maroccana* (4x) were similarly differentiated (Rutherford, 1984, 1989). Nonetheless, based on area disjunction that has promoted geographic isolation, *H. iberica* and *H. maderensis* (Ackerfield & Wen, 2003), and *H. nepalensis* var. *nepalensis* and *H. nepalensis* var. *sinensis* have been distinguished (Ackerfield, 2001; Ackerfield & Wen, 2002). Polyploidy is a frequent phenomenon in the genus *Hedera*, in which diploid, tetraploid, hexaploid, and octoploid populations are known (Vargas et al., 1999), and polyploidization events followed by geographic isolation have been identified as prominent processes in promoting the diversification of species (Valcárcel et al., 2017).

On the basis of trichome morphology, geographical distribution, and ploidy level, four species of *Hedera* have been recognized in Asia, namely, *H. colchica* Koch (8x), *H. nepalensis* Koch (2x), *H. pastuchovii* Woronow (6x), and *H. rhombea* (Miq.) Bean (2x), all of which are characterized by scale-like trichomes. Furthermore, in Central Europe, two species with stellate trichomes are known, namely, *H. helix* L. (2x), which has multiangulate erect trichomes, and *H. hibernica* (Kirch.) Bean (4x), which bears bifurcate stellate trichomes. The most diverse region in terms of *Hedera* species is the Mediterranean region, in which the following seven taxa have been described: *H. azorica* Carr. (2x), which has stellate hairs, and *H. algeriensis* Hibberd (4x), *H. canariensis* Willd (2x), *H. iberica* McAllister (6x), *H. maderensis* Koch ex Rutherford (6x), *H. maroccana* (2x), and *H. pastuchovii* ssp. *cyprica* McAllister (6x), which all possess scale-like trichomes (McAllister & Marshall, 2017).

In addition to numerous morphological studies (Ackerfield, 2001; Ackerfield & Wen, 2002, 2003; Kost et al., 2003; Lum & Maze, 1989; Valcárcel & Vargas, 2010), molecular studies, which started in the late 1990s, have provided new insights into taxonomical relationships, phylogeography, and evolution of the genus *Hedera* (Ackerfield & Wen, 2003; Green et al., 2011; Grivet & Petit, 2002; Lowry et al., 2004; Valcárcel et al., 2003, 2017; Valcárcel & Vargas, 2012; Vargas et al., 1999). Phylogenetic and phylogeographic studies have confirmed Asia to be the center of origin for the genus, with the Mediterranean area being considered a secondary center of diversification (Valcárcel et al., 2003, 2017; Valcárcel & Vargas, 2012). However, these molecular studies have not been able to conclusively clarify the phylogenetic lineages of all taxa, owing to the low genetic variability between species within the genus.

In Central Europe, *H. helix* is considered to be the only native ivy (Rose, 1996; Soó & Jávorka, 1951). In addition to *H. helix*, however, several species are mass cultivated and have horticultural importance, including the Western European *H. hibernica*

and the Central Asian *H. colchica*. Furthermore, *H. azorica*, *H. maroccana*, and *H. rhombea* are cultivated to a lesser extent, although mainly in private collections or botanical gardens. However, during the early 2000s, the spread of an ivy that differs from the known species with respect to several traits was detected in Hungary (Bényei-Himmer et al., 2017). Initially, this taxon was regarded to be a variety of the tetraploid *H. hibernica* (Udvardy & Bényei-Himmer, 1999), although subsequent cytological analyses (Lengyel et al., 2007) revealed it to be diploid ($2x = 48$). On the basis of an assessment of morphological, cytological, and phenological traits, the newly identified species has been described under the name *H. crebrescens* (Bényei-Himmer et al., 2017). According to field observations, this species is spreading spontaneously in several parts of Hungary, and has also been observed in other neighboring countries, namely, Western Ukraine, Slovakia, Austria, Germany, and The Netherlands. Owing to its high reproductive capacity (high rate of viable seed production, successful propagation by birds, and high germination rate) and vigorous growth, this species is considered to be a potentially invasive plant that is becoming naturalized in many parts of Central Europe, thereby threatening the habitats of the autochthonous *H. helix* (Bényei-Himmer et al., 2017). Indeed, this newly identified species has recently been included in the black list of the Hungarian flora (Bartha, 2020, p. 20).

With reference to earlier phylogenetic studies on the genus, we conducted a molecular study in which we sought to reveal the taxonomical position and phylogenetic relationships of *Hedera crebrescens*. Highlighting the importance of species' identification, we aimed at distinguishing this species from other ivy taxa to prevent its excessive growth and invasive spread throughout the habitats of the native *H. helix*.

2. Material and Methods

2.1. Plant Material and GenBank Sequences

Hedera crebrescens Himmer-Höhn, is a diploid taxon that was first described in 2017 by Bényei-Himmer and coauthors (Bényei-Himmer et al., 2017), after a 20-year-long survey of specimens. *Hedera crebrescens* can be distinguished from its presumed closest relatives *H. helix* and varieties of *H. hibernica* based on certain pronounced vegetative and generative traits. *Hedera crebrescens* has characteristic reddish green shoots. Leaves on the horizontally creeping shoots are triangular, slightly trilobed with a cordate leaf base, whereas those on the vertically emerging shoots are large-lobed (three to five; 10–16 cm) with overlapping bases (Figure 1). On the vegetative shoots, the apical lobes of the leaves are wide and dome-shaped, whereas leaves on the generative shoots are heart-shaped with a cordate leaf base. The stellate trichomes are multiangulate (7–12 rays) and typically appressed. The inflorescence is short (3–5 cm) with only a few or no lateral umbels. In the apical umbels, the flowers are crowded. Beneath the apical umbel, there is a solitary flower that rarely produces fruit. The densely packed fruits are black and contain three–five seeds. *Hedera crebrescens* also differs from *H. helix* and *H. hibernica* with respect to its vigorous growth and the slight frost sensitivity of its leaves (Bényei-Himmer et al., 2017).

From the total of 63 *Hedera* samples that were examined in the present study, we sampled eight specimens: five samples of *H. crebrescens* and one of each of the three assumed relatives *H. helix*, *H. hibernica*, and *H. azorica*. The samples were selected from the Ivy Collections of the Soroksár Botanical Garden, the Buda Arboretum in Budapest, the downtown area of Budapest, and the outskirts of the city of Zebegény, Hungary (also see Table S1). Sequences of these samples were aligned with *Hedera* sequences from the GenBank reported in previous studies (Table S2). For plastid regions, we selected 26 sequences from Green et al. (2011), whereas for nuclear regions, we selected 27 sequences from Vargas et al. (1999), three sequences from Valcárcel et al. (2003), and one sequences from Li et al. (2013). In this regard, it should be mentioned that the samples used for the chloroplast and nuclear analyses in these previous studies are not identical.



Figure 1 The holotype specimen of *Hedera crebrescens* (Bényei-Himmer et al., 2017).

2.2. DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from fresh leaves (20–25 mg) using a DNeasy Mini Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's recommendations. With reference to the phylogenetic study conducted by Green et al. (2011), we used universal plastid primers to amplify five noncoding chloroplast regions, namely, the *matk* (psbA5'R-matK8F) intergenic spacer, *trnH-psbA* (psbA-trnH^{GUG}) intergenic spacer, *psbB-psbH* (psbB-psbH) intergenic spacer, *rpl16* (rpl16F71-rpl16R1516) intron, and *rpoB-trnC^{GCA}* (rpoB-trnC^{GCA}R) intergenic spacer (Shaw et al., 2005; Taberlet et al., 1991). With reference to Vargas et al. (1999), we amplified the internal transcribed spacer ITS1-5.8S-ITS2 region using the ITS5/ITS4 primer pair (White et al., 1990) (Table 1).

PCR amplifications were performed using 24.5- μ L reactions mixture containing 1 μ L genomic DNA (20–30 ng), 18.7 μ L Milli-Q ultrapure water (Merckmillipore, Billerica, MA, USA), 2.5 μ L (10 \times) Dream Taq Green PCR buffer (ThermoFisher, Waltham, MA, USA), 0.5 μ L (10 mM) dNTP mix (ThermoFisher), 1 μ L (2.5 mM) MgCl₂, 0.5 μ L (10 mM) of each primer (Biocenter Kft., Szeged, Hungary), 0.25 μ L (1%) bovine serum albumin (ThermoFisher), 0.5 μ L (2%) dimethyl sulfoxide (Reanal, Budapest, Hungary), and 0.05 μ L (0.5 unit) Dream Taq GREEN DNA polymerase (ThermoFisher).

The thermocycling conditions used for amplification of the plastid regions were based on those described by Ramsey et al. (2005) with some modifications. In the case of certain primers, it was necessary to optimize the protocols for gradient PCR. The final thermocycling conditions were as follows: an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 30 s of denaturation at 94 °C, 40 s of annealing (temperature dependent on the primer pairs, shown in Table 1), and 2.5 min of extension at 72 °C, with a final extension step at 72 °C for 7 min. Thermocycling of the ITS region were performed by modifying the protocol of White et al. (1990).

Table 1 Description and amplification conditions of the six primer pairs (five chloroplast and one nuclear) used for the molecular study.

Name	Forward/Reverse	Sequence (5'→3')	T_a (° C)
psbA5'R	F	AACCATCCAATGTAAAGACGGTTT	50
matk8F	R	TCGACTTTCTTGTGCTAGAACTTT	
psbA	F	GTTATGCATGAACGTAATGCTC	59
trnH ^{GUG}	R	CGCGCATGGTGGATTACAATCC	
psbB	F	TCCAAAAANKKGGAGATCCAAC	57
psbH	R	TCAAYRGTYTGTGTAGCCAT	
rpL16F71	F	GCTATGCTTAGTGTGTGACTCGTTG	52
rpL16R1516	R	CCCTTCATTCTTCCTCTATGTTG	
rpoB	F	CKACAAAAYCCYTCRAATTG	53
trnC ^{GCA} R	R	CACCCRGATTYGAAGTGGGG	
ITS5	F	GGAAGTAAAAGTCGTAACAAGG	56
ITS4	R	TCCTCCGCTTATTGATATGC	

T_a – annealing temperature.

The amplifications were carried out with an initial denaturation step of 2.5 min at 95 °C, followed by 30 cycles of 30 s of denaturation at 95 °C, 30 s of annealing at 56 °C, and 2 min of extension at 72 °C, with a final extension step at 72 °C for 10 min.

Amplified products were purified using a CleanSweep PCR Purification (Applied Biosystems, Waltham, MA, USA) kit according to the instructions provided by the manufacturer. Cleaned products were sequenced from both directions using an ABI PRISM 3100 Genetic Analyzer automated DNA sequencer.

2.3. Sequence Alignment and Phylogenetic Analysis

We used BioEdit 7.2.5 (Hall, 1999) sequence alignment editor software to edit the sequence chromatograms, with visual inspection being performed for all polymorphic sites detected. Alignments were performed using ClustalW (Thompson et al., 1994) as implemented in BioEdit. The matrices of the five plastid sequences were concatenated into a single matrix using the SeaView 4.6.4 program (Gouy et al., 2010).

Phylogenetic relationships between the *Hedera* species were examined based on maximum likelihood (ML) and Bayesian approaches. ML analysis was performed using the RAxML-NG web server (<http://embnet.vital-it.ch/raxml-bb/>) (Kozlov et al., 2019) with 10 random starting trees and an autoMRE bootstrap search with 0.03 cut-off. The concatenated alignment was treated as a partitioned dataset. Evolutionary models were selected based on jModelTest results (Posada, 2008). On the basis of Bayesian information criterion values, the F81 model was used for the five chloroplast regions, with *Fatsia japonica* and *Travesia suindica* being used as outgroups. Bayesian phylogenetic trees were reconstructed based on MrBayes v.3.2.7a (Ronquist et al., 2012) using the GTR+I+ Γ model (general time-reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites) of sequence evolution. Four Markov chain Monte Carlo (MCMC) chains, one heated (temperature = 0.05) and three unheated, were run for 2,000,000 generations, sampling at every 1,000 generations. Information on gaps (indels) within the aligned sequence matrices were coded as binary characters. Gaps were coded following the simple indel coding algorithm (Simmons & Ochoterena, 2001) using the program FastGap 1.2 (Borchsenius, 2009).

PopART (Leigh & Bryant, 2015) with implemented Templeton–Crandall–Singh (TCS) statistical parsimony network analysis (Clement et al., 2002) was used to evaluate genealogical relationships among sequences. Each insertion/deletion was considered to be a single mutation event, and all indels were therefore coded as single positions in the final alignments. The connection limit for the TCS analysis was 95% and gaps were treated as a fifth state.

3. Results

3.1. Sequence Variation

On the basis of sequence alignments of the selected five chloroplast markers, 40 new sequences were generated for the four examined *Hedera* taxa. The five concatenated chloroplast datasets of the 34 samples contained 3,783 base pairs after alignment, with 27 variable and 17 informative sites within *Hedera* (Table 2). As has previously been reported for chloroplast markers (Green et al., 2011; Grivet & Petit, 2002; Valcárcel et al., 2003), we found that sequences showed a low degree of polymorphism, with a variability of only 0.7% being detected in plastid regions. Although originating from different geographical locations, we were unable to detect any variation in the chloroplast sequences of the different *H. crebrescens* specimens. At a microsatellite loci in the *psbA-3' trnK-matK* region, the *H. crebrescens* sequences had a species-specific insertion, which was not present in any of the other studied sequences. *Hedera crebrescens* had 12 T bases at this loci (7–11 T bases in other species). However, this was the only site that showed variability compared with those of the other species.

Compared with plastid regions, nuclear ITS sequences contained a proportionally greater number of polymorphic sites, showing 3.7% variability. Among all *Hedera* samples, we detected 22 variable and 14 informative sites. *Hedera crebrescens* was found to vary from the other three examined species with respect to two SNP loci. Moreover, of these two sites, one showed no variation among *H. crebrescens* samples, whereas the other site showed variation in one of the five samples.

Table 2 Characteristics of the aligned *Hedera* sequences.

Region	Length (bp)	Variable sites	Informative sites
<i>psbA-3' trnK-matK</i>	719–740	5	2
<i>psbA-trnH</i>	426–445	6	6
<i>psbB-psbH</i>	559–617	3	2
<i>rpL16</i>	824–851	9	6
<i>rpoB-trnC</i>	940–1,130	4	1
ITS1-5S8-ITS2	577–592	22	14

3.2. Haplotype Network

Unrooted haplotype genealogies were estimated based on the substitution polymorphisms (including indels coded as single characters) observed within the five plastid regions (Figure 2). TCS analysis of the 34 sequences of the 15 *Hedera* taxa yielded 20 haplotypes. The connection of haplotypes within the network followed a geographical structuring. In contrast, *H. helix* specimens were characterized by different haplotypes (eight specimens formed seven haplotypes) of different lineages. Each of the five *H. crebrescens* samples constituted an independent haplotype, which were placed at a single mutation step distance from the *H. helix* H1 specimen sampled in Hungary.

3.3. Chloroplast Phylogenetic Reconstruction

Phylogenetic analysis of the concatenated chloroplast matrix strongly supported the monophyly of *Hedera* (Figure 3, Figure S1). The strict consensus ML tree grouped *Hedera* sequences into two well-supported clades (99%), one of which included Western Mediterranean species (*H. maroccana*, *H. canariensis*, and *H. iberica*) and one sample of *H. helix*, whereas the other clade was divided into two clades, one of which separated two other Western Mediterranean taxa (*H. azorica* and *H. maderensis*) and the second contained the remainder of the taxa with weaker support. One clade contained the Western Mediterranean *H. algeriensis*, the Eastern Mediterranean *H. pastuchowii* subsp. *cypria*, and three *H. helix* specimens. The five *H. crebrescens* samples formed a single group and were connected to three other *H.*

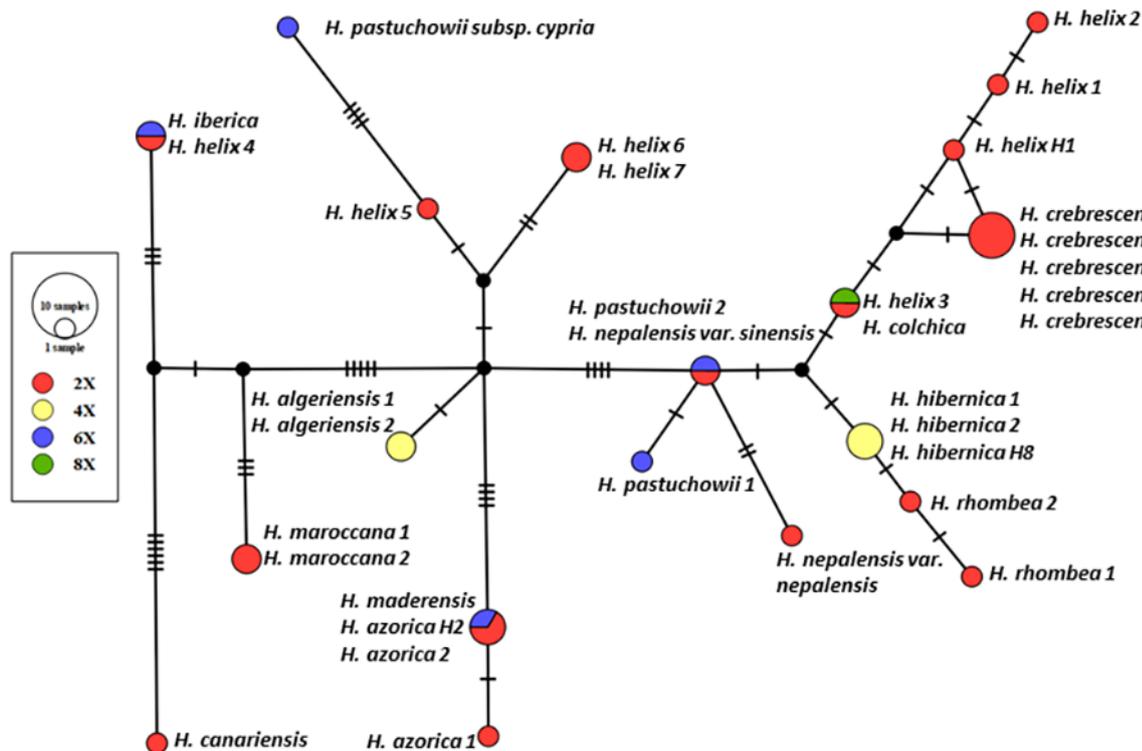


Figure 2 The haplotype network obtained based on Templeton–Crandall–Singh analysis of five concatenated cpDNA regions of *Hedera* samples. Black dots indicate missing intermediate haplotypes that were not observed in the analyzed sample set. Hash marks on branches represent mutation steps (number of base pair changes) between haplotypes. The different colors indicate the ploidy levels (red = 2x, yellow = 4x, blue = 6x, and green = 8x).

helix samples. The Western European *H. hibernica*, the Asian species *H. colchica*, *H. nepalensis*, *H. pastuchowii*, and *H. rhombea*, a single *H. helix* specimen, were separated with high support from the clade containing *H. crebescens* and *H. helix* samples.

3.4. ITS Phylogenetic Reconstruction

Nuclear phylogeny also supported the monophyly of the genus and grouped taxa into two well-defined clades, one containing diploids and the other containing the polyploid taxa (Figure 4, Figure S2). Owing to the limited resolution of the diploid clade, as has been found in previous studies (Valcárcel et al., 2003, 2017; Valcárcel & Vargas, 2012; Vargas et al., 1999), we were unable to use ITS phylogeny as a basis for drawing clear conclusion regarding the phylogenetic position of *H. crebescens*. However, nuclear phylogeny showing that *H. crebescens* belongs to the diploid clade is consistent with the findings of previous cytological studies (Bényei-Himmer et al., 2017).

4. Discussion

Although several studies (Ackerfield & Wen, 2003; Green et al., 2011; Grivet & Petit, 2002; Valcárcel et al., 2003, 2017; Valcárcel & Vargas, 2012; Vargas et al., 1999) conducted in recent decades have examined the phylogeny of the genus *Hedera*, certain lineages have remained insufficiently well clarified. In some cases, species segregation has been based on low numbers of variable loci, whereas hybridization, polyploidization, rapid diversification, and recent evolutionary events may have further contributed to complicating the detection of molecular relationships within the genus (Ackerfield & Wen, 2003; Green et al., 2011; Valcárcel et al., 2003, 2014, 2017; Vargas et al., 1999).

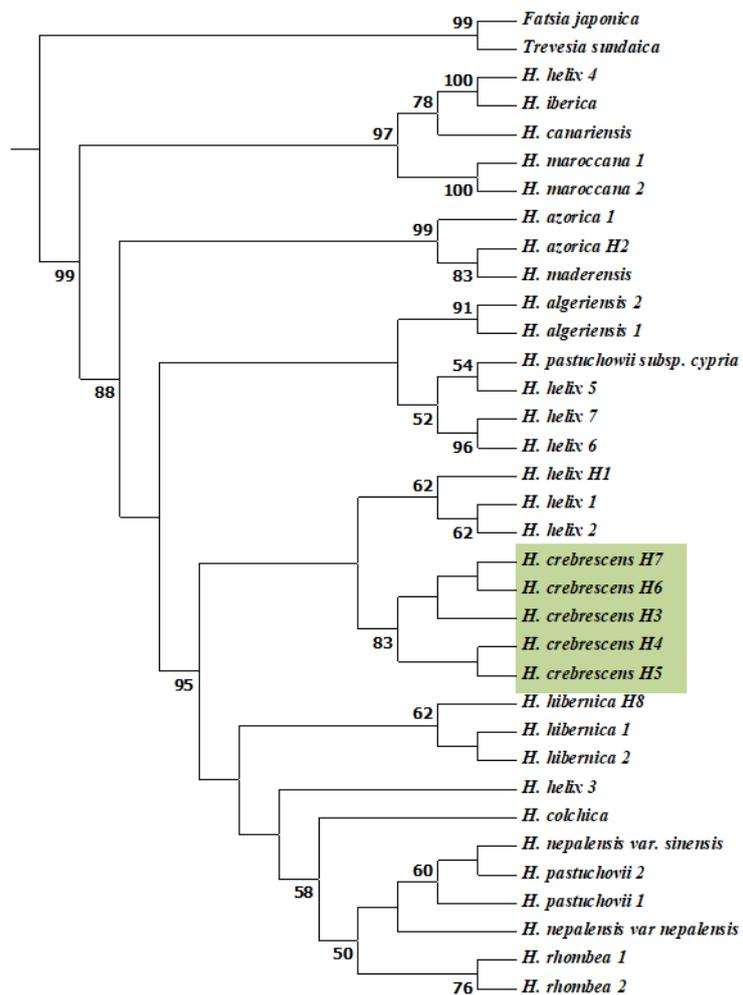


Figure 3 A consensus tree of the five concatenated cpDNA regions generated using the maximum likelihood method. Numbers above branches show the bootstrap values from 200 replications (bootstrap values less than 50% are not shown).

In the present study, we found that phylogeny based on plastid variation supported the monophyly of *Hedera*, although the phylogenetic trees did not show a strong and readily interpretable topology, and some subclades exhibited weak statistical support. Our consensus tree based on the analyses of five chloroplast regions showed a topology similar to that previously obtained based on phylogenetic cpDNA analyses (Green et al., 2011; Valcárcel et al., 2003). Previous molecular studies have established that, with the exception of *H. helix*, Mediterranean species are well separated from the Asian and European species. In contrast, overall phylogenetic relationships among European and Asian species, including *H. crebrescens*, tended to be poorly resolved due to the low statistical supports and limited resolution at lower levels. *Hedera helix* was found to be polyphyletic, as specimens were placed separately in the ML tree as well as in the haplotype network. This is consistent with the findings of previous studies that have revealed differences in lineages and chlorotypes within *H. helix* (Green et al., 2011; Grivet & Petit, 2002; Valcárcel et al., 2003). The long-term cultivation of multiple species and cultivars can promote the mixing of genetic lineages owing to hybridization events and recent gene flow. On the basis of molecular evidence, the genus *Hedera*, including *H. helix*, has an Asian origin and there has been recent diversification in the Mediterranean region (Valcárcel et al., 2003, 2017; Valcárcel & Vargas, 2012). It is assumed that Central European taxa preserve imprints of the eastern genetic lineage, whereas *H. helix* has further extended its distribution toward the Mediterranean, where mixing of genetic lineages has occurred. Accordingly, the observed polyphyly of *H. helix*, due to the diversity of its genetic makeup, may cause uncertainties with respect

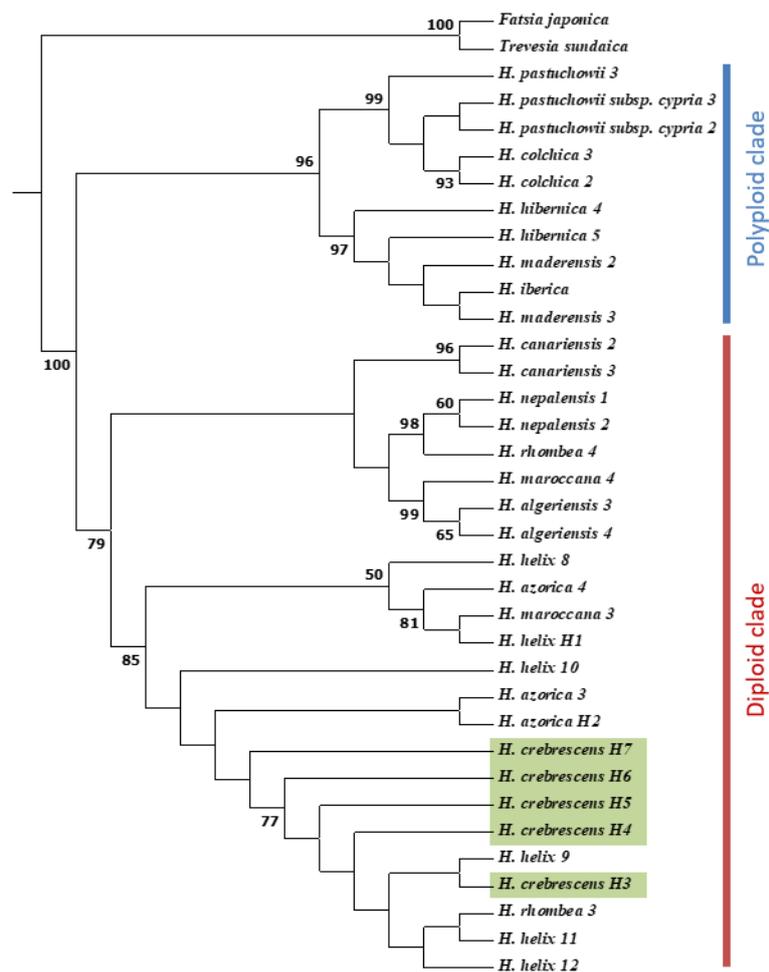


Figure 4 A consensus tree of the nrDNA ITS sequences generated using the maximum likelihood method. Numbers above branches show the bootstrap values from 200 replications (bootstrap values less than 50% are not shown).

to the molecular classification of the derived taxa. Although we found that the clade containing all European and Asian species had low support, all *H. crebrescens* specimens grouped together. On the basis of our analysis of five chloroplast regions, it appears that *H. crebrescens* is related to European populations of *H. helix* and *H. hibernica* and to certain Asian species (*H. colchica*, *H. rhombea*, *H. pastuchowii*, and *H. nepalensis*). However, on the basis of trichome morphology, which has previously been considered a taxonomically important trait for differentiating ivies, a close relationship with Asian taxa appears unlikely, due to the fact that unlike all Asian species, which have reddish scale-like trichomes, *H. crebrescens* has white stellate trichomes (Lum & Maze, 1989; McAllister, 1981; Rutherford et al., 1993). However, clarifying unequivocally the taxonomic position of *H. crebrescens* will clearly necessitate a more comprehensive survey of the entire *Hedera* genome. Nevertheless, the invariability we observed among *H. crebrescens* specimens of different geographical proveniences and its species-specific haplotype (unique loci that are not present in other species), provide support for its taxonomical autonomy. The previously hypothesized identity of *H. crebrescens* as *H. hibernica* (Udvardy & Bényei-Himmer, 1999) can be refuted on the basis of molecular data, and also on previously obtained cytological results, as *H. crebrescens* is a diploid species. The closest relatives based on the chlorotypes were found to be in a lineage including *H. helix* specimens. However, the significant morphological, phenological, and ecological differences preclude identifying *H. crebrescens* as *H. helix*. Moreover, the leaves of these two taxa, on both vegetative and generative shoots, differ morphologically, and the reproductive organs also exhibit distinct characters

(Bényei-Himmer et al., 2017). *Hedera crebescens* is readily recognizable from its vigorous growth and large-lobed leaves on the emerging shoots, with dome-shaped apical lobe and overlapping leaf bases. The flattened fruits of this species are also characteristic, as they are tightly packed in the apical umbel of the inflorescence, which typically comprises a single rudimentary lateral umbel (Figure 1). The vigorous growth, high seed and fruit production, and a germination rate that is twice that of *H. helix* (Bényei-Himmer et al., 2017), are all conducive to the prolific spread of this species. Furthermore, its striking, dense, fleshy fruits prove to be particularly attractive to birds, highly contribute to the plant's dispersal. Given this collection of favorable features, *H. crebescens* has the potential to supplant *H. helix* in its native habitats. Moreover, the propensity for hybridization among *Hedera* species provides ample opportunities to introduce foreign genetic material into native populations. Indeed, hybridization in gardens, where large numbers of species and cultivars typically coexist, has previously been reported (Valcárcel et al., 2003), and might favor the selection of new lineages prone to garden escape.

By drawing attention to the newly described species *H. crebescens* and highlighting its molecular background and relationships, we consider that the findings of this study provide a preliminary basis for forecasting the risks associated with the spread of this potentially invasive species in Central Europe. Identification and monitoring of this species will also be required in neighboring countries.

5. Supporting Material

The following supporting material is available for this article:

- Figure S1. A consensus tree of the five concatenated cpDNA regions generated using the Bayesian method.
- Figure S2. A consensus tree of the nrDNA ITS sequences generated using the Bayesian method.
- Table S1. Location information for the sampled *Hedera* material.
- Table S2. List of the sequences used in the study with respective location, voucher, and GenBank accession information.

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References

- Ackerfield, J. (2001). Trichome morphology in *Hedera* (Araliaceae). *Edinburgh Journal of Botany*, 58(2), 259–267. <https://doi.org/10.1017/s0960428601000622>
- Ackerfield, J., & Wen, J. (2002). A morphometric analysis of *Hedera* L. (the ivy genus, Araliaceae) and its taxonomic implications. *Adansonia*, 24(2), 197–212.
- Ackerfield, J., & Wen, J. (2003). Evolution of *Hedera* (the ivy genus, Araliaceae): Insights from chloroplast DNA data. *International Journal of Plant Sciences*, 164, 593–602. <https://doi.org/10.1086/375423>
- Bartha, D. (2020). *Black list. Invasive tree and shrub species of Hungary*. University of Sopron Press.
- Bényei-Himmer, M., Tóth, E. G., Lengyel, S., Pintér, I., & Bisztray, G. D. (2017). *Hedera crebescens* (Araliaceae) a newly identified diploid taxon and triploid ivies from Hungary. *Studia Botanica Hungarica*, 48(2), 225–252. <https://doi.org/10.17110/StudBot.2017.48.2.225>
- Bevill, R. L., & Louda, S. M. (1999). Comparisons of related rare and common species in the study of plant rarity. *Conservation Biology*, 13(3), 493–498. <https://doi.org/10.1046/j.1523-1739.1999.97369.x>
- Borchsenius, F. (2009). *FastGap 1.2* [Computer software]. http://www.aubot.dk/FastGap_home.htm
- Clement, M., Snell, Q., Walke, P., Posada, D., & Crandall, K. (2002). TCS: Estimating gene genealogies. In *Proceedings of the First IEEE International Workshop on High Performance Computational Biology*. <https://doi.org/10.1109/IPDPS.2002.1016585>

- Duncan, R. P., & Young, J. R. (2000). Determinants of plant extinction and rarity 145 years after European settlement of Auckland, New Zealand. *Ecology*, 81(11), 3048–3061. [https://doi.org/10.1890/0012-9658\(2000\)081](https://doi.org/10.1890/0012-9658(2000)081)
- Genovesi, P., & Shine, C. (2004). *European strategy on invasive alien species: Convention on the Conservation of European Wildlife and Habitats (Bern Convention) (No. 18-137)*. Council of Europe.
- Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 27(2), 221–224. <https://doi.org/10.1093/molbev/msp259>
- Green, A. F., Ramsey, T. S., & Ramsey, J. (2011). Phylogeny and biogeography of ivies (*Hedera* spp., Araliaceae), a polyploid complex of woody vines. *Systematic Botany*, 36(4), 1114–1127. <https://doi.org/10.1600/036364411x605100>
- Grivet, D., & Petit, R. J. (2002). Phylogeography of the common ivy (*Hedera* sp.) in Europe: Genetic differentiation through space and time. *Molecular Ecology*, 11(8), 1351–1362. <https://doi.org/10.1046/j.1365-294x.2002.01522.x>
- 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.
- Jacobsen, P. (1954). Chromosome numbers in the genus *Hedera* L. *Hereditas*, 40, 252–254.
- Kost, E. A., Rudakova, V. S., Volkova, S. A., & Shipunov, A. B. (2003). Morphological leaf variability of West Caucasian *Hedera* L. (Araliaceae) species. *Wulfenia*, 10, 51–59.
- 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(21), 4453–4455. <https://doi.org/10.1093/bioinformatics/btz305>
- Leigh, J. W., & Bryant, D. (2015). PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6(9), 1110–1116. <https://doi.org/10.1111/2041-210x.12410>
- Lengyel, S., Pintér, I., Bisztray, G., Höhn, M., & Bényeiné-Himmer, M. (2007). Citogenetikai vizsgálatok honos és kultúr borostyánoknál [Cytological evaluation of natural and cultivated ivies]. In *XIII Scientific Days in Plant Breeding* (p. 120). Hungarian Academy of Sciences (MTA).
- Li, R., & Wen, J. (2013). Phylogeny and biogeography of *Dendropanax* (Araliaceae), an amphipacific disjunct genus between tropical/subtropical Asia and the Neotropics. *Systematic Botany*, 38(2), 536–551. <https://doi.org/10.1600/036364413x666606>
- Lowry, P. P., Plunkett, G. M., van Wyk, B.-E., & Tilney, M. (2004). Generic relationships in Araliaceae: Looking into the crystal ball. *South African Journal of Botany*, 70(3), 382–392. [https://doi.org/10.1016/S0254-6299\(15\)30221-0](https://doi.org/10.1016/S0254-6299(15)30221-0)
- Lum, C., & Maze, J. (1989). A multivariate analysis of the trichomes of *Hedera* L. *Watsonia*, 17(4), 409–418.
- McAllister, H., & Marshall, R. (2017). *Hedera. The complete guide*. Royal Horticultural Society.
- McAllister, H. A. (1981). New work on ivies. *International Dendrological Society Year Book*, 1981, 106–109.
- McAllister, H. A., & Rutherford, A. (1990). *Hedera helix* L. and *H. hibernica* (Kirchner) Bean (Araliaceae) in British Isles. *Watsonia*, 18, 7–15.
- Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, 25(7), 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Ramsey, T. F. (2005). *Invasion of English ivy (Hedera spp., Araliaceae) into Pacific Northwest forests*. University of Washington.
- Robbins, W. J. (1957). Gibberellic acid and the reversal of adult *Hedera* to a juvenile state. *American Journal of Botany*, 44(9), 743–746. <https://doi.org/10.1002/j.1537-2197.1957.tb08259.x>
- Robbins, W. J. (1960). Further observations on juvenile and adult *Hedera*. *American Journal of Botany*, 47(6), 485–491. <https://doi.org/10.1002/j.1537-2197.1960.tb10617.x>
- Rogler, C. E., & Hackett, W. P. (1975). Phase change in *Hedera helix*: Induction of the mature to juvenile phase change by gibberellin A₃. *Physiologia Plantarum*, 34(2), 141–147. <https://doi.org/10.1111/j.1399-3054.1975.tb03809.x>
- Ronquist, F., Teslenko, M., Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematics Biology*, 61, 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rose, P. Q. (1996). *The gardener's guide to growing ivies*. Timber Press.
- Rutherford, A. (1984). The history of the Canary Islands ivy and its relatives. *Ivy Journal*, 10, 13–18.
- Rutherford, A. (1989). The ivies of Andalusia (southern Spain). *Ivy Journal*, 15, 7–17.
- Rutherford, A., McAllister, H. A., & Mill, R. R. (1993). *New ivies from the Mediterranean area and Macaronesia*. Plantsman.

- Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E., & Small, R. L. (2005). The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, 92(1), 142–166. <https://doi.org/10.3732/ajb.92.1.142>
- Simmons, M. P., Ochoterena, H., & Carr, T. G. (2001). Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analysis. *Systematic Biology*, 50(3), 454–462. <https://doi.org/10.1080/106351501300318049>
- Soó, R., & Jávorka, S. (1951). *A magyar növényvilág kézikönyve* [Handbook of the Hungarian flora]. Akadémiai Kiadó.
- Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17(5), 1105–1109. <https://doi.org/10.1007/BF00037152>
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680. <https://doi.org/10.1093/nar/22.22.4673>
- Udvardy, L., & Bényei-Himmer, M. (1999). Escape of Irish ivy [*Hedera hibernica* (Kirchner) Bean] as a woody evergreen weed. *Kertészeti és Élelmiszeripari Egyetem Közleményei*, 59, 161–164.
- Valcárcel, V., Fiz, O., & Vargas, P. (2003). Chloroplast and nuclear evidence for multiple origins of polyploids and diploids of *Hedera* (Araliaceae) in the Mediterranean basin. *Molecular Phylogenetics and Evolution*, 27(1), 1–20. [https://doi.org/10.1016/s1055-7903\(02\)00364-0](https://doi.org/10.1016/s1055-7903(02)00364-0)
- Valcárcel, V., Fiz-Palacios, O., & Wen, J. (2014). The origin of the early differentiation of ivies (*Hedera* L.) and the radiation of the Asian Palmate group (Araliaceae). *Molecular Phylogenetics and Evolution*, 70, 492–503. <https://doi.org/10.1016/j.ympev.2013.10.016>
- Valcárcel, V., Guzmán, B., Medina, N. G., Vargas, P., & Wen, J. (2017). Phylogenetic and paleobotanical evidence for late Miocene diversification of the Tertiary subtropical lineage of ivies (*Hedera* L., Araliaceae). *BMC Evolutionary Biology*, 17(1), Article 146. <https://doi.org/10.1186/s12862-017-0984-1>
- Valcárcel, V., & Vargas, P. (2010). Quantitative morphology and species delimitation under the general lineage concept: Optimization for *Hedera* (Araliaceae). *American Journal of Botany*, 97(9), 1555–1573. <https://doi.org/10.3732/ajb.1000115>
- Valcárcel, V., & Vargas, P. (2012). Phylogenetic reconstruction of key traits in the evolution of ivies (*Hedera* L.). *Plant Systematics and Evolution*, 299(2), 447–458. <https://doi.org/10.1007/s00606-012-0734-1>
- Vargas, P., McAllister, H. A., Morton, C., Jury, S. L., & Wilkinson, M. J. (1999). Polyploid speciation in *Hedera* (Araliaceae): Phylogenetic and biogeographic insights based on chromosome counts and ITS sequences. *Plant Systematics and Evolution*, 219(3–4), 165–179. <https://doi.org/10.1007/bf00985577>
- Vilà, M., Corbin, J. D., Dukes, J. S., Pino, J., & Smith, S. D. (2007). Linking plant invasion to global environmental change. In J. G. Candel, D. Pataki, & L. Pitelka (Eds.), *Terrestrial ecosystems in a changing world* (pp. 95–96). Springer. https://doi.org/10.1007/978-3-540-32730-1_8
- White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). Academic Press. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>