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Phylogeny and Biogeography of Ivies (*Hedera* spp., Araliaceae), a Polyploid Complex of Woody Vines

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***Abstract*—**Ivy (*Hedera* spp., Araliaceae) is a polyploid complex of woody vines. Native to Eurasia and northern Africa, ivy is cultivated worldwide and has become an aggressive invader of North American forests. Despite its ecological impacts and economic significance to the horticultural industry, the taxonomy of *Hedera* is controversial and historical relationships are poorly defined. Here we characterize the phylogeny of *Hedera* based on the low-copy nuclear locus Granule-bound starch synthase I (*GBSSI*) and twelve non-coding cpDNA regions. Maximum parsimony and Bayesian analyses of both data sets identified *Hedera* as monophyletic. For *GBSSI*, we isolated eighteen haplotypes that were widely shared across species. There was no evidence of fixed heterozygosity or haplotype additivity in polyploids, suggesting possible autopolyploid origins. For cpDNA, we isolated sixteen haplotypes that were highly structured by geography. Haplotype diversity and phylogenetic structure were greatest in northern Africa and southern Europe. Thus, while most members of the Araliaceae reside in tropical and subtropical Asia, the early diversification of *Hedera* probably occurred in the Mediterranean Basin. Geographically-structured clades included diploid and polyploid species, suggesting that genome duplication has occurred repeatedly in the genus. Closely-related ivies often differed in leaf size and trichome morphology, indicating evolutionary lability of traits traditionally used for classification. Nonetheless, we recovered similar or identical DNA sequences within morphologically-defined species. Notable exceptions included southern populations of *H. helix* (*H. helix* subsp. *caucasigena* and *H. helix* subsp. *rhizomatifera*) that had cpDNA haplotypes distinct from those of central and northern Europe (*H. helix* subsp. *helix*).

***Keywords*—**cpDNA, chromosome evolution, low-copy nuclear gene, molecular systematics, phylogeography.

Ivy (*Hedera* spp., Araliaceae) is a polyploid complex of woody vines with evergreen foliage and distinctive juvenile and adult life stages (McAllister 1979, 1981; Rose 1996; Metcalfe 2005). Juvenile plants, which exhibit herbaceous growth and characteristically lobed leaves, form dense patches at ground level. The transition to the adult stage is triggered by climbing of a host structure such as a tree, cliff face, or wall. Mature plants develop woody growth and unlobed leaves, eventually producing umbels of inconspicuous hermaphroditic flowers on secondary branches three or more meters above ground level. Ivy has been used for decoration and landscaping since ancient times (Beach 1980; Fearnley-Whittingstall 1992), and a remarkable diversity of cultivars has been developed over the past three hundred years (Rose 1996). *Hedera* is now a staple of the European and North American horticultural industries, with > 8 million potted plants sold each year in the U. S. A. alone (Anonymous 2010).

Ivy has a complicated taxonomic history because of its unusual life cycle, broad geographic distribution, and frequent horticultural use (McAllister 1979; Beach 1980; Rutherford et al. 1993; Rose 1996; Ackerfield and Wen 2002, 2003). Taxonomic distinction was first afforded to juvenile and adult plants, described by Linnaeus (1753) as "*Hedera helix*" and "*H. arborea*." However, the recognition of ivy's two-part life cycle led to widespread application of *H. helix* L. to plants throughout Europe and Asia. Additional species were added to the genus during the 1800s and early 1900s, including *H. canariensis* Willd. (1808, based on specimens from the Canary Islands); *H. colchica* K. Koch (1853, based on specimens from Asia Minor); and *H. pastuchovii* G. Woronow (1932, based on specimens from Central Asia). In some cases, multiple species epithets were applied to the same taxon (McAllister 1979; Rose 1996).

Unification of ivy taxonomy was achieved in the late twentieth century, for several reasons. First, the creation of the American Ivy Society (1973) and the British Ivy Society (1974) enabled permanent accessioning of ivy stocks, including "wild-type" species as well as cultivars. Second, the application of biosystematic approaches, especially chromosome counts and common garden studies, provided a more rigorous foundation for delineation of species (McAllister and Rutherford 1983, 1990; Rutherford et al. 1993). Field collection efforts expanded the diversity of ivy taxa, leading to recognition of new species like *H. cypria* McAllister, *H. iberica* (McAllister) Ackerfield & J. Wen, and *H. maroccana* McAllister (Rutherford 1984, 1989). These taxa are among the most controversial of ivy species; some botanists have argued that they represent subspecific variants (Sulgrove 1984; Rose 1996).

Despite the longstanding economic importance of *Hedera* to the horticultural industry, historical relationships in the genus are poorly understood. Analyses performed with non-coding cpDNA (*atpB–rbcL*, *trnD–trnT*, *trnH–psbA*) and the high-copy nuclear region ITS yielded poorly-resolved trees with little statistical support (Vargas et al. 1999; Grivet and Petit 2002; Ackerfield and Wen 2003; Valcárcel et al. 2003). Production of well-resolved species-level phylogenies is essential for interpreting the biogeography of ivies as well as patterns of chromosomal and morphological evolution. Molecular data are also needed to corroborate species boundaries, which in many cases are based on subtle differences in leaf shape and trichome morphology or ploidy level (Rutherford et al. 1993; Ackerfield and Wen 2002, 2003). Some taxonomists have suggested that *Hedera* has experienced excessive taxonomic splitting on the basis of biologically insignificant or spurious character differences (Sulgrove 1984; Rose 1996; Schäfer 2002).

The issue has taken new significance due to the invasion of cultivated plants in North American forests (Reichard 2000). Along the Atlantic Seaboard and Pacific Coast, naturalized ivies form dense growth in forest understories and cover trees with evergreen foliage. Invasive ivy has been the subject of control programs as well as efforts to reduce its sale and distribution via the horticultural industry. In Oregon, for example, "*Hedera helix*" has been designated a class-B weed and is banned. This taxon has been delineated in several different ways, however, and naturalized populations within Oregon are comprised primarily of the tetraploid *H. hibernica* (Kirch.) Bean rather than diploid *H. helix* s. s. (Clarke et al. 2006a).

Management of "invasive ivy" will require consistent nomenclature and identification, and this can only be achieved by resolving taxonomic disagreements in the genus.

Here we characterize phylogenetic relationships within *Hedera* based on the low-copy nuclear gene granule-bound starch synthase I and twelve noncoding cpDNA regions, together comprising > 10 kb of aligned sequence data. Based on these data, we address the following questions: (1) Do specimens of morphologically-defined species form natural, monophyletic groupings? (2) Do polyploid *Hedera* have an auto- or allopolyploid origin? (3) Has polyploidy evolved recurrently? (4) Are the morphological traits used in traditional classification schemes conserved or labile? and (5) What are the geographic patterns of speciation in *Hedera*?

Materials and Methods

***Sampling*—**We included 27 specimens of *Hedera*, including all of the 13 taxonomically-recognized species in the genus (Rutherford et al. 1993; Ackerfield and Wen 2002, 2003; Table 1). Most plants were obtained from the American Ivy Society (Deerfield, New Jersey), a nonprofit organization that maintains ivy accessions for taxonomic research and cultivar development (Appendix 1). Six additional specimens of *H. helix*, *H. hibernica*, and *H. algeriensis* Hibberd were collected from North American populations. Outgroup sampling included five genera with close relationships to *Hedera* (Valcárcel et al. 2003; Mitchell and Wen 2004), including *Brassaia actinophylla* Endl., *Fatsia japonica* (Thunb.) Decne. & Planch., *Polyscias fruticosa* (L.) Harms., *Schefflera* sp., and *Trevesia sundaica* Miq. (Appendix 1).

Range maps for *Hedera* taxa have not previously been assembled so we collated distribution information from taxonomic references (McAllister 1981; McAllister and Rutherford 1983, 1990; Rutherford 1984, 1989; Rutherford et al. 1993; Rose 1996; Ackerfield 2001; Ackerfield and Wen 2002, 2003; Valcárcel et al. 2003) and regional floras (Ohwi 1965; Hara 1966; Poyarkova 1950; Press and Short 2002; Xiang and Lowry 2007) (Fig. 1; Table 1).

***DNA Extraction, Amplification and Sequencing*—**We used the DNeasy plant kit (Qiagen, Valencia, California) to isolate genomic DNA. Approximately 60 mg of fresh tissue was used for each extraction. For study of the *GBSSI*, we used primers GBSSI-1F and GBSSI-11R (Mitchell and Wen 2004) to amplify exon 10, fragments of exons 9 and 11, and two introns. We performed 25 μL PCR reactions with 2 μL genomic DNA, 0.25 μL of each primer (100 mM stock, 1μM final concentration), 5 μL HotStar HiFidelity® PCR buffer (5×, Qiagen) and 0.5 μL HotStar HiFidelity DNA polymerase (2.5 units/μL, Qiagen). Thermocycling was performed as described by Mitchell and Wen (2004). The resulting product was cloned into One Shot® TOP10F′ competent *E. coli* using TOPO TA cloning kits® (Invitrogen, Carlsbad, California). Cloning was performed following manufacturer guidelines and used 2–4 μL of PCR product and 0.5 μL pCR®2.1-TOPO® vector. Clones were screened using blue/white selection. Sixteen positive clones were selected for each plant specimen (496 total clones), and *GBSSI* inserts were then amplified via colony PCR with M13 forward and reverse primers under the following conditions: (1) 95°C for 4 minutes; (2) 30 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec; (3) final extension at 72°C for 7 minutes. Visualization on agarose gels confirmed that 282 clones harbored an appropriately-sized fragment. Six clones per specimen (108 total clones) were selected for sequencing.

Universal primers were used to amplify twelve non-coding chloroplast regions, including the *mak5–mak6* intron, *psbA5′R–matk8F* intergenic spacer, *psbA–trnH* intergenic spacer, *psbB–psbH* intergenic spacer, *rpL16* intron, *5′rpS12–rpL20* intron, *rpoB–trnC* intergenic spacer, *rps16F–rps16R* intergenic spacer, *trnL5–trnF* intergenic spacer, *trnS–trnfM* intergenic spacer, *trnT–trnD* intergenic spacer, and *ycf6R–trnC* intergenic spacer (Taberlet et al. 1991; Shaw et al. 2005). We performed 25 μL PCR reactions with 2 μL genomic DNA, 0.125 μL of each primer (100 mM stock, 0.5 μM final concentration), 0.5 μL dNTP mix (10 mM final concentration), 2.5 μL standard *Taq* reaction buffer (10×) and 0.1 μL standard *Taq* (5,000 units/mL, New England Biolabs, Ipswich, Massachusetts). Thermocycling and clean-up were performed as described previously by Ramsey et al. (2008).

Cycle-sequencing reactions for *GBSSI* and cpDNA noncoding regions were performed in a 12.5 μL volume that included 1.5 μL purified PCR product, 0.02 μL of primer (100 mM stock, 0.2 μM final concentration), 0.5 μL 5 M ultrapure betaine (USB, Cleveland, Ohio), 2.5 μL Big Dye buffer (Applied Biosystems, Foster City, California), and 0.5 μL Big Dye (version 3.1; Applied Biosystems). Big Dye reactions were performed in forward and reverse direction. Products were purified using Montage™ vacuum plates (Millipore, Billerica, Massachusetts) and sequenced on an ABI 3730 capillary sequencer (University of Rochester Functional Genomics Center). High-quality sequence data were obtained for all cpDNA regions in all sampled taxa; for *GBSSI,* 84 clones yielded readable sequence in 11 species. Sequences were manually edited using Sequencher™ (v. 4.1; Gene Codes, Ann Arbor, Michigan) and manually aligned using MacClade (v. 4.08, Sinauer Associates, Sunderland, Massachusetts).

***Phylogenetic Analyses*—**Sequence data were analyzed using maximum parsimony with PAUP* (v. 4.0b10; Swofford 2003) and Bayesian approaches with MrBayes (v. 3.1b; Huelsenbeck and Ronquist 2001). For Bayesian analysis, the model of sequence evolution was estimated using the Akaike information criterion (Posada and Buckley 2004) as implemented in the program MrModeltest (v. 2.2; Nylander 2004). An incongruence length difference test, implemented as the partition homogeneity test in PAUP* and performed with parsimony-uninformative characters excluded from the data matrix, did not identify incongruence among cpDNA regions ($p = 0.81$). Thus, phylogenetic analyses were based on the combined data set. The cpDNA data set was complete (no missing data among sampled specimens) but the *GBSSI* data set was incomplete (haplotypes missing for six specimens) (Appendix 1). Data sets and resulting phylogenies can be found in TreeBASE (study number S11014).

For parsimony analysis of *GBSSI*, indels were excluded and all nucleotide substitutions were weighted as equal and unordered. Heuristic searches were conducted using tree bisection and reconnection with 1,000 random additions, and we estimated bootstrap support for nodes on the trees using 1,000 replicates. For Bayesian analyses, indels were excluded from analysis. We selected a HKY substitution model based on analysis of the data set with MrModeltest. We used six Markov chains (five heated, one cold) for a total of four runs, each with 2,000,000 generations. A total of 20,000 trees were sampled at 100-generation intervals. We visualized output from MrBayes using the program Tracer (v. 1.4; Rambaut and Drummond 2007) to determine run length and burnin. Stabilization of log-likelihood values occurred at 100,000 generations (1,000 sampled trees). We excluded the first 1,000 trees as burnin, the remaining 19,000 trees were used to estimate Bayesian posterior probabilities. Analysis of non-coding cpDNA proceeded as above with the exception that the GTR + I + Γ model of evolution was selected by MrModeltest. Model testing was performed on the combined cpDNA data set because we recovered low sequence variation within individual cpDNA regions.

Relationships of *GBSSI* alleles within *Hedera* were evaluated with statistical parsimony, a network approach implemented by the TCS software package (v. 1.21; Clement et al. 2000). Unlike traditional phylogenetic reconstruction methods, network analysis accommodates data sets exhibiting low sequence variation, recombination, reticulate evolution, and multifurcation. Network analysis is thus useful for visualizing diversity and heterozygosity of haplotypes in polyploids (Doyle et al. 2002; Guggisberg et al. 2006; Wu et al. 2010). For each sampled plant, we tallied the number of distinct *GBSSI* haplotypes recovered and characterized allelic diversity as the ratio of distinct haplotypes to the total number of homologous (or homeologous) loci present in the genotype (i.e. two for diploid plants, four for tetraploids, six for hexaploids, eight for octoploids). Finally, for genotypes that were heterozygous for *GBSSI* alleles, we calculated the average number of mutational steps distinguishing the haplotypes.

Results

***Granule-Bound Starch Synthase I*—**Production of spurious sequence variation is a concern for studies of low-copy nuclear loci, as gene cloning provides many opportunities for PCR-mediated mutation and recombination to occur (Doyle et al. 2002). The *GBSSI* data set appeared to have few, if any, sequences generated by recombination or polymerase error. More than 40% of haplotypes were recovered from two or more independent PCR reactions, and among the eleven haplotypes recovered once, five shared base pair changes with another minor haplotype. Minor haplotypes were recovered primarily from polyploid taxa that, in principle, could harbor four or more haplotypes per locus. We never recovered more than two haplotypes from diploid plant specimens or four haplotypes from tetraploid plant specimens. For these reasons, we retained all *GBSSI* sequences that had high-quality,

Table 1. Geographic distribution and phenotypic characteristics of commonly-recognized *Hedera* taxa.

Taxon	Distribution	Habitat	Elev. (m)	Ploidy	Trichome	Juvenile leaves	Fruits
H. algeriensis Hibberd	Algeria	coastal forest, rocky slopes, ravines; waste areas, roadsides	0–500	4*x*	scale	large (8–11 cm × 8–10 cm); mostly glossy, shallowly 3-lobed; red petioles	black
H. azorica Carr.	Azores	coastal cliffs, low to mid-elevation forest, forest margins, ravines	0–1,000	2*x*	stellate	large (8–10 cm × 7–10 cm); matte, variable lobing; green petioles	black
H. canariensis Willd.	Canary Islands	coastal cliffs, low to mid-elevation forest, rocky areas	0–1,000	2*x*	scale	medium (6–8 cm × 6–8 cm); scarcely 3-lobed; red petioles	black
H. colchica K. Koch	Turkey, Caucasus	shady areas, low- to mid-elevation forest	0–1,500	8*x*	scale	variable (4–10 cm × 4–10 cm); leathery, sometimes heart-shaped; red petioles	black
H. cypria McAllister	Cyprus (Troodos Mts.)	mid- to high elevation forest, ravines, riparian areas	1,000–3,500	6*x*	scale	small (2–3 cm × 3–4 cm); triangular, unlobed, white veined; red petioles	black
H. helix L. subsp. *helix*	Europe	forest, ravines, rocky areas; hedges, old walls, gardens	0–2,500	2*x*	stellate	medium (4–7 cm × 4–7 cm); variable shape, lobing; green or red petioles	black
H. helix subsp. *caucausigena* Kleop.	Turkey, Caucasus	coastal to mid-elevation forest; hedges, old walls, waste areas	0–1,500	2*x*	stellate	small (3–5 cm × 5–7 cm) sagittate, shallowly lobed; green petioles	black
H. helix subsp. *rhizomatifera* McAllister	Spain	limestone substrates in low- to mid-elevation forest, riparian areas	0–1,000	2*x*	stellate	small (3–4 cm × 3–4 cm); thick, 3- to 5-lobed, white veined; red petiole	black
H. hibernica (Kirch.) Bean	Europe	coastal cliffs, low to mid-elevation forest, riparian areas; old walls	0–1,500	4*x*	stellate	medium (5–8 cm × 5–8 cm); variable shape and lobing; green petioles	black
H. hibernica 'Hibernica'	Europe (naturalized)	urban parks, hedges, gardens, waste areas	0–1,500	4*x*	stellate	large (7–10 cm × 7–10 cm); thick, shallowly 3–5 lobed; green petioles	black
H. iberica (McAllister) Ackerfield & J. Wen	Spain, Portugal	sheltered, humid sites in low to mid-elevation forest and forest margins	0–500	6*x*	scale	medium (5–7 cm × 6–8 cm); 3-lobed with extended middle; red petioles	black
H. maderensis K. Koch ex Rutherford	Madeira	coastal cliffs, low to mid-elevation forest, ravines; old walls	0–1,000	6*x*	scale	medium (3–5 cm × 3–5 cm); thick, 3- to 5-lobed; green petioles	black
H. maroccana McAllister	Morocco	calcareous and limestone substrates on cliffs, riparian areas	1,000–2,500	2*x*	scale	large (8–11 cm × 8–11 cm); 3- to 5-lobed, red veined; red petioles	black
H. maroccana 'Spanish Canary'	Morocco; Spain (naturalized)	low to mid-elevation forest; roadsides, hedges, gardens	0–1,000	2*x*	scale	large (8–11 cm × 8–11 cm); glossy, 3- to 5-lobed; red petioles	black
H. nepalensis K. Koch. var. *nepalensis*	Kashmir, Pakistan, Afghanistan, Nepal	mid- to high elevation forest, rocky slopes; old walls	1,000–3,000	2*x*	scale	small (2–4 cm × 3–5 cm); triangular, 3- to 5-lobed; green petioles	yellow or red
H. nepalensis K. Koch var. *sinensis* Rehder	China, Laos, Vietnam	coastal to high elevation forest, ravines, rocky slopes	0–3,500	2x	scale	small (3–5 cm × 4–6 cm); triangular, 3-lobed w/ ext. middle; green petioles	yellow or red
H. pastuchovii G. Woronow	Caucasus, Iran	low to mid-elevation forest, forest margins, glades	0–1,500	6*x*	scale	small (3–4 cm × 3–4 cm); thick, triangular, unlobed; green petioles	black
H. rhombea var. *formosana* (Nakai) Li	Taiwan	low to high elevation forest; hedges, roadsides	500–2,500	2*x*	scale	small (3–4 cm × 2–4 cm); delicate, 3- to 5-lobed; green petioles	black
H. rhombea var. *rhombea* (Miq.) Bean	Japan, Korea	coastal to low elevation forest and thickets; roadsides, waste areas	0–1,000	2*x*	scale	small (3–4 cm × 2–4 cm); 3- to 5-lobed; reddish new growth; red or green petioles	black

easily-interpretable chromatograms, including 84 sequences from *Hedera* species and eight sequences from outgroup taxa. The aligned data set contained 517 base pairs.

Within *Hedera*, there were 21 variable and six parsimony informative sites, yielding 18 unique haplotypes. Mean pairwise sequence divergence among haplotypes was 0.51%. We recovered a single, one-base indel that did not obscure differences between haplotypes. Outgroup taxa added 65 parsimony informative sites and six haplotypes to the *GBSSI* data set. Heuristic search of the *GBSSI* data set yielded seven most parsimonious trees (length = 114 steps; CI = 0.964; RI = 0.957). Differences among these trees involved outgroup placement, although *Fatsia* was always recovered as sister to a strongly monophyletic *Hedera*; Bayesian analyses produced a similar result (Fig. 2).

Within *Hedera*, two major haplotypes separated by one mutational step (hereafter, *A0* and *B0*) were most commonly

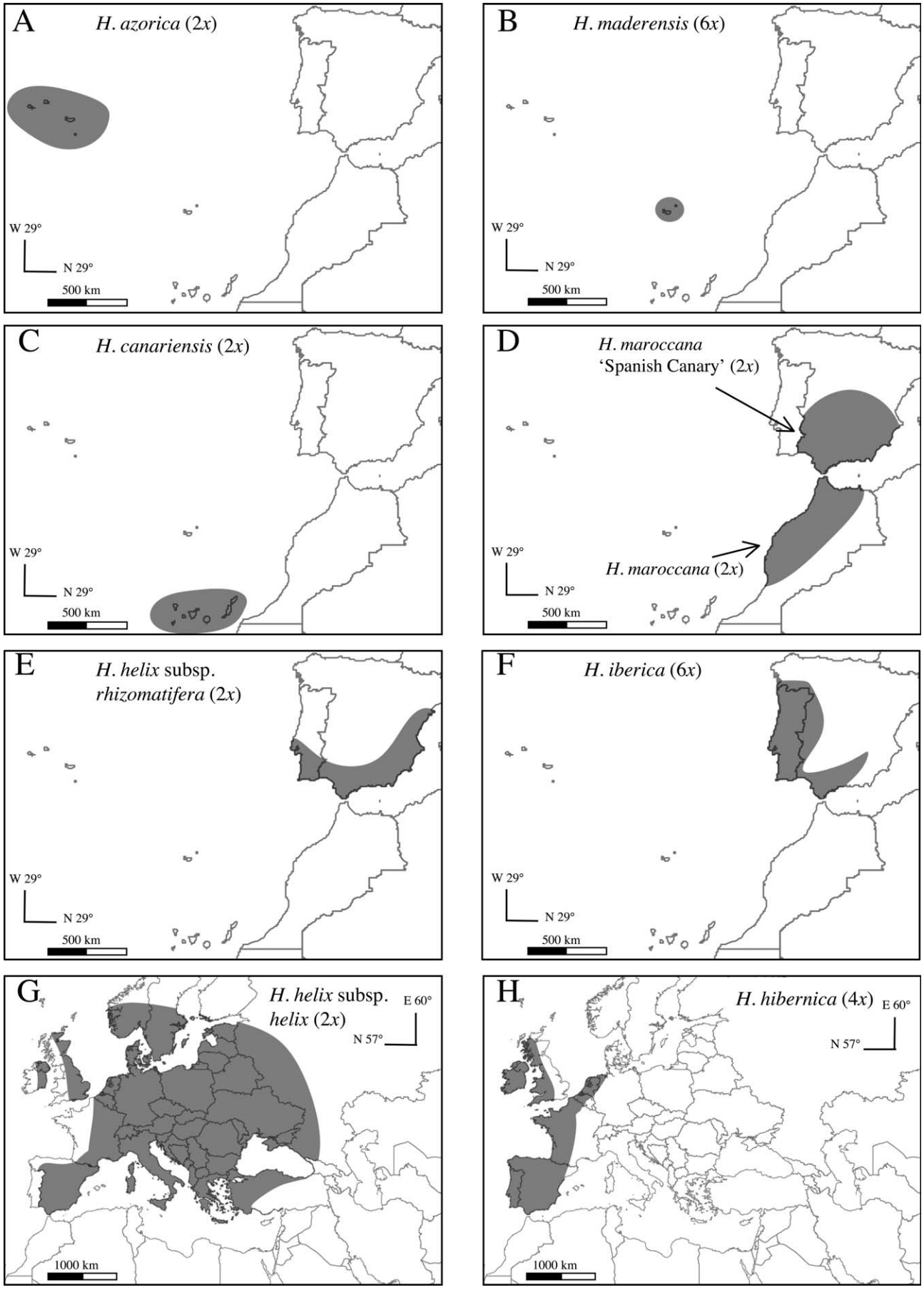

FIG. 1A Geographic distributions of *Hedera* taxa in Macaronesia (A-C), Europe (D-H).

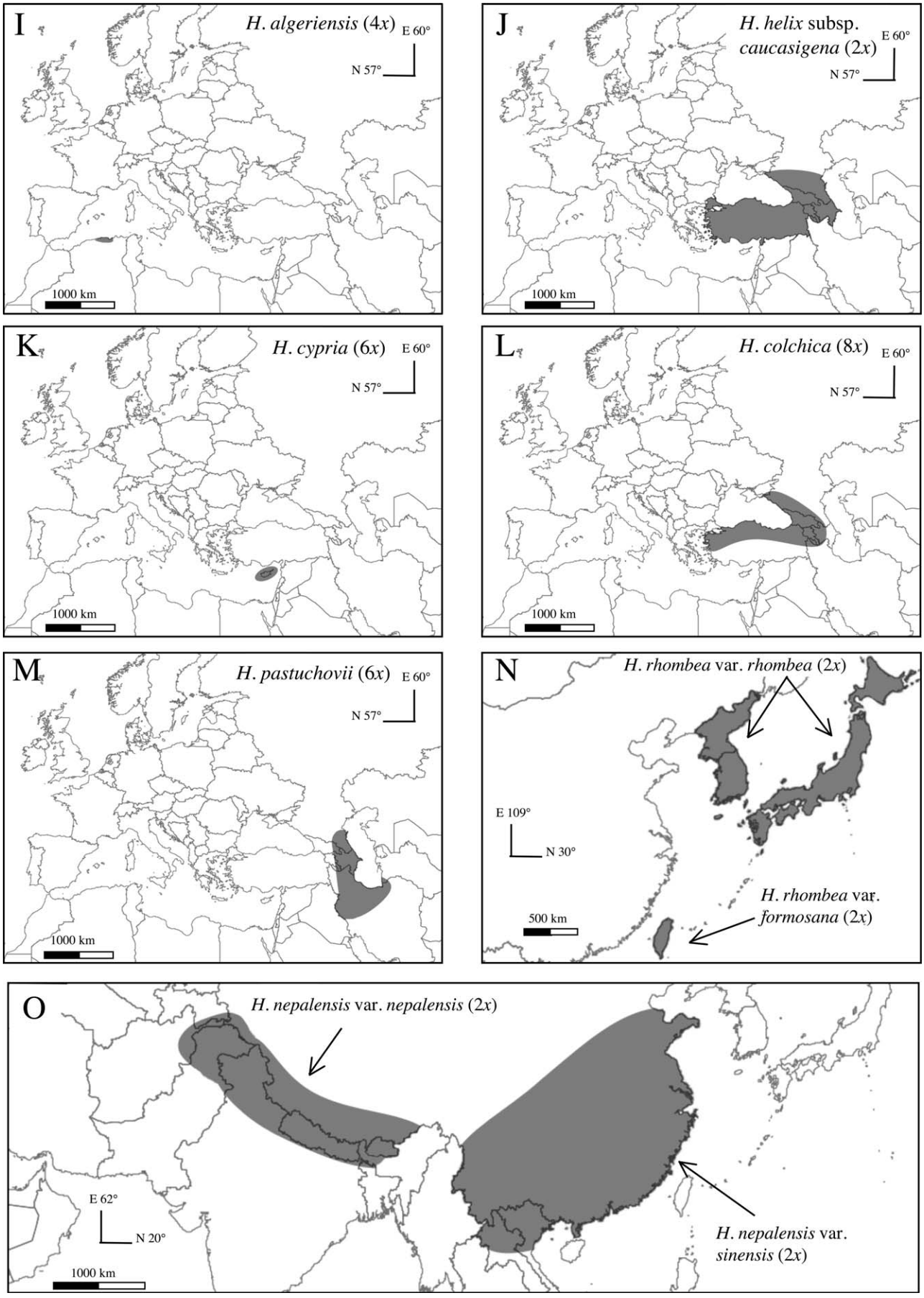

FIG. 1B Geographic distributions of *Hedera* taxa in Europe (J-K), northern Africa (D, I), and Asia (L-O).

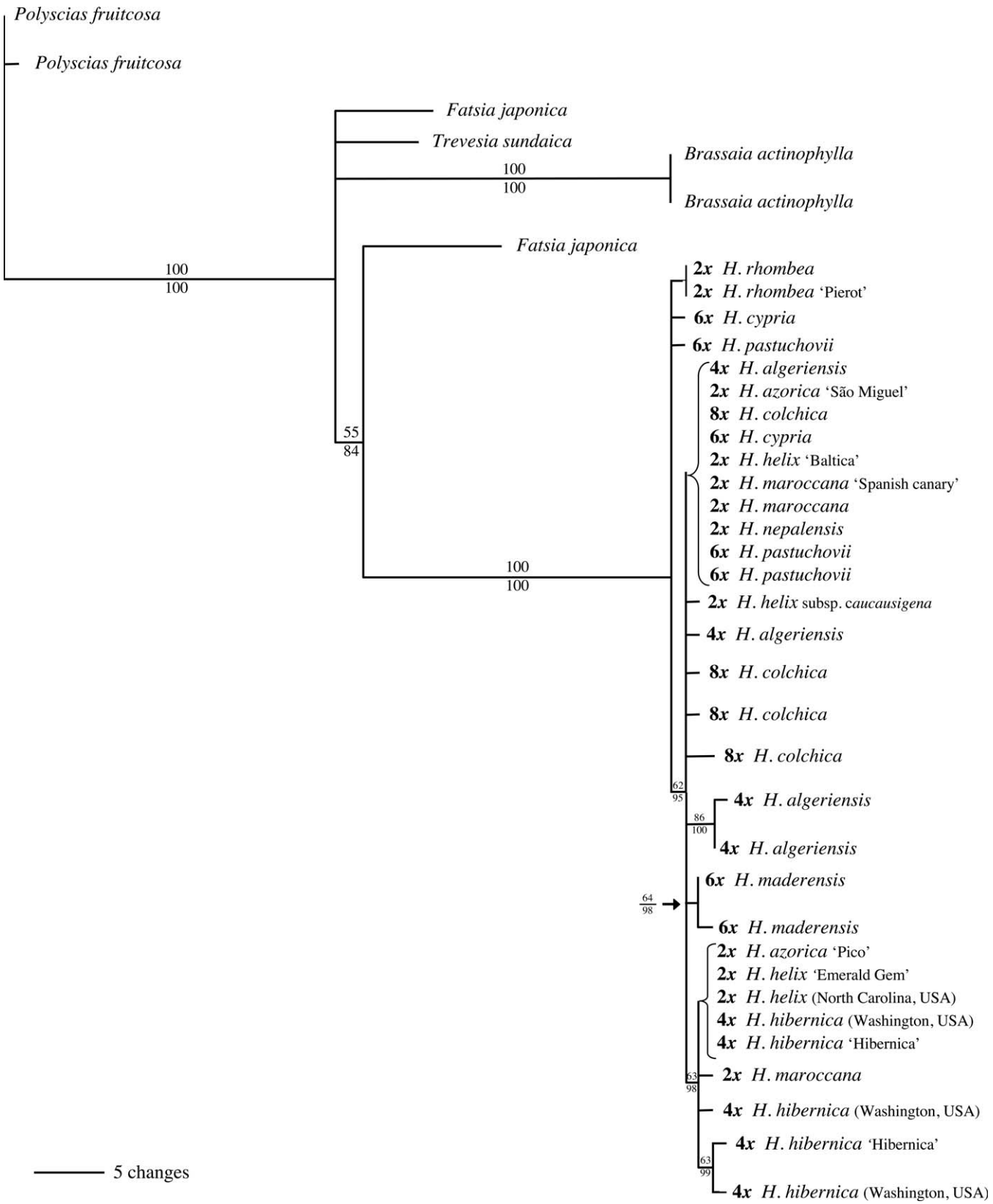

FIG. 2 Strict consensus phylogram of seven most parsimonious trees generated by maximum parsimony (length = 114 steps; CI = 0.964; RI = 0.957) based on the low-copy nuclear gene, *GBSSI*. Numbers above and below branches indicate maximum parsimony bootstrap values and Bayesian posterior probability values, respectively.

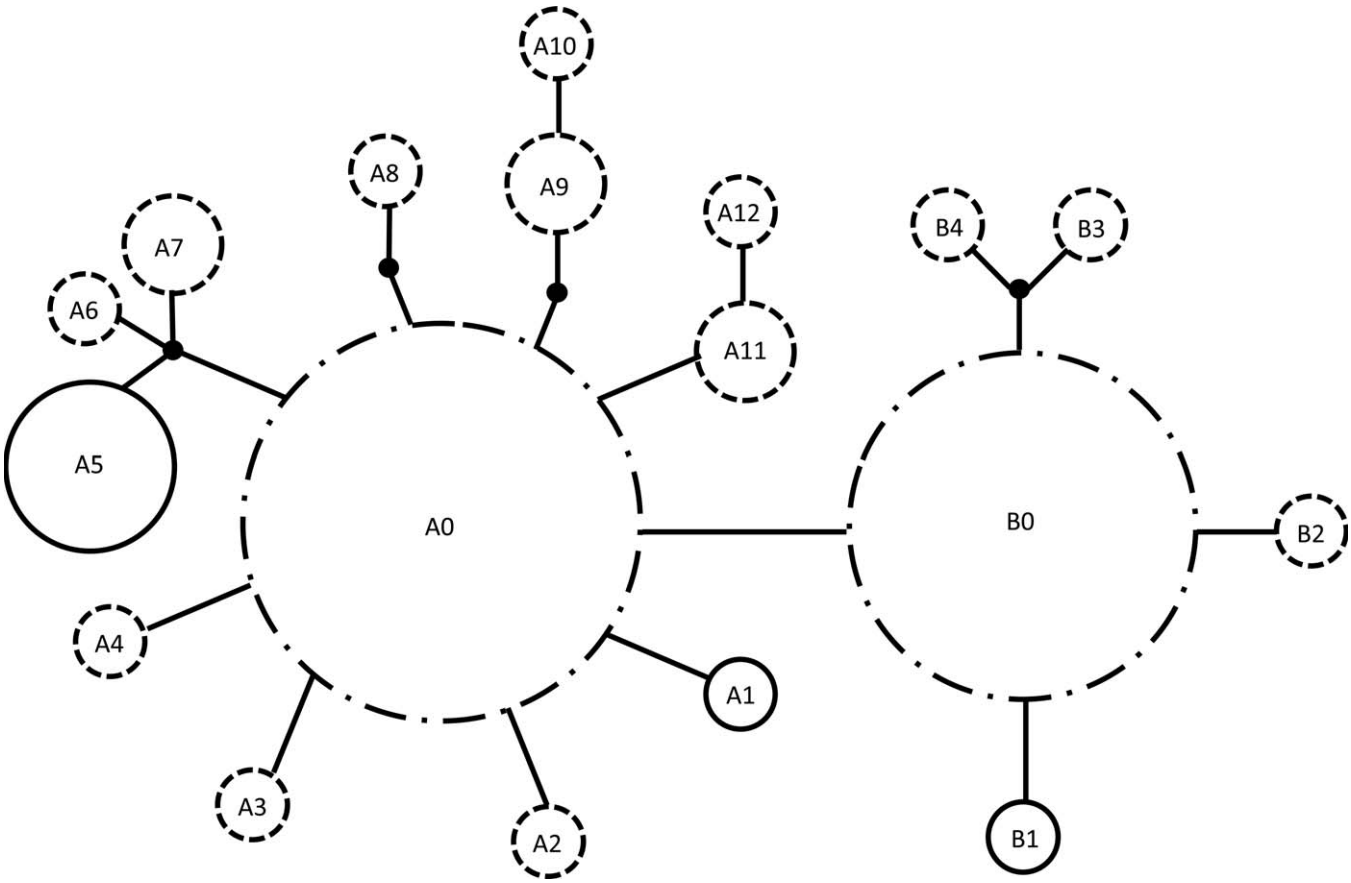

FIG. 3 Minimum-spanning network showing relationships among *GBSSI* alleles of *Hedera* species. The area of circle surrounding each haplotype corresponds to the total number of clones recovered for that DNA sequence (Table 2). Haplotypes surrounded by solid lines were only recovered from diploid species while haplotypes surrounded by dotted lines were only recovered from polyploid species. Haplotypes surrounded by dot-dash lines were found in both diploid and polyploid species.

found (Figs. 2, 3; Table 2). *A0* was recovered from 10 genotypes (including specimens of *H. algeriensis*, *H. azorica* Carr., *H. colchica*, *H. cypria*, *H. helix*, *H. maroccana*, *H. nepalensis* K. Koch, and *H. pastuchovii*) while *B0* was recovered from five genotypes (including specimens of *H. azorica*, *H. helix*, and *H. hibernica*) (Table 2). Sixteen minor *Hedera* haplotypes were one to four mutational steps removed from *A0* or *B0* and recovered from one or two specimens (Figs. 2, 3). Minor haplotypes, identified here by sequential number under the most similar major haplotype, clustered to some degree by species. For example, the two sampled specimens of *H. rhombea* (Miq.) Bean had an identical minor haplotype (*A5*) while all five haplotypes recovered from *H. hibernica* plants were within two mutational steps (*B0*, *B2*, *B3*, *B4*) (Table 2).

Polyploid genotypes harbored significantly more *GBSSI* haplotypes than diploid genotypes (mean 2.5 vs. 1.2 alleles; $n = 18$ genotypes, Mann-Whitney U test, $p = 0.02$). However, diploid and polyploid genotypes had similar measures of haplotype diversity (mean 0.6 vs. 0.5; $n = 18$ genotypes, Mann-Whitney U test, $p = 0.32$) and divergence (mean 1.5 vs. 1.9 mutational steps; $n = 9$ genotypes, Mann-Whitney U test, $p = 0.57$).

Non-Coding Chloroplast Regions—The aligned cpDNA data set contained 9,972 base pairs. Within *Hedera*, there were 47 variable and 33 parsimony informative sites, resulting in 16 unique haplotypes. Mean pairwise sequence divergence among cpDNA haplotypes was 0.12%. The twelve noncoding regions contributed the following numbers of parsimony-informative sites: *psbA-trnH*, six; *rpL16*, six; *ycf6R-trnC*, four; *rpoB-trnC*, three; *trnT-trnD*, three; *psbB-psbH*, two; *trnS-trnfM*, two; *rps16F-rps16R*, two; *psbA5′R-matk8F*, two; *matk5-matk6*, one; *5′rpS12-rpL20*, one; and *trnL5-trnF*, one. Outgroup taxa added 62 parsimony-informative characters and five haplotypes.

Heuristic search of the cpDNA data set yielded three most parsimonious trees (length = 279 steps; CI = 0.936; RI = 0.934) that differed primarily in outgroup placement (*Fatsia* vs. *Trevesia* as the sister group to *Hedera*) (Fig. 4). Monophyly of *Hedera* was strongly supported (100% bootstrap). Two divergent groupings were apparent within *Hedera*. The first, smaller clade consisted of taxa from the western Mediterranean Basin, including northwestern Africa (*H. maroccana*), the Canary Islands (*H. canariensis*), and the Iberian Peninsula (*H. helix* subsp. *rhizomatifera* McAllister and *H. iberica*) (Fig. 4; Table 1). The second, larger clade contained taxa from the central and eastern Mediterranean Basin (*H. algeriensis*, *H. cypria*, *H. helix* subsp. *caucasigena* Kleop.), Macaronesia (*H. azorica*, *H. maderensis* K. Koch ex Rutherford), northern and central Europe (*H. helix* subsp. *helix*, *H. hibernica*), and Asia (*H. colchica*, *H. nepalensis*, *H. pastuchovii*, *H. rhombea*) (Fig. 4; Table 1). Bayesian analysis generated a nearly identical tree topology as maximum parsimony except that the small, western Mediterranean clade (*H. canariensis*, *H. helix* subsp. *rhizomatifera*, *H. iberica*, *H. maroccana*) was recognized as sister to the *Fatsia* and *Trevesia* outgroups, thus making the genus *Hedera* paraphyletic rather than monophyletic (tree not shown).

TABLE 2. Occurrence of *GBSSI* haplotypes in *Hedera* taxa. Subscripts indicate numbers of alleles recovered from individual plant specimens.

Taxon	Ploidy	Alleles (clones)	*GBSSI* haplotype																	
			A0	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B0	B1	B2	B3	B4
H. algeriensis	4*x*	4(5)	$\surd_1$		$\surd_1$							$\surd_2$	$\surd_1$							
H. azorica 'Pico'	2*x*	1(5)														$\surd_5$				
H. azorica 'São Miguel'	2*x*	1(3)	$\surd_3$																	
H. colchica	8*x*	4(6)	$\surd_3$			$\surd_1$	$\surd_1$				$\surd_1$									
H. cypria	6*x*	2(2)	$\surd_1$						$\surd_1$											
H. helix 'Emerald Gem'	2*x*	1(6)														$\surd_6$				
H. helix 'Baltica'	2*x*	2(5)	$\surd_1$													$\surd_4$				
H. helix	2*x*	1(1)														$\surd_1$				
H. helix subsp. *caucausigena*	2*x*	1(3)		$\surd_3$																
H. hibernica	4*x*	3(7)														$\surd_5$		$\surd_1$		$\surd_1$
H. hibernica 'Hibernica'	4*x*	2(6)														$\surd_5$			$\surd_1$	
H. maderensis	6*x*	2(3)												$\surd_2$	$\surd_1$					
H. maroccana	2*x*	1(5)	$\surd_5$																	
H. maroccana	2*x*	2(6)	$\surd_5$														$\surd_1$			
H. nepalensis var. *sinensis*	2*x*	1(5)	$\surd_5$																	
H. pastuchovii	6*x*	1(5)	$\surd_5$																	
H. pastuchovii	6*x*	2(6)	$\surd_4$							$\surd_2$										
H. rhombea	2*x*	1(4)						$\surd_4$												
H. rhombea 'Pierot'	2*x*	1(2)						$\surd_2$												
Total clones		85	33	3	1	1	1	6	1	2	1	2	1	2	1	26	1	1	1	1

Within the two divergent clades of *Hedera*, samples grouped by geographic distribution and species designation (Fig. 4; Table 1). For example, well-supported groupings were evident for Asian taxa and for northern Macaronesian taxa. Traditional European taxa (*H. helix* subsp. *helix*, *H. hibernica*) also formed a well-supported clade. However, "*H. helix*" specimens from southern Spain (*H. helix* subsp. *rhizomatifera*) and southeastern Europe (*H. helix* subsp. *caucasigena*) were allied with the western and eastern Mediterranean ivies, rendering this species polyphyletic. Unlike the geographic structuring that was apparent in cpDNA phylogenies, ploidy level varied among close relatives (Fig. 4). For example, diploid, hexaploid, and octoploid species occur within the Asian clade while diploid and tetraploid species occur within the European group.

Discussion

Haplotype Variation and Geneology—Pairwise sequence divergence of *GBSSI* haplotypes (0.51%) and cpDNA haplotypes (0.12%) is less than typically reported in studies of comparably-sized genera (Mast et al. 2004; Weese and Johnson 2005; Kelly and Culham 2008; Tank and Olmstead 2008). Several factors may underlie the low sequence variation of ivy species. Speciation within *Hedera* may be rapid due to polyploidy, which appears to have evolved repeatedly in the genus (Fig. 4; Table 1). Ivy species may accumulate neutral genetic differences slowly because of slow maturation and long generation time. Moreover, *Hedera* may have been subject to more taxonomic "splitting" than other plant genera due to longstanding horticultural interest (Sulgrove 1984; Rose 1996). For example, ploidy level and slight differences in trichome morphology or leaf shape are critical for *Hedera* species delineations yet are often considered insufficient to distinguish species in other genera (Lewis 1980; Soltis et al. 2007).

While *GBSSI* haplotypes were widely shared across *Hedera* taxa, cpDNA haplotypes were generally unique to species and/or geographic regions. This finding probably reflects intrinsic differences for coalescence of nuclear haplotypes and cytoplasmic haplotypes. Expected times to coalescence for chloroplast haplotypes is equal to the effective haploid population size (N_e) while the expected time for a nuclear locus is $2N_e$ (diploid species), $4N_e$ (tetraploid species), $6N_e$ (hexaploid species), or $8N_e$ (octoploid species) (Hein et al. 2005). Due to complications of lineage sorting, characterization of species relationships in *Hedera* may only be feasible using haploid genomes (cpDNA, mtDNA) or population genetic analyses.

Origins of Polyploidy—Polyploidy is widespread within *Hedera*, unlike most members of the Araliaceae, but the incidence of auto- and allopolyploidy is poorly understood (Yi et al. 2004). McAllister and Rutherford (1990) inferred an allopolyploid origin of *H. hibernica* from diploid *H. helix* and *H. maroccana* on the basis of leaf morphology, trichome structure, and foliage odor. On the other hand, Jacobsen (1954) regarded *H. hibernica* as an autopolyploid form of the more widespread *H. helix* because of the species' morphological and chromosomal similarities. Vargas et al. (1999) identified allopolyploids (*H. hibernica*, *H. maderensis*, *H. pastuchovii*) and autopolyploids (*H. algeriensis*, *H. colchica*, *H. cypria*) based on nucleotide polymorphisms in nrDNA (ITS). Ackerfield and Wen (2003) describe incongruities between *Hedera* phylogenies generated by nrDNA and cpDNA that may reflect allopolyploidy.

The aforementioned sources of data have significant weaknesses. Leaf anatomy and morphology influences plant water relations, for example, so lobedness and trichome structure may reflect ecological adaptation rather than phylogenetic history. High-copy nuclear genes like ITS can misrepresent speciation in polyploid lineages (Álvarez and Wendel 2003; Feliner and Rosselló 2007). Inconsistency of phylogenetic signal between nrDNA and cpDNA genes may reflect lineage sorting, selective sweeps, or introgression rather than allopolyploidy (Wendel and Doyle 1999). These problems are exacerbated by the low sequence variation present in *Hedera*, such that fixed heterozygosity is inferred from a small number of double peaks in a DNA chromatogram and incongruity may reflect only a few base pair differences.

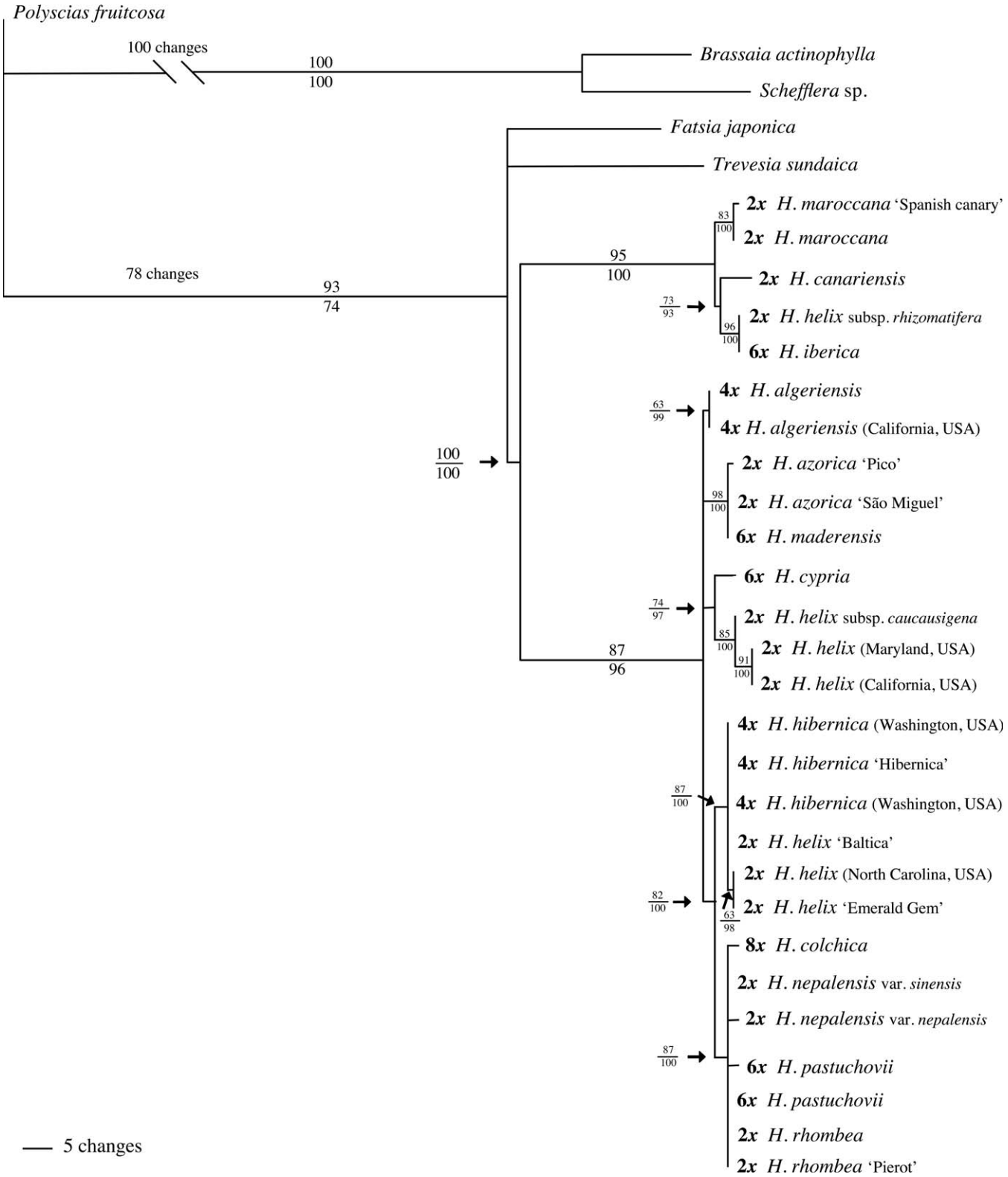

FIG. 4 Phylogram of one of three most parsimonious trees generated by maximum parsimony (length = 279 steps; CI = 0.936; RI = 0.931) based on 12 non-coding cpDNA regions. Numbers above and below branches indicate maximum parsimony bootstrap values and Bayesian posterior probability values, respectively.

Low-copy nuclear genes are recommended for study of polyploid complexes because cloning of intact haplotypes often distinguishes homologous and homeologous chromosomes, and thus auto- and allopolyploids (Doyle et al. 2002; Sang 2002). At face value, the *GBSSI* data appear consistent with autopolyploidy (Figs. 2, 3; Table 2). There is broad sharing of major and minor haplotypes among diploid and polyploid species. There is no indication that polyploids have fixed heterozygosity or harbor unusually divergent haplotypes. In some cases, we failed to recover haplotype profiles

anticipated for hypothesized allopolyploid species. For example, specimens of tetraploid *H. hibernica* did not have the *A0* haplotype characteristic of one putative diploid parent species, *H. maroccana*, but did have the *B0* haplotype characteristic of another putative diploid parent species, *H. helix*. Because *H. helix* and *H. hibernica* harbor identical cpDNA and low copy nuclear DNA haplotypes, occur sympatrically in Europe, and are morphologically similar, we suspect that *H. hibernica* has an autopolyploid origin from ancestral populations of diploid *H. helix* rather than an allopolyploid origin involving the allopatric, and highly divergent, *H. maroccana* and *H. helix* (Figs. 3, 4; Table 1). By similar logic, we can exclude *H. rhombea* and *H. helix* subsp. *caucausigena* Kleop. as progenitors of polyploids, because the minor haplotypes characteristic of these taxa were not recovered from extant polyploid species (Figs. 2, 3; Table 2).

Given the nature of the *GBSSI* data set, however, it is unlikely that we could distinguish haplotypes of homologous and homeologous chromosomes for many possible allopolyploid combinations. For example, three diploid species are characterized by the *A0* haplotype, and it would be impossible for us to distinguish auto- and allopolyploids derived from these taxa. While sequence variability in the *GBSSI* data is lower than desired, another problem is the abundance of minor haplotypes and lack of phylogenetic structure. The geneology of *GBSSI* is star-shaped in *Hedera*, as is typical of lineages undergoing rapid growth and demographic expansion (Ramsey et al. 2008). Mutation that is PCR-mediated may contribute to the apparent abundance of minor haplotypes but is unlikely to be the sole cause, as minor haplotypes were recovered from independent cloning reactions and we never found more than two haplotypes in diploid plant specimens. If low sequence divergence and phylogenetic structure is characteristic of nuclear genes within *Hedera*, determination of auto- and allopolyploid origins may be best accomplished by studies of inheritance patterns (Ramsey and Schemske 2002).

Irrespective of its origins, it appears that polyploidization has occurred repeatedly within *Hedera* (Fig. 4; Table 1). Ploidy variation is evident within the European clade (diploid and tetraploid species), Asian clade (diploid, hexaploid, and octoploid species), western Mediterranean clade (diploid and hexaploid species), eastern Mediterranean clade (diploid and hexaploid species), and Macaronesian clade (diploid and hexaploid species) inferred from cpDNA data, suggesting that polyploidy has evolved independently at least six times in the genus (Fig. 4). Interestingly, there are few independently-derived tetraploids, the most common polyploid level in most plant genera. Formation of hexaploid species almost always involves tetraploids (Ramsey and Schemske 1998, 2002), suggesting that the cytotype was previously more common in the genus or has gone undetected due to limited sampling among Mediterranean and Asian ivies.

Delineation of Species—We generally recovered similar or identical cpDNA sequences from morphologically-defined species recognized by McAllister and Rutherford (McAllister and Rutherford 1983, 1990; Rutherford 1984, 1989; Rutherford et al. 1993). Moreover, taxa delineated by Rutherford and McAllister harbored distinct cpDNA haplotypes or were clearly distinguished by ploidy level and/or morphology (Fig. 4; Table 1).

Among recent taxonomic changes to the genus, the splitting of *H. canariensis* s. l. into *H. algeriensis*, *H. azorica*, *H. maderensis*, *H. maroccana*, and *H. canariensis* s. s. is the most controversial (McAllister and Rutherford 1983; Rutherford et al. 1993). For example, Rose (1996) recognizes *H. algeriensis* and *H. maderensis* as subspecies of *H. canariensis* and treats *H. cypria* (introduced by Rutherford et al. 1993) as a variety of *H. pastuchovii*. The recent segregation of *H. iberica* from *H. maderensis* may be similarly debated (Ackerfield and Wen 2002, 2003). Our phylogenetic analyses provide strong support for splitting of *H. canariensis* s. l., which appears to be a polyphyletic taxon composed of allopatric lineages, as well as the separation of *H. cypria* and *H. pastuchovii* (Fig. 4; Table 1). Despite their morphological similarities and shared ploidy level, *H. iberica* and *H. maderensis* are distantly related.

Another controversial taxon is *H. hibernica*, a tetraploid species of coastal France, Spain, Belgium, the Netherlands, and the U. K. (Fig. 1; Table 1). Historically regarded as a subspecies or variety of *H. helix* s. l., *H. hibernica* was afforded species status on the basis of ploidy level, its distinctive ecological distribution, and subtle leaf size and trichome orientation features (Rutherford and McAllister 1983; Sulgrove 1984; McAllister and Rutherford 1990; but see Hatch 2010). *Hedera hibernica* 'Hibernica' is a wild-collected variant from the U. K. that is now widely planted, and sometimes naturalized, in Europe and North America (Table 1). We recovered identical *GBSSI* and cpDNA haplotypes from specimens of *H. helix* and *H. hibernica* (Figs. 2, 4; Table 2), suggesting that the species are poorly distinguished by DNA sequence data. On the other hand, in field studies we have found that *H. hibernica* rarely hybridizes with diploid *H. helix* and exhibits characteristic growth patterns in controlled experiments (A. Green, T. Ramsey, and J. Ramsey, unpubl. data). We thus favor recognition of *H. hibernica* as a taxonomic species distinct from its sister taxon, *H. helix* subsp. *helix*.

Hedera helix is the oldest named ivy and, with the possible exception of *H. nepalensis*, the taxon with the widest geographic distribution (Fig. 1; Table 1). Perhaps not surprisingly, our analyses suggest that *H. helix* s. l. is polyphyletic. *Hedera helix* populations from Spain (*H. helix* subsp. *rhizomatifera*) and the Caucasus (*H. helix* subsp. *caucasigena*) are divergent from the European *H. helix* subsp. *helix* as well as from each other (Figs. 1, 4; Table 1). *Hedera helix* subsp. *rhizomatifera* is allied to ivies of the western Mediterranean Basin (*H. canariensis, H. iberica*, *H. marrocana*) while *H. helix* subsp. *caucasigena* is allied to the geographically-proximate hexaploid species *H. cypria* (Figs. 1, 4; Table 1). Some North American specimens of "*H. helix*" clustered with *H. helix* subsp. *caucasigena* rather than *H. helix* subsp. *helix*.

The aforementioned results highlight the need for in-depth, population-level sampling in widespread species like *H. helix* s. l. and *H. nepalensis*. Our sampling scheme is modest, in most cases including two or three specimens per species, and focused on previously-described subspecific taxa (Table 1; Appendix 1). Another limitation of our study is the lack of phylogenetic resolution within geographically-defined clades. For example, the Asian species *H. colchica*, *H. nepalensis*, *H. pastuchovii*, and *H. rhombea* form a monophyletic grouping with strong statistical support but we have no inference of historical relationships amongst the four species (Fig. 4). In many cases, closely-related *Hedera* species appear to have distinct geographic distributions, ploidy levels, and morphologies (Figs. 1, 4; Table 1). For example, *H. azorica* and *H. maderensis* have identical cpDNA haplotypes but occur on different oceanic archipelagos and are strongly distinguished by leaf size and trichome morphology as well as ploidy level.

Critical analysis of species boundaries in *Hedera* will nonetheless require more extensive sampling and more variable molecular data than what is reported here.

Morphological Evolution—Extant species of *Hedera* share several phenotypic synapomorphies, including a woody vine growth form and heteroblastic foliage (Ackerfield and Wen 2002). Unlike several other genera of the Araliaceae, the monophyly of *Hedera* was never questioned on morphological grounds and is well-supported by molecular data (Ackerfield and Wen 2003; Valcárcel et al. 2003). *Hedera* species nonetheless exhibit considerable variability for leaf size, lobing, and texture; stem size and color; and trichome morphology and orientation (Ackerfield and Wen 2002, 2003; Table 1). Our analyses point to evolutionary lability of these traits (Fig. 4). For example, small- and large-leaved species exist within most geographic groupings delineated by cpDNA, including striking contrasts like *H. colchica* and *H. nepalensis* (Asian clade); *H. azorica* and *H. maderensis* (Macaronesian clade); and *H. maroccana* and *H. helix* subsp. *rhizomatifera* (western Mediterranean clade) (Figs. 1, 4; Table 1).

The evolution of trichomes deserves special mention. Most vegetative traits of *Hedera* are affected by environmental conditions (light, moisture) and development (age, position). Thus, leaf and stem features often differ statistically, rather than absolutely, between species (McAllister and Rutherford 1990; Rose 1996). In contrast, trichomes are thought to exhibit discrete differences and have been pursued as a reliable means of both species identification and phylogenetic inference (Lum and Maze 1989; Rutherford et al. 1993; Ackerfield 2001). For example, McAllister and Rutherford (1983) identified two principle groupings within *Hedera* on the basis of trichome morphology, distinguishing species with white trichome rays (*H. helix* s. l., *H. hibernica*, *H. azorica*) from species with reddish scale-like trichomes (all other species). Consideration of trichome characters has also influenced interpretation of ambiguous molecular results (Vargas et al. 1999; Valcárcel et al. 2003).

Our phylogenetic analyses suggest that trichomes are subject to the same evolutionary lability as other leaf and stem traits. Pronounced differences in trichome phenotype distinguish closely-related *Hedera* species, for example, *H. azorica* and. *H. maderensis* (Macaronesian clade) and *H. helix* subsp. *rhizomatifera* and *H. iberica* (western Mediterranean clade) (Figs. 1, 4; Table 1). Trichome characteristics are nonetheless useful for identifying some *Hedera* taxa. For example, *H. helix* and *H. hibernica* are distinguished by possession of bristling stellate hairs and adpressed stellate hairs, respectively (McAllister and Rutherford 1990).

Origin and Early Diversification—The family Araliaceae consists of ~50 genera and 1,350 species, most of which reside in tropical and subtropical Asia (Wen et al. 2001; Chandler and Plunkett 2004). *Hedera* is thus unusual in having a Eurasian distribution that is centered in the Mediterranean Basin, where eight of thirteen species occur (Rutherford et al. 1993; Ackerfield and Wen 2002, 2003; Table 1). Haplotype diversity and phylogenetic structure is most developed in the Mediterranean Basin and Macaronesia (Figs. 2, 4; Table 1). Only four species have an Asian distribution and these are allied to central and northern European species (Figs. 1, 4). Despite the broad geographic areas occupied by Asian and European species, these taxa had only six cpDNA haplotypes that were separated by small numbers of mutational steps. Like several other woody plant groups, the diversification of *Hedera* traces to Mediterranean regions of southern Europe and northern Africa (Pignatti 1978; Rodríguez-Sanchez et al. 2008).

Taxa of the highly divergent western Mediterranean clade reside in wooded areas of southern Spain, Portugal, Morocco, and Macaronesia (Figs. 1, 4, Table 1). Perhaps not coincidentally, these regions harbor remnants of "laurisilva," humid subtropical forest comprised of lauraceous evergreen hardwoods and other rare plants (Morales et al. 1996). Once widespread in southern Europe and northern Africa, laurisilvia forests retreated during the Pliocene as the Mediterranean Basin became increasingly arid. Laurisilva was extirpated from the central and eastern Mediterranean by the Late Pleistocene yet remnants persist in humid coastal and mountainous areas of the western Mediterranean Basin and Macaronesia (Morales et al. 1996). Spanish collections of *H. helix* subsp. *rhizomatiferra, H. iberica*, and *H. maroccana* are reported from areas harboring Pliocene relictual plant communities (Rutherford 1989).

Ivy fossils are of limited occurrence and subject to misidentification, in particular, labeling with "*Hedera*" based on superficial resemblance to modern-day ivies. Reports of *Hedera* from the Dakota Formation and other North American sites are almost certainly invalid (K. Johnson, Denver Museum of Nature and Science, pers. comm.). Among verified fossils, nearly all specimens were collected from Europe and Asia. For example, ivy megafossils (leaves and fruits) were found among fossils of mesic forest species in Macedonia (5.97 ± 0.07 mya) and Italy (7.25 ± 0.05–5.33 ± 0.05 mya) (Kovar-Eder et al. 2006). Fossilized ivy pollen has been dated to the late Miocene in Spain (11.61 ± 0.05–5.33 ± 0.05 mya; Muller 1981), the Pliocene in Germany (5.33–1.81 mya; Averdieck 1971) and the Holocene in Denmark (47,000 BP; Kolstrup 1991) and Britain (5,000–6,000 BP; Huntley 1983). Because of its broad historic distribution, and the association of fossil and extant species to mesic forest habitats, *Hedera* has been interpreted as a "tertiary relict," that is, a taxon with widespread occurrence in the northern hemisphere during warm periods of the Miocene and Early Pliocene that became geographically restricted during cooler periods of the Late Pliocene and Pleistocene (Rutherford 1989).

Recent fossil discoveries and phylogenetic analyses reported here support biogeographic hypotheses advocated by Valcárcel et al. (2003), in particular: (1) *Hedera* originated in temperate or subtropical regions of eastern Asia in the Mid- to Late Miocene; (2) early diversification of *Hedera* occurred in Mediterranean regions of Europe and Africa in the Late Miocene; (3) extinction or migration of Asian and northern European ivies occurred during periods of global cooling in the Late Pliocene and Pleistocene; and (4) northward and eastward dispersal of Mediterranean lineages in the Pleistocene produced the genetically depauperate but phenotypically diverse clade of European and Asian species. In this scenario, *H. helix* is a derived ecological specialist of cold, high-latitude climates. In contrast, poorly-studied endemic species of the Mediterranean Basin harbor most of the group's genetic diversity and reside near the center of its historical distribution (Figs. 1, 4; Table 1).

Macaronesia—The Macaronesian region includes five archipelagos (the Azores, Canary Islands, Cape Verde Islands, Salvage Islands, Madeira) that are well-studied by plant biogeographers (Baldwin et al. 1998; Vargas 2007). With islands resting between 100 and 1,500 km from mainland areas of northwest Africa and southwest Europe, Macaronesia has

developed a unique flora comprised of many endemic species (Pignatti 1978; Rodríguez-Sanchez et al. 2008). Macaronesia is floristically most similar to the Mediterranean Basin (Sunding 1979), and most Macaronesian endemics are derived from single colonization events from mainland sources (Francisco-Ortega et al. 1999, 2001a,b; Vargas et al. 1999). However, fleshy-fruited, bird-dispersed Macaronesian taxa, including hollies, junipers, olives, and strawberry trees, may have more complex histories (Valcárcel et al. 2003).

Ivies appear to have had two primary colonization events from mainland sources and one secondary colonization event (Figs. 1, 4; Table 1). *Hedera canariensis* belongs to the divergent western Mediterranean group (*H. maroccana*, *H. helix* subsp. *rhizomatifera*, *H. iberica*), as might be expected from the Canary Islands' close proximity to western Morocco (Fig. 1). Areas of western Morocco adjacent to the Canary Islands are floristically similar to Macaronesia and sometimes referenced as the "Macaronesian Enclave" of Morocco (Evers 1964). Land bridges intermittently connected the Canary Islands and Morocco during the Pliocene and Pleiostocene (Rothe 1974; Sunding 1979), suggesting that *H. canariensis* is derived by short-distance dispersal of a Mediterranean lineage or vicariant speciation of a widespread laurisilvan ancestor.

In contrast to the close relationship of *H. canariensis* and the western Mediterranean ivies, *H. azorica* and *H. maderensis* form a clade that is associated with ivies of Europe and Asia (Fig. 4). Madeira and the Azores are spatially closer to Morocco, southern Spain, and the Canary Islands than northern Europe or Asia (Fig. 1). Unlike the Canary Islands, however, Madeira and the Azores are true oceanic islands that have never been linked by land bridges to mainland areas (Evers 1964; Rothe 1974). In general, the floras of the Azores and Madeira show a stronger European than African influence (Sunding 1979; Press and Short 2002). Moreover, European birds, including pigeons, crows, thrushes, and other ivy fruvigores, occur in the Azores and Madeira as migrants, strays, or breeding populations (Metcalfe 2005; Clarke et al. 2006b).

The occurrence of *H. azorica* and *H. maderensis* in Macaronesia could reflect independent primary colonization events from closely-related mainland lineages. Secondary colonization ("island hopping") seems more likely, however, as the two species have identical cpDNA haplotypes that are separated by multiple mutation steps from haplotypes of other extant taxa (Fig. 4). Despite their shared history, *H. azorica* and *H. maderensis* are differentiated by trichome morphology, leaf shape, and ploidy level (Table 1). The emergence of the Madeiran archipelago pre-dates the emergence of the Azores (18.0 vs. 5.4 mya; Hess et al. 2000) but both island chains are probably older than the divergence of *H. azorica* and *H. maderensis*.

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Literature Cited

Ackerfield, J. 2001. Trichome morphology in *Hedera* (Araliaceae). *Edinburgh Journal of Botany* 58: 259–267.

Ackerfield, J. and J. Wen. 2002. A morphometric analysis of *Hedera* L. (the ivy genus, Araliaceae) and its taxonomic implications. *Adansonia* 24: 197–212.

Ackerfield, J. and J. Wen. 2003. Evolution of *Hedera* (the ivy genus, Araliaceae): insights from chloroplast DNA data. *International Journal of Plant Sciences* 164: 593–602.

Álvarez, I. and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

Anonymous. 2010. *Census of Horticultural Specialties (*2009*).* Document AC-07-SS-3. Washington DC: National Agricultural Statistics Service. http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Census_of_Horticulture/HORTIC.pdf.

Averdieck, F. R. 1971. Palynologische Untersuchungen zum Tertiär auf Sylt. *Meyniana* 21: 1–8.

Baldwin, B. G., D. J. Crawford, J. Francisco-Ortega, S. Kim, T. Sang, and T. F. Stuessy. 1998. Molecular phylogenetic insights on the origin and evolution of oceanic island plants. Pp. 410–441 in *Molecular systematics of plants II DNA sequencing*, eds. D. E. Soltis, P. S. Soltis, and J. J. Doyle. Boston: Kluwer Academic Publishers.

Beach, M. A. 1980. 7600 years of English ivy. *Light Garden* 18: 82–88.

Chandler, G. T. and G. M. Plunkett. 2004. Evolution in Apiales: nuclear and chloroplast markers in (almost) perfect harmony. *Botanical Journal of the Linnean Society* 144: 123–147.

Clarke, M., S. Reichard, and C. Hamilton. 2006a. Prevalence of different horticultural taxa of ivy (*Hedera* spp., Araliaceae) in invading populations. *Biological Invasions* 8: 149–157.

Clarke, T., C. Orgill, and T. Disley. 2006b. *Field guide to the birds of the Atlantic islands*. London: Christopher Helm.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1660.

Doyle, J. J., J. L. Doyle, A. D. H. Brown, and R. G. Palmer. 2002. Genomes, multiple origins, and lineage recombination in the *Glycine tomentella* (Leguminosae) polyploid complex: Histone *H3-D* gene sequences. *Evolution* 56: 1388–1402.

Evers, A. 1964. Das Entstehungsproblem der Makaronesichen Inseln und dessen Bedeutung für die Artentstehung. *Entomologische Blätter* 60: 81–87.

Feliner, G. N. and J. A. Rosselló. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution* 44: 911–919.

Fearnley-Whittingstall, J. 1992. *Ivies*. New York: Random House.

Francisco-Ortega, J., L. R. Goertzen, A. Santos-Guerra, A. Benabid, and R. K. Jansen. 1999. Molecular systematics of the *Asteriscus* alliance (Asteraceae: Inuleae) I: Evidence from the internal transcribed spacers of nuclear ribosomal DNA. *Systematic Botany* 24: 249–266.

Francisco-Ortega, J., J. C. Barber, A. Santos-Guerra, R. Febles-Hernandez, and R. K. Jansen. 2001a. Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: Evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. *American Journal of Botany* 88: 161–169.

Francisco-Ortega, J., S. J. Park, A. Santos-Guerra, A. Benabid, and R. K. Jansen. 2001b. Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. *Biological Journal of the Linnean Society. Linnean Society of London* 72: 77–97.

Grivet, D. and R. J. Petit. 2002. Phylogeography of the common ivy (*Hedera* sp.) in Europe: genetic differentiation through space and time. *Molecular Ecology* 11: 1351–1362.

Guggisberg, A., G. Mansion, S. Kelso, and E. Conti. 2006. Evolution of biogeographic patterns, ploidy levels, and breeding systems in a diploid-polyploid species complex of *Primula*. *The New Phytologist* 171: 617–632.

Hara, H. 1966. *The Flora of Eastern Himalaya*. Tokyo: University of Tokyo Press.

Hatch, L. C. 2010. *The IvyFile*. Raleigh: Cultivar.org New Ornamentals Society.

Hein, J., M. H. Schierup, and C. Wiuf. 2005. *Gene genealogies, variation and evolution: a primer in coalescent theory*. Oxford: Oxford University Press.

Hess, J., J. W. Kadereit, and P. Vargas. 2000. The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD), and intersimple sequence repeats (ISSR). *Molecular Ecology* 9: 857–868.

Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Huntley, B. 1983. *Hedera, Ilex* and *Viscum* – Ivy, Holly, and Mistletoe. Pp. 230–234 in *An atlas of past and present pollen maps for Europe: 0–13000 years ago*, eds. B. Huntley and H. J. B. Birks. Cambridge: Cambridge University Press.

Jacobsen, P. 1954. Chromosome numbers in the genus *Hedera* L. *Hereditas* 40: 252–255.

Kelly, L. J. and A. Culham. 2008. Phylogenetic utility of *MORE AXILLARY GROWTH4* (*MAX4*)-like genes: a case study in *Digitalis/Isoplexis* (Plantaginaceae). *Plant Systematics and Evolution* 273: 133–149.

Kolstrup, E. 1991. A Late Pleistocene "initial" vegetation at Vrøgum, west Jutland (Denmark). *Palaeogeography, Palaeoclimatology, Palaeoecology* 88: 53–67.

Kovar-Eder, J., Z. Kvacek, E. Martinetto, and P. Roiron. 2006. Late Miocene to Early Pliocene vegetation of southern Europe (7–4 Ma) as reflected in the megafossil plant record. *Palaeogeography, Palaeoclimatology, Palaeoecology* 238: 321–339.

Lewis, W. H. 1980. Polyploidy in species populations. Pp. 103–144 in *Polyploidy: Biological relevance* ed. W. H. Lewis. New York: Plenum Press.

Linnaeus, C. 1753. *Hedera*. P. 202 in *Species Plantarum*, vol.1.

Lum, C. and J. Maze. 1989. A multivariate analysis of the trichomes of *Hedera* L. *Watsonia* 17: 409–418.

Mast, A. R., D. M. S. Feller, S. Kelso, and E. Conti. 2004. Buzz-pollinated *Dodecatheon* originated from within the heterostylous *Primula* subgenus *Auriculastrum* (Primulaceae): A seven-region cpDNA phylogeny and its implications for floral evolution. *American Journal of Botany* 91: 926–942.

McAllister, H. A. 1979. The species of ivy. *Ivy Exchange Newsletter* 1: 4–5.

McAllister, H. 1981. New work on ivies. *International Dendrological Society Year Book* 1981: 106–109.

McAllister, H. A. and A. Rutherford. 1983. The species of ivy. *Ivy Journal* 9: 45–54.

McAllister, H. A. and A. Rutherford. 1990. *Hedera helix* L. and *H. hibernica* (Kirchner) Bean (Araliaceae) in the British Isles. *Watsonia* 18: 7–15.

Metcalfe, D. J. 2005. *Hedera helix* L. *Journal of Ecology* 93: 632–648.

Mitchell, A. and J. Wen. 2004. Phylogenetic utility and evidence for multiple copies of granule-bound starch synthase I (*GBSSI*) in Araliaceae. *Taxon* 53: 29–41.

Morales, D., M. S. Jiménez, A. M. González-Rodriguez, and J. Cermák. 1996. Laurel forests in Tenerife, Canary Islands I. The site, stand structure and stand leaf area distribution. *Trees (Berlin)* 11: 34–40.

Muller, J. 1981. Fossil pollen records of extant angiosperms. *Botanical Review* 47: 1–142.

Nylander, J. A. 2004. MrModelTest 2.2. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

Ohwi, J. 1965. *Hedera*. P. 663 in *Flora of Japan*. Washington D. C.: Smithsonian Press.

Pignatti, S. 1978. Evolutionary trends in Mediterranean flora and vegetation. *Vegetation* 37: 175–185.

Posada, D. and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.

Poyarkova, A. I. 1950. *Hedera*. Pp. 5–14 in *Flora of the USSR Volume XVI Umbelliflorae*, ed. B. K Shishkin. Moscow: Academy of Sciences of the USSR. English translation by R. Lavoot, Jerusalem: Israel Program for Scientific Translations, 1973.

Press, J. R. and M. J. Short. 2002. *Hedera*. Pp. 237–238 in *Flora of Madeira*. London: HMSO.

Rambaut, A. and A. J. Drummond. 2007 Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer.

Ramsey, J. and D. W. Schemske. 1998. Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467–501.

Ramsey, J. and D. W. Schemske. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* 33: 589–639.

Ramsey, J., A. Robertson, and B. Husband. 2008. Rapid adaptive divergence in New World *Achillea*, an autopolyploid complex of ecological races. *Evolution* 62: 639–653.

Reichard, S. 2000. *Hedera helix*. Pp. 212–216 in *Invasive plants of California's wildlands*, eds. C. C. Bossard, J. M. Randall, and M. C. Hoshovsky. Berkeley: University of California Press.

Rodríguez-Sánchez, F., R. Pérez-Barrales, F. Ojeda, P. Vargas, and J. Arroyo. 2008. The Strait of Gibraltar as a melting pot for plant biodiversity. *Quaternary Science Reviews* 27: 2100–2117.

Rose, P. Q. 1996. *The gardener's guide to growing ivies*. London: David and Charles.

Rothe, P. 1974. Canary Islands: origin and evolution. *Naturwissenschaften* 61: 526–533.

Rutherford, A. and H. McAllister. 1983. *Hedera hibernica* and its cultivars. *The Ivy Journal* 9: 23–32.

Rutherford, A. 1984. The history of the Canary Island ivies and its relatives. *The Ivy Journal* 10: 13–18.

Rutherford, A. 1989. The ivies of Andalusia (southern Spain). *The Ivy Journal* 15: 7–17.

Rutherford, A., H. A. McAllister, and R. R. Mill. 1993. New ivies from the Mediterranean area and Macronesia. *Plantsman (London, England)* 15: 115–128.

Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121–147.

Schäfer, H. 2002. Araliaceae. P. 136 in *Flora of the Azores*. Weikersheim: Margraf Verlag.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling, and 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.

Soltis, D. E., P. S. Soltis, D. W. Schemske, J. F. Hancock, J. N. Thompson, B. C. Husband, and W. S. Judd. 2007. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *Taxon* 56: 13–30.

Sulgrove, S. 1984. The great ivy debate: the status of *Hibernica*. *The Ivy Journal* 10: 33–45.

Sunding, P. 1979. Origins of the Macaronesian Flora. Pp. 1–40 in *Plants and islands*, ed. D. Bramwell. London: Academic Press.

Swofford, D. L. 2003. PAUP*: Phylogenetic analysis using parsimony (and other methods). Version 4.0 beta 10. Sunderland: Sinauer Associates.

Taberlet, P., G. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Tank, D. C. and R. G. Olmstead. 2008. From annuals to perennials: Phylogeny of subtribe Castillejinae (Orobanchaceae). *American Journal of Botany* 95: 608–625.

Vargas, P. 2007. Are Macaronesian islands refugia of relict plant lineages? a molecular survey. Pp. 297–314 in *Phylogeography in southern European refugia*, eds. S. J. Weiss and N. Ferrand. Dordrecht: Springer Press.

Vargas, P., H. A. McAllister, C. Morton, S. L. Jury, and M. J. Wilkinson. 1999. Polyploid speciation in *Hedera* (Araliaceae): phylogenetic and biogeographic insights based on chomosome counts and ITS sequences. *Plant Systematics and Evolution* 219: 165–179.

Valcárcel, V., O. Fix, and P. Vargas. 2003. Chloroplast and nuclear evidence for multiple origins of polyploids and diploids of *Hedera* (Araliaceae) in the Mediterranean Basin. *Molecular Phylogenetics and Evolution* 27: 1–20.

Weese, T. L. and L. A. Johnson. 2005. Utility of NADP-dependent isocitrate dehydrogenase for species-level evolutionary inference in angiosperm phylogeny: a case study in *Saltugilia*. *Molecular Phylogenetics and Evolution* 36: 21–41.

Wen, J., G. M. Plunkett, A. D. Mitchell, and S. J. Wagstaff. 2001. The evolution of Araliaceae: a phylogenetic analysis based on ITS sequences of nuclear ribosomal DNA. *Systematic Botany* 26: 144–167.

Wendel, J. F. and J. J. Doyle. 1999. Phylogenetic incongruence: window into genome history and molecular evolution. Pp. 265–295 in *Molecular systematics of plants II: DNA sequencing*, eds. D. S. Soltis, P. S. Soltis, and J. J. Doyle. Boston, Dordrecht, London: Academic Publishers.

Wu, L., X. Cui, R. I. Milne, Y. Sun, and J. Liu. 2010. Multiple autopolyploidizations and range expansion of *Allium przewalskianum* Regel. (Alliaceae) in the Qinghai-Tibetan Plateau. *Molecular Ecology* 19: 1691–1705.

Xiang, Q. and P. P. Lowry. 2007. *Hedera* Linnaeus. *Flora of China* 13: 441.

Yi, T., P. P. Lowry, G. M. Plunkett, and J. Wen. 2004. Chromosomal evolution in Araliaceae and close relatives. *Taxon* 53: 987–1005.

Appendix 1. Taxa used in the present study with collection information, voucher numbers, and GenBank accessions. Propagated clones of ingroup taxa are maintained by the American Ivy Society ("AIS"; Deerfield, New Jersey) or by the Ramsey Lab ("RL"; University of Rochester, Rochester, New York) with indicated accession numbers. Voucher specimens were prepared from plants grown in an experimental garden in 2008-2009 and are deposited at WTU. Data are presented in the following sequence: taxon name; field collection locality (or site of cultivation); collector; live plant accession number; specimen voucher information; and GenBank

accession numbers for the *mak5–mak6* intron, *psbA5′R–matk8F* intergenic spacer, *psbA–trnH* intergenic spacer, *psbB–psbH* intergenic spacer, *rpL16* intron, *5′rpS12–rpL20* intron, *rpoB–trnC* intergenic spacer, *rps16F–rps16R* intergenic spacer, *trnL5–trnF* intergenic spacer, *trnS–trnfM* intergenic spacer, *trnT–trnD* intergenic spacer, *ycf6R–trnC* intergenic spacer, and granule-bound starch synthase I (*GBSSI*) partial gene sequence. "—" indicates no sequence for the DNA region.

Hedera algeriensis Hibberd; Algeria: Kabyle Mountains, by J. Whitehead; AIS 88-188; *T. Ramsey 374854* (WTU); HQ220470, HQ220279, HQ220566, HQ220534, HQ220374, HQ220215, HQ220438, HQ220310, HQ220342, HQ220406, HQ220247, HQ220598, HQ234505-HQ234509. ***Hedera azorica*** Carr.; Azores: Pico, by Botanical Society of the British Isles Expedition; AIS 82-254 ('Pico'); *T. Ramsey 374856* (WTU); HQ220471, HQ220280, HQ220567, HQ220535, HQ220375, HQ220216, HQ220439, HQ220311, HQ220343, HQ220407, HQ220248, HQ220599, HQ234513-HQ234517. ***Hedera azorica*** Carr.; Azores: São Miguel, by anonymous; AIS 82-259 ('São Miguel'); *T. Ramsey 374857* (WTU); HQ220472, HQ220281, HQ220568, HQ220536, HQ220376, HQ220217, HQ220440, HQ220312, HQ220344, HQ220408, HQ220249, HQ220600, HQ234510-HQ234512. ***Hedera helix*** L.; U. S. A. (naturalized population): Maryland, Anne Arundel Co., by A. Green; RL MD06-C5; *T. Ramsey 374867* (WTU); HQ220473, HQ220282, HQ220569, HQ220537, HQ220377, HQ220218, HQ220441, HQ220315, HQ220345, HQ220409, HQ220250, HQ220601, —. ***Hedera helix*** L.; U. S. A. (naturalized population): California, Santa Barbara Co., by A. Green; RL CA07-E2; *T. Ramsey 374866* (WTU); HQ220474, HQ220283, HQ220570, HQ220538, HQ220378, HQ220219, HQ220442, HQ220314, HQ220346, HQ220410, HQ220251, HQ220602, —. ***Hedera algeriensis*** Hibberd; U. S. A. (naturalized population): California, Santa Cruz Co., by A. Green; RL CA07-N4; *T. Ramsey 374855* (WTU); HQ220475, HQ220284, HQ220571, HQ220539, HQ220379, HQ220220, HQ220443, HQ220313, HQ220347, HQ220411, HQ220252, HQ220603, —. ***Hedera canariensis*** Willd.; Canary Islands: Tenerife, La Mercedes, by Glasgow Naturalist Expedition; AIS 94-052; *T. Ramsey 374858* (WTU); HQ220476, HQ220285, HQ220572, HQ220540, HQ220380, HQ220221, HQ220444, HQ220316, HQ220348, HQ220412, HQ220253, HQ220604, HQ234518. ***Hedera colchica*** K. Koch; U. S. A. (cultivated): Pennsylvania, Longwood Gardens; AIS 94-058 ('My Heart'); *T. Ramsey 374859* (WTU); HQ220477, HQ220286, HQ220573, HQ220541, HQ220381, HQ220222, HQ220445, HQ220317, HQ220349, HQ220413, HQ220254, HQ220605, HQ234519-HQ234524. ***Hedera cypria*** H. McAllister; Cyprus: Troodos Mountains, by anonymous; AIS 03-079; *T. Ramsey 374860* (WTU); HQ220478, HQ220287, HQ220574, HQ220542, HQ220382, HQ220223, HQ220446, HQ220318, HQ220350, HQ220414, HQ220255, HQ220606, HQ234525, HQ234526. ***Hedera helix*** L. **subsp**. ***helix***; U. S. A. (cultivated): New Jersey, American Ivy Society; AIS 87-139 ('Emerald Gem'); *T. Ramsey 374861* (WTU); HQ220479, HQ220288, HQ220575, HQ220543, HQ220383, HQ220224, HQ220447, HQ220319, HQ220351, HQ220415, HQ220256, HQ220607, HQ234527-HQ234530, HQ234540. ***Hedera helix*** L. **subsp**. ***helix***; U. S. A. (cultivated): New Jersey, American Ivy Society; AIS 83-063 ('Baltica'); *T. Ramsey 374862* (WTU); HQ220480, HQ220289, HQ220576, HQ220544, HQ220384, HQ220225, HQ220448, HQ220320, HQ220352, HQ220416, HQ220257, HQ220608, HQ234531-HQ234535. ***Hedera helix*** **subsp**. ***rhizomatifera*** H. McAllister; Spain: Huelva, Aracena, by A. Rutherford; AIS 04-053; *T. Ramsey 374863* (WTU); HQ220481, HQ220290, HQ220577, HQ220545, HQ220385, HQ220226, HQ220449, HQ220321, HQ220353, HQ220417, HQ220258, HQ220609, HQ234536. ***Hedera helix*** **subsp**. ***caucasigena*** Kleop.; Caucauses: Sukhoms, by P. Zwijnenburg; AIS 90-079; *T. Ramsey 374864* (WTU); HQ220482, HQ220291, HQ220578, HQ220546, HQ220386, HQ220227, HQ220450, HQ220322, HQ220354, HQ220418, HQ220259, HQ220610, HQ234537-HQ234539. ***Hedera helix*** L.; U. S. A. (naturalized population): North Carolina, Wake Co., by A. Green; RL NC06-D1; *T. Ramsey 374865* (WTU); HQ220483, HQ220292, HQ220579, HQ220547, HQ220387, HQ220228, HQ220451, HQ220323, HQ220355, HQ220419, HQ220260, HQ220611, HQ234541. ***Hedera hibernica*** (Kirch.) Bean; U. S. A. (naturalized population): Washington, King Co., by T. Ramsey; RL L94; *T. Ramsey 374868* (WTU); HQ220484, HQ220293, HQ220580, HQ220548, HQ220388, HQ220229, HQ220452, HQ220324, HQ220356, HQ220420, HQ220261, HQ220612, HQ234542-HQ234546. ***Hedera hibernica*** (Kirch.) Bean ('Hibernica'); UK: Scotland, Clydesbank, by O. Kernaghan; AIS 06-023; *T. Ramsey 374869* (WTU); HQ220485, HQ220294, HQ220581, HQ220549, HQ220389, HQ220230, HQ220453, HQ220325, HQ220357, HQ220421, HQ220262, HQ220613, HQ234547-HQ234552. ***Hedera hibernica*** (Kirch.) Bean; U. S. A. (naturalized population): Washington, King Co., by A. Green; RL I91-G1; *T. Ramsey 374870* (WTU); HQ220486, HQ220295, HQ220582, HQ220550, HQ220390, HQ220231, HQ220454, HQ220326, HQ220358, HQ220422, HQ220263, HQ220614, HQ234553. ***Hedera iberica*** (H. McAllister) Ackerfield & J. Wen; Spain: Cadiz, by H. McAllister; AIS 04-003; *T. Ramsey 374871* (WTU); HQ220487, HQ220296, HQ220583, HQ220551, HQ220391, HQ220232, HQ220455, HQ220327, HQ220359, HQ220423, HQ220264, HQ220615, —. ***Hedera maderensis*** Koch ex Rutherford; Madeira: Funchal, by D. McClintock; AIS 91-097; *T. Ramsey 374872* (WTU); HQ220488, HQ220297, HQ220584, HQ220552, HQ220392, HQ220233, HQ220456, HQ220328, HQ220360, HQ220424, HQ220265, HQ220616, HQ234554-HQ234556. ***Hedera maroccana*** H. McAllister; Spain (naturalized population): Andalucia, by H. McAllister; AIS 88-009 ('Spanish Canary'); *T. Ramsey 374873* (WTU); HQ220489, HQ220298, HQ220585, HQ220553, HQ220393, HQ220234, HQ220457, HQ220329, HQ220361, HQ220425, HQ220266, HQ220617, HQ234557-HQ234561. ***Hedera maroccana*** H. McAllister; Morocco: Middle Atlas Mountains, by International Dendrological Expedition; AIS 88-008; *T. Ramsey 374874* (WTU); HQ220490, HQ220299, HQ220586, HQ220554, HQ220394, HQ220235, HQ220458, HQ220330, HQ220362, HQ220426, HQ220267, HQ220618, HQ234562-HQ234567. ***Hedera nepalensis*** K. Koch. **var.** ***sinensis*** Rehder; China, by Missouri Botanical Garden Expedition; AIS 88-259; *T. Ramsey 374875* (WTU); HQ220491, HQ220300, HQ220587, HQ220555, HQ220395, HQ220236, HQ220459, HQ220331, HQ220363, HQ220427, HQ220268, HQ220619, HQ234568-HQ234572. ***Hedera nepalensis*** K. Koch. **var**. ***nepalensis***; Nepal, by USDA Plant Introduction Expedition; AIS 88-258; *T. Ramsey 374876* (WTU); HQ220492, HQ220301, HQ220588, HQ220556, HQ220396, HQ220237, HQ220460, HQ220332, HQ220364, HQ220428, HQ220269, HQ220620, —. ***Hedera pastuchovii*** G. Woronow; Iran: Mt. Elburtz, by R. Lancster & A. Ala; AIS 82-118; *T. Ramsey 374877* (WTU); HQ220493, HQ220302, HQ220589, HQ220557, HQ220397, HQ220238, HQ220461, HQ220333, HQ220365, HQ220429, HQ220270, HQ220621, HQ234573-HQ234577. ***Hedera pastuchovii*** G. Woronow; U. S. A. (cultivated): New Jersey, American Ivy Society; AIS 88-264; *T. Ramsey 374878* (WTU); HQ220494, HQ220303, HQ220590, HQ220558, HQ220398, HQ220239, HQ220462, HQ220334, HQ220366, HQ220430, HQ220271, HQ220622, HQ234578-HQ234583. ***Hedera rhombea*** (Miq.) Bean; U. S. A. (cultivated): Pennsylvania, Longwood Gardens; AIS 82-260; *T. Ramsey 374879* (WTU); HQ220495, HQ234504, HQ220591, HQ220559, HQ220399, HQ220240, HQ220463, HQ220335, HQ220367, HQ220431, HQ220272, HQ220623, HQ234584-HQ234587. ***Hedera rhombea*** (Miq.) Bean; U. S. A. (cultivated): New Jersey, American Ivy Society; AIS 88-149 ('Pierot'); *T. Ramsey 374880* (WTU); HQ220496, HQ220304, HQ220592, HQ220560, HQ220400, HQ220241, HQ220464, HQ220336, HQ220368, HQ220432, HQ220273, HQ220624, HQ234588, HQ234589. ***Fatsia japonica*** Thunb.; U. S. A. (cultivated): Washington, University of Washington campus arboretum; *T. Ramsey 374849* (WTU); HQ220497, HQ220305, HQ220593, HQ220561, HQ220401, HQ220242, HQ220465, HQ220337, HQ220369, HQ220433, HQ220274, HQ220625, HQ234590, HQ234591. ***Brassaia actinophylla*** Endl.; U. S. A. (cultivated): Connecticut, University of Connecticut, EEB greenhouse; UCONN 198500235; *T. Ramsey 374850* (WTU); HQ220498, HQ220306, HQ220594, HQ220562, HQ220402, HQ220243, HQ220466, HQ220339, HQ220371, HQ220434, HQ220275, HQ220627, HQ234594, HQ234595. ***Polyscias fruticosa*** (L.) Harms; U. S. A. (cultivated): Connecticut, University of Connecticut, EEB greenhouse; UCONN 198500224; *T. Ramsey 374851* (WTU); HQ220499, HQ220307, HQ220595, HQ220563, HQ220403, HQ220244, HQ220467, HQ220338, HQ220370, HQ220435, HQ220276, HQ220626, HQ234592, HQ234593. ***Schefflera*** **sp.**; U. S. A. (cultivated): Massachusetts, Wheaton College greenhouse; *T. Ramsey 374852* (WTU); HQ220500, HQ220308, HQ220596, HQ220564, HQ220404, HQ220245, HQ220468, HQ220340, HQ220372, HQ220436, HQ220277, HQ220628, —. ***Trevesia sundaica*** Miq.; U. S. A. (cultivated): Connecticut, University of Connecticut, EEB greenhouse; UCONN 199100029; *T. Ramsey 374853* (WTU); HQ220501, HQ220309, HQ220597, HQ220565, HQ220405, HQ220246, HQ220469, HQ220341, HQ220373, HQ220437, HQ220278, HQ220629, HQ234596.