Phylogeny, morphology, and biogeography of *Haplophyllum* (Rutaceae), a species-rich genus of the Irano-Turanian floristic region

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Abstract *Haplophyllum* A. Juss. is one of the most species-rich, but poorly-known genera of Rutaceae (citrus family), reaching maximum species diversity in Turkey, Iran, and Central Asia. Many of its species exhibit a narrow geographic range ("narrow endemics"), which makes them particularly vulnerable to extinction. Despite its importance for the characterization of the Irano-Turanian floristic region, the evolution of species diversity in *Haplophyllum* has never been examined in a phylogenetic and biogeographic context. We generated gene trees from DNA sequences of four regions of the chloroplast genome for 118 accessions, representing 66% of the species diversity of the genus. Additionally, *Haplophyllum* was examined morphologically. The phylogenetic analyses showed that several species of the genus do not form reciprocally monophyletic groups. Optimization of morphological characters on the chloroplast DNA phylogeny indicated that most of the species, in particular those with a widespread geographic distribution, can only be diagnosed by combinations of homoplasious character states. Homoplasy notwithstanding, the main morphological characters traditionally used to classify the genus are consistent with the molecular phylogeny of *Haplophyllum*. Finally, the Mediterranean representatives of *Haplophyllum* were found to be embedded within a clade that includes primarily Irano-Turanian species, suggesting multiple invasions of the Mediterranean basin from the east.

Keywords biogeography; *Haplophyllum*; Irano-Turanian floristic region; morphology; phylogeny

Supplementary Material Tables S1 and S2 are available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

Haplophyllum A. Juss. is one of the most species-rich, but poorly known genera of Rutaceae. As currently circumscribed, it includes 68 species (Townsend, 1986; Navarro & al., 2004; Soltani & Khosravi, 2005) and reaches maximum species diversity in Turkey, Iran, and Central Asia (the latter region being bordered by the Caspian Sea in the west, China in the east, Iran and Afghanistan in the south, and Russia in the north). Many species of *Haplophyllum* exhibit a narrow geographic range (i.e., "narrow endemics"), a feature that makes them particularly vulnerable to extinction. Despite its importance for the characterization of the Irano-Turanian floristic region (Zohary, 1973; Takhtajan, 1986), the evolution of species diversity in *Haplophyllum* has never been examined in a phylogenetic and biogeographic context.

Haplophyllum is distributed from Morocco and Spain in the west to the Heilongjiang Province of China in the east. In the west it extends north to Romania and south to Somalia and the Hadhramaut area and in the east it extends north to the Lake Baikal region (Fig. 1) (Townsend, 1986). Its range spans five different floristic regions: the Irano-Turanian, Mediterranean, Saharo-Arabian, and Sudano-Zambezian regions (Fig. 1) (Takhtajan, 1986). The main centre of diversity of *Haplophyllum* is the Irano-Turanian region—in particular, Iran, Turkey, and Central Asia—which harbours 60% of the species diversity. Thirty species of *Haplophyllum* are present in Iran, fourteen of which are endemic to the country (Joharchi, 2008). Fewer species occur in the other three floristic regions, most notably in the Mediterranean region, which contains 13% of the species diversity (Fig. 1).

Characteristic of many species of *Haplophyllum* is their highly restricted geographic distribution, sometimes consisting of a single mountain range (Townsend, 1986). For example, *H. telephioides* is found in a few mountains of central Anatolia; *H. viridulum* occurs in a small area of the Fars province of Iran; and *H. eugenii-korovinii* is restricted to the Karatau mountains of Kazakhstan, where it is very rare (Townsend, 1986). Overall, 54% of the species have a relatively narrow range as compared to the most widespread species, which constitute 18% of the total; the remaining species exhibit an intermediate distribution. Additionally, several endemic species of *Haplophyllum* occur in small, disjunct populations across their narrow range (G. Salvo, S. Manafzadeh, pers. obs.). These factors make many species and populations of the genus potentially in danger of extinction, a fact that has been recognized with the inclusion of nine species in the *Red Data Book of Iran* (Jalili & Jamzad, 1999). Conversely, some species of *Haplophyllum* have

a very widespread distribution. For example, *H. tuberculatum* stretches from Morocco to western Pakistan, broadly spanning the distribution of the entire genus; *H. buxbaumii* is found from Morocco to western Iran, including many islands in the eastern Mediterranean Sea (Townsend, 1986).

Haplophyllum species are perennial herbs, sometimes low shrubs, which grow mainly on sandy, stony, or rocky hill slopes in arid areas (Townsend, 1986). Morphologically, they can be broadly characterized by the presence of cymose and bracteate inflorescences with five sepals and creamy-white to bright yellow petals, ten stamens with free filaments expanded below and pubescent on the inner surface, three to five connate carpels, and five-lobed capsules (Townsend, 1986).

Haplophyllum has been studied from a morphological (Jussieu, 1825; Spach, 1849; Boissier, 1867; Engler, 1896; Vvedensky, 1949; Townsend, 1986) and phytochemical (e.g., Mester & Vicol, 1971; Pascual-Villalobos & Robledo, 1999; Shaiq & al., 2001; Nazrullaev & al., 2002; Prieto & al., 2002) point of view. The most comprehensive morphological analysis of the genus was published by Townsend (1986), who also proposed a classification and a tentative scheme of species relationships. Phytochemically, Rutaceae as a whole are notable for their vast array of secondary chemical compounds (e.g., alkaloids, lignanes, glycosides, flavonoids; Price, 1963). Mester & Vicol (1971) performed a thorough phytochemical analysis of *Haplophyllum* by focusing on the distribution of different classes of alkaloids. However, on the basis of these two sources of data—morphology and phytochemistry—both the generic status of the genus and its subdivision into different sections have been questioned.

In the most comprehensive classification of Rutaceae, based mainly on morphological characters, Engler (1896, 1931) treated *Haplophyllum* as a subgenus of *Ruta* L. This view was dismissed by subsequent systematic works, which emphasized the distinctiveness of the former taxon with respect to both morphological and phytochemical features. Townsend (1986) listed a series of morphological traits differentiating *Haplophyllum* from closely related genera (e.g., pollen structure, seed shape, petal margins) and Mester & Vicol (1971) discovered the presence of secondary metabolites in *Haplophyllum*, such as the alkaloids robustine, haplopine, and skimmianine, which are not found in any other genus of Rutaceae.

Several authors have attempted to subdivide *Haplophyllum* into different sections by means of morphological characters (Spach, 1849; Boissier, 1867; Engler, 1896; Vvedensky, 1949; Townsend, 1986). Of these, only the last two authors adopted explicit criteria, rather than generic statements, to support their classifications. Vvedensky (1949) divided the genus into four sections according to carpel number, fruit opening, and ovule number (Table 1). The first two features, together with petal colour, plant architecture, ovary shape, and stamen form, were used by Townsend (1986) to divide the genus into three sections (Table 1). In his assessment of the taxonomic value of different morphological characters, Townsend (1986) noted that the ovary and stamens provide the most useful characters to infer species relationships within *Haplophyllum*. However, both classifications have been criticized, because they lack morphological traits that are consistent across all species of the proposed sections (Mester & Vicol, 1971).

The single phylogenetic study of *Haplophyllum* available so far included only six of the 68 species and focused on the Iberian representatives of the genus (Navarro & al., 2004). More recently, Salvo & al. (2008) performed a phylogenetic analysis of tribe Ruteae, which includes *Haplophyllum* and closely related genera, based on chloroplast (cp) DNA sequences. This study, comprising a limited sample of 22 species of *Haplophyllum*, corroborated the monophyly of the genus, but did not address species relationships within it.

From a biogeographic point of view, *Haplophyllum* was used to characterize the Irano-Turanian region (Zohary, 1973,

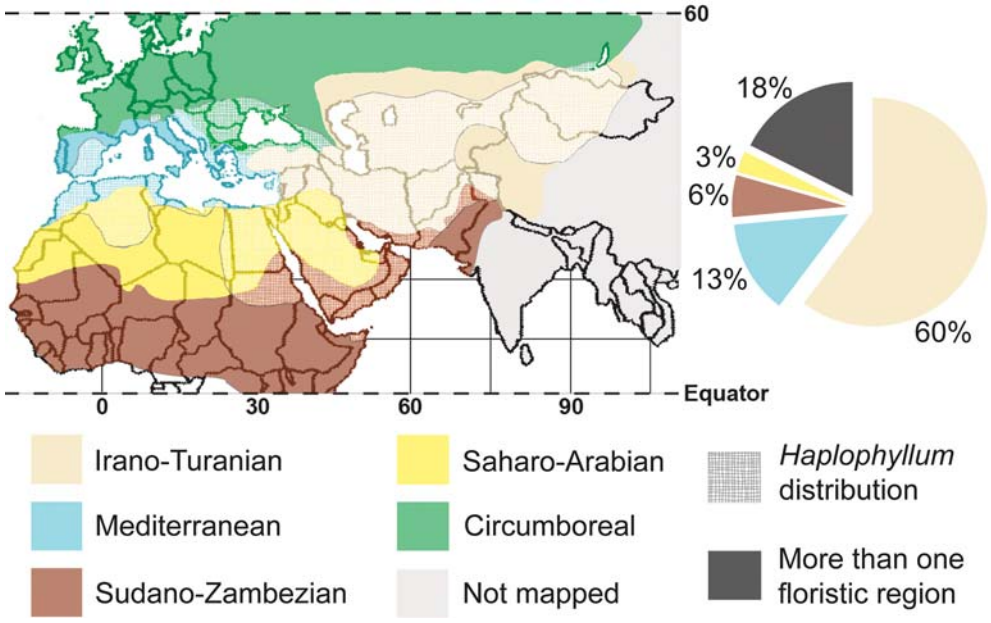


Fig. 1. Left, Map showing the geographic distribution of *Haplophyllum* (after Townsend, 1986) and the five floristic regions in which its species occur (after Takhtajan, 1986); **right,** pie chart showing the percentage of species found in each floristic region. Note that there are no species restricted to the circumboreal floristic region only.

in relation to his Western Irano-Turanian subregion; Takhtajan, 1986, in relation to his Western Asiatic subregion), because many of its species are restricted to this geographic area. For similar reasons, Grubov (1959) mentioned *Haplophyllum* in the characterization of Central Asia. In its most common delimitation, the Irano-Turanian region extends from central and eastern Anatolia to the Tien Shan and Altai mountain ranges, reaching the Gobi desert, and includes parts of the Sinai peninsula, Lebanon, Jordan, Israel, and Palestine, most of Syria and Iran, northern Iraq, north-eastern Afghanistan, parts of northern Pakistan and northern India, and Central Asia (Fig. 1) (Takhtajan, 1986; Davis & al., 1994). Based on either floristic similarities or phylogenetic evidence, some authors suggested that the Irano-Turanian region served as a key source for the colonization of neighbouring areas, most notably the Mediterranean region (Zohary, 1973; Quézel, 1978, 1985, 1995; Ribera & Blasco-Zumeta, 1998; Thompson, 2005; Mansion & al., 2008, 2009), while others argued more generally that the present arid floras of Eurasia, the Mediterranean region, North Africa, and even South Africa originated from Central Asia (Bobrov, 1965, 1966; Pyankov & al., 2002).

While a detailed knowledge of the evolution of species diversity in *Haplophyllum* could yield useful insights into the biogeographic role of the Irano-Turanian region, the genus has never been comprehensively examined from a phylogenetic/biogeographic point of view. In order to start filling this gap of knowledge, we generated sequence data for 66% of the species diversity of *Haplophyllum* and addressed the following questions: (1) Are the different species of *Haplophyllum* monophyletic? (2) Does our inferred cpDNA phylogeny support Vvedensky's (1949) or Townsend's (1986) classifications? (3) Do species from the same floristic region form monophyletic groups? (4) Did the Irano-Turanian region serve as a source for the colonization of the Mediterranean region? (5) What are the phylogenetic relationships between the narrow endemics and the geographically widespread species?

■ MATERIALS AND METHODS

Taxon sampling. — Forty-five out of 68 species of *Haplophyllum* were sampled, including species with a very narrow distribution occurring in remote areas. For geographically widespread taxa, and/or taxa that are difficult to diagnose morphologically, multiple accessions per species (two to eleven) were sampled. All three sections of Townsend (1986) were sampled. Five outgroup taxa were selected according to previous phylogenetic results: *Cneoridium dumosum*, *Aegle marmelos*, *Citrus reticulata*, *Poncirus trifoliata*, and *Glycosmis citrifolia* (Salvo & al., 2008). The final matrix contained 118 accessions. Included material, voucher information, sources, and GenBank/EBI accession numbers are listed in Appendix 1.

Table 1. The two most comprehensive classifications of *Haplophyllum*.

Section	Species	Diagnostic characters
Vvedensky (1949)		
Peganoides	*H. dauricum*	Ovary: (2–)3(–4)-locular Ovules: 2 in each cell Capsule: dehiscent
Polyoon	*H. pilosum* *H. suaveolens* *H. armenum* *H. bucharicum* *H. affine*	Ovary: 5-locular Ovules: 4–12 in each cell Capsule: dehiscent
Oligoon	Remaining species (greatest bulk of the genus)	Ovary: 5-locular Ovules: 2 in each cell Capsule: dehiscent
Achaenococcum	*H. latifolium* *H. acutifolium*	Ovary: 5-locular Ovules: 2 in each cell Capsule: indehiscent
Townsend (1986)		
Peganoides	*H. gilesii* *H. dauricum*	Habit: suffrutescent perennials Flower colour: yellow or greenish-yellow Ovary: 3-locular, rarely 2- or 4–5-locular Capsule: dehiscent
Indehiscentes	*H. acutifolium* *H. latifolium*	Habit: much branched, bushy perennials Flower colour: yellow Ovary: 5-locular Capsule: indehiscent
Haplophyllum	Remaining species (greatest bulk of the genus)	Habit: Perennials, suffrutescent or herbaceous below Flower colour: white, creamy, greenish, reddish or pale to bright yellow Ovary: 5-locular Capsule: dehiscent

Character sampling. — To allow for inclusion of the new molecular data in a global dataset of Rutaceae, the following cpDNA markers were chosen: the *matK* gene, the *trnK* gene, the *rpl16* intron, and the *trnL-trnF* intergenic spacer. These markers enabled us to produce unequivocal alignments and provided sufficient resolution at our level of investigation.

DNA extraction, amplification and sequencing. — Prior to DNA extraction, silica-dried leaf material (15–20 mg) was ground using glass beads and a MM 3000 shaker (Retsch GmbH, Haan, Germany). Total genomic DNA was extracted using DNeasy Plant Mini Kits from Qiagen AG (Basel, Switzerland), following the manufacturer's instructions. The *matK* and *trnK* cpDNA coding regions were amplified using primers 1F and 1R (Sang & al., 1997). The *rpl16* intron was amplified using primers F71 and R1516 (Baum & al., 1998). The *trnL-trnF* spacer was amplified with primers c and f (Taberlet & al., 1991). All PCR reactions were 20 µl in volume. Each reaction included 9.2 µl of ddH_2O, 2 µl of Taq-Buffer (10×, 15 mM $MgCl_2$), 1.6 µl of $MgCl_2$ (25 mM), 3.2 µl of dNTP (1.25 mM), 0.2 µl of Taq-Polymerase (5 U/µl), 1 µl of BSA, 0.4 µl of each primer (forward and reverse), and 2 µl of DNA template. Amplification of the *matK* region consisted of 2 min at 94°C followed by 30 cycles of: 1.5 min denaturation (94°C), 2 min annealing (53°C), and 3 min extension (72°C). After the last cycle, the temperature was kept at 72°C for the last 15 min of extension and then lowered to 4°C. Amplification of both the *rpl16* and *trnL-trnF* regions consisted of 2 min at 94°C followed by 35 cycles of: 0.5 min denaturation (94°C), 1 min annealing (52°C), and 1.75 min extension (72°C). After the last cycle the temperature was kept at 72°C for 10 min of extension and then lowered to 4°C. All PCR and cycle sequencing reactions were run on a TGradient thermocycler (Biometra, Göttingen, Germany). In order to detect amplified DNA target regions and possible contamination, PCR products were separated on 1% agarose gels, stained with ethidium bromide, and viewed under UV light. Successfully amplified products were purified with the GFX PCR DNA and Gel Band purification Kit (Bioscience Amersham, Otelfingen, Switzerland), following the manufacturer's recommendations.

Cycle sequencing reactions were carried out using the BigDye Terminator Mix (Applied Biosystems, Inc., Foster City, California, U.S.A.) and the same primers as above. The sequencing protocol consisted of 24 cycles of 10 s denaturation (96°C), 5 s annealing (50°C), and 4 min elongation (60°C). Products were run on an ABI 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer's protocol. For each region both strands were sequenced.

Phylogenetic analyses. — Sequences were edited and assembled using Sequencher v.4.2 software (Gene Codes Corp., Ann Arbor, Michigan, U.S.A.). Base positions were individually double-checked for agreement between the complementary strands. All sequences were visually aligned in MacClade v.4.06 (Maddison & Maddison, 2000) using the similarity criterion (e.g., Simmons, 2004). Regions of ambiguous alignment were excluded from the analysis (Kelchner, 2000). Gap positions were treated as missing data, unequivocally aligned gaps being coded as presence/absence of characters with the software GapCoder (Young & Healy, 2003) and then added as binary characters to the data matrix.

Four data partitions were defined, corresponding to the four loci of the chloroplast genome examined in this study. The individual partitions were initially analysed separately to establish whether there were any well-supported, incongruent clades among the respective trees. Since no such incongruence was detected, the sequences of the four loci were combined in a single dataset. The combined matrix was analysed using both maximum parsimony (MP) and Bayesian MCMC inference (BI; Yang & Rannala, 1997). Parsimony analyses were conducted using PAUP* v.4.0b10 (Swofford, 2001). All changes were treated as unordered and equally weighted (Fitch, 1971). Tree search was performed using the following protocol: (1) a heuristic search was carried out with 1000 replicates of random taxon addition sequence and 10 trees held at each step, and tree bisection-reconnection branch swapping (TBR) on best trees only, with no more than 100 trees saved per replicate; (2) the best trees found in (1) were then used as starting trees for a second heuristic search using TBR branch swapping until all swapping options were explored, and saving multiple trees (MULTREES option in effect). The STEEPEST DESCENT option was used in both (1) and (2). Relative support for each node obtained by MP was assessed using bootstrap re-sampling (Felsenstein, 1985). The following protocol was employed: heuristic search, 1000 bootstrap replicates, ten random addition sequence replicates with three trees held at each step, TBR swapping with STEEPEST DESCENT and saving no more than 50 trees per replicate.

Bayesian inference of phylogeny was performed with MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). First, the model of evolution most suitable for each individual cpDNA region was determined with the Akaike information criterion (AIC; Akaike, 1974) in Modeltest v.3.06 (Posada & Crandall, 1998). Subsequently, five partitions corresponding to the four loci (only nucleotide characters) and the coded gap characters were specified, and the commands "lset NST = 6, RATES = gamma" and "lset coding = variable" were entered in MrBayes for the former and the latter, respectively. Six independent runs with four Monte Carlo Markov chains (one cold and three incrementally heated; TEMP = 0.1) run for 5×10^6 generations each, with trees sampled every 1000th generation, were performed. The first 1 to 2.5×10^6 sampled trees of each run were discarded as "burn-in", after checking for stability on the log-likelihood curves using the software Tracer v.1.4 (Rambaut & Drummond, 2007) and after visual inspection of the split (clade) frequencies using the software AWTY (Wilgenbusch & al., 2004). The remaining 22,000 trees were used to build a 50% majority rule consensus tree.

Morphological data. — A matrix was constructed for 27 discrete morphological characters scored using herbarium material (G, FAR, LE, MA, P, TAK, TARI, TBI, W, Z) and Townsend's (1986) monograph, for the same 45 species of *Haplophyllum* used in the phylogenetic analyses and for its sister group, *Cneoridium dumosum* (Appendix 2; Table S1). These characters represent vegetative (characters 1–10), inflorescence (including both stamen and pistil features; 11–26), and fruit (27) morphology (Appendix 2). When possible, morphological

characters were assessed for several specimens of each species. All characters were treated as unordered; 23 characters were binary and 6 were multistate (Appendix 2). Autapomorphies were not included in the matrix. Missing data and polymorphic character states represented 3.2% and 1.1% of the data matrix entries, respectively (Table S1).

Morphological analyses. — Initially, the matrix was analysed using cladistic methods in PAUP*; however, the resulting tree was poorly resolved and weakly supported, even after tree searching was performed using successive weighting (Farris, 1969; results not shown). This is a known problem of reconstructing phylogenies using morphological data only (Scotland & al., 2003). Since the morphological matrix consisted of categorical data, a multiple correspondence analysis (MCA; Benzecri, 1992; Venables & Ripley, 2002) was carried out using the statistical software-package SPSS for Windows Rel. 11.0.1, in order to visualize the joint properties of the 27 morphological variables in two dimensions.

Character mapping analyses. — To assess the fit of each morphological character onto the inferred molecular phylogeny, all morphological characters were mapped onto a subset of the post–burn-in Bayesian trees. The subset was created by sampling a tree every 100 trees from the original set of trees, yielding a total of 220 trees. The 50% majority rule consensus of these trees was identical to the one from the original set of trees, indicating that our subset was representative of the original set of trees. Four taxa belonging to the outgroup were pruned from the 220 trees, leaving only the sister group of *Haplophyllum*, *Cneoridium dumosum*. The fit of each character onto a tree was assessed using the rescaled consistency index (RC; Farris, 1989). This index has been shown to be superior to both the consistency and retention indexes in assessing fit of characters onto a phylogeny (Kitching & al., 1998). The character mapping analyses were implemented in PAUP* and Mesquite v.2.7.1 (Maddison & Maddison, 2008) using parsimony as the optimization procedure and treating character state transitions as unordered.

■ RESULTS

Phylogenetic analyses. — The combined molecular matrix consisted of 3849 characters, of which 561 were parsimony-informative. The MP analysis yielded 9400 most parsimonious trees of 1485 steps, with a consistency index (CI) of 0.66 and a retention index (RI) of 0.86. The AIC, as implemented in Modeltest, selected the following models of evolution: GTR+G for the *matK* region, TVM+G for both *rpl16* and *trnL-trnF*, and TIM+G for *trnK*. The 50% majority rule consensus tree obtained from the Bayesian analysis is shown in Fig. 2A. This tree is slightly more resolved than the strict consensus tree found from the MP analysis of the same matrix. Branch support values, in terms of both bootstrap percentages (BP) and posterior probabilities (PP), were generally lower along the backbone of the tree and higher towards the tips. Two main strongly supported (i.e., BP $\geq$ 70 and PP $\geq$ 0.95; Hillis & Bull, 1993; Zander, 2004) clades can be identified: clade A, including only Irano-Turanian species, such as the characteristic *H. acutifolium* and *H. robustum*, and clade B, containing species from different floristic regions, including Mediterranean representatives and also the widespread species *H. buxbaumii* and *H. tuberculatum* (Fig. 2A). Many species represented by multiple accessions were either poorly resolved or non-monophyletic, but only a few cases of non-monophyly were strongly supported. Neither the Irano-Turanian and Mediterranean representatives nor the species occurring in more than one floristic region formed monophyletic groups (Fig. 2A).

Morphological analyses. — The results of the MCA are displayed in Fig. 2B. The first and second dimensions explained 23% and 16% of the total variance, respectively. The characters that contributed the most to the first and second dimensions were characters 6 (0.603), 13 (0.557), 23 (0.514), and characters 13 (0.663), 15 (0.638), 22 (0.630), 18 (0.519), respectively (Appendix 2).

Character mapping analyses. — The results of the character mapping analyses are summarized in Fig. 3 (see also Appendix 2). A lot of variation in mean RC values was found across characters, with "stem branching" (character 5, RC = 0.024) and "number of carpels" (character 21, RC = 0.257) receiving the lowest and highest value, respectively. In the vegetative-morphology category, the characters that showed the best fit onto the tree were "sterile axillary shoots" (character 3, RC = 0.136) and "number of stems" (character 1, RC = 0.128). In the inflorescence-morphology category, the features that received the highest RC values were: "number of carpels" (character 21, RC = 0.257), "number of ovules" (character 24, RC = 0.193), "indumentum of filament" (character 18, RC = 0.130), and "dark dorsal vitta/tinge on petal" (character 14, RC = 0.116). Overall, the characters that Vvedensky (1949) and Townsend (1986) valued the most in their classifications of *Haplophyllum* (Table 1), namely "number of carpels", "number of ovules", and "capsule dehiscence" (character 27, RC = 0.238), showed the best fit onto the molecular tree (Fig. 3). Details of the mapping of these three characters onto the molecular phylogeny are shown in Fig. 4.

■ DISCUSSION

The main goal of our study was to provide an initial estimate of the evolutionary relationships of *Haplophyllum* derived from cpDNA sequences and morphology, within the conceptual framework of the phylogenetic species concept sensu Baum (1992). According to this concept, "taxa (including species) are viewed as monophyletic or exclusive groups of organisms" (Baum & Donoghue, 1995: 569). Species are thus defined within the historical dimension provided by the phylogeny at hand (i.e., diachronistically) and, in order to be recognized as natural entities, they must be monophyletic (Rieppel, 2010). While we are aware of the debate on multiple species concepts (e.g., Hennig, 1966; Cracraft, 1989; Baum & Donoghue, 1995; Freudenstein, 1998; Rieppel, 2010), an exhaustive discussion of the pros and cons of each position is beyond the goal of the present paper.

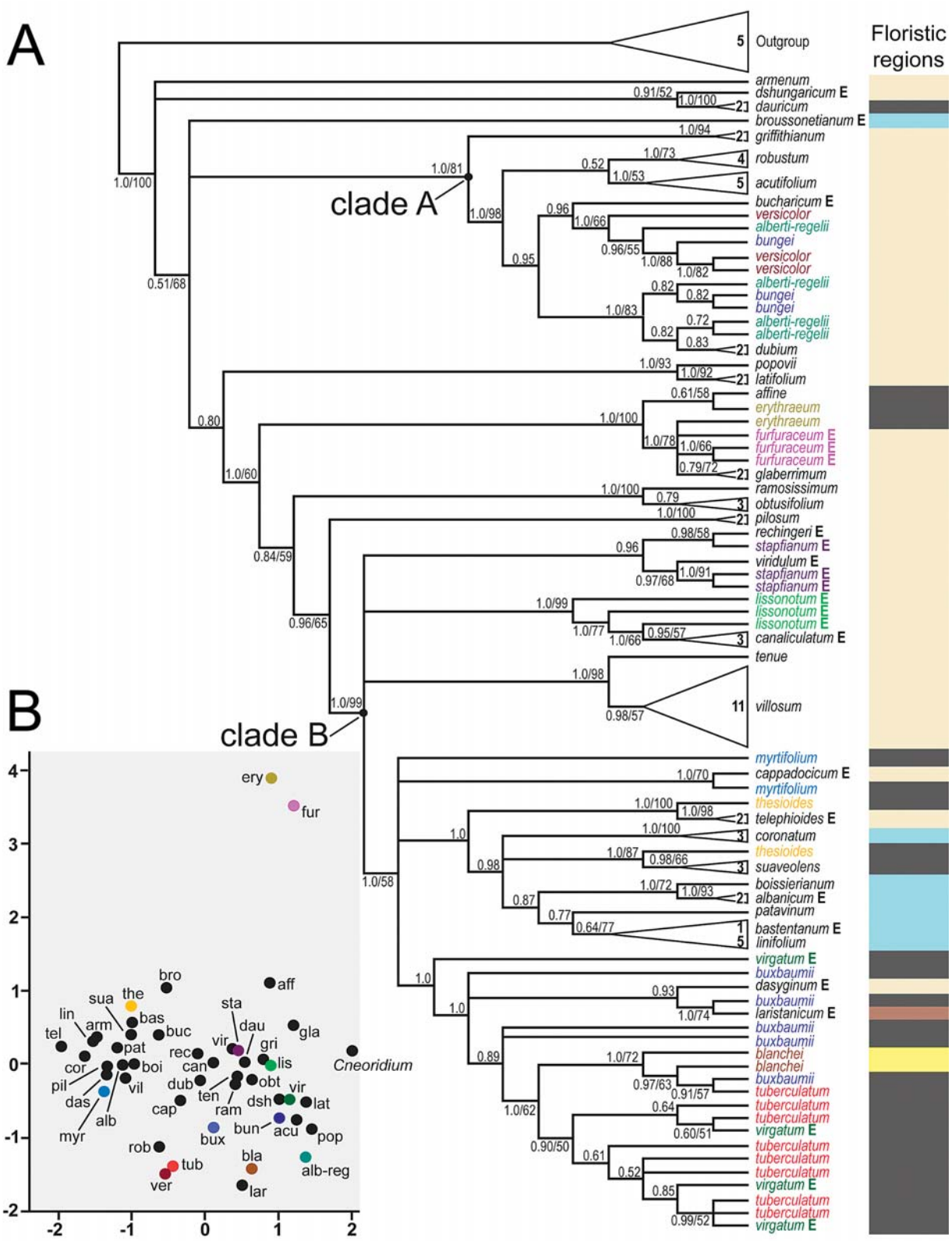


Fig. 2. A, Fifty-percent majority rule consensus tree obtained from the Bayesian analysis on the combined molecular dataset (*matK*, *rpl16*, *trnK*, *trnL-trnF*). Numbers next to branches indicate posterior probabilities (PP; >0.50) and bootstrap percentages calculated under maximum parsimony (BP; >50). Taxa with coloured text are inferred to be non-monophyletic; taxa followed by an "E" exhibit a narrow geographical range. Coloured boxes indicate the floristic region (after Takhtajan, 1986) in which each species is found (see Fig. 1 for colour legend). **B,** Scatter plot of the first and second dimensions (x- and y-axes, respectively) of the multiple correspondence analysis of the 27 morphological characters for 45 species of *Haplophyllum* (marked with the first three letters of the species name) and its sister group *Cneoridium dumosum*.

Systematics. — The phylogenetic analyses indicated that several taxonomic species of *Haplophyllum* were non-monophyletic. Even though most of the inferred cases of non-monophyly were weakly supported, possibly resulting at least in part from inadequate phylogenetic signal (Syring & al., 2007), a few instances of species-level paraphyly and polyphyly were strongly supported (Fig. 2A). These raise concerns about species circumscription within *Haplophyllum* and potential discrepancies between gene trees and species trees. Commonly cited, causative factors responsible for species non-monophyly and gene tree versus species tree incongruence are: imperfect taxonomy (e.g., Goodwillie & Stiller, 2001), introgressive hybridization (e.g., Shaw & Small, 2005), incomplete lineage sorting (e.g., Bouillé & Bousquet, 2005), unrecognized amplification of paralogous loci (e.g., Alvarez & al., 2005), and recombination among divergent alleles (e.g., Schierup & Hein, 2000). Although the phylogeny inferred in the present study is based on cpDNA markers only, making it difficult to assess the relative contribution of the above-mentioned factors to our study-group, our phylogenetic results, coupled with evidence from morphology, distribution, and ecology, represent a useful first step towards addressing the issue of species circumscription and identity in *Haplophyllum*.

Haplophyllum tuberculatum, *H. buxbaumii*, *H. virgatum*, and *H. blanchei* were inferred to be non-monophyletic, although branch support in the clade that includes their accessions was generally low (Fig. 2A). Moreover, the level of intra-specific polymorphism within these taxonomic species was similar to the level of inter-specific divergence between them (Table S2). For example, the average absolute number of nucleotide substitutions between the accessions of *H. buxbaumii*, and between these and the accessions of *H. tuberculatum*, *H. virgatum*, and *H. blanchei*, was 13.5 and 14.5, respectively. Such high levels of intra-specific polymorphism are similar to those found in other studies at the genus level that included sequences from multiple, infra-specific accessions (e.g., Widmer & Baltisberger, 1999; Särkinen & al., 2011). *Haplophyllum tuberculatum*, *H. buxbaumii*, and *H. blanchei* are morphologically similar (Fig. 2B). *H. tuberculatum* and *H. buxbaumii* exhibited the same character states for 17 out of the 27 scored morphological characters (Table S1). They are the species with the most widespread distribution within *Haplophyllum* and with the highest level of intra-specific morphological variability, which led Townsend (1986) to recognize two "morphs" within *H. tuberculatum* and two subspecies in *H. buxbaumii*. As a matter of fact, Townsend (1966a: 99) stated that the circumscription of *H. tuberculatum* is "the most difficult problem to be solved in the genus". *Haplophyllum blanchei* is difficult to separate from *H. tuberculatum* on the basis of morphology, the main distinguishing features being the bright-magenta-coloured flowers and distinctly fused filaments of *H. blanchei* (Townsend, 1986). Furthermore, the geographic ranges of these two species overlap. The taxonomic status of *H. virgatum* is unclear (Townsend, 1986). From a morphological standpoint, this species is difficult to separate from *H. canaliculatum*. In fact, in *Flora Iranica* Townsend (1966b) reduced these two species to synonymy. In the morphological matrix, although *H. virgatum* shared the same states with *H. canaliculatum* with respect to three vegetative characters, it possessed the same states as *H. tuberculatum*, *H. buxbaumii*, and *H. blanchei* for eight characters (Table S1).

Within the problematic clade formed by *H. tuberculatum*, *H. buxbaumii*, *H. virgatum*, *H. blanchei*, *H. laristanicum*, and *H. dasyginum* (Fig. 2A), only the sister relationship between one accession of *H. buxbaumii* and *H. laristanicum* (74 BP, 1.0 PP), and the clade including *H. blanchei* and one accession of *H. tuberculatum* and *H. buxbaumii* each (72 BP, 1.0 PP) were strongly supported. *H. laristanicum* is a rare narrow endemic restricted to a small part of southern Iran. Its range is included within the range of *H. tuberculatum*, but it is geographically separated from the range of *H. buxbaumii*. Morphologically, *H. laristanicum* bears a resemblance to some

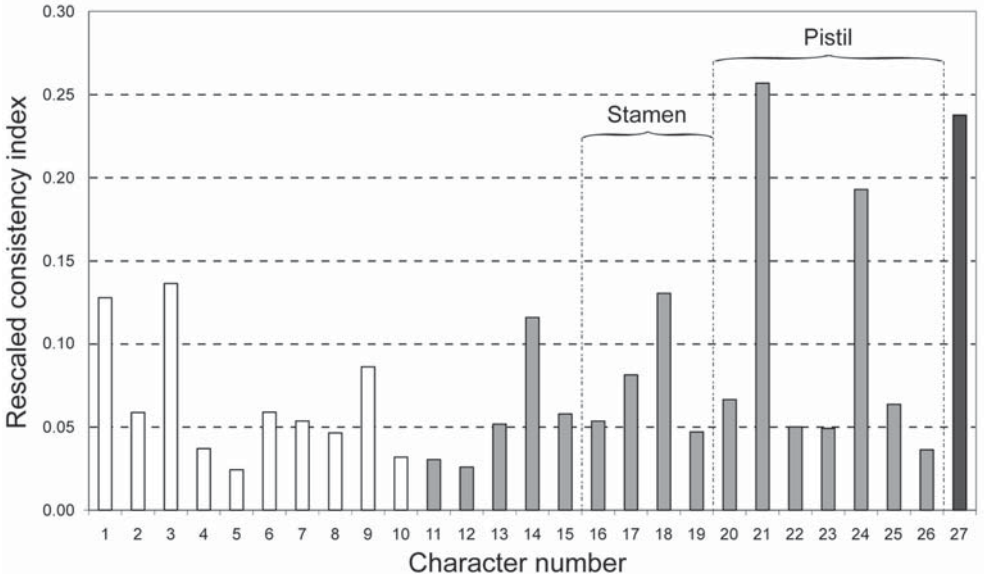


Fig. 3. Mean rescaled consistency (RC) index of each of the 27 morphological characters optimized onto 220 trees derived from the Bayesian analysis of 118 cpDNA sequences of *Haplophyllum* and outgroup taxa. Bars indicate the following categories of characters: vegetative morphology (white bars), inflorescence morphology (including features of both stamen and pistil; gray bars), and fruit morphology (black bar). Character numbers refer to those in Appendix 2.

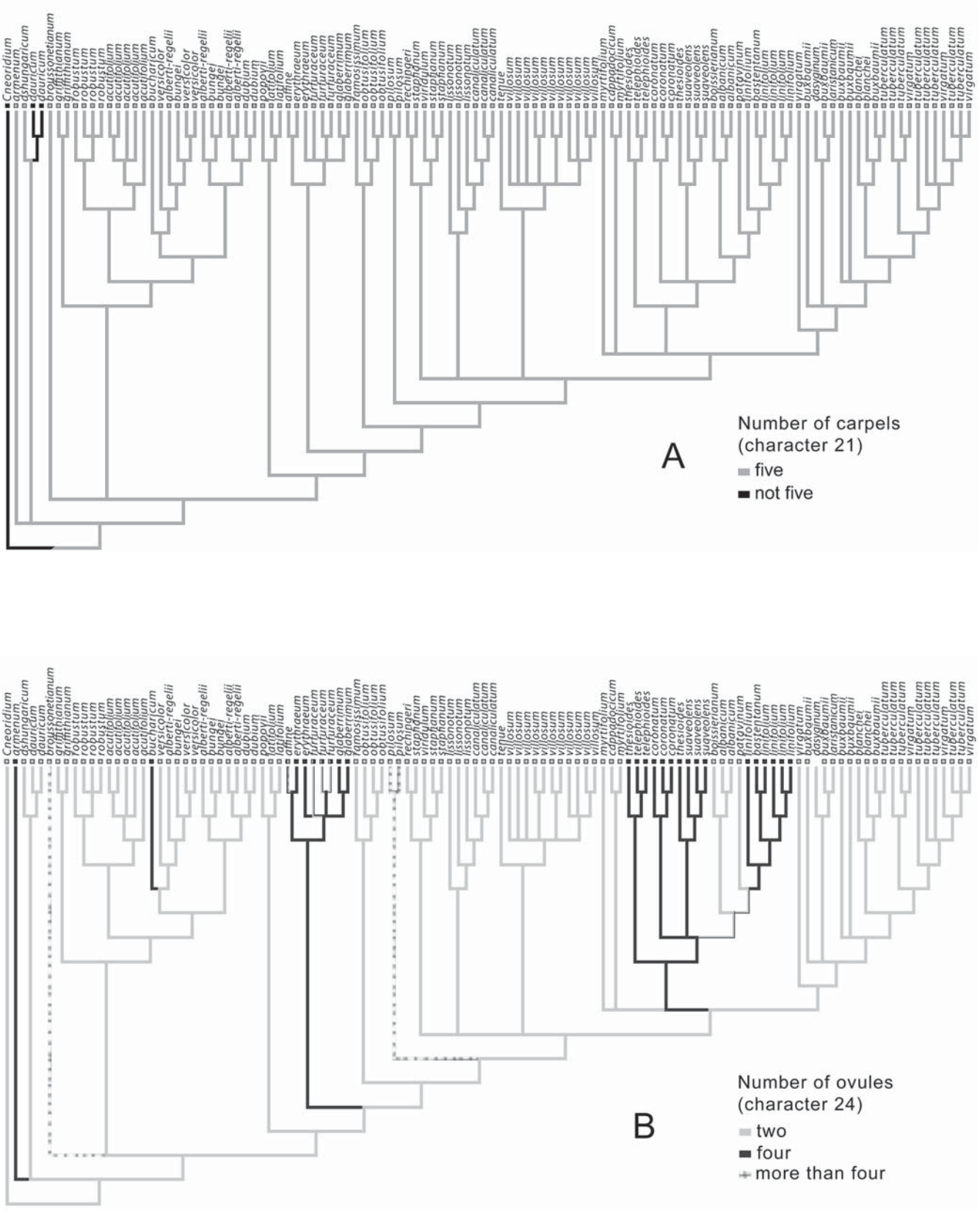
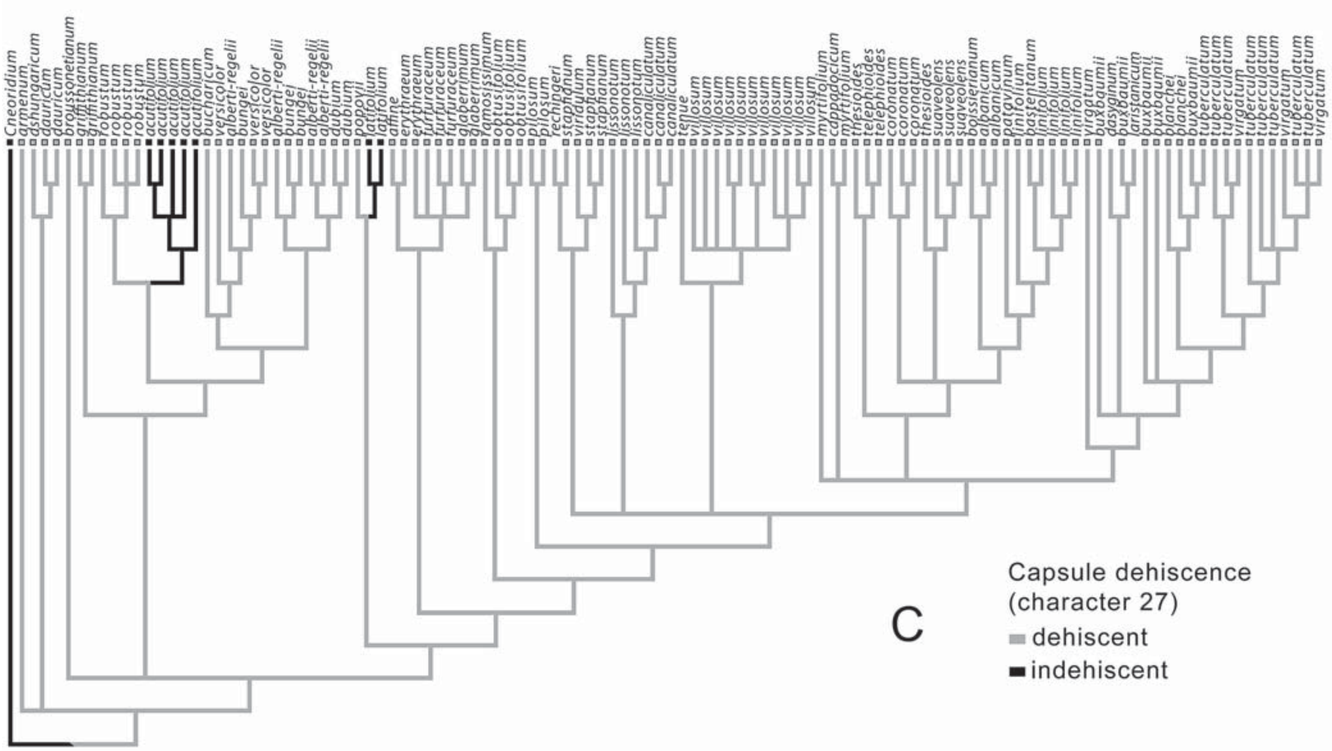


Fig. 4. Evolution of three selected morphological characters optimized onto the molecular phylogeny shown in Fig. 2A. **A,** Number of carpels; **B,** number of ovules; **C,** capsule dehiscence.



forms of *H. tuberculatum*, which have similarly fused filaments (Townsend, 1986). In the morphological matrix, *H. laristanicum* shared identical states with *H. buxbaumii* and *H. tuberculatum* for four characters, respectively (Table S1).

In conclusion, due to the interdigitation of accessions from different species, similar levels of intra-specific polymorphism and inter-specific divergence, high levels of intra-specific morphological variability, morphological character conflict, and overlapping geographic ranges, species circumscription and phylogenetic relatedness within this problematic clade remain unclear. Taxonomic "lumping" may partially be responsible for the inferred phylogenetic interdigitation of the accessions of *H. tuberculatum* and *H. buxbaumii*, due to the possible presence of unrecognized, "cryptic" species. A more thorough infraspecific sampling, including several individuals and populations representing the entire geographic and morphological variation of each species, is needed to disentangle relationships within this "species complex".

The main finding of the MCA was a sharp morphological separation between *H. furfuraceum* and *H. erythraeum*, and the remaining species of the genus (Fig. 2B). These two species share the presence of a characteristic farinose indumentum on the sepals, petals, and ovary (Appendix 2). However, in the inferred phylogeny, *H. furfuraceum* and *H. erythraeum* did not form a monophyletic group, but were intermingled with *H. affine* and *H. glaberrimum* (Fig. 2A). In Townsend's (1986) tentative scheme of species relationships *H. furfuraceum*, *H. erythraeum*, and *H. affine* are clustered together, whereas *H. glaberrimum* is placed in a very distant position from these three species.

The lack of monophyly in *H. bungei*, *H. alberti-regelii*, and *H. versicolor* is strongly supported: one accession of *H. bungei* was inferred to be sister to two accessions of *H. versicolor* with 88 BP and 1.0 PP, whereas the remaining two accessions of *H. bungei* were part of a strongly supported clade comprising representatives of *H. alberti-regelii* and *H. dubium* (83 BP, 1.0 PP; Fig. 2A). From a morphological point of view, *H. bungei* and *H. alberti-regelii* are more similar to each other than they are to *H. versicolor* (Fig. 2B). Therefore, the accession of *H. bungei* inferred to be sister to *H. versicolor* seems to be in a spurious phylogenetic position.

Another case of strongly supported species non-monophyly is represented by *H. thesioides*. One accession of this species was inferred to be sister to *H. telephioides* with strong support (100 BP, 1.0 PP), whereas for the other accession a sister relationship with *H. suaveolens* was strongly supported (87 BP, 1.0 PP; Fig. 2A). Morphologically and ecologically, *H. thesioides* is different from *H. telephioides* but similar to *H. suaveolens* (Fig. 2B). Townsend (1986: 297) stated: "This species [*H. thesioides*] and *H. suaveolens* have been much confused in herbaria." *Haplophyllum telephioides* exhibits a characteristic plant architecture and petal colouration, not encountered in the other two species, and is confined to rocky, limestone slopes, whereas *H. thesioides* and *H. suaveolens* have much broader habitat preferences. Moreover, the accession of *H. thesioides* inferred to be sister to *H. suaveolens* was collected from an area where the latter species occurs, but where *H. telephioides* is absent. Hence, it is possible that this accession represents a case of introgressive hybridization between *H. thesioides* and *H. suaveolens*. Such a process

has already been proposed for the origin of *H. ptilostylum* (Townsend, 1966a).

The phylogenetic results also identified several instances of strongly supported species monophyly: *H. dauricum* (100 BP, 1.0 PP), *H. griffithianum* (94 BP, 1.0 PP), *H. robustum* (73 BP, 1.0 PP), *H. latifolium* (92 BP, 1.0 PP), *H. pilosum* (100 BP, 1.0 PP), *H. telephioides* (98 BP, 1.0 PP), *H. coronatum* (100 BP, 1.0 PP), and *H. albanicum* (93 BP, 1.0 PP) (Fig. 2A). Some of these species, especially the narrow endemics (see below), can be diagnosed by a set of morphological features, which, however, are not exclusive to them. For example, *H. dauricum* is a small, suffrutescent plant and has a distinctive plant architecture, with numerous, slender stems arising from a stout, woody base; features that are also encountered in *H. bucharicum*. Together with *H. gilesii*, this species is the only one with a 3-locular (rarely 2- or 4–5-locular) ovary (Table 1). *Haplophyllum robustum* is easy to recognize in the field, due to its broad lanceolate leaves and stout, erect stems, usually un-branched below the inflorescence, reaching up to 80 cm in height (G. Salvo, S. Manafzadeh, pers. obs.). Such characteristics are also encountered in *H. latifolium* and *H. popovii*. The typical lanate indumentum of *H. pilosum* occurs also in *H. villosum*, *H. telephioides*, *H. suaveolens*, and *H. coronatum*, although to a lesser extent. The prominent, apical appendages found in the ovary of *H. albanicum* individuals are also present in *H. coronatum*, *H. broussonetianum*, *H. pilosum*, *H. telephioides*, *H. suaveolens*, *H. linifolium*, *H. balcanicum*, *H. armenum*, and *H. patavinum*, and others, although the shape of this feature varies slightly among species (Townsend, 1986). In essence, only by means of combinations of homoplasious, morphological character states (i.e., changing more than once across the *Haplophyllum* phylogeny) are we able to diagnose the different species of the genus. In fact, the character mapping analyses detected high levels of homoplasy across most of the scored morphological characters (Fig. 3). A similar situation has been found in other taxonomically complex plant groups (e.g., Moylan & al., 2004; Norup & al., 2006).

Haplophyllum species with a widespread distribution are often more difficult to diagnose morphologically as compared to narrow endemics. For example, the degree of fusion of the filaments, which is a very important character for the classification of the genus, is variable only in the broadly distributed *H. tuberculatum*, which includes individuals with filaments that are either free or joined at the base (Townsend, 1986). Likewise, in *H. buxbaumii* the form of the apex of the ovary, which is another crucial taxonomic character, is variable, with individuals either lacking or possessing an apical appendage on the ovary (Townsend, 1986). On the contrary, species with a narrow range, such as *H. telephioides* or *H. bucharicum*, can be easily diagnosed by clear morphological features, even though sometimes these are also present in a few other species. A dark green line along the dorsal side of the petals, for example, is very prominent in *H. telephioides*, although not restricted to it (Townsend, 1986). Similarly, a woody, frequently gnarled base of the stem is very distinct in *H. bucharicum* (Townsend, 1986).

Such extensive morphological polymorphism in some broadly ranging species groups has long been observed by taxonomists and has posed several problems for the delimitation of species boundaries (Mayr, 1942; Wilson & Brown, 1953). This common observation represents an interesting link between biogeography and systematics. It is likely that narrow endemics are more specialized in their ecological requirements, as compared to species with a widespread distribution. This specialization may consist in the acquisition of unique morphological features (i.e., apomorphic character states), which are the result of adaptation to local environmental conditions and make the narrow endemics "diagnosable".

Neither Vvedensky's (1949) nor Townsend's (1986) classification of *Haplophyllum* were supported by our phylogenetic findings (Table 1; Fig. 2A). *Haplophyllum acutifolium* and *H. latifolium*, placed by both systematists in the same section, were not inferred to be sister to one another. The former was found to be sister to *H. robustum*, although with low support (<50 BP, 0.52 PP); the latter exhibited a strongly supported sister relationship to *H. popovii* (93 BP, 1.0 PP). Additionally, Townsend's (1986) *H.* sect. *Haplophyllum* and Vvedensky's (1949) *H.* sect. *Polyoon* and sect. *Oligoon* did not form monophyletic groups. Unfortunately, the validity of *H.* sect. *Peganoides* sensu Townsend (1986) could not be ascertained, since we were unable to obtain samples of *H. gilesii*, a species endemic to the Kashmir region.

The main morphological characters used by both systematists to divide the genus into sections—namely, "number of carpels", "capsule dehiscence", and "number of ovules"—exhibited the lowest levels of homoplasy when optimized on the inferred phylogeny (Fig. 3). For example, *H. dauricum* and *Cneoridium dumosum* are the only sampled taxa that do not have five carpels, whereas all the others do (Fig. 4A; Appendix 2; Table S1). Similarly, *H. acutifolium*, *H. latifolium*, and *Cneoridium dumosum* are the only taxa with indehiscent capsules (Fig. 4C; Appendix 2; Table S1). "Number of ovules", which was used for classificatory purposes by Vvedensky (1949) only, shows a more complex pattern; however, in this case too, the least common character state (more than four ovules) is only found in *H. pilosum* and *H. broussonetianum*, and the next, less common state (four ovules) exhibits great phylogenetic structure (Fig. 4B; Appendix 2; Table S1). Such unbalanced distribution of characters states, with most of the taxa represented by one state and only a few taxa by the other state(s), means that opportunities for state transitions are few and hence levels of homoplasy low (Sanderson & Donoghue, 1989). Overall these findings emphasize the important taxonomic value of the three mentioned characters within *Haplophyllum*. More generally, morphological features of the inflorescence and fruit provide the most useful taxonomic characters to infer species relationships within the genus (Fig. 3). This fact was noted by Townsend (1986: 3) who stated: "The ovary furnishes some of the most useful characters in classifying the genus."

Biogeographic patterns. — The phylogenetic results indicated that species from the same floristic region do not form monophyletic groups (Fig. 2A). Even though the Mediterranean representatives of the genus do not cluster together, they are embedded within a clade that includes primarily Irano-Turanian species and species that occur in more than one floristic region (Fig. 2A), suggesting that multiple invasions of

the Mediterranean region from the east took place during the evolution of the genus. A pattern of migration from western Asia into the Mediterranean basin has been inferred for the origin of *Arum* and *Biarum*, two genera of Araceae restricted primarily to the Mediterranean region (Mansion & al., 2008). Similarly, an Anatolian origin has been inferred for *Anchusa*, *Borago*, and *Echium*, three genera of Boraginaceae that comprise members endemic to the western Mediterranean region (Mansion & al., 2009).

Both the geographically widespread species and the narrow endemics were found to be intermingled across the phylogeny (Fig. 2A). A few terminal clades containing a widespread species and a narrow endemic were inferred: *H. dauricum*/*H. dshungaricum* (although weakly supported); *H. boissierianum*/*H. albanicum* (strongly supported); *H. myrtifolium*/*H. cappadocicum*, *H. thesioides*/*H. telephioides*, and *H. buxbaumii*/*H. laristanicum* (although the widespread species of these three last clades were non-monophyletic; Fig. 2A). An expanded taxon sampling within such clades, especially with respect to the narrow endemics, will enable us to verify whether the narrow endemics and the widespread taxa form reciprocally monophyletic sister pairs or whether the narrow endemics are nested within paraphyletic widespread taxa. These different phylogenetic patterns have implications for the origin of the narrow endemics and the geography of speciation (e.g., Bush, 1975; Lynch, 1989; but see Losos & Glor, 2003). The former pattern is compatible with an allopatric mode of speciation if the sister species display little or no overlap in their geographic ranges or with a sympatric mode of speciation if the geographic ranges of the sister species overlap (Barraclough & Vogler, 2000). The latter pattern would point to a peripatric mode of speciation if the narrow endemic species is geographically isolated from the widespread species (e.g., Harrison, 1991).

For example, the widespread *H. boissierianum* and the narrow endemic *H. albanicum* exhibit a strongly supported sister relationship (Fig. 2A) and an overlapping geographic range, with the range of the latter species contained within the range of the former one. One possible interpretation of these observations is that the two species originated via a sympatric mode of speciation. This view is supported by their different ecological preferences: *H. boissierianum* occurs on rocky and stony places, on hill slopes, along roads, in open *Pinus* woodlands, and on limestone or serpentine soil, whereas *H. albanicum* is restricted to rocky and stony habitats with limestone soil (Townsend, 1986). An alternative scenario would involve allopatric speciation followed by secondary contact.

■ CONCLUSION

The present study represents a first step towards disentangling species relationships in a taxonomically complex and biogeographically important genus by means of phylogenetic and morphological analyses. The phylogenetic analyses identified both cases of strongly supported species monophyly and instances of species non-monophyly. The morphological assessment showed that the different species of the genus, especially those with a widespread distribution, cannot be readily diagnosed by sets of unique character states. Character mapping analyses indicated that the main morphological characters traditionally used to classify the genus are consistent with the molecular phylogeny of *Haplophyllum*. Our initial, phylogeny-based interpretation of biogeographic patterns suggests that the Mediterranean representatives of *Haplophyllum* arrived from the east multiple times.

The inferred phylogenetic framework lays the foundations for future studies that will focus on selected, problematic clades (e.g., the *H. tuberculatum*/*H. buxbaumii* clade). To gain a deeper understanding of evolutionary and biogeographic processes within such clades, it will be necessary to expand the current infra-specific sampling and perform more detailed molecular (examining haplotype variation, for example) and morphological analyses. Additionally, sampling the nuclear genome will be a requisite in order to understand the biological processes underlying species non-monophyly in *Haplophyllum*.

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Appendix 1. Sampled accessions of *Haplophyllum* and outgroup taxa, including source, voucher information, and GenBank accession numbers for the four cpDNA regions studied (sequences that could not be amplified are indicated by a "–").

Taxon; code; source; voucher (herbarium); GenBank accession numbers: *matK*, *rpl16*, *trnL-trnF*, *trnK*

Haplophyllum acutifolium (DC.) G. Don; acu1W; Iran, Gorgan, Golestan national park, Almeh valley; 1999-02041 (W); HM163962, HM163862, HM163761, HM163657. ***H. acutifolium*;** acuF1469; Iran, Qazvin Prov., Qazvin to Takestan, 14 km before Takestan; *F. Ghahremaninejad 1469* (Z); EF489076, EF489150, EF489224, HM163658. ***H. acutifolium*;** acuF1488; Iran, Khorassan Province, Chenaran, Freizi, 30 km S of Chenaran, 1700 m; *F. Ghahremaninejad 1488* (Z); HM163963, HM163863, HM163762, HM163659. ***H. acutifolium*;** acuZel0505221; Iran, Khorasan Prov., Ashkhaneh, Tange Raz; *Zeltner 05.05.22 1a & b* (Z); HM163964, HM163864, HM163763, HM163660. ***H. acutifolium*;** acuZel0505232; Iran, Khorasan Prov., Ashkhaneh, Robat e Barah Bil; *Zeltner 05.05.23 2* (Z); HM163965, HM163865, HM163764, HM163661. ***H. affine*** (Aitch. & Hemsl.) Korovin; aff1615LE; Turkmenistan; *Litvinova N.P. & Nikizienko E.V. 1615* (LE); HM163966, HM163866, HM163765, HM163662. ***H. albanicum*** (Bald.) Bornm.; alb85697LE; Macedonia; *E. Mayer 85697* (LE); HM163967, HM163867, HM163766, HM163663. ***H. albanicum*;** albSelvi5; Albania, Drisht, Scutari region; Selvi, Coppi, *Cecchi 5* (Z); HM163968, HM163868, HM163767, HM163664. ***H. alberti-regelii*** Korovin; albert220LE; Tajikistan; *V.P. Bochantzev 220* (LE); HM163969, HM163869, HM163768, HM163665. ***H. alberti-regelii*;** albert2W; Afghanistan, Baghlan, Surkh Kotal, ca. 15 km NW of Pule-Khumri; 1976-00031 (W); HM163970, –, HM163769, HM163666. ***H. alberti-regelii*;** albert391LE; Uzbekistan; *V.P. Bochantzev 391* (LE); HM163971, HM163870, HM163770, –. ***H. alberti-regelii*;** albert3W; Afghanistan, Badakhshan, 15 miles NE of Kesem, road to Faizabad; 1973-13349 (W); HM163972, HM163871, HM163771, HM163667. ***H. armenum*** Spach; armeT13; Georgia; *s.n.* (TBI); HM163973, HM163872, HM163772, HM163668. ***H. bastentanum*** F.B. Navarro, Suár.-Sant. & Blanca; bastGDA47502; Spain; 47502 (GDA); EF489097, EF489171, EF489245, HM163669. ***H. blanchei*** Boiss.; blan6W; Iraq, desertum occidentale, inter Ramadi et Rutba 260 km; 16372 (W); HM163974; HM163873; HM163773; HM163670. ***H. blanchei*;** blan7W; Jordanien, Amman, Nordostjordanische Basaltwüste, Hammada, ca. 50 km W of Azraq; 2004-20318(W); HM163975, HM163874, HM163774, HM163671. ***H. boissierianum*** Vis. & Pančič; boissSelvi2; Albania, Krume, Mt. Pastrik, Region of Kukes; *Selvi, Coppi, Cecchi 2* (Z); HM163976, HM163875, HM163775, HM163672. ***H. broussonetianum*** Coss.; broussW; Marokko, Todra-schlucht; 1999-06842 (W); HM163977, HM163876, HM163776, HM163673. ***H. bucharicum*** Litv.; buch211Z; Uzbekistan, betw. Shurab and Darhand; *Manafzadeh & Salvo 211* (Z); HM163978, HM163877, HM163777, HM163674. ***H. bungei*** Trautv.; bun207Z; Uzbekistan, Shafrikan-Shuruk village, 51 km after Shafrikan (Botanical desert station), SW Kizil Kum; *Manafzadeh & Salvo 207* (Z); HM163979, HM163878, HM163778, HM163675. ***H. bungei*;** bun2119LE; Uzbekistan; *R.V. Kamelin 2119* (LE); HM163980, HM163879, HM163779, HM163676. ***H. bungei*;** bun431LE; Kazakhstan, western part; *I.N. Saffronova & al. 431* (LE); –, HM163880, HM163780, –. ***H. buxbaumii*** (Poir.) G. Don; bux12W; Iraq, Hamam Ali; 1974-06575 (W); HM163981, HM163881, HM163781, HM163677. ***H. buxbaumii*;** bux13W; Iraq, Rasheed; 1970-1918 (W); HM163982, HM163882, HM163782, HM163678. ***H. buxbaumii*;** bux14W; Turkey, 3 km S of Caykavak pass C5, Nigde; 1991-9560 (W); HM163983, HM163883, HM163783, HM163679. ***H. buxbaumii*;** buxMA557457; Tunisia; 557457 (MA); EF489095, EF489169, EF489243, HM163680. ***H. buxbaumii*;** buxTurkey; Turkey, 1km before Nizip; *Gabriele* 19 May 2006 b (Z); HM163984, HM163884, HM163784, HM163681. ***H. canaliculatum*** Boiss.; canF1454; Iran, Fars Prov., Shiraz to

Appendix 1. Continued.

Kharameh, km 13; *F. Ghahremaninejad 1454* (Z); EF489077, EF489151, EF489225, HM163682. ***H. canaliculatum*;** canSM50; Iran, Fars Prov., between Bidshahr and Kavian, near Banarooye; *Manafzadeh 5* (Z); HM163985, HM163885, HM163785, HM163683. ***H. canaliculatum*;** canZel0505092; Iran, Paste chenar region, 10 km SE of Sarvestan; *Zeltner 05.05.09 2* (Z); HM163986, HM163886, HM163786, HM163684. ***H. cappadocicum*** Spach; cap16W; Turkey, Eski Malata; 1965-19219 (W); HM163987, HM163887, HM163787, HM163685. ***H. cappadocicum*;** cor17W; Turkey; 1970-16869 (W); HM163988, HM163888, HM163788, HM163686. ***H. coronatum*** Griseb.; cor19W; Greece, Thessalien, ca. 28 km WNW of Kalambaka; 2000-14981 (W); HM163989, HM163889, HM163789, HM163687. ***H. coronatum*;** corMA353234; Macedonia; 353234 (MA); EF489081, EF489155, EF489229, HM163688. ***H. dasyginum*** C. Towns.; dasyW; Iran, Hamadan Prov. Aq Bulaq; 1961-1137 (W); HM163990, HM163890, HM163790, HM163689. ***H. dauricum*** (L.) G. Don; daur354LE; Mongolia; *V.I. Grubov & al. 354* (LE); HM163991, HM163891, HM163791, HM163690. ***H. dauricum*;** daurOyumaa1; Mongolia; *Oyumaa 1* (Z); EF489099, EF489173, EF489247, HM163691. ***H. dshungaricum*** Rubtzov; dshun616LE; Kazakhstan, mountains in the eastern part; *M. Piminov & al. 616* (LE); HM163992, HM163892, HM163792, HM163692. ***H. dubium*** Korovin; dub22W; Afghanistan, Faryab province, 7 miles E of Maimana, road to Belcheragh; 1973-13418 (W); HM163993, HM163893, HM163793, HM163693. ***H. dubium***; dub49LE; Turkmenistan; *V.P. Bochantzev 49* (LE); HM163994, HM163894, HM163794, HM163694. ***H. erythraeum*** Boiss.; ery24W; Afghanistan, Farah province, 21.5 miles E of Dilaram, road to Kandahar; 1973-13384(W); HM163995, HM163895, HM163795, HM163695. ***H. erythraeum*;** eryZel0505101; Iran, Fars Prov., Reserve de Onagres; *Zeltner 05.05.10 1* (Z); HM163996, HM163896, HM163796, HM163696. ***H. furfuraceum*** Bunge ex Boiss.; fur104Z; Iran, Shahrood-Ramiyan road, near to military campus, 85 km to Azadshahr; *Manafzadeh & Salvo 104* (Z); HM163997, HM163897, HM163797, HM163697. ***H. furfuraceum*;** fur26W; Iran, C Damghan-Semnan, in deserto gypsaceo, 2–7 km supra Sorkheh, prope Semnan; 1983-07483 (W); HM163998, HM163898, HM163798, HM163698. ***H. furfuraceum*;** furFAR14508; Iran, Khorasan Prov., between Mashad & Sarakhs, Chaahak hills; 14508 (FAR); EF489093, EF489167, EF489241, HM163699. ***H. glaberrimum*** Bunge ex Boiss.; gla28W; Iran, C. Kavir (Kavir protected region), Mobarakiyeh 40 km a Varamin, eridiem versus; 1975-13435 (W); HM163999, HM163899, HM163799, HM163700. ***H. glaberrimum*;** glaFAR34555; Iran, Khorasan Prov., SW of Sabz e vaar, Parvand; 34555 (FAR); EF489082, EF489156, EF489230, HM163701. ***H. griffithianum*** Boiss.; gri182LE; Tajikistan; *V.P. Bochantzev 182* (LE); –, HM163900, HM163800, –. ***H. griffithianum*;** gri30W; Afghanistan, Kalifghan, Kataghan Prov.; 1980-16250 (W); HM164000, HM163901, HM163801, HM163702. ***H. laristanicum*** C. Towns.; lariW; Iran, Lar prov.; 1958-2917 (W); –, HM163902, HM163802, –. ***H. latifolium*** Kar. & Kir.; lat1971LE; Kazakhstan, southern part; *R.V. Kamelin 1971* (LE); HM164001, HM163903, HM163803, HM163703. ***H. latifolium*;** latMA642325; Uzbekistan; 642325 (MA); EF489094, EF489168, EF489242, HM163704. ***H. linifolium*** (L.) G. Don; lin11758LE; Marocco; *J. Lewalle 11758* (LE); HM164002, HM163904, HM163804, –. ***H. linifolium*;** lin31W; Spain, Prov. Huesca, in collibus siccis 10 km a candasnos meridiem versus, subste. Calcif; 1994-09998 (W); HM164003, HM163905, HM163805, HM163705. ***H. linifolium*;** lin32W; Spain, Madrid, entre Aranjuez y Valdelagua. Base del cwrro cavina; 1995-01052 (W); HM164004, HM163906, HM163806, HM163706. ***H. linifolium*;** linGDA47314; Spain; 47314 (GDA); HM164005, HM163907, HM163807, HM163707. ***H. linifolium*;** linMA684635; Spain, Almeria; 684635 (MA); EF489079, EF489153, EF489227, HM163708. ***H. lissonotum*** C. Towns.; lisSM30; Iran, Hormozgan Prov., Bastak-Lar road (5 km after Bastak); *Manafzadeh 3* (Z); HM164006, HM163908, HM163808, HM163709. ***H. lissonotum*;** lisSM40; Iran, Hormozgan Prov., Bastak-Lar road (8km after Bastak); *Manafzadeh 4* (Z); HM164007, HM163909, HM163809, HM163710. ***H. lissonotum*;** lisZel0505112; Iran, Hormozgan Prov., Mount. Genu; *Zeltner 05.05.11 2 & 3* (Z); HM164008, HM163910, HM163810, HM163711. ***H. myrtifolium*** Boiss.; myr36W; Turkey, Malayta, Yeshilyurt; miozäner Mergel; 1965-19217 (W); HM164009, HM163911, HM163811, HM163712. ***H. myrtifolium*;** myr37W; Turkey, distr. Asaray, in valle ihlara; 2004-07473 (W); HM164010, HM163912, HM163812, HM163713. ***H. obtusifolium*** (Ledeb.) Ledeb.; obt106Z; Iran, Ghoochan-Dargaz, 22 km to Dargaz, Bibi gherghez; *Manafzadeh & Salvo 106* (Z); HM164011, HM163913, HM163813, HM163714. ***H. obtusifolium*;** obt38W; Iran, NE, ca. 7 km NW of Soolegerd; 1999-02467 (W); –, HM163914, HM163814, HM163715. ***H. obtusifolium*;** obtFAR16876; Iran, Khorasan Prov., South of Daragaz, Gherkhghez hills; 16876 (FAR); EF489098, EF489172, EF489246, –. ***H. patavinum*** (L.) G. Don; patMA353204; Bosnia and Herzegovina; 353204 (MA); EF489085, EF489159, EF489233, –. ***H. pilosum*** Stschegleev ex Turcz.; pil204Z; Uzbekistan, border between Kattakurgan and past Kargan; *Manafzadeh & Salvo 204* (Z); HM164012, HM163915, HM163815, HM163716. ***H. pilosum*;** pilFAR18330; Iran, Khorasan Prov., between Torbat e Heydarieh & Gonabad, Lut e Omrani, Ziarat; 18330 (FAR); HM164013, HM163916, HM163816, HM163717. ***H. popovii*** Korovin; popo44W; Afghanistan, E. khosht, in montibus S yakubi, substr. Kalkschiefer; 1968-202 (W); –, HM163917, HM163817, –. ***H. ramosissimum*** (Paulsen) Vved.; ram1968LE; Uzbekistan, Karakalpakstan; *Sherbaev 1968* (LE); –, HM163918, HM163818, –. ***H. rechingeri*** C. Towns.; rechSM90; Iran, Bakhtiari Prov., Boroojen to Sefiddasht, 2km to Zarrinshahr junction; *Manafzadeh 9* (Z); HM164014, HM163919, HM163819, –. ***H. robustum*** Bunge; rob100Z; Iran, Tehran-Semnan, south of Tehran, 6km to Eyvanaki; *Manafzadeh & Salvo 100* (Z); HM164015, HM163920, HM163820, HM163718. ***H. robustum*;** rob108Z; Iran, Kashan, Aran-Bidgol, Maranjab area, 5 km after Aran; *Manafzadeh & Salvo 108* (Z); HM164016, HM163921, HM163821, HM163719. ***H. robustum*;** rob206Z; Uzbekistan, Navai, 30 km after Navai- Shafreghan, M-37 (Tashkent- Buchara); *Manafzadeh & Salvo 206* (Z); HM164017, HM163922, HM163822, HM163720. ***H. robustum*;** robFAR17725; Iran, Khorasan Prov., between Torbat e Heydarieh & Gonabad, Lut e Omrani; 17725 (FAR); EF489089, EF489163, EF489237, HM163721. ***H. stapfianum*** Hand.-Mazz.; stapfianumSM20; Iran, Kerman Prov., Sirjan-Bandar road (270km to Bandar); *Manafzadeh 2* (Z); HM164018, HM163923, HM163823, HM163722. ***H. stapfianum*;** staF1426; Iran, Fars Prov., Shiraz to Kharameh, km 21; F. *Ghahremaninejad 1426* (Z); EF489078, EF489152, EF489226, HM163723. ***H. stapfianum*;** staF1448; Iran, Fars Prov., beginning of the road Shiraz to Zarghan; *F. Ghahremaninejad 1448* (Z); HM164019, HM163924, HM163824, HM163724. ***H. suaveolens*** (DC.) G. Don; sua49W; Bulgaria, Rousse region, Danube plain; 2005-05940 (W); –, HM163925, HM163825, HM163725. ***H. suaveolens*;** sua63W; Bulgaria, Central rhodope mts., above the town of Assenovgrad; 2007-05771 (W); HM164020, HM163926, HM163826, –. ***H. suaveolens*;** suaMA692105; Macedonia; 692105 (MA); EF489086, EF489160, EF489234, HM163726. ***H. telephioides*** Boiss.; tel50W; Turkey, E Ozkonak, B5 Nevshehir; 1990-06922 (W); HM164021, HM163927, HM163827, HM163727. ***H. telephioides*;** telTurkey; Turkey, Yaylaci; *Gabriele 21 May 2006 b* (Z); HM164022, HM163928, HM163828, HM163728. ***H. tenue*** Boiss.; ten51W; Armenia, vayots dzor province, eghegnadzor district, E part; 2006-03911 (W); HM164023, HM163929, HM163829, HM163729. ***H. thesioides*** (Fisch. ex DC.) G. Don; thes52W; Turkey, 22 km W Tarakli; 1990-07118 (W); HM164024, HM163930, HM163830, HM163730. ***H. thesioides*;** thesTurkey; Turkey, Bozgüney; *Gabriele 21 May 2006 x* (Z); HM164025, HM163931, HM163831, HM163731. ***H. tuberculatum*** (Forssk.) Adr. Juss.; tub60W; Egypt, NW/C part of the oasis 6-10 km NNW of Mt…; 2006-12053 (W); HM164026, HM163932, HM163832, HM163732. ***H. tuberculatum*;** tub61W; Tunisia, SE, ca. 35 km E Medenine; 2007-07253 (W); –, HM163933, HM163833, HM163733. ***H. tuberculatum*;** tubTAR172376; Iran, Semnan Prov., Sorkheh, Lasjerd; 72376 (TARI); HM164027, HM163934, HM163834, –. ***H. tuberculatum*;** tubZel0505081a; Iran, Fars Prov., Firuzabad, Palace of Ardeshir's I; *Zeltner 05.05.08 1a* (Z); HM164028, HM163935, HM163835, HM163734. ***H. tuberculatum*;** tubZel0505081b; Iran, Fars Prov., Firuzabad, Palace of Ardeshir's I; *Zeltner 05.05.08 1* (Z); HM164029, HM163936, HM163836, HM163735. ***H. tuberculatum*;** tubZel0505152; Iran, Kerman Prov., Shahdad; *Zeltner 05.05.15 2a & b* (Z); HM164030, HM163937, HM163837, HM163736. ***H. tuberculatum*;** tubZel1; Iran, Lali, 5 km avant; *Zeltner & Mansion 3.5.2007 sn1* (Z); HM164031, HM163938, HM163838, HM163737. ***H. tuberculatum*;** tubZel2; Iran, Choghazanbil; *Zeltner & Mansion 3.5.2007 sn2* (Z); HM164032, HM163939, HM163839, HM163738. ***H. versicolor*** Fisch. & Mey.; ver203Z; Uzbekistan, 6 km before Samarkand; *Manafzadeh & Salvo 203* (Z); HM164033, HM163940, HM163840, HM163739. ***H. versicolor*;** ver209Z; Uzbekistan, border between Guzar and Dekanabad regions, 15 km from Pachkamar village, coming from Guzar; *Manafzadeh & Salvo 209* (Z); HM164034, HM163941, HM163841, HM163740. ***H. versicolor*;** verFAR23840; Iran, Khorasan Prov., Sarakhs, between Doulatabad and Polekhatoon; 23840 (FAR); EF489090, EF489164, EF489238, HM163741. ***H. villosum*** (M. Bieb.) G. Don; vil59W; Georgia, E, ca. 40 km SE of Tziteli-Tzkaro, S limit of Shirak plateau, Vashlovan valley, Pantishar gorge; 2008-02417 (W); –, HM163942, HM163842, HM163742. ***H. villosum*;** vil9LE; Caucasus region; *Menitzki & Popova 9* (LE); –, HM163943, HM163843, –. ***H. villosum*;** vilMA417870; Azerbaijan; 417870 (MA); EF489096, EF489170, EF489244, –. ***H. villosum*;** vilSM110; Iran, East Azarbayjan Prov., Khaje to Ahar road (6km after Khaje); *Manafzadeh 11* (Z); HM164035, HM163944, HM163844, HM163743. ***H. villosum***; vilSM120; Iran, East Azarbayjan Prov., Kaleybar (Orliban Dam); *Manafzadeh 12* (Z); HM164036, HM163945, HM163845, HM163744. ***H. villosum*;** vilSM130; Iran, East Azarbayjan Prov., Kaleybar (Orliban Dam); *Manafzadeh 13* (Z); HM164037, HM163946, HM163846, HM163745. ***H. villosum*;** vilSM140; Iran, East Azarbayjan Prov., Bandar e Sharafkhaneh-Tasooj road, Heris Village; *Manafzadeh 14* (Z); HM164038, HM163947, HM163847, HM163746. ***H. villosum*;** vilSM150; Iran, East Azarbayjan Prov., Sarab to Heris Village, after

Appendix 1. Continued.

Asbforooshan junction; *Manafzadeh 15* (Z); HM164039, HM163948, HM163848, HM163747. ***H. villosum*;** vilSM160; Iran, Ardebil Prov., Ardebil-Nir road (12 km to Nir); *Manafzadeh 16* (Z); HM164040, HM163949, HM163849, HM163748. ***H. villosum*;** vilSM170; Iran, Ardebil Prov., Ardebil-Nir road (12 km to Nir); *Manafzadeh 17* (Z); HM164041, HM163950, HM163850, HM163749. ***H. villosum*;** vilT; Georgia, Transcaucasia; *s.n.* (TBI); HM164042, HM163951, HM163851, HM163750. ***H. virgatum*** Spach; virgF1428; Iran, Fars Prov., Shiraz to Kharameh, km 21; *F. Ghahremaninejad 1428* (Z); HM164043, HM163952, HM163852, HM163751. ***H. virgatum*;** virgF1437; Iran, Fars Prov., Shiraz to Kazerrun, Parishan Lake; *F. Ghahremaninejad 1437* (Z); HM164044, HM163953, HM163853, HM163752. ***H. virgatum*;** virgSM60; Iran, Fars Prov., Jahrom-Shiraz road (85km to Shiraz); *Manafzadeh 6* (Z); HM164045, HM163954, HM163854, HM163753. ***H. virgatum*;** virgSM70; Iran, Fars Prov., Dahak Village, 100 km to solar Powerhouse; *Manafzadeh 7* (Z); HM164046, HM163955, HM163855, HM163754. ***H. viridulum*** Soják; virSM80; Iran, Fars Prov., Shiraz-Fasa road (Miyanjangal), opposite of emamzadeh Esmail; *Manafzadeh 8* (Z); HM164047, HM163956, HM163856, HM163755. ***Aegle marmelos*** Corrêa; Aeg; Eastern Asia; *Chase 1340* (K); HM163957, HM163857, HM163756, HM163653. ***Citrus reticulata*** Blanco; Citr; Switzerland, Zürich Botanic Gardens, living collection, cult. 19790418; *Sandro Wagen 48* (Z); HM163958, HM163858, HM163757, –. ***Cneoridium dumosum*** Hook. f.; Cneo; U.S.A., Oak Crest Park, California; *Alexander Kocyan 154* (Z); HM163959, HM163859, HM163758, HM163654. ***Poncirus trifoliata*** (L.) Raf.; Ponc; Switzerland, Zürich Botanic Gardens, living collection, cult. 19760414; *Sandro Wagen 7* (Z); HM163960, HM163860, HM163759, HM163655. ***Glycosmis citrifolia*** Lindl.; Glyc; Taiwan, Taipei; *Yih-Han Chang 3310* (Z); HM163961, HM163861, HM163760, HM163656.

Appendix 2. Morphological characters and states selected for this study.

Vegetative morphology

1. Number of stems: (1) one, (2) more than one
2. Inflorescence form in each stem: (1) lax/broad, (2) dense/compact
3. Sterile axillary shoots: (0) absent, (1) present
4. Glands on stem: (0) invisible under a microscope (×40), (1) visible
5. Stem branching: (1) branched under the inflorescence, (2) unbranched
6. Stem indumentum: (0) glabrous, (1) hairy scattered, (2) hairy dense
7. Indumentum of leaf margin: (0) glabrous, (1) hairy
8. Indumentum of leaf: (0) glabrous, (1) hairy
9. Tuberculate glands on leaf: (0) absent, (1) present
10. Petiole: (0) absent, (1) present

Inflorescence morphology

11. Indumentum of inflorescence: (0) glabrous, (1) hairy
12. Form of bract: (1) linear, (2) broad
13. Indumentum of sepal: (0) glabrous, (1) farinose, (2) hairy
14. Dark dorsal vitta/tinge on petal: (0) absent, (1) present
15. Indumentum of petal: (0) glabrous, (1) farinose, (2) hairy

Stamen

16. Form of filament: (1) abruptly expanding from base to apex, (2) gradually expanding
17. Attachment between filaments: (1) monadelphous, (2) free
18. Indumentum of filament: (0) glabrous, (1) hairy in central portion, (2) hairy in lower half
19. Form of anther: (1) oval, (2) oblong

Pistil

20. Apical appendage on ovary: (0) absent, (1) present
21. Number of carpels: (1) five, (2) not five
22. Indumentum of ovary: (0) glabrous, (1) farinose, (2) hairy
23. Glands of ovary: (0) non-tuberculate, (1) tuberculate
24. Number of ovules: (2) two, (4) four, (5) more than four
25. Form of style: (1) slender, (2) stout
26. Indumentum of style: (0) glabrous, (1) hairy

Fruit morphology

27. Capsule dehiscence: (1) dehiscent, (2) indehiscent

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Phylogeny, morphology, and biogeography of *Haplophyllum* (Rutaceae), a species-rich genus of the Irano-Turanian floristic region

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***Taxon* 60: pp–pp**

Table S1. Morphological matrix for the 45 species of *Haplophyllum* and its sister species, *Cneoridium dumosum*, included in this study. Character numbers refer to those presented in Appendix 2. Missing data are indicated by a "?"; polymorphic character states are indicated with a "&".

Taxon/Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Haplophyllum acutifolium	2	1	0	1	1	0	1	0	0	1	1	2	0	0	0	1	2	1	1	0	1	0	0	2	2	0	2
H. affine	2	2	1	1	2	0	0	0	0	0	0	2	0	0	0	2	2	0	2	1	1	0	0	4&5	2	0	1
H. albanicum	2	2	1	1	2	2	?	?	0	1	1	1	2	0	0	2	2	2	1	1	1	2	1	2	1	0	1
H. alberti-regelii	1	1	?	1	1	0	0	0	0	0	0	1	2	0	0	1	1	1	2	0	1	0	0	2	1	0	1
H. armenum	2	2	1	0	2	2	0	0	0	1	1	1	2	1	2	2	2	1	1&2	1	1	2	1	4	1	0	1
H. bastentanum	2	2	1	?	?	2	0	0	0	0	1	1	2	0	2	2	2	2	2	?	1	2	?	4	1	0	1
H. blanchei	2	1	1	1	1	2	1	1	0&1	0	1	1	0	0	0	1	1	1	1&2	0	1	0	0	2	1	0	1
H. boissierianum	2	1	1	1	2	2	0	0	0	1	1	1	2	1	0	2	2	2	1	1	1	2	1	2	1	0	1
H. broussonetianum	2	2	1	1	?	1	0	0	0	1	0&1	1	2	0	0	2	2	0	1	1	1	0	1	5	2	0	1
H. bucharicum	2	1	1	0	2	1	1	0	0	?	1	1	2	0	0	2	2	2	2	1	1	0	0	4	2	0	1
H. bungei	2	1	1	1	1	0	0	0	0	1	0	2	0	0	0	1	1	1	2	1	1	0	1	2	1	0	1
H. buxbaumii	2	1	1	1	2	?	1	1	0	1	1	2	0	0	0	1	2	1	1	0	1	2	0	2	1	0	1
H. canaliculatum	2	2	1	1	2	0	0	0	0	1	1	2	0	0	0	2	2	1	2	0	1	2	0	2	2	0	1
H. cappadocicum	2	1	1	0	1	2	1	0	0	1	1	1	2	0	0	2	2	1	2	0	1	2	1	2	2	0	1
H. coronatum	2	2	1	0	2	?	1	1	0	1	1	1	2	0	2	2	2	2	2	1	1	2	1	4	1	0	1
H. dasygynum	2	2	?	1	2	1	1	1	0	?	1	1	2	0	0	?	?	?	?	?	?	?	?	?	?	?	?
H. dauricum	2	2	1	1	2	?	0	0	0	0	1	2	2	0	0	1	2	2	2	0	2	0	0	2	1	0	1
H. dshungaricum	2	1	1	1	2	0	0	0	0	?	0	1	0	0	0	1	2	1	2	1	1	0	0	2	1	1	1
H. dubium	2	1	1	0	2	0	0	0	0	1	1	1	2	0	0	1	1&2	2	2	1	1	2	0	2	1	0	1
H. erythraeum	2	2	1	1	1	0	0	0	0	0	1	2	1	0	1	2	2	0	2	1	1	1	0	4	2	0	1
H. furfuraceum	2	1	1	1	2	0	0	0	0	0	1	1	1	0	1	1	2	0	2	1	1	1	0	2&4	2	0	1
H. glaberrimum	2	1	1	1	1	0	0	0	0	0	0	2	0	0	0	2	2	0	2	0	1	0	0	4	1	0	1
H. griffithianum	2	1&2	1	1	2	0	0	0	0	0	0	1	0	0	0	2	2	1	1&2	1	1	0	0	2	1	0	1
H. laristanicum	?	1	?	0	1	?	1	1	0	0	1	?	2	0	0	1	1	1	2	0	1	0	0	2	1	0	?
H. latifolium	2	1	0	1	2	0	0	0	0	1	0	2	0	0	0	1	2	1	2	0	1	0	1	2	2	0	2
H. linifolium	2	2	1	1	2	1	1	1	0	0	1	1	2	1	0	2	2	2	2	1	1	2	1	4	1	0	1
H. lissonotum	2	1	1	1	2	0	0	0	0	0	0	1	0	0	0	2	2	1	2	0	1	2	0	2	2	0	1
H. myrtifolium	2	2	1	0	2	1	1	1	0	0	1	2	2	0	2	2	2	1	1	1	1	2	1	2	1	0	1
H. obtusifolium	2	1	0	1	2	2	0	0	1	0	0	2	0	0	0	2	2	1	2	1	1	2	0	2	2	0	1
H. patavinum	2	2	1	0	2	2	0	0	0	1	1	1	2	1	2	2	2	2	2	1	1	0	1	2	1	0	1
H. pilosum	2	2	0	1	2	2	1	1	0	1	1	1	2	1	0	2	2	1	1&2	1	1	2	1	5	2	0	1
H. popovii	2	1	0	1	1	0	0	0	0	1	0	1	0	0	0	1	1&2	1	2	0	1	0	0	2	1	0	1
H. ramosissimum	2	1	?	0	1	?	0	0	1	0	1	1	0	0	0	2	2	1	1&2	1	1	2	0	2	2	0	1
H. rechingeri	2	2	1	1	2	0	0	0	0	1	1	1	2	0	2	2	2	1	2	1	1	0	0	2	1	0	?
H. robustum	1	1	0	1	2	?	1	1	1	1	1	1	2	0	2	1&2	2	2	2	1	1	2	0	2	1	0	1
H. stapfianum	2	2	1	1	2	0	0	0	0	1	0	1	0	0	0	2	2	2	2	1	1	0	0	2	1	0	1
H. suaveolens	2	2	1	1	2	2	1	0	0	0	1	2	0	1	2	2	2	1	2	1	1	2	1	4	1	0	1
H. telephioides	2	2	1	0	2	?	1	1	0	1	1	2	2	1	2	2	2	2	1	1	1	2	1	4	1	0	1
H. tenue	2	2	0	1	2	0	0	0	0	1	1	2	2	0	0	1	2	1	2	1	1	0	0	2	1	0	1
H. thesioides	2	2	1	1	2	1	1	0	0	0	1	2	2	1	0	2	2	2	2	1	1	0	1	4	2	0	1
H. tuberculatum	2	1	1	1	2	1	1	1	1	0	1	1&2	2	0	0	2	1	1	2	0	1	2	1	2	1	1	1
H. versicolor	2	1	?	1	1	2	1	1	0	1	1	1	2	0	2	1	1	1	2	1	1	2	1	2	1	1	1
H. villosum	2	2	1	1	2	?	1	1	0	1	1	1	2	0	0	2	2	2	2	1	1	2	1	2	1	0	1
H. virgatum	2	1	1	1	1	0	0	0	0	0	1	1	0	0	0	1	2	1	2	0	1	0	0	2	1	0	1
H. viridulum	2	1	1	1	2	0	0	0	0	0	0	2	2	0	2	2	2	2	2	1	1	2	0	2	1	1	1
Cneoridium dumosum	2	1	?	1	?	0	0	0	0	0	0	?	0	0	0	2	2	0	1&2	0	2	0	0	2	1	0	2

Table S2. Absolute number of nucleotide substitutions between selected accessions.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*H. blanchei*	–																				
2	*H. blanchei*	8	–																			
3	*H. buxbaumii*	21	25	–																		
4	*H. buxbaumii*	17	15	12	–																	
5	*H. buxbaumii*	16	18	17	15	–																
6	*H. buxbaumii*	2	4	14	14	10	–															
7	*H. buxbaumii*	14	12	19	9	14	11	–														
8	*H. dasyginum*	12	18	13	9	11	12	12	–													
9	*H. laristanicum*	11	14	8	13	12	4	11	10	–												
10	*H. tuberculatum*	7	11	22	18	19	6	15	15	9	–											
11	*H. tuberculatum*	8	5	15	5	11	4	4	8	9	9	–										
12	*H. tuberculatum*	10	8	20	10	15	7	7	13	9	11	0	–									
13	*H. tuberculatum*	15	13	24	14	19	12	10	17	10	17	3	3	–								
14	*H. tuberculatum*	14	18	23	19	20	14	17	16	8	15	7	8	7	–							
15	*H. tuberculatum*	12	10	21	11	16	9	9	14	9	13	2	0	5	8	–						
16	*H. tuberculatum*	12	10	21	11	16	9	9	14	9	14	1	1	3	8	2	–					
17	*H. tuberculatum*	12	10	21	11	16	9	9	14	9	14	1	1	3	8	2	0	–				
18	*H. virgatum*	20	24	23	19	20	19	18	16	14	22	14	17	23	22	20	20	20	–			
19	*H. virgatum*	14	12	23	13	18	11	11	16	10	16	2	3	1	6	4	2	2	22	–		
20	*H. virgatum*	13	11	22	12	17	10	10	15	9	14	3	0	6	9	1	3	3	21	5	–	
21	*H. virgatum*	14	12	23	13	18	11	11	16	10	16	2	3	3	8	4	2	2	22	2	5	–