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RESEARCH ARTICLE

An updated infrageneric classification based on phylogenomics and character evolution in *Hydrangea* (Hydrangeaceae)

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Abstract The genus *Hydrangea* s.l. (Hydrangeaceae), a favorite among gardeners, comprises more than 80 species that are important understory plants in natural temperate and subtropical forests of the Northern Hemisphere. These species are hypervariable in morphology, especially in indumentum and floral characters, warranting a thorough exploration of the complex evolutionary history of morphological characters of the genus. Ideally, this is done within a robust phylogenetic framework with extensive taxon sampling. In this study, we propose a comprehensive taxonomic reassessment of *Hydrangea* based on a reconstruction of its molecular phylogeny based mainly on the chloroplast genome (plastome) and the most representative taxon sampling reported to date. Phylogenomic reconstruction yielded five well-resolved major clades and three newly recognized subclades. Inspection of the 28 characters employed in traditional taxonomic revisions revealed homoplasy in many of these traits. Our results show that characters such as growth habit, woody stem type, number of enlarged marginal sepals, style and stamen number, fruit shape, and ovary position are useful for circumscribing infrageneric divisions. Unique synapomorphies are limited. For example, herb and subshrub growth habits are associated with all the species in *H.* sect. *Deinanth* and sect. *Cardiandra*, respectively. Character state combinations are proposed for distinguishing infrageneric taxa. Based on a robust phylogenetic framework, our findings suggest a complex evolutionary history of *Hydrangea* morphological characters. Following the criterion of monophyly and considering the morphological consistency and synapomorphy of single and combined characters to diagnose relevant lineages, we update the classification of *Hydrangea* into 5 subgenera and 19 sections. Our proposed taxonomic scheme, based on a robust phylogenetic framework and in-depth character study, provides an updated perspective on the infrageneric subdivision of *Hydrangea* s.l.

Keywords *Hydrangea*; monophyly; phylogeny; synapomorphy; taxon sampling; taxonomic revision

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Hydrangeas are among the most important understory plants in warm temperate and tropical forests, where they display shrubby, herbaceous, and root-climbing habits (Samain & al., 2010; Granados Mendoza & al., 2013; De Smet & al., 2015). The genus *Hydrangea* L. currently comprises 16 sections (De Smet & al., 2015) and approximately 80 species, mainly distributed in East Asia, Southeast Asia, South Asia, and North, Central, and South America. Species identification and the circumscription of a given species are known to be notoriously difficult in *Hydrangea* (De Smet & al., 2017;

Zhang & al., 2019), mainly due to continuous variation and overlap among its morphological characters (Wei, 1994; Hufford, 1995; Hao & Hu, 1996a,b). There is a considerable disparity between classifications based solely on morphological traits and those based exclusively on molecular evidence, especially since the molecular phylogenetic findings indicate the polyphyletic nature of *Hydrangea* s.str. (Wei & Bartholomew, 2001; De Smet & al., 2015).

In the morphological trait-based taxonomy, *Hydrangea* s.str. (relative to *Hydrangea* s.l.) proposed by De Smet & al. (2015) is classified within tribe Hydrangeae, which also comprises eight satellite genera in addition to *Hydrangea* s.str.,

i.e., *Broussaisia* Gaudich., *Cardiandra* Siebold & Zucc., *Decumaria* L., *Deinathe* Maxim., *Dichroa* Lour., *Pileostegia* Hook.f. & Thomson, *Platyocrater* Siebold & Zucc., and *Schizophragma* Siebold & Zucc (Huang & Bartholomew, 2001; Wei & Bartholomew, 2001). Compared with the more narrowly circumscribed *Hydrangea* s.str., in which growth habit is restricted to shrubs, the expanded taxon (i.e., with the eight satellite genera) shows marked variation in morphological characters, including growth habit (e.g., herb, liana, bush, shrub, or small tree) and floral traits (e.g., enlarged marginal flowers). However, there is accumulating molecular evidence that *Hydrangea* s.str. is actually a paraphyletic taxon with the eight satellite genera nested within the genus (Ge, 2003; Samain & al., 2010; Granados Mendoza & al., 2013; De Smet & al., 2015). This paraphyletic nature of *Hydrangea* may arise from significant diversification of morphological characters in geographically isolated branches of the tribe, resulting in the recognition of these branches as distinct genera (Stewart & al., 2010; De Smet & al., 2017; Sakaguchi & al., 2021), and leading to further difficulty in delimiting traditionally recognized genera.

To address this issue, a recent study revised the classification of Hydrangeaceae based on multiple plastid fragments and extensive sampling, following the principle of monophyly (De Smet & al., 2015). This revision involved merging the nine genera of Hydrangeaceae into a single genus, *Hydrangea* s.l., and further subdividing *Hydrangea* s.l. into 16 sections. This new classification resolved the paraphyletic status of the genus and better elucidated the phylogenetic relationships within Hydrangeaceae. However, the phylogenetic relationships of *Hydrangea* require further clarification. For example, sister relationships between *H.* sect. *Broussaisia* and *Hydrangea* I, and the clade composed of *H.* sect. *Heteromallae*, sect. *Schizophragma*, sect. *Decumaria*, sect. *Pileostegia*, sect. *Deinathe*, and sect. *Cardiandra* have received only moderate to low branch support. Furthermore, although Zhang & al. (2021a) improved on the inadequate taxon sampling of East Asian species in previous studies and identified several new clades based on a phylogenetic framework for *Hydrangea* s.l. inferred using three chloroplast regions and the internal transcribed spacer region, several relationships within the genus still remain unresolved. Based on plastome genomic data, two species within *H.* sect. *Decumaria*, *H. barbara* and *H. obtusifolia*, were found not to form a monophyletic clade (Fu & al., 2019). Specifically, *H. obtusifolia*, which is distributed in East Asia, was not included in the earlier molecular phylogenetic tree (De Smet & al., 2015). Therefore, to elucidate the phylogenetic relationships within *Hydrangea* accurately and unambiguously, it is necessary to expand the sampling to be more representative, which, together with molecular data, can adequately help address the complex evolutionary history of morphological characters in *Hydrangea* within a solid phylogenetic framework.

Although incorporating relevant satellite genera into a broad circumscription of *Hydrangea* is expected to result in a more stable classification (De Smet, 2020), the proposed

classification was not unanimously accepted by authors working in the group, illustrating the difficulty in uniting traditional classifications with molecular insights. Previous studies have shown an apparent overlap between several diagnostic characters that are traditionally used in circumscriptions, contradicting the monophyly of *Hydrangea*. Examples include seed morphology (Hufford, 1995, 1997), leaf venation (Hao & Hu, 1996a), and pollen morphology (Zhang & al., 2019), in the delimitation of infrageneric lineages based on morphological features. Therefore, it is necessary to address the incongruence between the phylogeny and the traditional treatment of *Hydrangea* and elucidate the complex evolutionary pattern of morphological characters in these taxa.

With the advent of phylogenomics, many remaining problems in systematics have gradually been resolved (Xiang & al., 2017; Guo & al., 2020), with large amounts of sequence data acting as a buffer to reduce random errors in phylogenetic reconstruction (Zou & Ge, 2008). For *Hydrangea*, such a robust reconstruction is possible due to the recent abundance of plastid genomes from the genus that have been sequenced (Fu & al., 2019; Sakaguchi & al., 2021; Yang & al., 2023). We assembled 65 plastid genomes of *Hydrangea* and constructed the most robust phylogenetic framework to date, primarily based on plastid genome data and complete lineage sampling. We also inferred the ancestral states of 28 key diagnostic morphological characters with important taxonomic value to reveal the complex evolutionary history of these characters. The objective of our study was to reveal the infrageneric relationships of *Hydrangea* by conducting extensive sequencing of Asian species along with two species distributed in North America, integrating multiple plastid sequences from key lineages distributed in the Americas in order to explore the complex evolutionary history of important morphological characters of *Hydrangea* and their taxonomic applications.

■ MATERIALS AND METHODS

Taxon sampling. — Extensive sampling of representative taxa for all infrageneric *Hydrangea* lineages was done to minimize potential sources of error associated with large-scale phylogenetic taxon sampling (Hillis, 1998; Hirt & al., 2017; Schönhuth & al., 2018). A total of 60 species of *Hydrangea* were analyzed, which represented c. 70% of all *Hydrangea* species (Appendix 1). Our taxon sampling included all 16 sections of *Hydrangea* s.l. proposed by De Smet & al. (2015): *H.* sect. *Asperae* (9/c. 16), sect. *Broussaisia* (1/1), sect. *Calyptanthae* (2/2), sect. *Cardiandra* (3/4), sect. *Chinenses* (4/c. 5), sect. *Cornidia* (3/–), sect. *Decumaria* (2/2), sect. *Deinathe* (2/2), sect. *Dichroa* (5/12), sect. *Heteromallae* (5/c. 10), sect. *Hirtae* (1/1), sect. *Hydrangea* (4/c. 4), sect. *Macrophyllae* (1/c. 2), sect. *Pileostegia* (2/3), sect. *Schizophragma* (6/c. 10), and sect. *Stylosae* (1/1). The phylogenetic positions of the nine sampled species, i.e., *Hydrangea*

davidii, *H. hypoglauca*, *H. kwangsiensis*, *H. kwangtungensis*, *H. lingii*, *H. linkweiensis*, *H. liukiensis*, *H. mangshanensis*, and *H. stenophylla*, have not been discussed previously. These species are primarily distributed in southern China and Japan. Appendix 1 provides information on locality, collection, and voucher specimens.

We obtained a total of 69 plastid genomes from GenBank, including 53 species of *Hydrangea* s.l. and 4 outgroup species (all belonging to Hydrangeaceae). Among these, 65 sequences of *Hydrangea* were assembled by extensive sampling in East Asia (Appendix 1). We also obtained the plastid sequences (*matK* intron, *ndhA* intron, *rpl32-ndhF* intergenic spacer [IGS], *rps16-trnK* IGS, *trnL-rpl32* IGS, *trnV-ndhC* IGS) of seven species (four primarily distributed in the Americas) and one variety to present a comprehensive phylogenetic framework for *Hydrangea* (Appendix 2).

DNA extraction, plastome sequencing, and assembly. —

We used the GetOrganelle v.1.7.5 toolkit (Jin & al., 2020) and NOVOPlasty v.2.6.3 *de novo* assembler (Dierckxsens & al., 2017) for *de novo* sequence assembly. In the GetOrganelle pipeline, plastomic reads extracted from complete genomic reads were assembled using the SPAdes v.3.10 assembler (Bankevich & al., 2012). For NOVOPlasty, we selected several published complete plastid sequences as references, e.g., *Hydrangea heteromalla* (NC_044842), *H. luteovenosa* (NC_035662), *H. paniculata* (MN380680), *Dichroa febrifuga* (NC_044841), and *Deinanthoe caerulea* (MN380658). Whole plastid genomes were annotated using the Geneious v.10.2.3 platform (<http://www.geneious.com>), followed by manual verification of the start and stop codons of protein-coding genes (PCGs). Then, the whole plastomes and 79 PCGs were aligned using MAFFT v.7.490 (Katoh & Standley, 2013) with the auto strategy.

For screening differential characteristics of *Hydrangea* plastid genomes, we used 65 aligned plastid whole-genome sequences in the DnaSP v.6.10.04 software package (Rozas & al., 2017) to calculate the nucleotide diversity (Π) based on the average number of nucleotide differences (K) and total number of mutations (Eta). The sliding window length was set to 600 bp and the step length to 200 bp. We then extracted and aligned the *rpl32-ndhF* IGS, *rps16-trnK* IGS, *trnL-rpl32* IGS, and *trnV-ndhC* IGS sequences from each plastid genome for phylogenetic analysis.

Phylogenetic analysis. — We used four aligned datasets (complete plastome, concatenation matrix of 79 PCGs, concatenation matrix of six plastid sequences, and concatenation matrix of 79 PCGs plus six plastid sequences [Appendix S1]) for phylogenetic analysis after evaluating the best-fit nucleotide substitution model using the Akaike information criterion in the jModelTest v.2.1.4 software (Darriba & al., 2012). For phylogenetic analysis, we used maximum likelihood (ML) analysis with a GTR + Γ model and 1000 bootstrap replicates, and Bayesian inference (BI) (Huelsenbeck & Ronquist, 2001) with a GTR + I + Γ model, sampling one tree every 1000 generations for 3×10^6 generations, implemented in the CIPRES Science Gateway (<https://www.phylo.org/>).

We analyzed the results obtained from BI runs using Tracer v.1.7 (Rambaut & al., 2018) to assess the convergence among concurrent runs, establish suitable burn-in periods, and confirm effective sampling sizes (ESS) for all parameters exceeding 200. Majority-rule consensus trees were constructed after removing the first 25% of the generations as burn-in.

Ancestral character state reconstruction. — We selected and coded 28 traditional morphological characters, including growth habit, vegetative characters, and reproductive characters, in accordance with previous studies (Hufford, 1997; Wei & Bartholomew, 2001), *Flora of North America* (FNA Editorial Committee, 2016), and *Flora of Japan* (Iwatsuki & al., 2001) (Fig. 1, suppl. Table S1). We examined a minimum of 20 specimens per species to determine the stable state for each morphological character based on herbarium specimens (e.g., herbaria PE and IBK). For species for which this approach was not applicable or was limited by the availability of specimens, we primarily assessed the quality of the specimen data from online biodiversity platforms such as GBIF. We used Mesquite v.3.61 software (Maddison & Maddison, 2019) to reconstruct ancestral character states for the *Hydrangea* species, but not for the outgroup species. Within our established phylogenetic framework, we applied the “Trace Character History” option and employed the Markov k-state one-parameter (Mk1) evolutionary model in the ML approach to study the evolution of characters. The morphological character matrix is presented in suppl. Table S2. The results were visualized using Mesquite and the inferred ancestral states are displayed on the nodes as pie charts.

■ RESULTS

Plastid genome structures. — In this study, 65 *Hydrangea* s.l. plastomes showed a typical quadripartite structure, including one large single-copy (LSC) region, one small single-copy (SSC) region, and a pair of inverted repeat (IR) regions, with similar lengths (suppl. Fig. S1). Our results showed that the structure of the plastomes was largely consistent with that of the reference and outgroup genomes. DnaSP analysis of the 65 plastomes demonstrated that nucleotide variability was significantly lower in the IR region than in the LSC and SSC regions (suppl. Fig. S1). In both single-copy regions, the following genes had Π values >0.03 : *ycf1*, *psbJ*, *ndhF-rpl32*, *ndhC-trnV(UAC)*, *trnK(UUU)-rps16*, and *rps15*.

Phylogenetic inference. — The results of the phylogenetic analysis indicated that the topologies based on the four datasets (complete plastome, concatenation matrix of 79 PCGs, concatenation matrix of six plastid sequences, and concatenation matrix of 79 PCGs plus 6 plastid sequences) using BI and ML methods were nearly identical, with overall strong support for the corresponding species (Fig. 2, suppl. Figs. S2–S7). However, the topology based on the six concatenated plastid sequences showed poor support and low resolution (suppl. Figs. S4, S5). The lengths of three datasets (complete plastome, concatenation matrix of 79 PCGs,



Fig. 1. Images of representative members of *Hydrangea* s.l., illustrating key morphological characters (suppl. Table S1) employed in traditional taxonomic revisions. **A**, *Hydrangea moellendorffii*; **B**, *H. tomentella*; **C**, *H. kwangsiensis*; **D**, *H. bretschneideri*; **E**, *H. tomentella*; **F**, *H. febrifuga*; **G**, *H. caerulea*; **H**, *H. longipes*; **I**, *H. paniculata*; **J**, *H. lingii*; **K**, *H. febrifuga*; **L**, *H. asperae*. — Photos taken by X.D. Yang (A, B, E, J, L), S.X. Yu (C, D, F, H, I, K), and K. Tan (G).

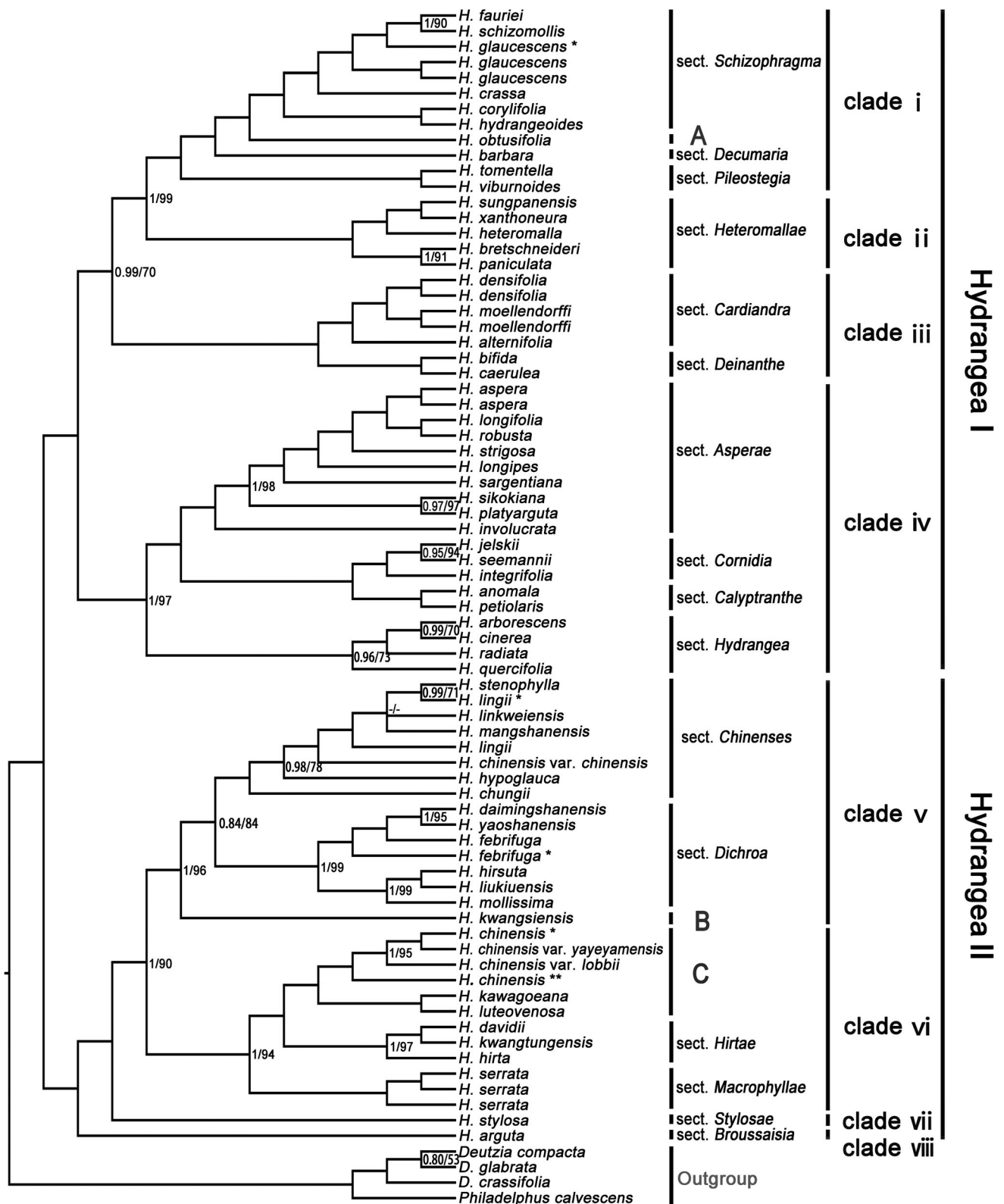


Fig. 2. Phylogenetic relationships of *Hydrangea* based on the concatenated nucleotide sequence of 79 protein-coding genes and six plastid sequences (*matK* intron, *ndhA* intron, *rpl32-ndhF* intergenic spacer [IGS], *rps16-trnK* IGS, *trnL-rpl32* IGS, *trnV-ndhC* IGS). The Bayesian inference (BI) tree is shown, with posterior probability and maximum likelihood (ML) bootstrap values at nodes. Nodes without a value had maximal support from both methods (ML and BI). The nomenclature and scope of each section (sect.) refers to the *Hydrangea* (Hydrangeaceae) classification of De Smet & al. (2015). *Hydrangea glaucescens** = *Schizophragma hypoglauca*. *Hydrangea lingii** = *Hydrangea vinicolor*. *Hydrangea febrifuga** = *Hydrangea yunnanensis*. *Hydrangea chinensis** = *Hydrangea macrosepala*. *Hydrangea chinensis*** = *Hydrangea scandens* var. *grosseserrata*.

and concatenation matrix of six plastid sequences) used for phylogenetic analysis after alignment are presented in suppl. Table S3. We used the topologies of the reconstructed BI tree based on the concatenation matrix of 79 PCGs plus six plastid sequences with excellent node support to explore the phylogenetic relationships and character evolution of *Hydrangea*, dividing the genus into two main lineages (*Hydrangea* I and *Hydrangea* II) (BI posterior probability [PP] = 1, ML bootstrap [BS] = 100) and five major clades with high support (Fig. 2).

Within the *Hydrangea* I lineage, there were four clades (i–iv) including 11 subclades corresponding to 10 sections and one newly recognized subclade (A) (Fig. 2). Clades i, ii, iii, and iv formed sister relationships successively. Clade i consisted of *Hydrangea* sect. *Schizophragma*, subclade A, sect. *Decumaria*, and sect. *Pileostegia*, all of which formed sister relationships in turn, with the highest support (PP = 1, BS = 100). Subclade A and *H.* sect. *Decumaria* formed a paraphyletic relationship. Clade ii contained only *H.* sect. *Heteromallae* (PP = 1, BS = 100), which was resolved as a sister to clade i (PP = 1, BS = 99). Two sections, *H.* sect. *Cardiandra* and sect. *Deinanthe*, were included in clade iii and formed a sister relationship with the highest support. Clade iv comprised four sections (*H.* sect. *Asperae*, sect. *Cornidia*, sect. *Calyptanthe*, sect. *Hydrangea*), with strong support (PP = 1, BS = 97). Within clade iv, the relationships of all subclades were well resolved, with high support. For example, *H.* sect. *Hydrangea* was derived first, followed by *H.* sect. *Cornidia* and sect. *Calyptanthe*, which formed sister relationships, first with each other, and then with *H.* sect. *Asperae* (Fig. 2).

Within the *Hydrangea* II lineage, four well-resolved clades including six sections and two newly identified subclades (B and C) were recognized with high support: clades v, vi, vii, and viii. The former two clades formed a sister relationship, and the latter two clades were derived successively. Clade v contained three highly resolved lineages, *Hydrangea* sect. *Chinenses*, sect. *Dichroa*, and the newly recognized subclade B, with each subclade receiving high support. However, the relationship of *H.* sect. *Chinenses* and sect. *Dichroa* received only moderate support (PP = 0.84, BS = 84). Clade vi also comprised three well-resolved clades, with *H.* sect. *Macrophyllae* derived first, followed by *H.* sect. *Hirtae* and subclade C, which formed a sister relationship. The three subclades A–C were newly recognized based on extensive sampling, notably subclade C, which mainly included *Hydrangea* species from East Asia.

Ancestral character state analysis. — We annotated the BI tree topology based on the concatenation matrix of 79 PCGs and six plastid sequences with character states and visualized the ancestral states as pie charts (Fig. 3, suppl. Figs. S8–S20). Most morphological characters examined in this study were identified according to their apparent ancestral states. Characters such as habit (chars. 1, 2; suppl. Tables S1, S2), leaf arrangement (char. 5), enlarged marginal flower sepals (chars. 15, 16), style (chars. 17, 18), ovary (chars. 21, 22),

capsule (chars. 23, 24), and seed (char. 26) were clearly clustered in one of several major clades on the phylogenetic tree (Fig. 3, suppl. Figs. S8, S10A, S15, S16A, S17B, S18, S19B). However, characters such as indument (chars. 3, 11, 12, 13, 14), blade (chars. 4, 6, 7, 8, 9, 10), stamen number (char. 20), and fruit shape (char. 25) were scattered among different clades of the tree (suppl. Figs. S9, S10B, S11, S12, S13, S14, S17A, S19A). Therefore, it was difficult to identify the ancestral states of indument characters associated with branchlets, petioles, blade midveins, and peduncles. In contrast, characters such as opposite leaves (char. 5:1), enlarged marginal flower sepals present (char. 15:1), superior ovary (char. 21:1), and protuberant apex of capsule (char. 23:1) were recognized as ancestral states (Fig. 3C, suppl. Figs. S10A, S15A, S18A). The probability of small shrubs (char. 1:2) being recognized as an ancestral state was significantly higher than that of other characters (Fig. 3A).

Comparison of character states at the genus level revealed that ancestral character states were well resolved for the major clades of the genus. For example, being a small shrub (char. 2:1) was identified unambiguously as an ancestral state of *Hydrangea* II (suppl. Fig. S8B). Both inferior ovary (char. 21:0) and truncated apex of capsule (char. 23:0) were recognized as ancestral states of clade iv (Fig. 3C, suppl. Fig. S18A), woody lianas (char. 2:0) as the ancestral state of clades i and ii (suppl. Fig. S8B), and alternate leaves (char. 5:0) as the ancestral state of *Hydrangea* sect. *Cardiandra* (suppl. Fig. S10A). The absence of enlarged marginal flower sepals (char. 15:0) is the ancestral state of *H.* sect. *Dichroa* (suppl. Fig. S15A1), and winged seeds (char. 26:1) is probably an ancestral state of *Hydrangea* I (suppl. Fig. S19B). It was also possible to recognize the ancestral states of several characters within major clades, which tended to be ambiguous at the generic level. For example, stamen number >15 (char. 20:1) is an ancestral state of clade iii (suppl. Fig. S17A), and ovoid or subglobose capsules (char. 25:0) were unambiguously identified as an ancestral state of several clades (suppl. Fig. S19A). However, the ancestral states of some characters remained ambiguous at the generic or subclade level, such as indument associated with branchlets, petioles, blades, blade midveins, and peduncles (chars. 3, 11, 12, 13, 14), as well as other blade characters (chars. 4, 6, 7, 8, 9, 10).

Association of morphological characters with the phylogeny. — At the subgeneric level, a combination of character states 1:2, 2:0, 5:2, 17:0, and 28:0 readily circumscribed clade i (Fig. 4). Clade ii was effectively delimited by combinations of character states such as 1:2, 2:2, 5:2, 15:2, 16:2, 17:2, 18:2, 21:2, 23:2, 24:0, and 28:1. Clade iii was circumscribed by the combination of character states 15:2, 18:2, 21:2, and 23:2. Clade iv was delimited by combinations of character states such as 1:2, 5:2, 17:2, 18:0, 21:0, 23:0 and 28:1. It was difficult to find reliable morphological characters for the delimitation of clade v. However, the combination of character states 1:2, 2:1, 5:2, 17:2, 18:2, 21:2, and 23:2 may circumscribe *Hydrangea* II to some extent. At the sectional level, most sectional clades were well circumscribed by character combinations. For example, the

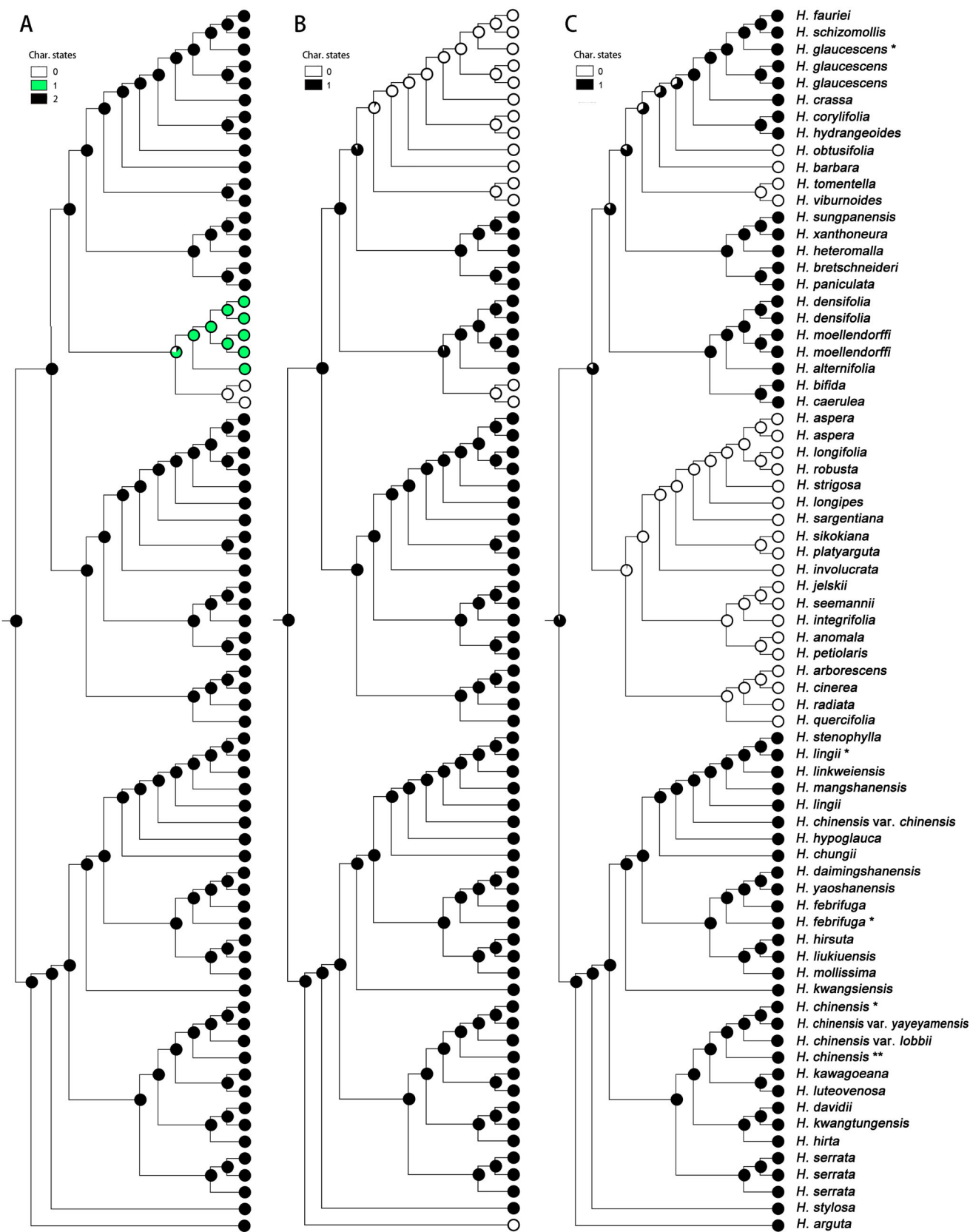


Fig. 3. Ancestral state reconstruction of three characters. **A**, Growth habit (char. 1); **B**, Extent of style union (char. 17), **C**, Ovary position (char. 21). — Pie charts at stem nodes represent reconstructed ancestral character states. Character states shown in the legend correspond to those described in suppl. Tables S1 and S2.

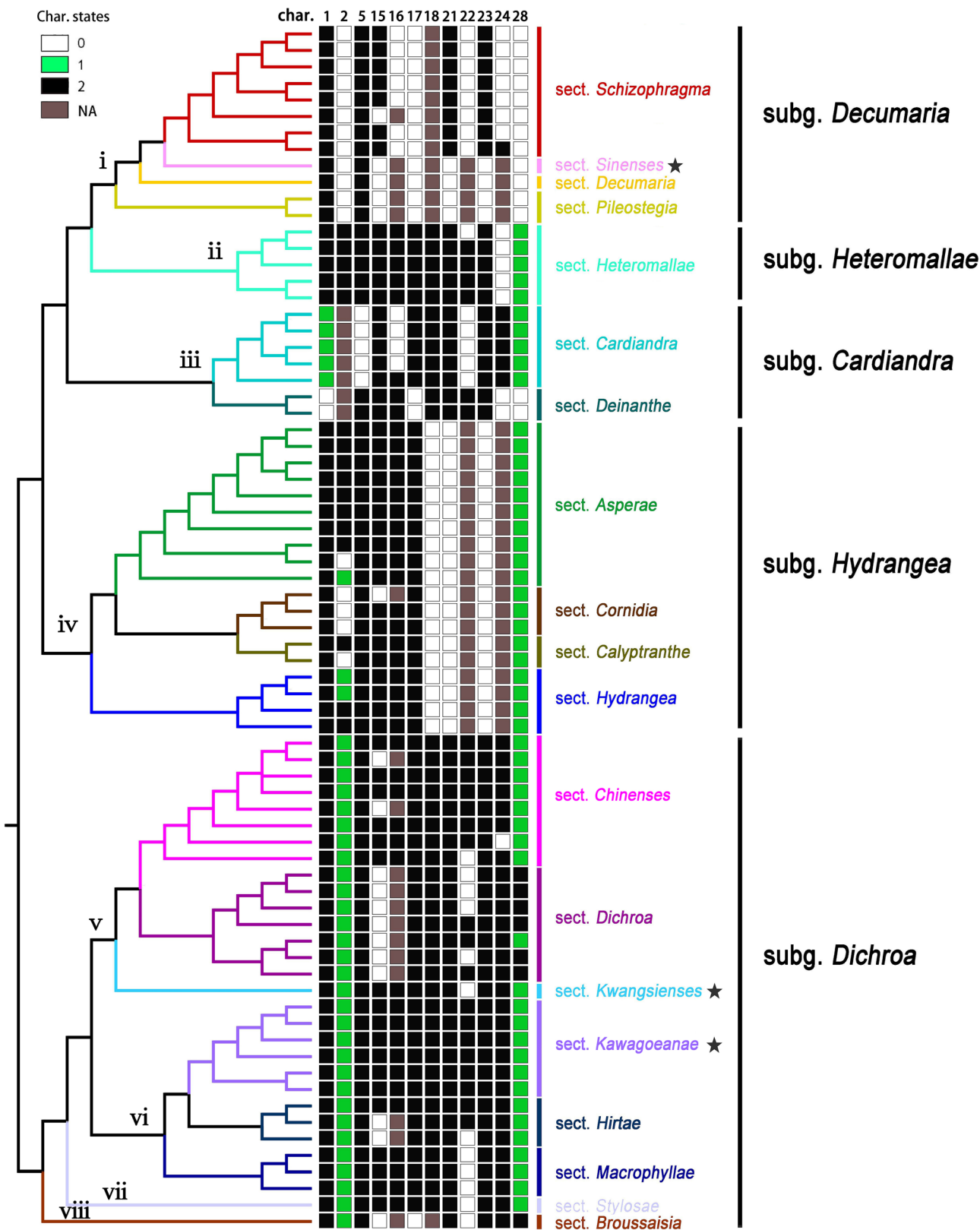


Fig. 4. States of 12 characters for each species and a summary of the proposed infrageneric classification of *Hydrangea*. The characters corresponding to the numbers are: growth habit (char. 1), woody stem type (char. 2), leaf arrangement (char. 5), enlarged marginal flower sepals (char. 15), number of enlarged marginal flower sepals (char. 16), extent of style union (char. 17), number of ununited styles (char. 18), ovary position (char. 21), extent of superior ovary (char. 22), capsule apex (char. 23), shape of capsule protuberance (char. 24), and fruit dehiscence type (char. 28). Character states shown in the legend correspond to those described in suppl. Tables S1 and S2. Asterisks indicate three sections first proposed in this study.

combination of character states 1:2, 2:0, 5:2, 17:0, 21:2, 22:0, 23:2, and 28:0 readily delimited *Hydrangea* sect. *Schizophragma* (Fig. 4). The combination of character states 1:2, 2:2, 5:2, 15:2, 16:2, 17:2, 18:2, 21:2, 23:2, 24:0, and 28:1 was important for defining *H.* sect. *Heteromallae*, whereas character states 1:2, 5:2, 15:2, 16:2, 17:2, 18:0, 21:0, 23:0, and 28:1 distinguished *H.* sect. *Asperae* from all others except the basal taxon (Fig. 4). Character states 1:2, 2:1, 5:2, 15:0, 17:2, 18:2, 21:2, 23:2, and 24:2 were important combinations of traits for describing *H.* sect. *Dichroa*. Character states 7:1, 12:1, 14:2, 20:0, 25:0, 26:0, and 27:0 were useful for delimiting *H.* sect. *Hirtae*, and character states 3:0, 4:1, 7:1, 11:0, 12:0, 13:0, 19:0, and 27:0 for *H.* sect. *Macrophyllae* (suppl. Fig. S21).

Characters related to growth habit, leaves, enlarged marginal flower sepals, styles, stamen numbers, ovary position, fruits, and seed wings are important for infrageneric delimitation in *Hydrangea* (Fig. 4). Five clades exhibited unique growth habits, such as climbing shrubs in clade i and large shrubs in clade ii, whereas important leaf characters such as the number of secondary veins and shape of the blade base, distinguish sections in clades ii, iv, and v. Three to five sepals on enlarged marginal flowers within an inflorescence represented the main state of *Hydrangea*, whereas 0, 1, or 2 sepals distinguished *H.* sect. *Schizophragma*, clade ii, and *H.* sect. *Dichroa*, respectively. Number of stamens >15 was an important distinctive trait in clade iii, and the style number, ovary position, and fruit apex were useful to distinguish clades iv and v, and other clades as well. Campanulate to turbinate or obconic capsules were key morphological characters of clade i. Most species in clade v (*Hydrangea* II) had wingless seeds, which distinguished these species from *Hydrangea* I. Other characters did not distinguish clades well, either due to their widespread distribution across the phylogenetic tree or because they were shared among most species with the same ancestral state; however, these characters may be used as partial identification traits between some sections or species, e.g., branchlet indument, petioles, leaf surfaces, leaf midveins, and peduncles (suppl. Fig. S21).

DISCUSSION

Phylogeny of *Hydrangea*. — In this study, we inferred the infrageneric phylogeny of the genus *Hydrangea* including all previously discovered lineages (McClintock, 1957; Samain & al., 2010; De Smet & al., 2015) and three newly recognized clades, primarily using whole-genome (plastome) sequences mainly from East Asian species, complemented by multiple plastid sequences mainly from American species. We expect this phylogeny to provide the basis for the most robust framework for in-depth analysis of the taxonomic relationships among all lineages, and the character evolution of a number of traits in this genus. The phylogenetic relationships proposed for *Hydrangea* in this study are largely aligned with those from previous research based on multiple plastid

sequences (Samain & al., 2010; Granados Mendoza & al., 2013; De Smet & al., 2015); however, our more robust support enabled a deeper resolution of the phylogenetic positions of challenging species. This tree also resolved the paraphyly of *Hydrangea* II noted by De Smet & al. (2015) by showing that clade viii (*H.* sect. *Broussaisia*) forms a sister relationship with the remaining clades of *Hydrangea* II (Fig. 2). In addition, our tree identified three new clades (subclades A, B, and C), whose members are mainly from East Asia, with distinctive phylogenetic positions and high support (Fig. 2). Importantly, for the phylogenetic positions of *H.* sect. *Cornidia*, sect. *Calypttranthe*, and sect. *Asperae*, our tree supports the results of Granados Mendoza & al. (2021), who constructed the phylogenetic relationships of *Hydrangea* based on sequences from two low-copy nuclear genes, whereas our phylogenetic analysis is based on plastid markers.

Our findings also agree with those of De Smet & al. (2015), indicating that *Hydrangea* sect. *Cornidia* and sect. *Calypttranthe* first form a sister relationship with each other and are monophyletic with *H.* sect. *Asperae*. However, for *Hydrangea* I, De Smet & al. (2015) recognized subclade *H.* sect. *Hydrangea* as having diverged first, whereas our study resolved subclade *H.* sect. *Hydrangea* as embedded in clade iv and forming a sister relationship with the remaining species of the clade (Fig. 2). Phylogenetic relationships at the sectional level among clades i, ii, and iii were also well resolved in our tree. With the exception of *H. barbara* and the newly sampled *H. obtusifolia*, which formed a monophyletic relationship with *H.* sect. *Schizophragma* as described by Fu & al. (2019), other sectional relationships among clades i, ii, and iii concurred with the results of De Smet & al. (2015). The phylogenetic positions of *H. radiata* and *H. quercifolia* were resolved as taxa positioned at the base of subclade *H.* sect. *Hydrangea*, partially addressing the ambiguity regarding the phylogenetic position of these two species.

Compared to previous studies, support appeared to increase at some nodes, and the phylogenetic relationships between different sections of *Hydrangea* II were well resolved. For example, within clade v our tree showed that *Hydrangea* sect. *Dichroa* formed a sister relationship with *H.* sect. *Chinenses* (Fig. 2), which contradicts the results of De Smet & al. (2015). Previously confirmed *H.* sect. *Chinenses* was now confirmed as paraphyletic (*H.* sect. *Chinenses* and subclade C, Fig. 2) coinciding with the extension of our sampling area into south China. Within the subclade containing *H.* sect. *Chinenses*, the phylogenetic positions of six species were shown for the first time, i.e., *H. stenophylla*, *H. vinicolor*, *H. linkweiensis*, *H. mangshanensis*, *H. lingii*, and *H. hypoglauca*; these six species were monophyletic together with *H. chinensis* var. *chinensis* and *H. chungii*. Despite being considered a synonym of *H. lingii* by Wei & Bartholomew (2001), *H. vinicolor* did not form a monophyletic group with it in our tree. Furthermore, *H. chinensis* was also confirmed to be polyphyletic with *H. chinensis* var. *chinensis*, *H. chinensis* var. *yayeyamensis*, and *H. chinensis* var. *lobbii* appearing on different clades (Fig. 2). In clade v, the

newly sampled taxon *H. kwangsiensis* (subclade B) formed a sister relationship with *H.* sect. *Dichroa* and sect. *Chinenses*, with high support (PP = 1, BS = 96, Fig. 2). In addition, *H. macrosepala* was previously identified as a synonym of *H. chinensis* (Wei & Bartholomew, 2001); however, they have now been well resolved and placed in different subclades of *Hydrangea* II, with high support.

Complex evolutionary history of morphological characters. — It has long been recognized that hyper-variation among the morphological characters of *Hydrangea* s.l. presents challenges for identifying traits that may be used for systematic taxonomic revisions (Maximovicz, 1867; Chen, 1954; Wei, 1994). Our study demonstrated that most characters show a scattered distribution on the phylogenetic tree and are characterized by a complex evolutionary history, with many having multiple independent origins (Fig. 3, suppl. Figs. S8–S20). For example, characters such as the small-shrub growth habit (char. 2:1), absent enlarged marginal flower sepals (char. 15:0), inferior ovary (char. 21:0), and truncated capsule apex (char. 23:0) all have multiple origins on the phylogenetic tree (Fig. 3C, suppl. Figs. S8B, S15A, S18A). Although some characters, including growth habit (char. 1), enlarged marginal flower sepals (char. 15), number of ununited styles (char. 18), and fruit shape (char. 25), showed obvious evolutionary trends (Fig. 3A, suppl. Figs. S15A, S16A, S19A), others did not, e.g., petiole length (char. 4), blade length (char. 6), leaf texture (char. 7), leaf lateral veins (char. 8), leaf margin (char. 9), and leaf base (char. 10) (suppl. Figs. S9B, S10B, S11, S12). In particular, the indument associated with branchlets, petioles, blades, blade midveins, and peduncles (chars. 3, 11, 12, 13, 14) showed a scattered distribution pattern on the phylogenetic tree, with ambiguous ancestral states and unclear evolutionary trends (suppl. Figs. S9A, S13, S14). In addition, although the presence of sepals on enlarged marginal flowers was found to be an essential character for distinguishing *H.* sect. *Schizophragma*, there was no such unique character for *H. crassa*, complicating the delimitation of this section (suppl. Fig. S15A). Characters exhibiting complex evolutionary patterns may have been influenced by environmental changes (e.g., geographical isolation), further confusing clade or section boundaries, and thus rendering taxonomic circumscriptions ambiguous (De Smet & al., 2017; Sakaguchi & al., 2021).

This study confirmed a few evolutionary trends proposed in a previous study (Wei, 1994). For example, our study found that the shrub character was as an ancestral state and the liana growth form was identified as apomorphic (suppl. Fig. S8B), which agrees with Wei (1994), who inferred that species with climbing stems or lianas (char. 2:1) were more advanced than those with the erect-shrub growth habit. However, many characters described in traditional taxonomic revisions did not show clear evolutionary trends in our study. Wei (1994) also proposed that species with a one-half to two-thirds sized superior ovary are more primitive than those with a one-third sized superior ovary or a completely inferior ovary. However, we found that it was difficult to distinguish these ovary sizes

among species and that the superior ovary (char. 21:1) is an ancestral state, with no apparent evolutionary trends in ovary size (Fig. 3C). Nevertheless, the inferior ovary (char. 21:0) was identified as a diagnostic character for delimiting clade iv, although several species derived early in clade i were also characterized by an inferior ovary, as it originated twice on the phylogenetic tree (Fig. 3C). In the genus *Hydrangea*, a previous study (Hufford, 1997) also found multiple enlarged marginal sepals as an ancestral state, although this was based on the traditional classification of the tribe Hydrangeae. In *H.* sect. *Dichroa*, the indehiscent berry-like fruit (char. 28:2; suppl. Fig. S20B) was previously recognized as an important diagnostic character for delimiting the traditional genus *Dichroa*; however, our results identified this character as homoplastic.

Taxonomic significance of morphological characters. — Although as many as 28 traditionally used morphological characters were examined in this study, only a few were identified as synapomorphies at the generic level. For example, growth habit (char. 1) was identified as an important character for delimiting *Hydrangea* sect. *Cardiandra* and sect. *Deinanth* for only certain states of the character, i.e., herb (char. 1:0) and subshrub (char. 1:1) were found to be synapomorphies of these sections, respectively (Fig. 3A). The character leaf alternation (char. 5:0) was also recognized as a synapomorphy of *H.* sect. *Cardiandra* (suppl. Fig. S10A). However, the remaining large proportion of morphological characters used in traditional taxonomic revisions were scattered on different clades of the phylogenetic tree, many of which were homoplasies. Previous studies have found that seed and leaf characters were of limited value in infrageneric delimitation (Hufford, 1995; Hao & Hu, 1996a), and we confirmed them to be homoplastic, as both characters evolved multiple times independently. McClintock (1957) proposed the taxonomic status of genus *Platyterater* with segregation of *H.* sect. *Asperae* into three genera, two of which contained only a single species. However, this taxonomic arrangement was later refuted by molecular-based classification (De Smet & al., 2015). Therefore, it is difficult to circumscribe taxa at either the subgeneric or sectional level based on a particular state of a character. We addressed this challenge by exploring characters that are more in line with molecular phylogenetics, with the aim of effectively delineating infrageneric taxa.

Besides synapomorphies, the combination of several independent morphological characters is also useful to circumscribe taxa at different levels (Zhang & al., 2021b; Gillespie & al., 2022; Wei & Zhang, 2022). In this study, we explored some combinations of morphological characters to delimit infrageneric taxa. We found that some subgenera could be readily distinguished on the basis of a combination of three to five morphological characters. For example, a combination of shrub (char. 1:2) and liana (char. 2:0) growth habits and united styles (char. 17:0) was valuable for delimiting *Hydrangea* subg. *Decumaria* (stat. nov.) (clade i; Fig. 3, suppl. Fig. S8). Clade ii, *H.* subg. *Heteromallae* (stat. nov.; sect. *Heteromallae*) was distinguished from the remaining clades based on

large-shrub growth habit (char. 2:2) combined with a conical fruit protuberance (char. 24:0) (suppl. Figs. S8B, S18B). *Hydrangea* subg. *Cardiandra* (stat. nov.) contains two sections, *H. sect. Cardiandra* and *H. sect. Deinanthe*, which are characterized by the herbaceous (char. 1:0) or subshrub (char. 1:1) growth habit, combined with >15 stamens (char. 20:1) (Fig. 3A, suppl. Fig. S17A). *Hydrangea* subg. *Hydrangea* (clade iv) could also be circumscribed using a combination of three characters: ununited styles (char. 17:1), inferior ovary (char. 21:0), and truncated fruit apex (char. 23:0) (Fig. 3, suppl. Fig. S18A). In addition, it was possible to delimit *H. subg. Dichroa* (stat. nov.) from related subgenera using the combination of the growth habit large shrub (char. 2:2), inferior ovary (char. 21:1), and protuberant fruit apex (char. 23:1) (Fig. 3C, suppl. Figs. S8B, S18A).

At the section level, a large proportion of the sections evaluated in this study could not be circumscribed by combinations of only a few characters, but required a larger number of combined characters, which makes infrageneric taxonomic delineation difficult. For example, *Hydrangea* sect. *Schizophragma* was readily circumscribed by the combination of a liana growth habit (char. 2:0), absence of sepals in enlarged marginal flowers (char. 15:0), and coniform superior fruit protuberance (char. 24:0) (suppl. Figs. S8B, S15A, S18B). For *H. sect. Dichroa*, the indehiscent berry-like fruit (char. 28:2), absence of sepals in enlarged marginal flowers (char. 15:0) and small shrub growth habit (char. 2:1) circumscribed all species except *H. liukuensis* (suppl. Figs. S8B, S15A, S20B). Moreover, indehiscent berry-like fruit (char. 28:2), combined with united styles (char. 17:0) are key characters circumscribing *H. sect. Broussaisia* (Fig. 3B, suppl. Fig. S20B). Thus, compared to subgenera, it is more difficult to find reliable character combinations for circumscribing sections. Future studies should explore novel morphological characters with high systematic value based on our robust phylogenetic framework.

A new infrageneric classification of *Hydrangea* s.l. —

Based on the principles of monophyly and synapomorphy, as well as key diagnostic characters, we redefined several previously proposed sections and identified three lineages at the section level for the first time. Given the need to revise the classification of *Hydrangea* using novel combinations of morphological characters and the current challenges in reliably defining sections based on morphological characters, working at the subgenus level may be the optimal approach to integrate molecular and morphological evidence to update the classification (Villaverde & al., 2020), thereby promoting the practical application of molecular phylogenetic frameworks in biodiversity research. According to our results, the genus *Hydrangea* s.l. is divided into 5 subgenera and 19 sections, among which the 5 subgenera and 3 sections are newly proposed (Fig. 4; suppl. Table S4). The five subgenera include *H. subg. Decumaria* (stat. nov.), subg. *Heteromallae*, subg. *Cardiandra* (stat. nov.), subg. *Hydrangea*, and subg. *Dichroa* (stat. nov.), corresponding to clades i, ii, iii, iv and v–viii, respectively. The three new proposed sections are *H. sect.*

Sinenses (sect. nov.), *H. sect. Kawagoeanae* (sect. nov.), and *H. sect. Kwangsienses* (sect. nov.), corresponding to subclades A–C, respectively.

Hydrangea sect. *Sinenses* contains only one species, *H. obtusifolia*, and forms a sister relationship with *H. sect. Schizophragma*, as first recognized in this study. Based on our expanded sampling, certain species within the previously proposed *H. sect. Chinenses* (e.g., *H. luteovenosa* and *H. chinensis* var. *lobbii*) were found to be monophyletic with *H. kawagoeana*, *H. chinensis* var. *yayeyamensis*, *H. macrosepala*, and *H. scandens* var. *grosseserrata*, forming a distinct clade. We support the establishment of this clade as a new section called *H. sect. Kawagoeanae*. *Hydrangea* sect. *Chinenses* comprises two known species *H. chinensis* and *H. chungii* (De Smet & al., 2015), as well as *H. stenophylla*, *H. lingii*, *H. linkweiensis*, *H. mangshanensis*, and *H. hypoglaucha*, representing six additional specimens primarily from southern and eastern China. Although they do not form a monophyletic group, *H. sect. Chinenses* and *H. sect. Kawagoeanae* show relatively consistent morphological characters. Further research is needed to define morphological traits that distinguish these sections. *Hydrangea* sect. *Kwangsienses* is recognized in this study for the first time; it is characterized by a distinct combination of morphological characters and forms a sister-group relationship with *H. sect. Chinenses* and *H. sect. Dichroa* (Fig. 4). We extended the delimitation of *H. sect. Hirtae* by adding representative samples of two species, *H. shaochingii* and *H. davidii*. The detailed taxonomic framework of *Hydrangea* s.l. is as follows.

Hydrangea L., Sp. Pl.: 397. 1753 – Type: *H. arborescens* L.

Key to the subgenera of *Hydrangea*

1. Small, erect shrubs, rarely large shrubs and subshrubs; styles 3–4, rarely 5 or 6, slender; superior ovary; berry; projected apex of fruits not conical; seed (nearly) wingless.....subg. *Dichroa*
1. Mostly large erect or climbing shrubs, subshrubs or perennial herbs; styles united or 1–3, rarely 4; completely or mostly inferior ovary, rarely more than half superior ovary; capsule; projected apex of fruits truncate or conical, rarely not conical; seed winged, rarely wingless 2
2. Lianas or climbing shrubs; entire leaf blade margin, rarely serrate; inflorescences contain all fertile flowers or enlarged marginal sepals 1 or occasionally 2; styles united or 1 subg. *Decumaria*
2. Large, erect shrubs, subshrubs or perennial herbs, rarely climbing shrubs; serrate or roughly serrate blade margin; inflorescences with fertile and enlarged marginal sepals 2–5; styles 2–4, rarely connate 3
3. Subshrubs or perennial herbs; leaves 4–8 crowded upper or apically on stem, cuneate leaf base; stamens more than 15; subinferior ovary, rarely more than half superior; projected apex of fruit not conical, rarely conical.....subg. *Cardiandra*

3. Large, erect shrubs, rarely climbing; leaves not crowded on stem, leaf bases broadly cuneate, nearly round or shallowly cordate; stamens 8–15; inferior or more than half superior ovary; projected apex of fruit truncate or conical.....4
4. Ovary usually nearly half or more superior; styles 3–4; fruit nearly globose; projected apex of fruit conical.....subg. *Heteromallae*
4. Ovary completely inferior; styles mostly 2; urn-shaped or turbinate fruit, rarely globose; apex of fruit truncate.....subg. *Hydrangea*

Hydrangea subg. *Cardiandra* (Siebold & Zucc.) X.D.Yang & S.X.Yu, **stat. nov.** $\equiv$ *Cardiandra* Siebold & Zucc., Fl. Jap. 1: 119. 1839 – Type: *H. alternifolia* Siebold. = *Deinanth* Maxim. in Mém. Acad. Imp. Sci. Saint Pétersbourg, Sér. 7, 10(16): 2. 1867.

Diagnostic characters. – Subshrubs or perennial herbs with rhizomes. Leaves alternate or opposite, papery, or membranous, with margin serrate or roughly serrate. Inflorescences terminal cymes or panicles with bisexual flowers. Enlarged marginal sepals 2–4. Stamens numerous or extremely numerous. Ovary subinferior or nearly half superior. Styles 2–3 or 5-connate. Capsule ovoid or oblate. Seeds numerous, small, winged at both ends.

Taxa and distribution. – Two sections, *Hydrangea* sect. *Cardiandra* (Siebold & Zucc.) Y.De Smet & Samain and sect. *Deinanth* (Maxim.) Y.De Smet & Samain, restricted to China and Japan. Comprising c. 6 species.

Hydrangea sect. *Cardiandra* (Siebold & Zucc.) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *Cardiandra* Siebold & Zucc., Fl. Jap. 1: 119. 1839 – Type: *H. alternifolia* Siebold.

Hydrangea sect. *Deinanth* (Maxim.) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *Deinanth* Maxim. in Mém. Acad. Imp. Sci. Saint Pétersbourg, Sér. 7, 10(16): 2. 1867 – Type: *H. bifida* (Maxim.) Y.De Smet & C.Granados.

Hydrangea subg. *Decumaria* (L.) X.D.Yang & S.X.Yu, **stat. nov.** $\equiv$ *Decumaria* L., Sp. Pl., ed. 2: 1663. 1763 – Type: *H. barbara* (L.) B.Schulz.

= *Pileostegia* Hook.f. & Thomson in J. Proc. Linn. Soc., Bot. 2: 57. 1857.

= *Schizophragma* Siebold & Zucc., Fl. Jap. 1: 58. 1838.

Diagnostic characters. – Evergreen climbing shrub or deciduous woody liana, usually with aerial roots. Leaves opposite, petiolate, with entire or serrate margins. Inflorescences terminal panicles or cymes with bisexual flowers. Enlarged marginal flowers with entire sepal 1(3) or absent. Calyx tube adnate to ovary. Ovary (sub)inferior. Capsule campanulate, urn-shaped or obconical, with truncate or conical projected apex. Seed winged.

Taxa and distribution. – Four sections, *Hydrangea* sect. *Decumaria* (L.) Y.De Smet & Samain, sect. *Pileostegia*

(Hook.f. & Thomson) Y.De Smet & Samain, sect. *Schizophragma* (Siebold & Zucc.) Y.De Smet & Samain and sect. *Sinenses* X.D.Yang & S.X.Yu, mainly in China, India, Vietnam, Japan and the southeastern United States. Comprising c. 15 species.

Hydrangea sect. *Decumaria* (L.) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *Decumaria* L., Sp. Pl., ed. 2: 1663. 1763 – Type: *H. barbara* (L.) B.Schulz.

Hydrangea sect. *Pileostegia* (Hook.f. & Thomson) Y.De Smet & Samain in Taxon 64(4): 751. 2015 $\equiv$ *Pileostegia* Hook.f. & Thomson in J. Proc. Linn. Soc., Bot. 2: 57. 1857 – Type: *H. viburnoides* (Hook.f. & Thomson) Y.De Smet & C.Granados.

Hydrangea sect. *Schizophragma* (Siebold & Zucc.) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *Schizophragma* Siebold & Zucc., Fl. Jap. 1: 58. 1838 – Type: *H. hydrangeoides* (Siebold & Zucc.) B.Schulz.

Hydrangea sect. *Sinenses* X.D.Yang & S.X.Yu, **sect. nov.** – Type: *H. obtusifolia* (Hu) Y.De Smet & C.Granados.

Diagnostic characters. – Climbing shrub, 2–5 m tall. Leaf blade obovate to elliptic, 3.5–7 $\times$ 2–3.5 cm, leathery, base cuneate, margin entire, (sub)glabrous, apex obtuse to acute. Enlarged marginal flowers absent. Calyx tube turbinate, lobes ovate to ovate-deltoid. Stamens 20–30. Ovary 5–10-lobed, (sub)inferior. Capsule campanulate to turbinate, apex truncate. Seed winged.

Taxa and distribution. – Monotypic, *Hydrangea obtusifolia* (Hu) Y.De Smet & C.Granados, distributed mainly in south-central China and southeast China.

Hydrangea subg. *Dichroa* (Lour.) X.D.Yang & S.X.Yu, **stat. nov.** $\equiv$ *Dichroa* Lour., Fl. Cochinch.: 301. 1790. – Type: *H. hirta* (Thunb.) Siebold.

Diagnostic characters. – Mainly small shrubs, usually 0.3–2 m tall, rarely up to 4 m. Leaves opposite, rarely alternate distally, petiolate, papery or membranous, with apex acuminate or acute and caudate apiculate. Inflorescences cymes or panicles with bisexual or unisexual flowers. Enlarged marginal sepals 3–4 or absent. Stamens 8–12, rarely up to 20, with subequal length. Ovary 1/3 to 2/3 superior. Styles 3–4, rarely 5–6. Capsule or berry with nonconical projected apex. Seeds netted veined, wingless, or sometimes with very short wings.

Taxa and distribution. – Eight sections, *Hydrangea* sect. *Broussaisia* (Gaudich.) Y.De Smet & Samain, sect. *Chinenses* Y.De Smet & Samain, sect. *Dichroa* (Lour.) Y.De Smet & Samain, sect. *Hirtae* Y.De Smet & Samain, sect. *Kawagoeanae* X.D.Yang & S.X.Yu, sect. *Kwangsienses* X.D.Yang & S.X.Yu, sect. *Macrophyllae* (E.M.McClint.) Y.De Smet & Samain and sect. *Stylosae* Y.De Smet & Samain, restricted to Asia. Comprising more than 28 species.

Hydrangea sect. *Broussaisia* (Gaudich.) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *Broussaisia* Gaudich., Voy. Uranie: 479. 1830 – Type: *H. arguta* (Gaudich.) Y.De Smet & C.Granados.

Hydrangea sect. *Chinenses* Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *H.* sect. *Petalanthae* Maxim. in Mém. Acad. Imp. Sci. Saint Pétersbourg, Sér. 7, 10 (16): 6. 1867, nom. illeg. (Art. 22.2) – Type: *H. chinensis* Maxim.

Hydrangea sect. *Dichroa* (Lour.) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *Dichroa* Lour., Fl. Cochinch.: 301. 1790 – Type: *H. febrifuga* (Lour.) Y.De Smet & C.Granados.

Hydrangea sect. *Hirtae* Y.De Smet & Samain in Taxon 64(4): 750. 2015 – Type: *H. hirta* (Thunb.) Siebold.

Hydrangea sect. *Kawagoeanae* X.D. Yang & S.X. Yu, sect. nov. – Type: *H. kawagoeana* Koidz.

Diagnostic characters. – Shrub, 1–2(–4) m tall. Leaf blade oblong to broadly lanceolate, papery or herbaceous, secondary veins 3–7 on both sides of midvein, base cuneate. Enlarged marginal sepals 4 or 5. Stamens 10. Ovary superior. Styles 3–4. Capsule with nonconical projected apex. Seeds wingless or with short membranous wings.

Taxa and distribution. – Comprising more than 4 species, e.g., *Hydrangea kawagoeana* Koidz., *H. luteovenosa* Koidz., *H. macrosepala* Hay., *H. scandens* (L.f.) Ser., distributed predominantly in Taiwan and Japan.

Hydrangea sect. *Kwangsienses* X.D. Yang & S.X. Yu, sect. nov. – Type: *H. kwangsiensis* Hu.

Diagnostic characters. – Shrub, 1–3 m tall. Leaf blade lanceolate or broadly lanceolate, slightly asymmetric, papery, secondary veins 6–8(–11) on both sides of midvein, margin slightly reflexed and subentire or sparsely denticulate, apex caudate-acuminate. Enlarged marginal sepals (3 or) 4 (or) 5. Stamens 10, equal to or slightly longer than petals. Ovary ca. 1/5 superior. Styles 3, spreading to suberect, subulate. Capsule with nonconical projected apex. Seed wingless or sometimes with short wings at one or both ends.

Taxa and distribution. – Monotypic, *Hydrangea kwangsiensis* Hu, distributed mainly in central and southern China.

Hydrangea sect. *Macrophyllae* (E.M.McClint.) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *H.* subsect. *Macrophyllae* E.M.McClint. in J. Arnold Arbor. 37: 374. 1956 – Type: *H. macrophylla* (Thunb.) Ser.

$\equiv$ *Hortensia* sect. *Hortensia* H.Ohba & S.Akiyama in J. Jap. Bot. 91: 345–350. 2016 – Type: *Hortensia opuloides* Lam.

Hydrangea sect. *Stylosae* Y.De Smet & Samain in Taxon 64(4): 750. 2015 – Type: *H. stylosa* Hook.f. & Thomson.

Hydrangea subg. *Heteromallae* (Rehder) X.D. Yang & S.X. Yu, stat. nov. $\equiv$ *H.* subsect. *Heteromallae* Rehder in Sargent, Pl. Wilson. 1: 37. 1911 – Type: *H. heteromalla* D.Don.

Diagnostic characters. – Shrubs or small trees, 1–10 m tall. Branchlets brown to grey-brown. Leaves opposite, petiolate, papery; secondary veins 6–10 pairs; margins serrate. Corymbose or paniculate cymes with bisexual flowers. Enlarged marginal flowers with entire or denticulate sepals 4. Styles 3 or 4 with base usually connate, erect, or slightly spreading. Stamens usually 10. Ovary 1/2 or 1/3 superior. Capsule with conical projected apex. Seeds longitudinally veined with long wings at both ends.

Taxa and distribution. – Monotypic, *Hydrangea* sect. *Heteromallae* (Rehder) C.F. Wei, distributed mainly in Asia, including China, Japan, Nepal, northeastern India, Sikkim and Bhutan. Comprising c. 10 species.

Hydrangea sect. *Heteromallae* (Rehder) C.F. Wei in Guihaia 14(2): 111. 1994 $\equiv$ *H.* subsect. *Heteromallae* Rehder in Sargent, Pl. Wilson. 1: 37. 1911 $\equiv$ *Heteromalla* (Rehder) H.Ohba & S. Akiyama in J. Jap. Bot. 91(6): 349. 2016 – Type: *H. heteromalla* D.Don.

Hydrangea subg. *Hydrangea* – Type: *H. arborescens* L.

Diagnostic characters. – Shrubs or small trees, rarely climbing shrubs, 1–6 m tall, sometimes up to 15 m. Branchlets brown or reddish brown. Leaves opposite, petiolate, usually papery, with (roughly) serrate margins. Secondary veins 6–10 pairs, sometimes up to 13 pairs, (slightly) curved. Inflorescences cymes corymbose with bisexual flowers. Enlarged marginal sepals often 4, rarely absent. Styles 2, rarely 3. Ovary completely inferior. Capsule apex truncated. Seeds longitudinally veined and winged at both ends.

Taxa and distribution. – Four sections, *Hydrangea* sect. *Asperae* (Rehder) Y.De Smet & Samain, sect. *Calyptanthae* Maxim., sect. *Cornidia* (Ruiz & Pav.) Engl. and sect. *Hydrangea*, dominantly in Asia and the Americas. Comprising more than 32 species.

Hydrangea sect. *Asperae* (Rehder) Y.De Smet & Samain in Taxon 64(4): 750. 2015 $\equiv$ *H.* subsect. *Asperae* Rehder in Sargent, Pl. Wilson. 1: 39. 1911 – Type: *H. aspera* Buch.-Ham. ex D.Don.

$\equiv$ *Platycrater* Siebold & Zucc., Fl. Jap. 1: 64, t. 27. 1835 – Type: *P. arguta* Siebold & Zucc.

Hydrangea sect. *Calyptanthae* Maxim. in Mém. Acad. Imp. Sci. Saint Pétersbourg, Sér. 7, 10(16): 6. 1867 – Type: *H. scandens* Maxim.

Hydrangea sect. *Cornidia* (Ruiz & Pav.) Engl., Nat. Pflanzenfam. III(2a): 76. 1891 $\equiv$ *Cornidia* Ruiz & Pav., Fl. Peruv. Prodr.: 53, pl. 35. 1794 – Type: *H. preslii* Briq.

Hydrangea sect. *Hydrangea* – Type: *H. arborescens* L.

CONCLUSION

Based on extensive taxon and DNA sampling, this study constructed a robust phylogenetic framework for *Hydrangea* s.l. and recognized several new clades for the first time, resulting in a more comprehensive delimitation of infrageneric divisions than was previously available. We examined morphological characters commonly used in traditional taxonomic revisions and found that, with the exception of a few characters identified as synapomorphies, most were homoplasious. Given the difficulty of circumscribing infrageneric divisions based on synapomorphies alone, we combined several characters to define subgenera and sections. Finally, based on the principles of monophyly and synapomorphy, as well as combinations of key diagnostic characters, we proposed an updated classification that divides *Hydrangea* s.l. into 5 subgenera and 19 sections, of which 5 subgenera and 3 sections are newly proposed. Based on our in-depth analysis of systematically significant morphological characters within our robust phylogenetic framework, we believe that the results in this study are crucial for the further understanding of the genus *Hydrangea*.

AUTHOR CONTRIBUTIONS

SXY, SRG, EFS and XDY designed the research, SXY supervised the project; SXY, XDY, TTX and YFL collected the materials and carried out the experiments; XDY, TTX, TGG and SXY performed the data analyses; XDY, SXY, SRG, EFS and TGG drafted the manuscript; SXY, XDY, SRG, EFS, TGG, TTX and YFL revised the manuscript. All authors read and approved the final manuscript.

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Appendix 1. Voucher information and GenBank accession numbers for taxa used.

Voucher information is given for accessions using the following order: taxon name, collection country (province/state), collector(s) and collection number (herbarium code), GenBank accession number for plastid genome.

Hydrangea alternifolia Siebold, Japan: Honshu, M. Amano, 1287 (PE), OR102021; *Hydrangea anomala* D.Don, China: Sichuan, W.B. Xu & al., Y12190 (PE), OR102020; *Hydrangea aspera* Buch.-Ham. ex D.Don, China: Yunnan, W.B. Xu & al., QTP132 (PE), OR102019; *Hydrangea aspera* Buch.-Ham. ex D.Don, China: Yunnan, W.B. Xu & al., QTP207 (PE), OR102018; *Hydrangea barbara* (L.) Bernd Schulz, voucher not available, NC_044832; *Hydrangea bifida* (Maxim.) Y.De Smet & C.Granados, Japan: Tokyo, T. Yamazaki, 6807 (PE), OR102017; *Hydrangea bretschneideri* Dippel, China: Hebei, S.X. Yu & al., Y12027 (PE), OR102016; *Hydrangea caerulea* (Stapf) Y.De Smet & C.Granados, China: Hubei, X.C. Zhang & al., 12014 (PE), OR102015; *Hydrangea chinensis* Maxim., China: Taiwan, S.T. Chiu & al., 1719658 (PE), OR102013; *Hydrangea chinensis* Maxim., Japan: Kyushu, M. Furuse, 13168 (PE), OR102012; *Hydrangea chinensis* Maxim. var. *chinensis*, China: Jiangxi, X.D. Yang, Y15136 (PE), OR102014; *Hydrangea chinensis* var. *yayeyamensis* (Koidz.) T.Yamaz., Japan: Okinawa, Koji Yonekura & Keiko Yasuda, 11327 (PE), OR102011; *Hydrangea chungii* Rehder, China: Fujian, X.D. Yang, Y15246 (PE), OR102010; *Hydrangea corylifolia* (Chun) Y.De Smet & C.Granados, China: Zhejiang, Anonymous, 0838 (PE), OR102009; *Hydrangea crassa* (Hand.-Mazz.) Y.De Smet & C.Granados, China: Guangxi, G.M. Feng, 7268 (PE), OR102008; *Hydrangea daimingshanensis* (Y.C.Wu) Y.De Smet & C.Granados, China: Guangxi, P. Yang & al., 450125200528012LY (IBK), OR102007; *Hydrangea davidii* Franch., China: Sichuan, W.B. Xu & al., Y12180 (PE), OR102006; *Hydrangea densifolia*

Appendix 1. Continued.

(C.F.We) Y.De Smet & C.Granados, China: Taiwan, *Y.C. Kao*, 56 (PE), OR102005; *Hydrangea densifolia* (C.F.We) Y.De Smet & C.Granados, voucher not available, NC_044804; *Hydrangea fauriei* (Hayata) Y.De Smet & C.Granados, China: Taiwan, *H.L. Jiang*, 745 (PE), OR102004; *Hydrangea febrifuga* (Lour.) Y.De Smet & C.Granados, China: Jiangxi, *X.D. Yang*, Y15278 (PE), OR102002; *Hydrangea febrifuga* (Lour.) Y.De Smet & C.Granados, China: Yunnan, *Qinghai-Tibet Plateau Expedition*, 8894 (PE), OR102003; *Hydrangea glaucescens* (Rehder) Y.De Smet & C.Granados, China: Guangxi, *X.D. Yang*, 20210716 (PE), OR102000; *Hydrangea glaucescens* (Rehder) Y.De Smet & C.Granados, China: Guangxi, *Z.Z. Chen*, 51278 (IBK), OR102001; *Hydrangea glaucescens* (Rehder) Y.De Smet & C.Granados, China: Yunnan, *Northeast Yunnan Expedition*, 740 (PE), OR101999; *Hydrangea heteromalla* D.Don, China: Xizang, *W.B. Xu & al.*, QTP331 (PE), OR101998; *Hydrangea hirsuta* (Gagnep.) Y.De Smet & C.Granados, China: Guangxi, *Plant Expedition on Hongshui River*, 357 (PE), OR101997; *Hydrangea hirta* Siebold, Japan: Kanagawa, *T. Miyazaki*, 0806246 (PE), OR101996; *Hydrangea hydrangeoides* (Siebold & Zucc.) Bernd Schulz, Japan: Tottori, *K. Inoue*, 100750 (PE), OR101995; *Hydrangea hypoglauca* Rehder, China: Fujian, *X.D. Yang*, Y15235 (PE), OR101994; *Hydrangea integrifolia* Hayata, China: Taiwan, *B. Bartholomew*, 7731 (PE), OR101993; *Hydrangea involucrata* Siebold, Japan: Kanagawa, *T. Miyazaki*, 0807323 (PE), OR101992; *Hydrangea kawagoeana* Koidz., Japan: Kyushu, *T. Yamazaki*, 5909 (PE), OR101991; *Hydrangea kwangsiensis* Hu, China: Guangdong, *X.D. Yang*, Y15320 (PE), OR101990; *Hydrangea kwangtungensis* Merr., China: Guangxi, *Z.C. Lu*, 092311 (IBK), OR101989; *Hydrangea lingii* G.Hoo, China: Fujian, *X.D. Yang*, Y15243 (PE), OR101987; *Hydrangea lingii* G.Hoo, China: Hunan, *X.D. Yang*, Y15088 (PE), OR101986; *Hydrangea linkweiensis* Chun, China: Guangxi, *X.D. Yang*, Y16001 (PE), OR101985; *Hydrangea liukiensis* Nakai, Japan: Ryukyu, *K. Kondo*, 2633 (PE), OR101984; *Hydrangea longifolia* Hayata, China: Taiwan, *J.Y. Huang & al.*, 2792 (PE), OR101983; *Hydrangea longipes* Franch., China: Guangxi, *X.D. Yang*, Y15048 (PE), OR101982; *Hydrangea luteovenosa* Koidz., voucher not available, NC_035662; *Hydrangea mangshanensis* C.F.We, China: Hunan, *B.Z. Xiao*, 3600 (PE), OR101981; *Hydrangea moellendorffii* Hance, China: Jiangxi, *X.D. Yang*, 15155 (PE), OR101980; *Hydrangea moellendorffii* Hance, China: Guangxi, *Y. Liu & al.*, 450332151016006LY (IBK), OR101979; *Hydrangea mollissima* (Merr.) Y.De Smet & C.Granados, China: Hainan, 236-6 troops, 01747 (PE), OR101978; *Hydrangea obtusifolia* (Hu) Y.De Smet & C.Granados, China: Hubei, *Plant Survey Team of Shennongjia*, 20543 (PE), OR101977; *Hydrangea paniculata* Siebold, China: Guangxi, *X.D. Yang*, Y15016 (PE), OR101976; *Hydrangea petiolaris* Siebold & Zucc., Korea, *B.U. Oh & al.*, 2380957 (PE), OR101975; *Hydrangea platyarguta* Y.De Smet & Samain, voucher not available, NC_044806; *Hydrangea quercifolia* W.Bartram, U.S.A.: Oregon, *R.R. Halse*, 9590 (PE), OR101974; *Hydrangea radiata* Walter, U.S.A.: Connecticut, *E.H. Eames*, 06268 (PE), OR101973; *Hydrangea robusta* Hook.f. & Thomson, China: Jiangxi, *X.D. Yang*, Y15114 (PE), OR101972; *Hydrangea sargentiana* Rehder, China: Guizhou, *S.X. Yu & al.*, 8537 (PE), OR101971; *Hydrangea schizomollis* Y.De Smet & C.Granados, China: Chongqing, *Z.Y. Liu & al.*, 12976 (PE), OR101970; *Hydrangea serrata* (Thunb.) Ser., Japan: Fukui, *M. Furuse*, 51922 (PE), OR101968; *Hydrangea serrata* (Thunb.) Ser., Japan: Tokyo, *S. Okuyama*, 1296 (PE), OR101969; *Hydrangea serrata* (Thunb.) Ser., voucher not available, KU140669; *Hydrangea stenophylla* Merrill & Chun, China: Jiangxi, *X.D. Yang*, Y15110 (PE), OR101967; *Hydrangea strigosa* Rehder, China: Sichuan, *W.B. Xu & al.*, Y12187 (PE), OR101966; *Hydrangea sungpanensis* Hand.-Mazz., China: Sichuan, *W.B. Xu & al.*, Y12166 (PE), OR101965; *Hydrangea tomentella* (Hand.-Mazz.) Y.De Smet & C.Granados, China: Guangdong, *X.D. Yang*, Y15353 (PE), OR101964; *Hydrangea viburnoides* (Hook.f. & Thomson) Y.De Smet & C.Granados, China: Chongqing, *G.F. Li*, 64254 (PE), OR101963; *Hydrangea xanthoneura* Diels, China: Sichuan, *W.B. Xu & al.*, Y12192 (PE), OR101962; *Hydrangea yaoshanensis* (Y.C.Wu) Y.De Smet & C.Granados, China: Hunan, *X.D. Yang*, Y15061 (PE), OR101961; *Deutzia compacta* Craib, China: Yunnan, *T. Zhang & al.*, 10CS2104 (KUN), MN380704; *Deutzia crassifolia* Rehder, voucher not available, MG524993; *Deutzia glabrata* Kom., voucher not available, MN872800; *Philadelphus calvescens* (Rehder) S.M.Hwang, China: Yunnan, *Anonymous*, ZDQ17030 (Herbarium of Medicinal Plants and Crude Drugs of the College of Pharmacy and Chemistry, Dali University), MN486873.

Appendix 2. GenBank accession numbers for six plastid sequences for taxa used in this study.

Voucher information is given for accessions using the following order: taxon name, origin, GenBank accession number for *matK*, *ndhA*, *rpl32-ndhF*, *rps16-trnK*, *trnL-rpl32*, and *trnV-ndhC*. En-dash (–) indicates missing sequence.

Hydrangea arborescens L., North America, MK609001, LN830484, LN830559, GU217285, LN830604, LN830310; *Hydrangea arguta* (Gaudich.) Y.De Smet & C.Granados, Hawaii, JF308681, LN830487, LN830563, GU217311, LN830637, LN830344; *Hydrangea chinensis* var. *lobbii* (Maxim.) Kitam., China, Philippines, GU217330, LN830428, LN830503, GU217330, LN830578, LN830284; *Hydrangea cinerea* Small, North America, –, LN830455, –, GU217287, LN830605, –, *Hydrangea jelskii* Szyszyl., South America, –, LN830478, LN830553, –, LN830628, LN830334; *Hydrangea seemannii* L. Riley, Mesoamerica, GU217303, LN830444, LN830519, GU217303, LN830594, LN830300; *Hydrangea sikokiana* Maxim., Japan, GU217291, LN830446, LN830549, GU217291, LN830596, LT839023; *Hydrangea stylosa* Hook.f. & Thomson, Asia, GU217312, LN830462, LN830537, GU217312, LN830612, LN830318.