Zhengyia shennongensis: A new bulbiliferous genus and species of the nettle family (Urticaceae) from central China exhibiting parallel evolution of the bulbil trait

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Abstract *Zhengyia shennongensis* is described here as a new genus and species of the nettle family (Urticaceae) from Hubei province, central China. The phylogenetic position of *Z. shennongensis* is determined using DNA sequences of nuclear ribosomal ITS and three plastid regions (*rbcL*, *psbA-trnH*, *trnL-F*). *Zhengyia shennongensis* is readily distinguished from the related genera *Urtica*, *Hesperocnide*, and *Laportea* in the tribe Urticeae by its seed (oblong-globose or subglobose and not compressed achenes, surface densely covered with nipple-shaped protuberances) and stipule morphology (large leaf-like stipules with auriculate and amplexicaulous base and united with stem). Phylogenetic evidence indicates that *Zhengyia* is a distinct group related to *Urtica* (including *Hesperocnide*) species and *Laportea cuspidata* in tribe Urticeae. The bulbiliferous species of the tribe (*L. bulbifera*, *L. cuspidata*, *Z. shennongensis*) do not form a clade. This result indicates that the bulbil trait evolved in parallel within Urticeae. Our findings highlight the importance of shady and moist habitats in promoting species diversification and the parallel evolution of morphological traits that are likely to be adaptive.

Keywords bulbils; central China; new genus and species; parallel evolution; Urticaceae; Urticeae; *Zhengyia shennongensis*

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■ INTRODUCTION

Urticeae (= Urerareae Wedd.) is a moderately sized tribe of the nettle family (Urticaceae) with eleven genera and approximately 220 species. Its members are often found in humid habitats in forests or at forest margins and occur in both the Old and New Worlds (Friis, 1993; Hadiah & al., 2008; Cohn & Hadiah, 2009). The tribe is characterized by stinging hairs and pistillate flowers with four tepals, of which frequently one pair is larger than the other, and without staminodes (Friis, 1989, 1993). Because of the obvious morphological synapomorphies of Urticeae, it is not difficult to recognize a plant as being a member of this tribe. Moreover, phylogenetic analysis of Urticaceae using plastid DNA sequence data has shown that Urticeae (including *Poikilospermum* Zipp.) form a well-supported clade (Hadiah & al., 2008).

The Shennongjia National Nature Reserve (SNNR) is located in the Northwest of Hubei province, central China. Its unique geographical location and complex topology make it one of the most biodiverse areas in China (Ying, 2001; Xie, 2003). The Shennongjia Mountains are characterized by high mountains and deep valleys, a dense network of streams, and lush vegetation. The region is an important hot-spot for south-central Chinese biodiversity and contains many endemic plants (Myers & al., 2000). During our recent in-depth floristic explorations of the SNNR, an unusual taxon caught our attention. The plant was easily identified as belonging to Urticeae based on the presence of stinging hairs, stipules, and pistillate flowers with four tepals and without staminodes. In its paniculate inflorescences with many long branches and its four-lobed perianth with larger dorsal than ventral lobes in its female flowers, it superficially resembles *Urtica* L., a genus of about 30 species with a wide distribution in the northern temperate region (Chen & al., 2003). However, based on its alternate leaf arrangement, the presence of one to three woody bulbils in sterile axils, intrapetiolar stipules in the leaf axils, and oblique achenes with short stipes, we initially assigned the new taxon to *Laportea* Gaudich., a genus with 30 species confined to tropical and warm-temperate E Asia and eastern North America (Friis, 1993). Upon closer examination, however, it was clear that the set of morphological characters did not match *Urtica*, *Laportea* or any other genus of Urticeae (Table 1). The plant is described below as a new genus with only one species, *Zhengyia shennongensis* T. Deng, D.G. Zhang & H. Sun.

Bulbils are specialized propagules, allowing vegetative reproduction and dispersal, and many herbaceous plants can produce them (Wang & al., 2004; Walck & al., 2010). Presence or absence of bulbils has been recognized as a significant

morphological trait in species delimitation in Urticeae (Chen & al., 2003). The character is also useful to establish infrageneric taxa in genera of Urticeae (e.g., *Laportea*; Chen & al., 2003). To date, in Urticeae only two species of *Laportea* (*L. bulbifera* Weed., *L. cuspidata* Friis) have been reported to be bulbiliferous; they both grow in shady, moist conditions (Chen & al., 2003).

In the present study, we determine the phylogenetic position of the new taxon based on morphological data, especially surface features of the achene examined using scanning electron microscopy (SEM), and cytological and molecular data (the three plastid regions *rbcL*, *psbA-trnH* intergenic spacer [IGS], and *trnL-F* IGS; and nuclear ribosomal ITS [nrITS]). Based on the inferred phylogeny, we also provide a discussion of the evolution of the bulbil trait in Urticeae.

■ MATERIALS AND METHODS

Plant material. — Samples of *Z. shennongensis* were collected for morphological comparison from the only known population—Wushanhu, Hubei Province—during our field investigations in 2011 (Fig. 1). Leaves and mature seeds were also collected for SEM and cytological and molecular phylogenetic studies. For comparison with the seed morphology of *Z. shennongensis*, eight species of the closely related *U. mairei* H. Lév, *U. dioica* L., *U. fissa* E. Pritz., *U. urens* L., *L. bulbifera* Wedd., *L. cuspidata* Friis, *L. canadensis* Gaudich., and *Girardinia diversifolia* (Link) Friis were examined.

In order to determine phylogenetic relationships in Urticeae using molecular markers, we sampled 16 taxa (21 accessions) in addition to *Z. shennongensis*, including two species of *Dendrocnide* Miq. (two accessions), three subspecies of *Girardinia diversifolia* (three accessions), one species of *Hesperocnide* Torr. (one accession), three species of *Laportea* (five accessions), one species of *Poikilospermum* (one accession), and six species of *Urtica* (nine accessions). We also included three species, *Pilea plataniflora* C.H. Wright of Elatostemateae Gaudich., *Boehmeria spicata* Thunb. of Boehmerieae Gaudich., and *Fatoua villosa* Nakai of Moraceae as outgroups, based on a previous phylogenetic analysis using *rbcL* and *trnL* DNA sequence data (Hadiah & al., 2008). Voucher information and GenBank accession numbers for all specimens used in this study are listed in Appendix 1.

Seed observation. — The mature seeds of 90 individuals of the species listed above and our new taxon were mounted on aluminum stubs with double-sided adhesive tape, sputter-coated with gold to a maximum thickness of 20 μm, and examined using a KYKY-1000B scanning electron microscope (SEM; Science Instrument Company, Beijing, China) with a voltage of 30 kV. Microphotographs focused primarily on the center of the seeds. Seed morphology was also examined under the dissecting microscope (OLYMPUS BX53).

Cytological studies. — Root-tip meristems were obtained by germinating seeds on wet filter paper in Petri dishes at approximately 20°C. Root tips less than 1.5 cm long were cut and treated with 0.002 M 8-hydroxyquinoline at room temperature for 3–5 h before being fixed in Carnoy (glacial acetic acid: absolute ethanol = 1 : 3), macerated in a 1 : 1 mixture of 45% acetic acid and 1 M HCl for 2.5 min, and stained and squashed in Carbol Fuchsine. Karyotypes of mitotic chromosomes at metaphase were determined from at least five well-spread

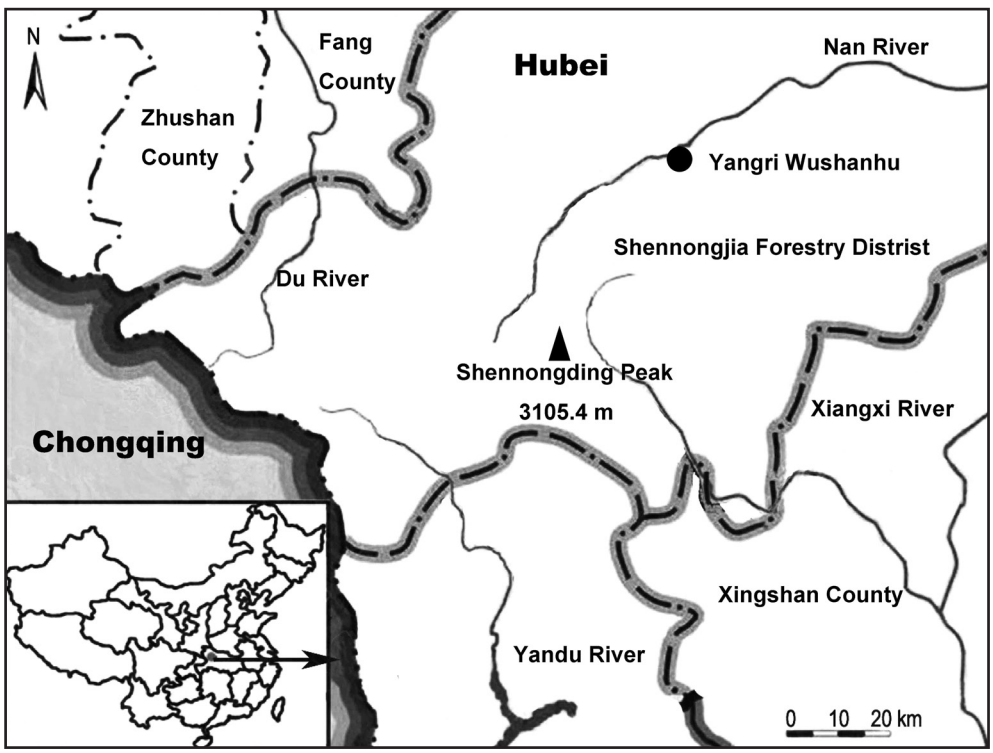

Fig. 1. Distribution of *Zhengyia shennongensis* T. Deng, D.G. Zhang & H. Sun. The circle indicates the type locality of *Z. shennongensis*.

metaphases of three different roots. The designation of the centromere position as median (m), submedian (sm) and subterminal (st) followed Levan & al. (1964).

DNA extraction, PCR amplification, and sequencing. — Total genomic DNA was isolated from silica gel-dried leaf material using the Universal Genomic DNA Extraction Kit (Takara, Dalian, China). Primer sets and protocols for PCR followed specifications in previous publications: *rbcL* (primers Z1 and 1204R; Zurawski & al., 1981; from G. Zurawski [DNAX Research Institute, Palo Alto, California, U.S.A.]), *psbA-trnH* IGS (psbA_F and trnH_R; Hamilton, 1999), *trnL-F* IGS (e and f; Taberlet & al., 1991), and nrITS (ITS1 and ITS4; White & al., 1990; Kim & al., 2010). Amplified DNA samples were analyzed by electrophoresis on 1.4% agarose gel, run in a 0.5× TBE buffer and detected by ethidium bromide staining. The PCR products were then purified using a QiaQuick gel extraction kit (Qiagen, Inc., Valencia, California, USA) and directly sequenced in both directions using the amplification primers on an ABI 3730 automated sequencer (Applied Biosystems, Forster City, California, U.S.A.).

Phylogenetic analyses. — DNA Baser v.3 (http://www.dnabaser.com) was used to evaluate the chromatograms for base confirmation and to edit contiguous sequences. Multiple-sequence alignment was performed with MAFFT v.6 (Katoh & al., 2009; available at http://www.genome.jp/tools/mafft) using the default alignment parameters. Gaps were coded as missing data. All datasets have been submitted to TreeBASE (http://www.treebase.org/; study accession number, S12631).

The phylogenetic reconstruction of the sequences was performed by maximum parsimony (MP) in PAUP* v.4.0b10 (Swofford, 2002). All characters were weighted equally and unordered. Each dataset was analyzed separately and then a simultaneous analysis was performed including all four regions. Before combining the datasets, the incongruence length difference (ILD) test was conducted to assess data congruency (Farris & al., 1995) using PAUP* and 10,000 heuristic search replications including only parsimony-informative characters. Most parsimonious trees were searched with a heuristic algorithm using tree bisection-reconnection branch swapping, MULPARS, and the alternative character state. Strict consensus trees were constructed from the most parsimonious trees. Bootstrap analyses (BP; 1000 pseudoreplicates) were conducted to examine the relative level of support for individual clades (Felsenstein, 1985). The consistency index (CI; Kluge & Farris, 1969) and retention index (RI; Farris, 1989) were calculated to measure the amount of homoplasy in the dataset.

Phylogenetic analyses of the nrITS and combined datasets were also conducted using Bayesian Markov chain Monte Carlo (MCMC) inference (BI; Yang & Rannala, 1997) using MrBayes v.3.12 (Ronquist & Huelsenbeck, 2003). Modeltest v.3.1 (Posada & Crandall 1998) was used to determine the optimal model of DNA evolution for the data based on the Akaike information criterion (AIC; Akaike, 1974). Four chains of the MCMC inference were run simultaneously, with sampling every 100 generations over a total of one million generations. The first 2500 trees (25%) of the sample trees from each run were discarded as determined by Tracer v.1.5 (Rambaut & Drummond, 2007). A Bayesian consensus tree was constructed from the remaining trees, yielding the posterior probability (PP) values for each clade.

The single most parsimonious topology obtained from the analysis of the combined molecular data (nrITS and three plastid DNA regions) was employed to reconstruct the evolution of the bulbil character. Character reconstruction was carried out under the assumption of unordered and unweighted character states with the Ancestral State Reconstruction Package in Mesquite v.2.75 (Maddison & Maddison, 2011) using unambiguous optimization.

■ RESULTS

Morphological characters. — Morphological characters of *Z. shennongensis* and related genera, including *Dendrocnide*, *Girardinia*, *Hesperocnide*, *Laportea*, *Poikilospermum*, and *Urtica*, are listed in Table 1. The seed characteristics of our new taxon, including shape and surface sculpturing, were found to be unique when compared to the other genera. The achene shape of *Z. shennongensis* was oblong-globose or subglobose and extremely asymmetrical, and no infraspecific variation was found (Fig. 2A). The seed surface of *Z. shennongensis* is densely covered with nipple-shaped protuberances but smooth and/or verrucose in the other genera (Fig. 2).

Chromosome counts and karyomorphology. — The chromosome number in mitotic metaphase cells was found to be $2n = 24$, and the karyotype formula is $2n = 6m+16sm+2st$ (Fig. 3).

Phylogenetic analyses. — The characteristics and statistics for nrITS, the three plastid regions, and the combined datasets for the MP analyses are presented in Table 2. Bayesian analyses of all datasets resulted in the same tree topologies as the corresponding MP analyses (data not shown). All MP trees were generally congruent with respect to well-supported clades, but there was an incongruence between the plastid and nrITS analyses concerning the position of *Z. shennongensis*. The combined plastid analysis resolved both *Z. shennongensis* samples as well-supported sister to *Urtica* and *Hesperocnide* species (BP = 92%, PP = 1.00), whereas in the nrITS tree the species was sister to a clade including *Urtica*, *Hesperocnide*, and *Laportea* species but with low statistical support (BP = 50%, PP = 0.64; data not shown).

ILD tests failed to identify significant conflict among the three partitions of the plastid dataset (*rbcL*, *psbA-trnH*, *trnL-F*; $P = 0.065$) and between the nrITS and the plastid datasets ($P = 0.052$). When all molecular datasets were combined, the single MP tree found was better resolved than any tree from separate analyses. Phylogenetic analysis of the combined dataset resulted in a single most parsimonious tree (tree length = 2302, CI = 0.597, RI = 0.756). In the MP tree, tribe Urticeae formed a monophyletic group (BP = 100%, PP = 1.00; Fig. 4). The two individuals of *Z. shennongensis* were sister to *Urtica* (including *Hesperocnide*) species, with high statistical support (BP = 92%, PP = 1.00). *Laportea cuspidata* was sister to the clade comprising *Zhengyia* and *Urtica*+*Hesperocnide* species (BP =

86%, PP = 1.00). The bulbiliferous species (*L. bulbifera*, *L. cuspidata*, and *Z. shennongensis*) were not closely related to each other (Fig. 4).

■ DISCUSSION

Systematic position of *Zhengyia shennongensis*. — According to the classification of Urticaceae by Friis (1989, 1993), our new taxon *Z. shennongensis* is a member of tribe Urticeae. In tribe Urticeae, the basic chromosome number most often is $x = 12$ and 13, and less often $x = 10$, 11, and 19 (e.g. Woodland & al., 1976, 1982; Friis, 1993). The chromosome number of *Z. shennongensis* was found to be $2n = 24$ ($x = 12$) in this study (Fig. 3). Thus, cytological evidence supports that our new species should be included in Urticeae. Moreover, our molecular phylogenetic results clearly confirmed that *Z. shennongensis* is part of Urticeae (Fig. 4).

In general, genera of Urticeae have been recognized primarily on the basis of stipule and fruit shape (Friis, 1993; Chen & al., 2003). *Zhengyia shennongensis* has a distinctive oblong-globose or subglobose achene with dense nipple-shaped

Table 1. Morphological comparison of *Zhengyia* with other genera in Urticeae.

Character	*Zhengyia*	*Dendrocnide*	*Girardinia*	*Hesperocnide*	*Laportea* I[d]	*Laportea* II[e]	*Poikilospermum*	*Urtica*
Habit[a,b]	robust herb	shrub	robust herb	herb	herb	herb	shrub or woody climber	herb
Bulbils[a,b]	present	absent	absent	absent	present	absent or present in *L. bulbifera*	absent	absent
Leaf arrangement[a,b]	alternate	alternate	alternate	opposite	alternate	alternate	alternate	opposite
Stipules[a,b]	intrapetiolar, auriculate-amplexicaulous base united with the stem, persistent	intrapetiolar, subulate or linear, deciduous	intrapetiolar, subulate or linear, deciduous	lateral, subulate or linear, persistent	intrapetiolar, subulate or linear, deciduous	intrapetiolar, subulate or linear, deciduous	intrapetiolar, subulate or linear, deciduous	lateral, subulate or linear, persistent
Perianth[a]	deeply 4-lobed, one pair larger	4-lobed, lateral ones slightly larger	ovoid-tubular, (2–) 3-toothed	almost tubular, minutely 2-toothed at the apex	4-lobed, strongly unequal, lateral larger	4-lobed, one minute or absent	clavate-tubular	deeply 4-lobed, one pair larger
Inflorescences	pairs	solitary	solitary or pairs	pairs	solitary	solitary	solitary	pairs
Stigmas[a,b]	short clavate	linear or ligulate	subulate, acute, minute	capitate-penicillate	linear, papillose on one side	linear, papillose on one side	capitate or ligulate	capitate-penicillate
Achene symmetry[a,b]	asymmetric	asymmetric	asymmetric	symmetric	asymmetric	asymmetric	asymmetric	symmetric
Achene shape[a]	oblong-globose or subglobose, not compressed	elliposidal to ovoid, compressed	broadly ovoid, compressed	ovate, compressed	ovoid to semicircular, compressed	ovoid to semicircular, compressed	oblong, ellipsoid or ovoid, compressed	ovoid, compressed
Achene surface[a,c]	with dense nipple-shaped protuberances	verrucose	verrucose	unknown	smooth	smooth or with stripes	verrucose	smooth or verrucose with sunken dots

[a] Friis (1993) and Chen & al. (2003).
[b] Based on herbarium collections and field observation.
[c] Based on SEM.
[d] *Laportea* I includes *L. cuspidata*.
[e] *Laportea* II comprises two species (*L. bulbifera*, *L. interrupta*).

protuberances on the surface (Fig. 2A). The species also differs from other genera of Urticeae by having large leaf-like stipules with an auriculate-amplexicaulous base united with the stem (Figs. 4, 5E). These differences are reflected in the MP tree using the combined dataset where the new genus occupies a distinct position in Urticeae with maximum support (BP = 100%, PP = 1.00; Fig. 4). Therefore, the recognition of *Zhengyia* at the rank of genus is warranted based on morphological and molecular evidence.

Our phylogenetic analyses showed that *H. tenella* Torr. was nested in the *Urtica* clade. This close relationship is morphologically supported by opposite leaves, lateral stipules, and straight achenes (Table 1). *Zhengyia shennongensis* is closest relative of the *Urtica*+*Hesperocnide* clade with high support (BP = 92%, PP = 1.00; Fig. 4). Several morphological characters (e.g., herbs with persistent stipules, deeply 4-lobed perianth, inflorescences with many long branches) support these relationships. Within this clade, *Z. shennongensis* can be easily distinguished from *Urtica* and *Hesperocnide* species by its alternate leaf arrangement (vs. opposite), large stipules inserted in the axil of leaves (vs. 2 or 4 rather small and narrow, lateral stipules), and extremely oblique achenes (vs. erect; Table 1; Fig. 4).

Our molecular data do not support the monophyly of *Laportea*. *Laportea cuspidata* (*Laportea* I) is a sister to the *Zhengyia*+*Urtica* (including *Hesperocnide*) lineage but not to the other species of *Laportea* (*Laportea* II; Fig. 4). *Zhengyia shennongensis* with one to three woody bulbils in sterile leaf axils (Fig. 5F) resembles *L. cuspidata*, and these two species share other morphological characters such as alternate leaves and oblique achenes. However, the stigma of *Z. shennongensis* is short and clavate, while that of *L. cuspidata* is linear (Table 1). In addition, the achene surface of *Z. shennongensis* differs from that of *L. cuspidata* in having markedly nipple-shaped protuberances (Fig. 2A, G).

Of the other genera in tribe Urticeae, *Z. shennongensis* is similar to *Girardinia* in that both are robust herbs with long stinging hairs (>5 mm). However, *Z. shennongensis* is distinguished from *Girardinia* by three morphological characters: the presence of bulbils in leaf axils, branched inflorescences, and the ornamentation of the achene surface (Table 1; Fig. 4). *Zhengyia shennongensis* can be easily distinguished from *Dendrocnide* and *Poikilospermum* by habit (herbs vs. shrub or trees). Moreover, our molecular evidence shows that *Z. shennongensis* is not closely related to *Girardinia*, *Dendrocnide*, and *Poikilospermum* (Fig. 4).

Parallel evolution of bulbils. — Many herbaceous plants form bulbils (Okagami, 1979). Bulbils serve as a means of clonal reproduction with the ability to colonize and sequester resources quickly after initial introduction, particularly in isolated populations (Callaghan & al., 1997; Abrahamson, 1980). Although bulbils are a valuable reproductive property, they are found in only three species (*L. bulbifera*, *L. cuspidata*, *Z. shennongensis*) of Urticeae. In the combined MP tree, the three bulbiliferous species did not group together but were placed in three different clades, each with maximal support except *L. bulbifera* (BP = 57%, PP = 0.79; Fig. 4). Two

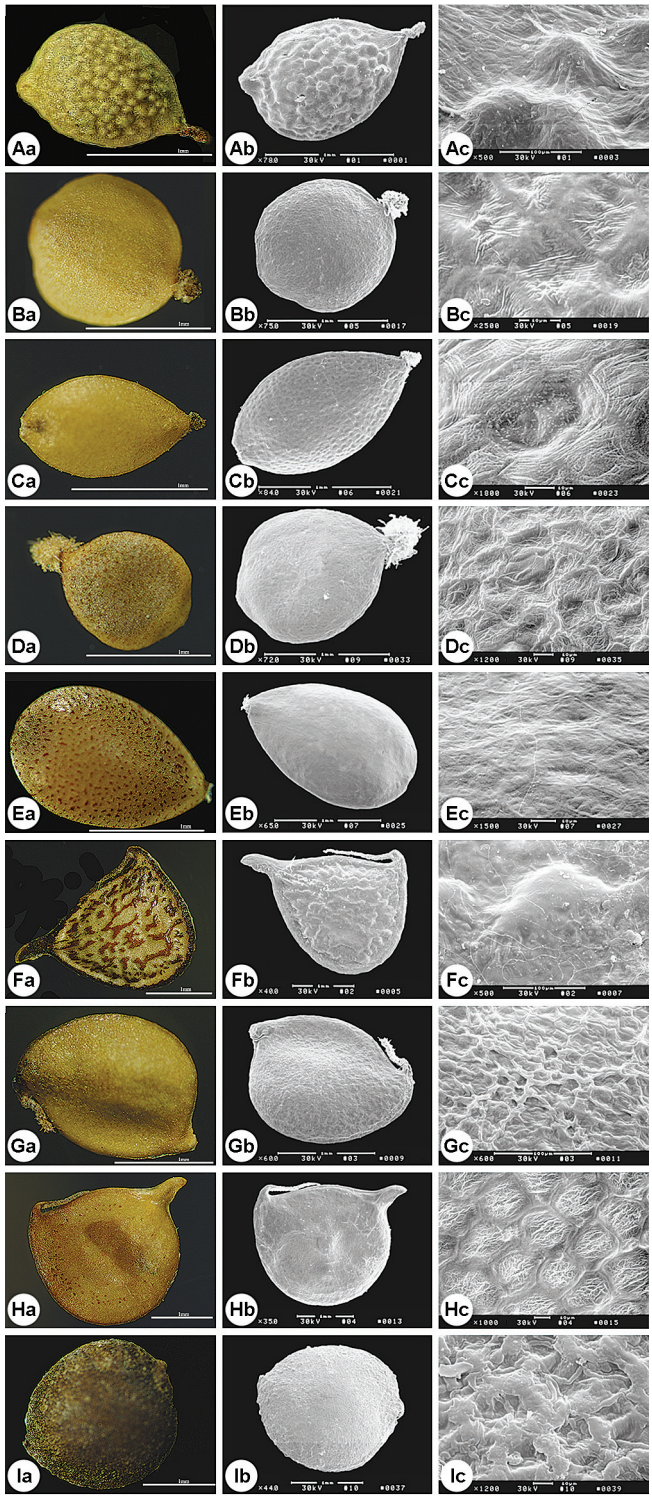

Fig. 2. Comparison of achene morphology and surface sculpting in tribe Urticeae. **A,** *Zhengyia shennongensis* (*Deng, Zhang & Sun 3431*; KUN); **B,** *Urtica mairei* (*Peng 1607*; KUN); **C,** *U. dioica* (*Cai 55102*; KUN); **D,** *U. fissa* (*Liu 16628*; KUN); **E,** *U. urens* (*Qingzhang Exped. 318*; KUN); **F,** *Laportea bulbifera* (*Zhangdian Exped. 2575*; KUN); **G,** *L. cuspidata* (*Qingzhang Exped. 13367*; KUN); **H,** *L. canadensis* (*Koyama 7401*; KUN); **I,** *Girardinia diversifolia* (*Nie & Deng 4248*; KUN). **a,** Dissecting microscope; **b,**SEM, low magnification; **c,** SEM, ultrastructure of seed surface.

reconstructions of bulbil evolution are equally parsimonious in our phylogenetic tree (Fig. 4). Either bulbils evolved three times independently in Urticeae or they evolved twice and were lost once.

Bulbils have been recorded in many families and also in different clades of single tribes (Givnish & al., 2000; Wang & al., 2004; Thomas & al., 2005; Kitahara & al., 2010) and may have originated in response to strong selection in shady, moist and pollinator-poor habitats (Wake & al., 2011), and indeed the three bulbiliferous species of Urticeae grow mainly in shady habitats along creeks, particularly on wet, dripping cliffs in valleys. This trait probably replaces propagation and dispersal by seeds or fruits. When compared with species without bulbils (e.g., *Urtica*, *Girardinia*), the bulbiliferous taxa appear to have less seed set as judged from our field observations and herbarium material, but statistical confirmation of this observation would require more detailed measurement. It might be that the wind-pollinated bulbiliferous taxa of Urticeae have evolved these propagules to cope with lack of seed set in the windless conditions of their extremely shady, humid habitats. Alternatively, the bulbils, which may be dispersed by gravity, water, animals, or birds (Thomas & al., 2005; Mizuki & Takahashi, 2009), may be better suited for shady habitats than seeds because they are much larger than normal seeds of Urticaceae and may store more nutrients needed for establishment.

■ TAXONOMIC TREATMENT

Zhengyia shennongensis T. Deng, D.G. Zhang & H. Sun, **gen. & sp. nov. –** Holotype: China, central China, Hubei province, Shennongjia Forest District (SNNR), Yangri town, Wushanhu, 31°32′37″ N, 110°50′35″ E, 450 m alt, 4 Sep 2011, *T. Deng, D.G. Zhang & H. Sun 2295* (KUN; isotypes: A, K, MO, PE). — Figures 5 and 6.

Description. – Perennial robust herbs with long stinging hairs. *Rhizomes* stoloniferous, up to 2 m long. *Stems* erect, 1–3 m tall, terete, not longitudinally angular or sulcate, slightly

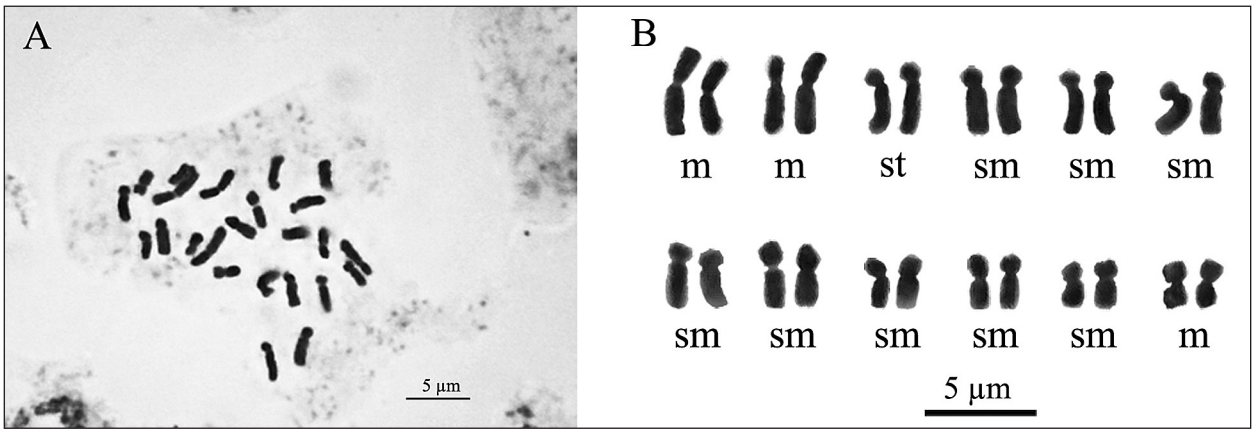

Fig. 3. Mitotic metaphase of *Zhengyia shennongensis* T. Deng, D.G. Zhang & H. Sun. **A,** Micrograph of metaphase chromosomes; **B,** karyotype of mitotic metaphase chromosomes.

Table 2. Tree statistics for the nrITS, *rbcL*, *psbA-trnH*, *trnL-F*, and combined datasets from maximum parsimony (MP) analysis.

					Combined	
Parameters	nrITS	*rbcL*	*psbA-trnH*	*trnL-F*	ptDNA	nrITS+ptDNA
Number of sequences (ingroup/outgroup)	26 (23/3)	26 (23/3)	26 (23/3)	26 (23/3)	26 (23/3)	26 (23/3)
Aligned length [bp]	744	1013	337	426	1776	2520
Variable characters (%)	427 (57.4)	160 (15.8)	225 (66.8)	178 (41.8)	563 (31.7)	990 (39.3)
Parsimony informative characters (%)	307 (41.3)	87 (8.6)	131 (38.9)	113 (26.5)	331 (18.6)	638 (25.3)
Number of trees (MP)	1	8	6	8	2	1
MP tree length	1169	260	537	303	1117	2302
Consistency index (CI)[a]	0.577	0.591	0.623	0.727	0.632	0.597
Retention index (RI)	0.743	0.809	0.707	0.878	0.783	0.756
Model selected (AIC)	GTR+I+G	GTR+I+G	GTR+G	GTR+G	GTR+I+G	GTR+I+G

[a] The consistency index is calculated excluding uninformative characters.

woody at base, ca. 2 cm in diam. Sterile leaf axils often with 13 woody bulbils, fawn, globose or ovoid, 3–6 mm in diam, often with adventitious roots. Upper stems and petioles densely covered with stinging hairs and white pubescent. *Stipules* greenish, leaf-like, herbaceous, persistent, solitary in leaf axils, united with stem at base; stipule cordate or triangular-ovate, 3–4 cm, margin subentire or minutely sparsely crenate, base auriculate-amplexicaulous, apex long caudate-acuminate, shallowly 2-cleft, basal veins 3. *Leaves* alternate; leaf blade broadly ovate, 13–27 × 10–26 cm, base shallowly cordate to

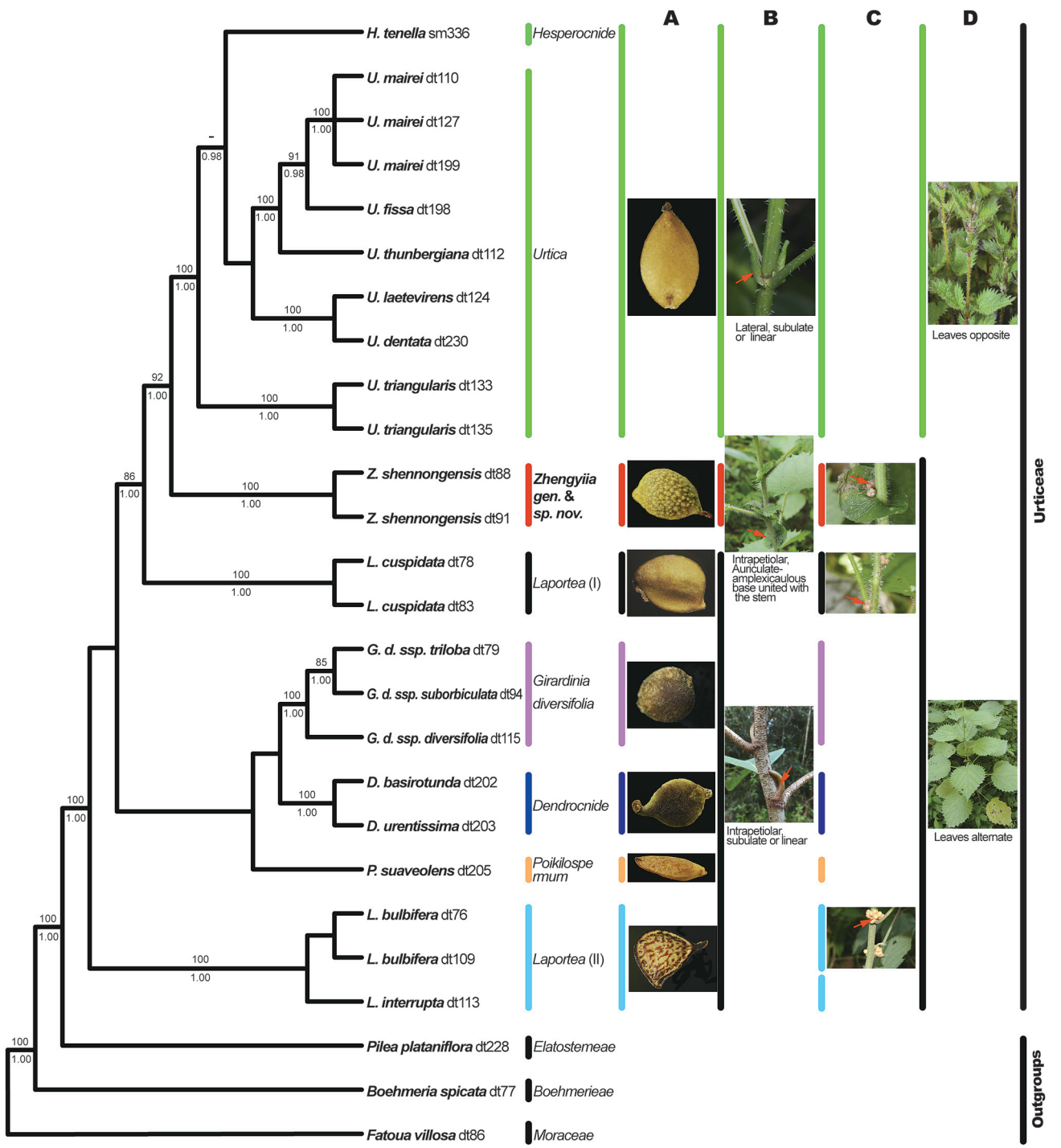

Fig. 4. Single most parsimonious tree (tree length = 2302, CI = 0.597, RI = 0.756) from the analysis of the combined nrITS and ptDNA sequences. Numbers above branches indicate bootstrap support (BP); numbers below branches are Bayesian posterior probabilities (PP); a dash (–) indicates that a node did not receive >80% BP in the MP analysis. **A,** achene shape; **B,** stipule position and shape (arrows indicate stipules); **C,** presence of bulbils (arrows indicate bulbils); **D,** leaf arrangement.

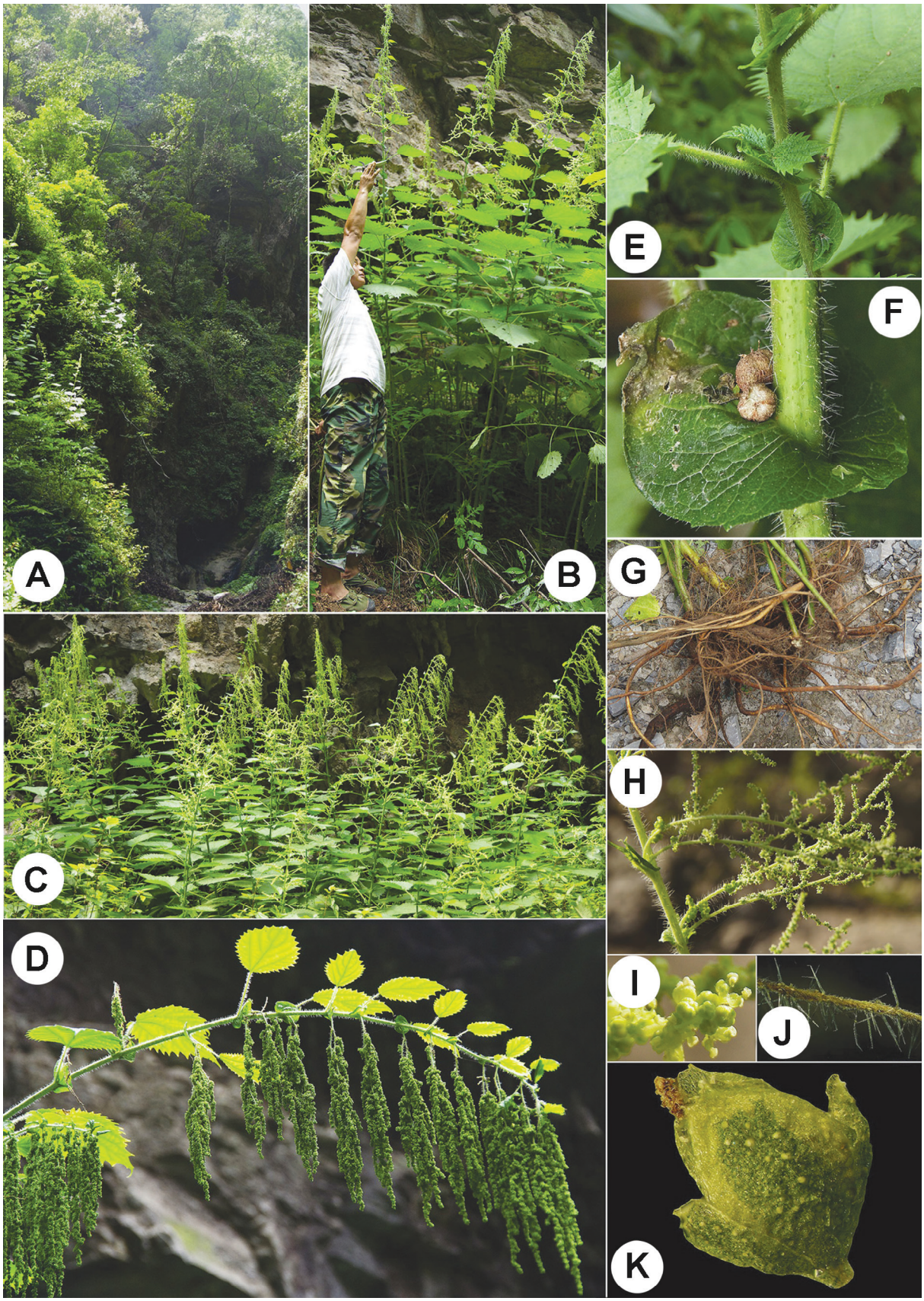

Fig. 5. Images of living plants of *Zhengyia shennongensis* T. Deng, D.G. Zhang & H. Sun. **A,** Habitat; **B,** habit; **C,** population; **D,** inflorescence; **E,** stipules; **F,** bulbils; **G,** root; **H,** inflorescence; **I,** staminate flower; **J,** pedicel; **K,** fruit.

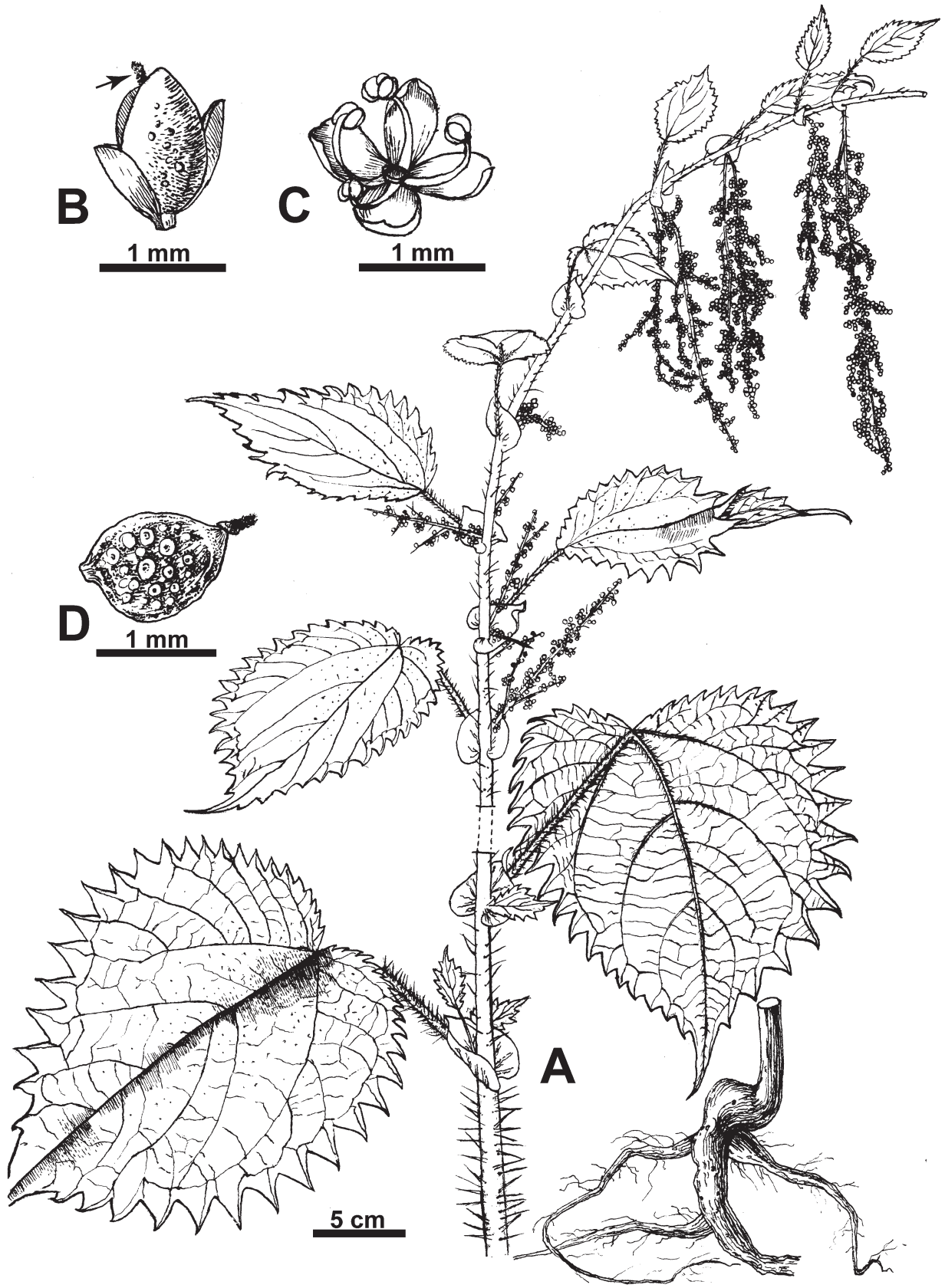

Fig. 6. Holotype of *Zhengyia shennongensis* T. Deng, D.G. Zhang & H. Sun, gen. & sp. nov., with details. **A,** Habit; **B,** pistillate flower (arrow indicates stigma); **C,** staminate flower; **D,** achene. — Drawn by X.-S. Zhang.

cordate, margin dentate or lobed; lobes deltoid, denticulate, slightly falcate; apex shortly acuminate; cystoliths minutely punctiform; lateral basal veins reaching middle lobes, secondary veins 4–6 on each side, reaching teeth or anastomosing before margin, adaxial surface with sparse, stinging and setulose hairs, abaxial surface densely setulose and sparsely armed with stinging hairs on veins. *Petiole* 12–16 cm. *Inflorescences* unisexual, in axillary pairs; paniculate with many long branches; male inflorescences in proximal axils, paniculate, erect, 15–25 cm; female inflorescence terminal or in subterminal leaf axils, pendulous, 20–30 cm, peduncle 2–4 cm. *Staminate flowers* ca. 1.5 mm, shortly pedicellate or subsessile; perianth lobes connate below middle, apex not corniculate; stamens 4, filaments incurved, longer than perianth, anthers peltate; pistillode terete, ca. 0.3 mm. *Pistillate flowers* ca. 1.3 mm, subsessile; perianth lobes 4, connate at base, strongly unequal, the 2 dorsal-ventral lobes larger, enclosing the ovary, elliptic-ovate, setulose, as long as achene; lateral lobes smaller, ovate-lanceolate, ca. 1/2 as long as dorsal lobe. *Ovary* ca. 1.1 mm, shortly stipitate, asymmetrically ovoid; stigma spirally winding, short clavate, ca. 0.4 mm. *Achene* yellowish green, oblong-globose or subglobose, ca. 1.2–1.5 mm, conspicuously oblique, with dense nipple-shaped protuberances on surface, enclosed by persistent enlarged dorsal-ventral perianth lobes; stipe ca. 0.1 mm.

Etymology. — *Zhengyia* is named in honor of Prof. Zhengyi Wu, a renowned Chinese botanist who has studied Chinese plants for over 70 years. He deserves this homage in recognition of his important contributions to the field of plant taxonomy and floristics, to his deep involvement in training new researchers and his tremendous contribution to our knowledge of the flora of China.

Distribution and habitat. — Despite extensive investigations in central China by the collectors of this taxon, the species has so far only been found in the area of Wushanhu Mountain in the SNNR, in the southwest part of Hubei province, central China (Fig. 1). The new species is probably calcicole. It prefers shady and wet habitats with deep humus-rich soil. It grows in small clusters in the valley and on limestone mountain slopes mainly at 500 to 600 m. These ancient limestone mountains in the region are deeply eroded and dissected by deep river valleys. The globose woody bulbils are probably associated with a rain-splash dispersal mechanism: when bulbils are released from parent plants, they are washed down the mountain slope by rainwater and have the potential to spread more widely via streams.

Conservation status. — Endangered, based on the occurrence in an area smaller than 5000 km² and known at fewer than five localities (IUCN, 2001).

Phenology. — The peak flowering period was observed in September and fruiting specimens were found in October and November.

Paratype. — China. Hubei province, Shennongjia Forest District (SNNR), Yangri town, Wugu Mountain, 110°50′35″ E, 31°32′37″ N, 450 m alt, 4 Sep 2011, *T. Deng, D.G. Zhang & H. Sun 2593* (KUN).

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Appendix 1. Voucher information and GenBank accession numbers for taxa used in this study. Information is presented in the following order: Species name, collection locality, abbreviation of OTU, collector and collection number (herbarium acronym), GenBank accession number for nrITS, *rbcL*, *psbA-trnH*, and *trnL-F*. (*SNJ Exped.* = Shennongjia Expedition).

INGROUP: ***Dendrocnide basirotunda*** (C.Y. Wu) Chew, China, Yunnan, dt202, *Deng 408* (KUN), KC284962, KC284988, KC285040, KC285014; ***Dendrocnide urentissima*** (Gagnep.) Chew, China, Yunnan, dt203, *Deng 409* (KUN), KC284963, KC284989, KC285041, KC285015; ***Girardinia diversifolia*** (Link) Friis subsp. ***diversifolia***, China, Yunnan, dt115, *Nie 4248* (KUN), KC284955, KC284981, KC285033, KC285007; ***Girardinia diversifolia*** subsp. ***suborbiculata*** (C.J. Chen) C.J. Chen & Friis, China, Hubei, dt094, *SNJ Exped. 20111107004* (KUN), KC284950, KC284976, KC285028, KC285002; ***Girardinia diversifolia*** subsp. ***triloba*** (C.J. Chen) C.J. Chen & Friis, China, Hubei, dt079, *SNJ Exped. 20110906024* (KUN), KC284945, KC284971, KC285023, KC284997; ***Hesperocnide tenella*** Torr., U.S.A., California, sm336, *B. Trusk 188* (US), KC284967, KC284993, KC285045, KC285019; ***Laportea bulbifera*** (Siebold & Zucc.) Wedd., China, Hubei, dt076, *SNJ Exped. 20110730020* (KUN), KC284942, KC284968, KC285020, KC284994; China, Yunnan, dt109, *Nie 3717* (KUN), KC284951, KC284977, KC285029, KC285003; ***Laportea cuspidata*** (Wedd.) Friis, China, Hubei, dt078, *SNJ Exped. 20110714028* (KUN), KC284944, KC284970, KC285022, KC284996; China, Hubei, dt083, *SNJ Exped. 20110723090* (KUN), KC284946, KC284972, KC285024, KC284998; ***Laportea interrupta*** (L.) Chew, China, Yunnan, dt113, *Nie 4263* (KUN), KC284954, KC284980, KC285032, KC285006; ***Poikilospermum suaveolens*** (Blume) Merr., China, Yunnan, dt205, *Deng 411* (KUN), KC284964, KC284990, KC285042, KC285016; ***Urtica dentata*** Hand.-Mazz., China, Hubei, dt230, *SNJ Exped. 20110724077* (KUN), KC284966, KC284992, KC285044, KC285018; ***Urtica fissa*** E. Pritz., China, Hubei, dt198, *SNJ Exped. 20111112001* (KUN), KC284960, KC284986, KC285038, KC285012; ***Urtica laetevirens*** Maxim., China, Hunan, dt124, *D.G. Zhang 134* (KUN), KC284956, KC284982, KC285034, KC285008; ***Urtica mairei*** H. Lév., China, Yunnan, dt110, *Nie 4292* (KUN), KC284952, KC284978, KC285030, KC285004; China, Sichuan, dt127, *Liu & Yuan MY-121* (KUN), KC284957, KC284983, KC285035, KC285009; China, Hunan, dt199, *Deng 406* (KUN), KC284961, KC284987, KC285039, KC285013; ***Urtica thunbergiana*** Siebold & Zucc., China, Yunnan, dt112, *Nie 4275* (KUN), KC284953, KC284979, KC285031, KC285005; ***Urtica triangularis*** Hand.-Mazz., China, Sichuan, dt133, *Qingzhang Exped. 5801* (KUN), KC284958, KC284984, KC285036, KC285010; China, Xizang, dt135, *Qingzhang Exped. 12157* (KUN), KC284959, KC284985, KC285037, KC285011; ***Zhengyia shennongensis*** T. Deng & al., China, Hubei, dt088, *SNJ Exped. 20110904001* (KUN), KC284948, KC284974, KC285026, KC285000; China, Hubei, dt091, *SNJ Exped. 20111107001* (KUN), KC284949, KC284975, KC285027, KC285001. **OUTGROUP:** ***Boehmeria spicata*** Thunb., China, Hubei, dt077, *SNJ Exped. 20110812020* (KUN), KC284943, KC284969, KC285021, KC284995; ***Pilea plataniflora*** C.H. Wright, China, Hubei, dt228, *SNJ Exped. 20110714007* (KUN), KC284965, KC284991, KC285043, KC285017; ***Fatoua villosa*** Nakai, China, Hubei, dt086, *SNJ Exped. 20110802047* (KUN), KC284947, KC284973, KC285025, KC284999.