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RESEARCH ARTICLE

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Seed morphology of *Hypericum* (Hypericaceae) in China and its taxonomic significance

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Abstract

The seed morphology of 40 taxa within the genus *Hypericum* (Hypericaceae) from China, representing 9 sections of the genus, was examined using both Light and Scanning Electron Microscopy to evaluate the taxonomic relevance of macro- and micromorphological features. Details articulating variation in seed size, color, shape, appendages, and seed coat ornamentation are described, illustrated, and compared, and their taxonomic importance is discussed. Seeds were generally brown in color and cylindric-ellipsoid to prolonged cylindric in shape. Seed size displayed wide variation, ranging from 0.37–1.91 mm in length and 0.12–0.75 mm in width. Seed appendages were observed as a characteristic morphological feature. Seed surface ornamentation has high phenotypic plasticity, and four types (reticulate, foveolate, papillose, and ribbed) can be recognized. In general, seed color and shape have limited taxonomic significance. However, some other features represent informative characters that can be used efficiently in distinguishing the studied taxa at the section and/or species levels. The findings illustrate that considerable taxonomic knowledge can be obtained by investigating the seed features of *Hypericum*, and the use of Scanning Electron Microscopy can reveal inconspicuous morphological affinities among species and play a role in taxonomic and systematic studies of the genus *Hypericum*.

Research Highlights

- Macro- and micro-morphological features of seeds of 40 *Hypericum* taxa from China were examined using Light and Scanning Electron Microscopy, providing the first broad study regarding seed morphology for *Hypericum* from China.
- Details and variations of seed size, shape, color, surface ornamentation, and appendages are fully presented.

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- Seed features and their variation have important taxonomic significance at the section and/or species levels within *Hypericum*.

KEYWORDS
Hypericeae, Malpighiales, micromorphology, seed surface ornamentation, taxonomy

1 | INTRODUCTION

Hypericum L. (Hypericaceae, Hypericeae) is the largest genus within Hypericaceae, comprising about 500 species of herbs, shrubs, and small trees (APG IV, 2016; Robson, 1981; Stevens, 2007). Various species of *Hypericum* have been used as traditional medicinal herbs in Europe, the Americas, Africa, and Asia. For example, *Hypericum perforatum* L. is a well-known species that is used to treat mild to moderate depression (Butterweck, 2003; Crockett & Robson, 2011), and is one of the best-selling herbal medicines in the United States and Europe. In China, a total of 30 species have been used as ethnomedicines. For instance, *H. ascyron* L., *H. attenuatum* Choisy, *H. erectum* Thunb., *H. japonicum* Thunb., *H. perforatum*, and *H. sampsonii* Hence are widely used to treat wounds, bruises, depression, hepatitis, snake bites, and hemorrhages (Galeotti, 2017; Zhang et al., 2020).

The genus *Hypericum* has a near-cosmopolitan distribution in various habitats, mostly occurring in temperate regions and tropical mountains (Robson, 1977). Two regions, Eurasia (ca. 230 spp.) and Andean South America (ca. 130 spp.), are considered as the major diversity centers of the genus (Meseguer et al., 2013; Nürk et al., 2013; Nürk & Blattner, 2010). As one of the richest countries for plant diversity in the world (Mittermeier et al., 1999; Myers et al., 2000), China has 71 species and 10 subspecies of *Hypericum*, of which 33 species are endemic (Li & Robson, 2007; Robson, 2012, 2016).

Morphologically, *Hypericum* mainly differs from other genera within Hypericaceae by having leaves with translucent or sometimes reddish to black glands, septicidal capsules, and seeds barely or not winged (Li & Robson, 2007; Robson, 2016; Stevens, 2007). It was long thought that the morphology-based delimitation of *Hypericum* was monophyletic, but molecular phylogenetic studies have gradually clarified the circumscription of the genus. Using plastid DNA (*matK*, *ndhF*, and *rbcL*) and mitochondrial (*matR*) nucleotide sequences, Ruhfel et al. (2011) suggested that *Lianthus* N. Robson, *Santomasia* N. Robson, *Thornea* Breedlove & E. M. McClint., and *Triadenum* Raf. should be merged into *Hypericum*. Later, Nürk et al. (2013) confirmed the non-monophyly of the traditionally circumscribed *Hypericum* based on nrDNA ITS phylogenetic data, and found the genus *Triadenum* is nested within *Hypericum*. Finally, based on previous molecular phylogenetic studies, Robson (2016) enlarged *Hypericum* to include *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum*, and divided the redefined *Hypericum* into two subgenera (*H.* subg. *Hypericum* N. Robson and *H.* subg. *Brathys* (Mutis ex L.f.) N. Robson) and 40 sections (Robson, 1985, 1987, 1990, 1996, 2001, 2002, 2006, 2010a, b, 2012, 2016). Unfortunately, monophyly for most sections were not supported either by morphology-based cladistics analyses (Nürk & Blattner, 2010) or molecular phylogenetics studies (Crockett et al., 2004; Meseguer et al., 2013; Nürk et al., 2013; Park & Kim, 2004; Ruhfel et al., 2011). These findings challenge the traditional infrageneric classification and therefore a taxonomic revision for the entire genus is needed based on multifaceted evidence.

Seed morphology can serve as a common source of taxonomic characters to identify sections or hypothesize relationships among taxa within *Hypericum* (Alonso et al., 2013; Meseguer & Sanmartín, 2012; Núñez, 1982; Szkudlarz & Celka, 2016). In his taxonomic treatment of *Hypericum*, Robson (1981) considered that seed morphology is one of the most important diagnostic characters that distinguishing *H.* sect. *Olympia* (Spach) Nyman, *H.* sect. *Campylopus* Boiss, *H.* sect. *Origanifolia* Stef. in God, *H.* sect. *Drosocarpium* Spach, *H.* sect. *Oligostema* (Boiss.) Stef. in God, and *H.* sect. *Crossophyllum* Spach (Robson, 2010a, 2012). Núñez (1982) reported seed characters from 30 taxa of *Hypericum* from the Iberian Peninsula and Balearic Islands and found that seed size, exotesta, and endotesta are important morphological characters for the infrageneric classification of *Hypercicum*. Later studies (Alonso et al., 2013; Bojňanský & Fargašová, 2007; Meseguer & Sanmartín, 2012; Szkudlarz, 2014; Szkudlarz & Celka, 2016) further demonstrated that seed morphology is a useful diagnostic character within *Hypericum* at different taxonomic levels. Although these studies provided some morphological data for seeds within *Hypericum*, scant data on seed morphology of the genus are available in comparison to some other large genera (e.g., Amini et al., 2011; Anjum, 2009; Arabi et al., 2017; Chernoff et al., 1992; Crow, 1979; Fawzi et al., 2010; Fedotova & Ardjanova, 1992; Güneş, 2012; Han et al., 2021; Ullah et al., 2019; Waheed et al., 2021). To date, only 37 species from Europe have detailed descriptions of seed morphology (Alonso et al., 2013; Núñez, 1982; Szkudlarz & Celka, 2016). And although China harbors high levels of species richness and endemism within *Hypericum*, only nine species have had their seed morphology described in detail (Sun et al., 2009). Thus, macro- and micro-features of seed morphology of Chinese *Hypericum* species are poorly documented. Therefore, a detailed morphological study is needed which can clarify diversity and variability of the seed morphological characters that may help to elucidate species relationships and relate such characters to the taxonomy and systematics of the genus.

The present study forms part of an ongoing taxonomic revision of the genus *Hypericum* in China (Bai et al., 2023). In this study, we examined the seed morphology of 40 taxa of *Hypericum* collected from China using both Light and Scanning Electron Microscopy

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(LM and SEM, respectively). The specific goals of this study are to (1) identify taxon-specific seed morphology, (2) clarify seed morphological variability for Chinese species, and (3) explore the utility of these characters for taxonomy and systematic implications for relationships within *Hypericum*.

2 | MATERIALS AND METHODS

2.1 | Sampling

In total, we sampled seeds of 40 taxa representing nine of eleven sections of *Hypericum* in China. Species of two sections (*H.* sect. *Takasagoya* (Y. Kimura) N. Robson and *H.* sect. *Humifusoideum* R. Keller) were not sampled. Mature seeds were collected in the field (five taxa) or sampled from Germplasm Bank of Wild Species in Southwest China (35 taxa). The list of species and their origins are given in Table 1.

2.2 | Light microscopy

Fifty seeds were investigated for each taxon. Fully mature and healthy seeds were carefully prepared for LM as described by Szkudlarz and Celka (2016). Photographs and morphological observations were taken using ultra-depth-of-field microscope VHX-6000 (KEYENCE Corporation, Japan) at 50× and 200× magnification, respectively. Seed size, shape, color, and appendage characteristics were observed and measured. The shape and color were determined following Bojňanský and Fargašová (2007) and Beentje (2010), and description of the appendages was based on Bojňanský and Fargašová (2007) and Robson (1981).

The length (L) and width (W) of seeds were measured using the image analysis software ImageJ (Schindelin et al., 2015). The length/width ratio, mean, and values of the standard deviation were calculated by the formula L/W, functions AVERAGE and STDEV in Microsoft Excel 2016, respectively. Quantitative characters were summarized by minimum–maximum (mean) ± standard deviation, and interspecific differences were analyzed by GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California, https://www.graphpad.com).

2.3 | Scanning electron microscopy

For SEM analyses, 10 seeds from each taxon were examined. Seeds were transferred into aluminum stubs with double-sided adhesive tape (Xiang et al., 2013). Samples were sputter-coated with gold, examined, and photographed. The seed epidermal cell shapes and surface ornamentation were observed and recorded with a ZEISS Sigma 300 Scanning Electron Microscope (Carl Zeiss AG, Jena, Germany) at an acceleration voltage of 7 kV. General seed terminology from Robson (1981) and Bojňanský and Fargašová (2007) were used to describe seed microstructure.

3 | RESULTS

The main features of the seed (shape, size, etc.) are summarized in Table 2. Selected LM and SEM micrographs are shown in Figures 1–6, and the variation of seed size among all examined taxa is provided in Figures 7–9.

3.1 | Seed color

In general, the seeds were brown (BR) and lustrous (Figure 1, [1–37]) for most investigated *Hypericum* species, except for *Hypericum scabrum* (Figure 1, [38]) and *H. hirsutum* (Figure 1, [39]), which had stellate hairs outside the surface. In addition, seed colors can be further divided into five subtypes: pale-brown (Figure 1, [4, 8, 22, 23, 39, 40]), dark-brown (Figure 1, [2, 7, 9–20]), blackish-brown (Figure 1, [28, 29, 31, 32]), brownish-yellow (Figure 1, [33, 34]), and reddish-brown (Figure 1, [27, 30, 38]).

Dark-brown (DB) was the most common color and was observed in 14 species (Figure 1, [2, 7, 9–20]) from *Hypericum* sect. *Ascyreia*. Pale-brown (PB) seeds were found in four species (Figure 1, [4, 8, 22, 23]) of *H.* sect. *Ascyreia*, and one species from both *H.* sect. *Taeniocarpium* (Figure 1, [39]) and *H.* sect. *H. sampsonii* (Figure 1, [40]). Blackish-brown (BB) seeds were easily distinguished from other types and found in four species from *H.* sect. *Hypericum* (Figure 1, [28, 29]) and *H.* sect. *Elodeoida* (Figure 1, [31, 32]). Reddish-brown (RB) seeds were observed in three species, *H. faberi* (Figure 1, [27]), *H. elodeoides* (Figure 1, [30]), and *H. scabrum* (Figure 1, [38]). Brownish-yellow (BY) seeds were only observed in two species of *H.* sect. *Trigynobrathys* (Figure 1, [33, 34]).

In some cases, seed color was somewhat variable among different individuals of the same species. For example, seeds of *Hypericum bellum*, *H. choisyanum*, *H. curvisepalum*, and *H. forrestii* were pale-brown to dark-brown (Figure S1, A–D, respectively); *H. uralum* had pale-brown to brown seeds (Figure S1E); and *H. sampsonii* had brownish-yellow to brown seeds (Figure S1F).

3.2 | Seed shape

The seeds were usually cylindric in shape, but in outline three types can be recognized: cylindric, prolonged cylindric, and cylindric-ellipsoid (Figure 1, [1–40], Table 2). Cylindric (CY) seeds were the most common type, found in 28 species from seven sections (Figure 1, [1, 6–19, 24–25, 27–32, 35–39]). Cylindric-ellipsoid (CY-EL) seeds were found in seven taxa belonging to *Hypericum* sect. *Ascyreia* (Figure 1, [2–5]), *H.* sect. *Trigynobrathy* (Figure 1, [33, 34]), and *H.* sect. *Sampsonia* (Figure 1, [40]). Prolonged cylindric (P-CY) seed was observed in four taxa from *H.* sect. *Ascyreia* (Figure 1, [20–23]), and *H. Ascyron* subsp. *gebleri* from *H.* sect. *Roscyna* (Figure 1, [26]). In addition, seeds of most species of *H.* sect. *Ascyreia* (Figure 1, [1–23]) and *H.* sect. *Roscyna* (Figure 1, [24–26]) had a winged apex and were carinate in lateral margins. Seeds of most species of *H.* sect. *Hypericum*, *H.*

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TABLE 1 Voucher information for *Hypericum* used in this study. Sectional classification of the species is according to Li and Robson (2007).

Section	Species	Voucher	Locality	Coordinates
Ascyreia	*H. acmosepalum* N. Robson	He DM & Feng YF, WSLJS827	Wenshan, Yunnan	23°20′26.60″ N, 104°04′26.30″ E
Ascyreia	*H. addingtonii* N. Robson	Gong YJ et al., 14CS9872	Gongshan, Yunnan	28°04′01.40″ N, 98°20′01.40″ E
Ascyreia	*H. augustinii* N. Robson	Zhao F & Zhao Y, XCL2215	Jianshui, Yunnan	23°57′45.96″ N, 102°59′36.56″ E
Ascyreia	*H. beanii* N. Robson	Zou FL, ZouFL0303	Bijie, Guizhou	26°49′42.00″ N, 104°16′57.00″ E
Ascyreia	*H. bellum* H.L. Li	Zhang DC et al., ZhangDC-07ZX-1977	Diqing, Yunnan	27°55′52.60″ N, 99°13′59.40″ E
Ascyreia	*H. choisyanum* Wall. ex N. Robson	Luo J et al., LiuJQ-09XZ-ML109	Nyingchi, Tibet	29°45′21.50″ N, 93°13′46.90″ E
Ascyreia	*H. cohaerens* N. Robson	Gan QL, GanQL1334	Shiyan, Hubei	32°06′12.83″ N, 109°42′52.39″ E
Ascyreia	*H. curvisepalum* N. Robson	Bai RZ et al., CSXCL1079	Yangbi, Yunnan	25°45′28.20″ N, 99°58′32.48″ E
Ascyreia	*H. forrestii* (Chitt.) N. Robson	Zhang DC et al., ZhangDC-07ZX-1873	Nyingchi, Tibet	29°57′27.50″ N, 94°47′13.40″ E
Ascyreia	*H. henryi* H. Lév. & Vaniot	Yang JR & Bi B, YDDXSA109	Lincang, Yunnan	24°04′04.60″ N, 99°36′11.00″ E
Ascyreia	*H. henryi* subsp. *hancockii* N. Robson	Chu YX & Tao GQ, Pbdws050	Honghe, Yunnan	23°07′50.00″ N, 103°53′05.00″ E
Ascyreia	*H. henryi* subsp. *uraloides* (Rehder) N. Robson	Li YL, YDDXS0041	Lincang, Yunnan	24°12′31.60″ N, 99°42′01.70″ E
Ascyreia	*H. hookerianum* Wight & Arn.	Yu XL & Zhao Y, BSGLGStc023	Baoshan, Yunnan	25°26′56.00″ N, 98°43′05.00″ E
Ascyreia	*H. lancasteri* N. Robson	Li YL, YDDXS0653	Lincang, Yunnan	24°07′16.20″ N, 99°39′12.00″ E
Ascyreia	*H. latisepalum* N. Robson	Guo YJ et al., 16CS14488	Nyingchi, Tibet	29°41′31.77″ N, 95°31′00.67″ E
Ascyreia	*H. longistylum* Oliv.	Gan QL, GanQL1329	Shiyan, Hubei	32°31′04.95″ N, 110°40′14.46″ E
Ascyreia	*H. maclarenii* N. Robson	Bai RZ et al., XCL2183	Wenchuan, Sichuan	30°53′32.36″ N, 102°59′39.44″ E
Ascyreia	*H. patulum* Thunb.	Liu J & Yuan M, MY-091	Panzhihua, Sichuan	27°03′24.20″ N, 101°57′45.60″ E
Ascyreia	*H. pseudohenryi* N. Robson	Li DZ, DZL479	Lijiang, Lincang	26°53′08.20″ N, 100°13′13.12″ E
Ascyreia	*H. reptans* Hook. f. & Thomson ex Dyer	Li DZ, DZL468	Gongshan, Yunnan	28°19′09.40″ N, 98°16′34.50″ E
Ascyreia	*H. stellum* N. Robson	Bai RZ & Zou L, BRZ41	Enshi, Hubei	30°51′48.93″ N, 110°17′30.98″ E
Ascyreia	*H. uralum* Buch.-Ham. ex D. Don	Guo YJ et al., 14CS9866	Gongshan, Yunnan	28°04′01.40″ N, 98°20′01.40″ E
Ascyreia	*H. wilsonii* N. Robson	Bai RZ & Zou L, BRZ40	Enshi, Hubei	30°49′29.23″ N, 110°14′50.73″ E
Roscyna	*H. ascyron* L.	Niu YL, NiuYL094	Shijiazhuang, Hebi	38°39′25.50″ N, 113°45′10.45″ E
Roscyna	*H. ascyron* subsp. *gebleri* (Ledeb.) N. Robson	Zheng BJ et al., ZhengBJ280	Heihe, Heilongjiang	48°41′17.62″ N, 128°01′34.81″ E
Roscyna	*H. przewalskii* Maxim.	Chen SL, Chensl1897	Rangtang, Sichuan	32°15′56.85″ N, 100°58′42.67″ E
Hypericum	*H. erectum* Thunb.	Chen GX, SCSB-HC-2007452	Xiangxi, Hunan	28°18′36.90″ N, 109°55′52.70″ E
Hypericum	*H. faberi* R. Keller	Gan QL., GanQL735	Shiyan, Hubei	31°55′50.71″ N, 109°39′06.51″ E
Hypericum	*H. perforatum* L.	Guo JY & Liu L, SHI2006286	Changji, Xinjiang	43°50′47.27″ N, 86°02′56.10″ E
Elodeoida	*H. elodeoides* Choisy	Yu WB et al., SCSB-W-1094	Zhaotong, Yunnan	27°16′37.80″ N, 103°01′44.30″ E
Elodeoida	*H. hengshanense* W.T. Wang	Huang CZ, HCZ00199	Chenzhou, Hunan	26°04′39.35″ N, 113°56′41.23″ E
Elodeoida	*H. kingdonii* N. Robson	Shui YM & Chen WH, 64604	Stone forest, Yunnan	24°41′19.90″ N, 103°28′30.50″ E
Monanthema	*H. himalaicum* N. Robson	Luo J et al., LiuJQ-09XZ-338	Nyingchi, Tibet	29°50′23.40″ N, 94°45′10.20″ E
Monanthema	*H. monanthemum* Hook. f. & Thomson ex Dyer	Li XJ, LiXJ495	Emeishan, Sichuan	29°31′21.00″ N, 103°20′07.00″ E
Monanthema	*H. wightianum* Wall. ex Wight & Arn.	Zhang SD et al., QJYS0243	Zhaotong, Yunnan	27°10′04.79″ N, 103°06′53.01″ E
Sampsonia	*H. sampsonii* Hance	Chen YS et al., Xuzd267	Lu'an, Anhui	31°05′57.00″ N, 116°34′08.00″ E
Trigynobrathys	*H. gramineum* G. Forst.	Xiong SR, NJWLS443	Dali, Yunnan	24°58′30.40″ N, 100°28′19.90″ E
Trigynobrathys	*H. japonicum* Thunb.	Chen YS et al., Xuzd069	Hefei, Anhui	31°46′07.00″ N, 117°32′58.00″ E
Hirtella	*H. scabrum* L.	Xu WB & Yang QL, SHI-2009084	Tarbagatay, Xinjiang	45°58′49.20″ N, 82°24′22.32″ E
Taeniocarpium	*H. hirsutum* L.	Liu L, SHI-A2007470	Ili, Xinjiang	43°44′23.70″ N, 83°25′02.87″ E

TABLE 2 Details of seed morphology characters in studied taxa of *Hypericum*.

Section	Taxon	Color	Shape	Testa cell shape	Surface ornamentation	Appendage	Length (mm)	Width (mm)	Length/width ratio
Ascyreia	*H. acmosepalum*	DB	CY	IT	LR	**	0.78–1.26 (1.02) ± 0.13	0.20–0.32 (0.28) ± 0.03	2.65–4.73 (3.73) ± 0.56
Ascyreia	*H. addingtonii*	DB	CY	IT	S-LR	**	0.85–1.19 (0.99) ± 0.09	0.29–0.40 (0.34) ± 0.03	2.36–3.36 (2.92) ± 0.32
Ascyreia	*H. augustinii*	DB	CY	IT	LR	**	0.90–1.31 (1.17) ± 0.09	0.28–0.41 (0.34) ± 0.03	2.61–4.23 (3.40) ± 0.34
Ascyreia	*H. beanii*	PB	P-CY	IT	LR	***	1.17–1.84 (1.46) ± 0.19	0.29–0.46 (0.35) ± 0.04	3.29–5.04 (4.16) ± 0.53
Ascyreia	*H. bellum*	PB-DB	CY	IT/IP	LR	**	0.61–0.97 (0.81) ± 0.08	0.23–0.42 (0.29) ± 0.03	1.84–3.47 (2.82) ± 0.33
Ascyreia	*H. choisyanum*	PB-DB	CY-EL	IT	LR	**	0.65–0.90 (0.77) ± 0.05	0.23–0.31 (0.27) ± 0.01	2.42–3.67 (2.86) ± 0.30
Ascyreia	*H. cohaerens*	DB	CY	IT	LR	**	1.00–1.34 (1.13) ± 0.08	0.23–0.46 (0.29) ± 0.04	2.26–4.70 (3.89) ± 0.42
Ascyreia	*H. curvisepalum*	PB-DB	CY-EL	IT/IP	LR	**	0.61–0.91 (0.75) ± 0.06	0.23–0.36 (0.27) ± 0.02	2.06–3.48 (2.75) ± 0.3
Ascyreia	*H. forrestii*	PB-DB	P-CY	IT	S-LR	**	0.90–1.43 (1.12) ± 0.15	0.26–0.36 (0.32) ± 0.03	2.99–4.59 (3.79) ± 0.43
Ascyreia	*H. henryi*	DB	CY	IT/IP	LR	**	0.83–1.40 (1.14) ± 0.29	0.25–0.37 (0.31) ± 0.05	2.66–4.64 (3.69) ± 0.38
Ascyreia	*H. henryi* subsp. *hancockii*	DB	P-CY	IT/IP	LR	**	1.16–1.65 (1.41) ± 0.11	0.24–0.38 (0.31) ± 0.03	3.15–5.80 (4.64) ± 0.61
Ascyreia	*H. henryi* subsp. *uraloides*	DB	CY-EL	IT	SR	**	0.46–1.04 (0.65) ± 0.12	0.18–0.31 (0.24) ± 0.03	2.00–3.56 (2.66) ± 0.37
Ascyreia	*H. hookerianum*	DB	CY	IT	LR	**	0.95–1.46 (1.12) ± 0.16	0.24–0.32 (0.28) ± 0.02	3.12–5.22 (4.01) ± 0.61
Ascyreia	*H. lancasteri*	PB	CY	IT	LR	**	0.70–0.99 (0.86) ± 0.08	0.25–0.36 (0.31) ± 0.03	2.14–3.45 (2.84) ± 0.37
Ascyreia	*H. latisepalum*	DB	CY	IT/IP	LR	**	1.04–1.53 (1.30) ± 0.11	0.24–0.35 (0.29) ± 0.02	3.51–5.45 (4.49) ± 0.47
Ascyreia	*H. longistylum*	DB	CY	IT/IP	S-LR	**	0.99–1.38 (1.20) ± 0.11	0.27–0.42 (0.32) ± 0.04	2.69–4.75 (3.80) ± 0.52
Ascyreia	*H. maclarenii*	DB	CY	IT	LR	**	1.02–1.54 (1.27) ± 0.11	0.26–0.43 (0.33) ± 0.03	3.05–4.86 (3.89) ± 0.41
Ascyreia	*H. patulum*	DB	CY	IT	LR	**	0.95–1.48 (1.17) ± 0.12	0.26–0.36 (0.31) ± 0.02	2.86–4.84 (3.80) ± 0.42
Ascyreia	*H. pseudohenryi*	PB	P-CY	IT/IP	S-LR	***	1.30–1.91 (1.63) ± 0.15	0.27–0.39 (0.32) ± 0.03	3.97–6.41 (5.03) ± 0.48
Ascyreia	*H. reptans*	PB	CY-EL	IT/IP	RE	**	0.54–0.87 (0.72) ± 0.06	0.28–0.40 (0.32) ± 0.03	1.71–2.77 (2.23) ± 0.24
Ascyreia	*H. stellum*	DB	CY	IT	S-LR	**	1.00–1.46 (1.19) ± 0.10	0.23–0.36 (0.29) ± 0.03	3.11–5.46 (4.06) ± 0.47
Ascyreia	*H. uralum*	PB-BR	CY	IT/IP	RE/SR	**	0.37–0.49 (0.43) ± 0.03	0.22–0.29 (0.26) ± 0.02	1.33–2.00 (1.69) ± 0.17
Ascyreia	*H. wilsonii*	DB	CY	IT/IP	LR	**	1.00–1.26 (1.12) ± 0.08	0.30–0.39 (0.34) ± 0.02	2.77–3.81 (3.23) ± 0.29
Roscyna	*H. ascyron*	BR	CY	IT	RE	**	1.05–1.50 (1.24) ± 0.09	0.30–0.49 (0.38) ± 0.04	3.00–3.47 (3.23) ± 0.26
Roscyna	*H. ascyron* subsp. *gebleri*	BR	P-CY	IT	RE	***	1.19–1.81 (1.47) ± 0.14	0.24–0.41 (0.33) ± 0.04	3.61–5.95 (4.56) ± 0.54
Roscyna	*H. przewalskii*	BR	CY	IT	CR	**	1.09–1.50 (1.36) ± 0.09	0.39–0.55 (0.47) ± 0.03	2.40–3.61 (2.94) ± 0.20
Hypericum	*H. erectum*	BB	CY	IEP/RO	FO	*	0.59–0.74 (0.69) ± 0.04	0.28–0.37 (0.33) ± 0.02	1.74–2.37 (2.11) ± 0.14
Hypericum	*H. faberi*	RB	CY	IEP	RE	*	0.51–0.67 (0.59) ± 0.03	0.20–0.28 (0.25) ± 0.02	1.90–2.91 (2.39) ± 0.23
Hypericum	*H. perforatum*	BB	CY	IEP	FO	*	0.85–1.18 (1.05) ± 0.06	0.36–0.50 (0.44) ± 0.03	2.09–2.86 (2.38) ± 0.16
Elodeoida	*H. elodeoides*	RB	CY	IT	RE	*	0.41–0.54 (0.47) ± 0.03	0.20–0.27 (0.24) ± 0.02	1.86–2.01 (1.92) ± 0.03
Elodeoida	*H. hengshanense*	BB	CY	IT/IP	RE	*	0.80–1.03 (0.90) ± 0.06	0.29–0.39 (0.34) ± 0.02	2.21–3.00 (2.58) ± 0.18
Elodeoida	*H. kingdonii*	BB	CY	IE	FO	*	0.45–0.63 (0.60) ± 0.04	0.25–0.33 (0.29) ± 0.02	1.64–2.10 (1.91) ± 0.12
Monanthema	*H. himalaicum*	BR	CY	IEP	RE	*	0.43–0.64 (0.53) ± 0.04	0.23–0.30 (0.27) ± 0.02	1.65–2.26 (1.96) ± 0.14
Monanthema	*H. monanthemum*	BR	CY	IEP	RE	*	0.54–0.72 (0.64) ± 0.04	0.23–0.32 (0.28) ± 0.02	1.95–2.65 (2.33) ± 0.16

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TABLE 2 (Continued)

Section	Taxon	Color	Shape	Testa cell shape	Surface ornamentation	Appendage	Length (mm)	Width (mm)	Length/width ratio
Monanthema	*H. wightianum*	BR	CY	IEP/EL	FO	*	0.40–0.62 (0.49) ± 0.04	0.20–0.31 (0.25) ± 0.02	1.67–2.48 (2.00) ± 0.14
Sampsonia	*H. sampsonii*	PB	CY-EL	IE	RI	*	0.93–1.21 (1.09) ± 0.07	0.38–0.53 (0.44) ± 0.03	2.26–2.50 (2.44) ± 0.04
Trigynobrathys	*H. gramineum*	BY	CY-EL	IE	RS	*	0.45–0.58 (0.52) ± 0.03	0.17–0.29 (0.24) ± 0.02	1.78–2.90 (2.22) ± 0.25
Trigynobrathys	*H. japonicum*	BY	CY-EL	IE	F-RS	*	0.38–0.57 (0.48) ± 0.04	0.12–0.26 (0.21) ± 0.02	1.97–3.63 (2.31) ± 0.33
Hirtella	*H. scabrum*	RB	CY	-	PA	-	1.38–1.85 (1.57) ± 0.10	0.60–0.75 (0.68) ± 0.04	2.15–2.60 (2.31) ± 0.11
Taeniocarpium	*H. hirsutum*	PB	CY	-	PA	-	0.88–1.18 (1.07) ± 0.07	0.28–0.42 (0.36) ± 0.04	2.79–3.24 (2.99) ± 0.13

Abbreviations: ***, with visible winged apex and carinate lateral margins; **, with winged apex and carinate lateral margins; *, with beaked apex; –, not; BB, blackish-brown; BR, brown; BY, brownish-yellow; CR, coarse reticulate; CY, cylindric; CY-EL, cylindric-elliptic; DB, dark-brown; EL, elliptic; FO, foveolate; F-RS, finely ribbed-scalariform; IE, irregular elliptic; IEP/EL, irregular elliptic polygonal or elliptic; IEP, irregular elliptic polygonal; IP, irregular polygonal; IT/IP, irregular tetragonal or polygonal; IT, irregular tetragonal; LR, linear-reticulate; PA, Papillose; PB, pale-brown; P-CY, prolonged cylindric; PO, polygonal; RB, reddish-brown; RE, reticulate; RI, ribbed; RO, rounded; RS, ribbed-scalariform; S-LR, shallowly linear-reticulate; SR, scalariform-reticulate.

sect. *Elodeoida*, *H.* sect. *Trigynobrathys*, *H.* sect. *Sampsonia*, and *H.* sect. *Monanthema* have a beaked apex (Figure 1, [27–37, 40]), but the beak is more pronounced in *H.* sect. *Trigynobrathys* (Figure 1, [33, 34]).

3.3 | Seed surface ornamentation

Four patterns were recognized based on seed-surface ornamentation (sculpturing), including reticulate, foveolate, ribbed, and papillose, but the shape of testa cells in different ornamentation types is usually different (Table 2; Figures 2–6). Reticulate (RE) surface is the most common type and was observed in *Hypericum* sect. *Ascyreia* (Figures 2 and 3; Figure 4, a1–g2), *H.* sect. *Roscyna* (Figure 4h1, h2; Figure 5, a1–b2), *H.* sect. *Hypericum* (Figure 5d1, d2), *H.* sect. *Elodeoida* (Figure 5, f1–g2), and *H.* sect. *Monanthema* (Figure 6, a1–b2). The reticulate surface usually consists of irregular tetragonal or polygonal testa cells, and the arrangement, width, and depth of epidermal cells varied in different taxa. For example, shallow, linear, irregular tetragonal or polygonal testa cells were found in *H. addingtonii*, *H. forrestii*, *H. longistylum*, *H. pseudohenryi*, and *H. stellum* (Figure 2b1, b2; Figure 3a1, a2, h1, h2; Figure 4c1, c2, e1, e2, respectively), forming shallowly linear-reticulate (S-LR). Linear, irregular tetragonal or polygonal testa cells were observed in *H. acmosepalum*, *H. augustinii*, and so forth. (Figure 2a1, a2, c1–h2; Figure 3, b1–c2, e1–g2; Figure 4a1–b2, g1, g2, respectively), forming linear-reticulate (LR). Scalariform, irregular tetragonal or polygonal testa cells were found in *H. henryi* subsp. *uraloide* and *H. uralum* (Figure 3d1, d2; Figure 4f1, f2, respectively), forming scalariform-reticulate (SR). Coarse, irregular tetragonal testa cells were only found in *H. przewalskii* (Figure 5b1, b2), forming coarse reticulate (CR).

Foveolate surface was composed of irregular elliptic or rounded cells, which was evident in *H. erectum* (Figure 5c1, c2), *H. perforatum* (Figure 5e1, e2), *H. kingdonii* (Figure 5h1, h2), and *H. wightianum* (Figure 6c1, c2). Ribbed surfaces were formed by the longitudinal thickening of cell walls, and based on the arrangement and width of cells, ribbed (RI), ribbed-scalariform (RS), and finely ribbed-scalariform (F–RS) were observed in *H. sampsonii*, *H. japonicum*, and *H. gramineum* (Figure 6, d1–f2, respectively). Papillose (PA) initially consists of convex epidermal cells, but during seed coat drying the outer wall collapses; this was found in *H. hirsutum* (Figure 6g1, g2) and *H. scabrum* (Figure 6h1, h2).

3.4 | Seed size

The length (i.e., longest diameter) of the seeds ranged from 0.37–0.49 (0.43 ± 0.03 average) mm in *Hypericum uralum* to 1.30–1.91 (1.63 ± 0.15) mm in *H. pseudohenryi* (Figure 7, Table 2), and their width (i.e., shortest diameter) varied from 0.12–0.26 (0.21 ± 0.02) mm in *H. japonicum* to 0.60–0.75 (0.68 ± 0.04) mm in *H. scabrum* (Figure 8, Table 2). The length/width ratio ranged from 1.33–2.00 (1.69 ± 0.17) mm in *H. uralum* to 3.97–6.41 (5.03 ± 0.48) mm in *H. pseudohenryi* (Figure 9, Table 2).

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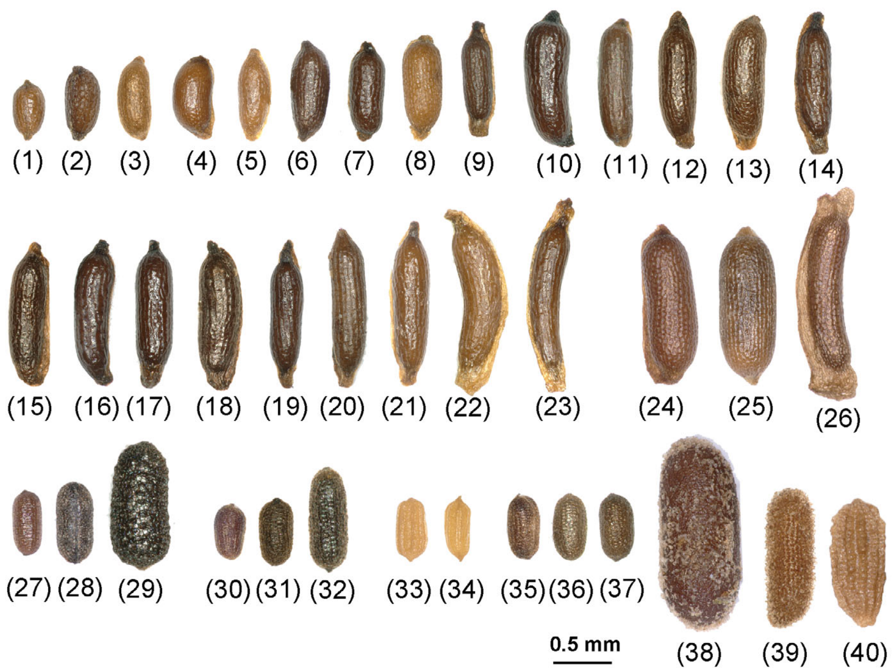

FIGURE 1 Seed macromorphology of *Hypericum* under the light microscopy (LM). (1) *H. uralum*, (2) *H. henryi* subsp. *uraloide*, (3) *H. curvisepalum*, (4) *H. reptans*, (5) *H. choisyanum*, (6) *H. bellum*, (7) *H. latisepalum*, (8) *H. lancasteri*, (9) *H. acmosepalum*, (10) *H. addingtonii*, (11) *H. longistylum*, (12) *H. wilsonii*, (13) *H. augustinii*, (14) *H. henryi*, (15) *H. stellum*, (16) *H. patulum*, (17) *H. cohaerens*, (18) *H. maclarenii*, (19) *H. hookerianum*, (20) *H. henryi* subsp. *hancockii*, (21) *H. forrestii*, (22) *H. beanii*, (23) *H. pseudohenryi*, (24) *H. ascyron*, (25) *H. przewalskii*, (26) *H. ascyron* subsp. *gebleri*, (27) *H. faberi*, (28) *H. erectum*, (29) *H. perforatum*, (30) *H. elodeoides*, (31) *H. kingdonii*, (32) *H. hengshanense*, (33) *H. japonicum*, (34) *H. gramineum*, (35) *H. wightianum*, (36) *H. monanthemum*, (37) *H. himalaicum*, (38) *H. scabrum*, (39) *H. hirsutum*, (40) *H. sampsonii*.

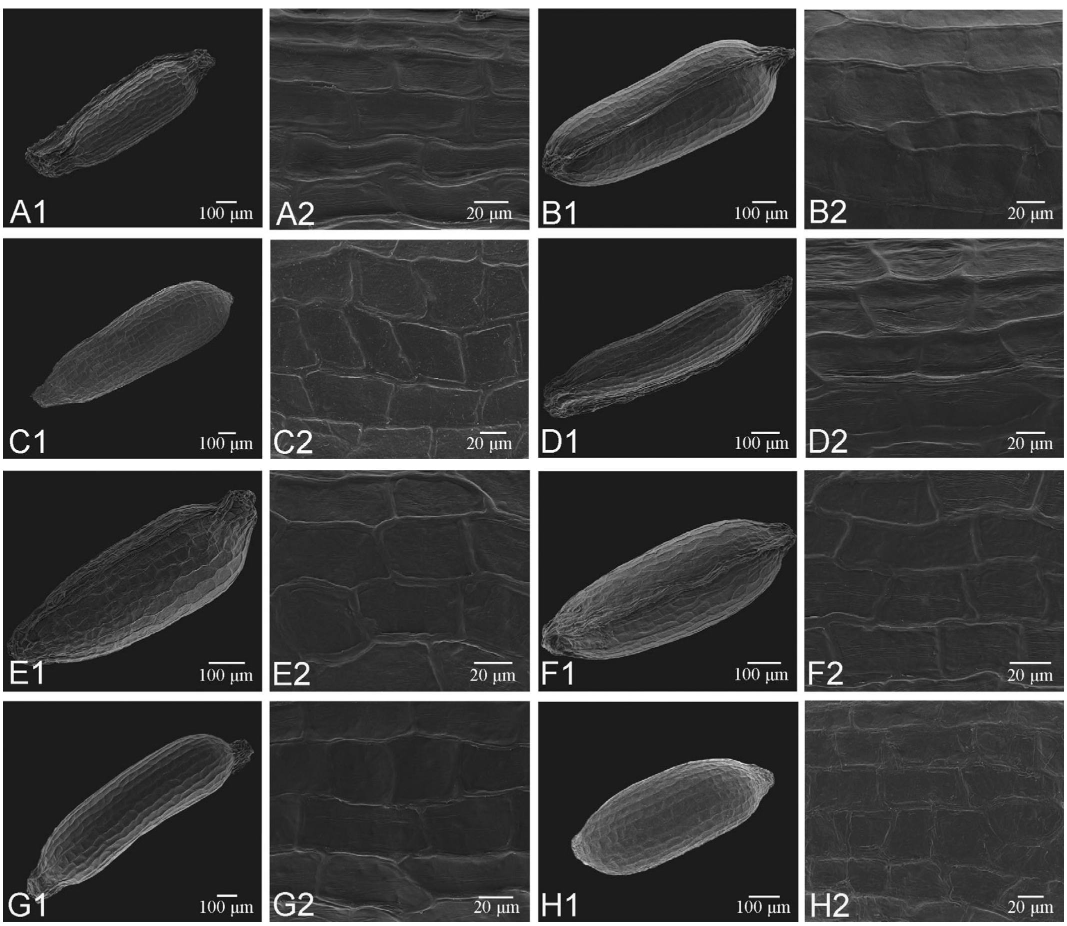

FIGURE 2 Seed micromorphology of *Hypericum* taxa under the scanning electron microscopy (SEM). (a1, a2) *H. acmosepalum*; (b1, b2) *H. addingtonii*; (c1, c2) *H. augustinii*; (d1, d2) *H. beanii*; (e1, e2) *H. bellum*; (f1, f2) *H. choisyanum*; (g1, g2) *H. cohaerens*; (h1, h2) *H. curvisepalum*.

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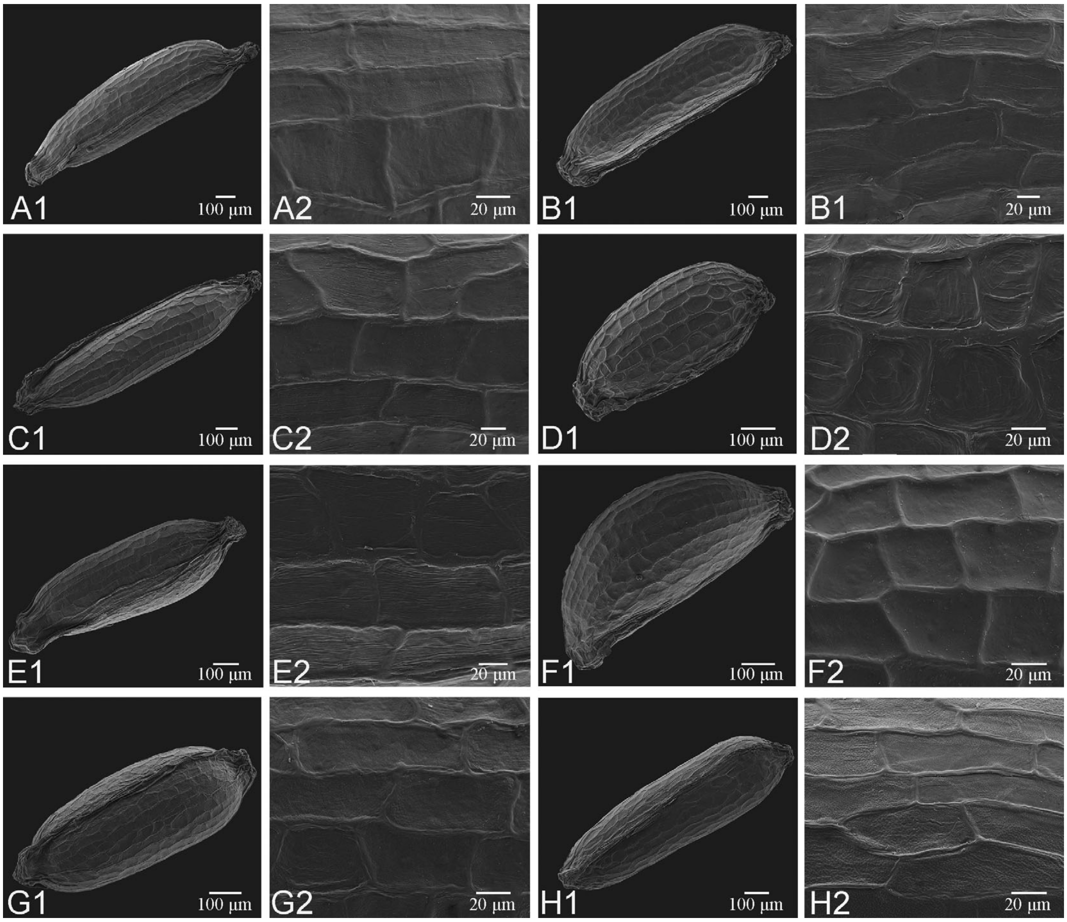

FIGURE 3 Seed micromorphology of *Hypericum* taxa under the scanning electron microscopy (SEM). (a1, a2) *H. forrestii*; (b1, b2) *H. henryi*; (c1, c2) *H. henryi* subsp. *hancockii*; (d1, d2) *H. henryi* subsp. *uraloide*; (e1, e2) *H. hookerianum*; (f1, f2) *H. lancasteri*; (g1, g2) *H. latisepalum*; (h1, h2) *H. longistylum*.

4 | DISCUSSION

Seed morphological traits provide important information for the classification and evolution of seed plants and can play a major role in species delimitation (Corner, 1976; Dahlgren, 1980). These traits are often the subject of morphological studies to resolve various taxonomic complexities (Gabr, 2014; Ghimire et al., 2015; Han et al., 2021; Kanwal et al., 2022; Kramina & Polevova, 2022; Luqman et al., 2019; Nardi et al., 2016; Veiskarami et al., 2018). In *Hypericum*, seed morphology has been considered an important character for solving taxonomic problems at the section and species levels (Alonso et al., 2013; Corner, 1976; Meseguer & Sanmartín, 2012; Núñez, 1982; Robson, 1981; Szkudlarz & Celka, 2016).

In this study, we present a comprehensive investigation of the seed morphology of 40 *Hypericum* taxa native to China. We found that some characters, for example, seed appendages, seed size, and surface ornamentation, display a high-level of variability and have a fairly high taxonomic value in the genus.

The presence and shape of seed appendages are of taxonomic value in *Hypericum*. For example, seeds from *Hypericum* sect. *Ascyreia* and *H.* sect. *Roscyna* have a conspicuous winglet at the apex and are carinate in lateral margins (Figure 1, [1–26]). In all other sections, seeds only have a slight beak at the apex (Figure 1, [27–37, 40]) or lack appendages (Figure 1, 38, 39), suggesting a close relationship between sections *Ascyreia* and *Roscyna*. Indeed, previous molecular phylogenetic studies (Crockett et al., 2004; Nürk et al., 2013; Pilepić et al., 2010) also suggested that these two sections form a clade, indicating that conspicuous winged apex and carinate lateral margins are potential synapomorphies for this clade. In addition, *H.* sect. *Ascyreia* and *H.* sect. *Roscyna* can easily be differentiated from other sections based on external morphology, for example, stamens in 5 fascicles (vs. 3 fascicles) and an ovary with 5 styles (vs. 3 styles), and black glands absent in leaves and sepals (vs. black glands obviously present in leaves and sepals) (Li, 1990; Li & Robson, 2007). Therefore, seed appendages provide an additional indicative character that distinguishes *H.* sect. *Ascyreia* and *H.* sect. *Roscyna* from other sections in Chinese *Hypericum*.

Seeds of most species of *Hypericum* sect. *Hypericum*, *H.* sect. *Elodeoida*, *H.* sect. *Trigynobrathys*, *H.* sect. *Sampsonia* and *H.* sect. *Monanthema* have a beak at the seed apex (Figure 1, [27–37, 40]), and especially in *H.* sect. *Trigynobrathys*, the beak is conspicuous (Figure 1, [33, 34]). This character also corresponds to external morphology of

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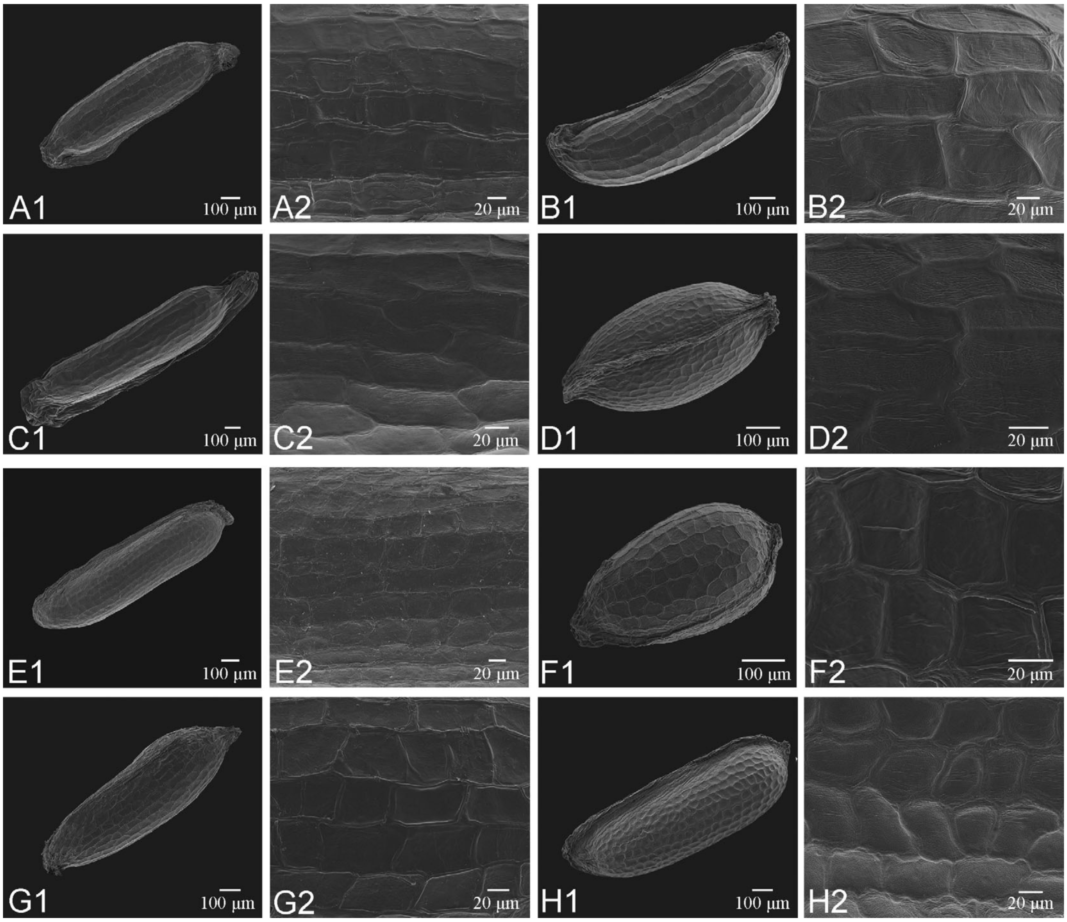

FIGURE 4 Seed micromorphology of *Hypericum* taxa under the scanning electron microscopy (SEM). (a1, a2) *H. maclarenii*; (b1, b2) *H. patulum*; (c1, c2) *H. pseudohenryi*; (d1, d2) *H. reptans*; (e1, e2) *H. stellum*; (f1, f2) *H. uralum*; (g1, g2) *H. wilsonii*; (h1, h2) *H. ascyron*.

those sections. For example, species of *H.* sect. *Trigynobrathys* do not have black glands on any part of the plant. On the contrary, species from *H.* sect. *Hypericum*, *H.* sect. *Elodeoida*, *H.* sect. *Sampsonia*, and *H.* sect. *Monanthema* have dense or irregular black glands on the leaves, sepals, and anthers (Li & Robson, 2007). Seeds of two sections, that is, *H.* sect. *Hirtella* and *H.* sect. *Taeniocarpium*, do not have appendages at the apex (Figure 1, [38, 39]). Species of *H.* sect. *Hirtella* and *H.* sect. *Taeniocarpium* have some unique morphological features that distinguish them from other sections such as tuberculate stems in *H.* sect. *Hirtella* and indumentum on stems of *H.* sect. *Taeniocarpium* (vs. smooth/glabrous stems in other sections) (Li & Robson, 2007). Here, we found that seed appendages are also absent in these two sections, providing an additional reliable taxonomic character that distinguishes them from other sections. In conclusion, appendages have taxonomic value at the section level, and further attention should be paid to this character in future taxonomic studies.

Seed size is a quantitative trait, and in this study, we observed at least 50 seeds for each taxon. We found that seed size has taxonomic utility at the species level. *Hypericum* sect. *Ascyreia* is the largest section of *Hypericum* in China and species delimitation in this section is notoriously difficult (Robson, 1985, 2012). Robson (1985, 2012) divided the section into 5 species groups based on various characters (e.g., the presence/absence of intramarginal vein on leaves, erect/outcurved of sepals, etc.). Based on field investigation, however, we found that these characters display great inter- and intraspecies variability among species groups. In contrast, seed size is a stable character within those species and can be used to distinguish some species. For example, *H. uralum* and *H. henryi* from the *H. patulum* group are morphologically similar species and some variable characters (i.e., sepal shape and stem frondose or not) are typically used to distinguish them. Here, we found that seed size is a useful feature to reliably distinguish *H. uralum* (0.40–0.46 × 0.24–0.28 mm; Figure 1, [1]) and *H. henryi* (0.85–1.43 × 0.26–0.36 mm; Figure 1, [14]). In addition, seed size is also an important character that distinguish *H. choisyanum* (0.72–0.82 × 0.26–0.28 mm; Figure 1, [5]) and *H. maclarenii* (1.16–1.38 × 0.30–0.36 mm; Figure 1, [18]) from the *H. maclarenii* group, which were traditionally differentiated by sepal shape and the petiole length. Seed size also displayed taxonomic values in other species groups and certain sections (e.g., Figure 1, [9, 23], [6, 7, 10, 21, 22], [27, 28, 29], etc.), and our study further illustrates the seed size is another character that can be used for species delimitation in *Hypericum*.

Seed surface ornamentation is probably the most important character used for the infrageneric taxonomy of the genus

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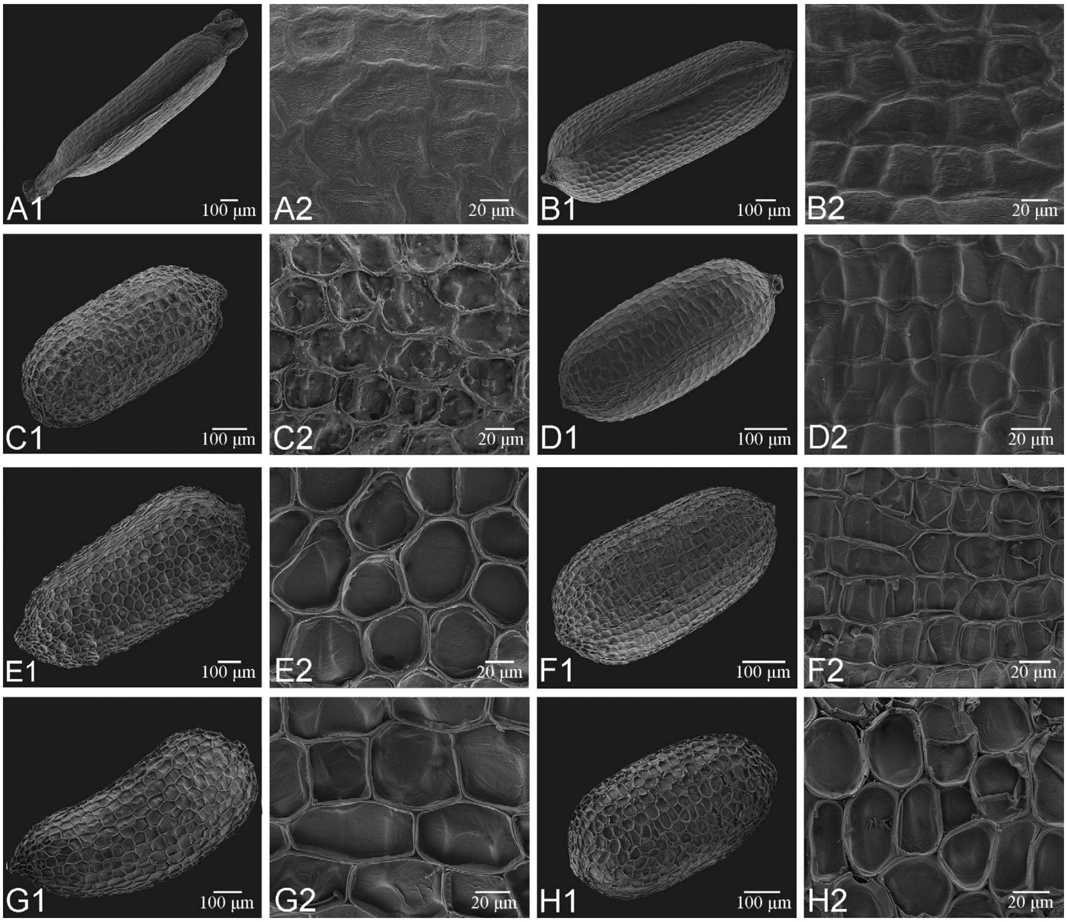

FIGURE 5 Seed micromorphology of *Hypericum* taxa under the scanning electron microscopy (SEM). (a1, a2) *H. ascyron* subsp. *gebleri*; (b1, b2) *H. przewalskii*; (c1, c2) *H. erectum*; (d1, d2) *H. faberi*; (e1, e2) *H. perforatum*; (f1, f2) *H. elodeoides*; (g1, g2) *H. hengshanense*; (h1, h2) *H. kingdonii*.

Hypericum. Robson (1977, 2012) has considered that seed surface ornamentation is the main distinguishing character between *Hypericum* sect. *Origanifolium* and *H.* sect. *Drosocarpium*. In this study, we sampled seeds of 40 taxa representing 9 sections. In general, species in the same section exhibit similar seed surface ornamentation. For example, *H.* sect. *Ascyreia* usually has linear-reticulate seed surface ornamentation, *H.* sect. *Roscyna* has reticulate seed surface ornamentation, *H.* sect. *Sampsonia* has ribbed seed surface ornamentation, and *H.* sect. *Trigynobrathys* has ribbed-scalariform seed surface ornamentation, indicating ornamentation is useful for taxonomy in Chinese *Hypericum* at the sectional level. On the other hand, surface ornamentation provides additional evidence for section delimitation. For example, Robson (2001) subdivided the *H.* sect. *Hypericum* sensu lato into 6 sections, of which *H.* sect. *Elodeoida*, *H.* sect. *Monanthema*, and *H.* sect. *Hypericum* sensu stricto were considered most closely related sections. However, monophyly for those sections were not supported by molecular phylogenetic studies (Meseguer et al., 2013; Nürk et al., 2013; Nürk & Blattner, 2010). Here, we found that species for those sections also have similar seed surface ornamentation, ranging from reticulate to foveolate (Figure 5, c1–h2; Figure 6, a1–c2), and no relevant difference has been observed. Therefore, previous molecular phylogenetic studies as well as seed characters presented in this study suggested that the circumscription of these sections needs to be reconsidered.

In China, *Hypericum* sect. *Taeniocarpium* and *H.* sect. *Hirtella* are well defined and distinct based on several morphological characters, including elongate inflorescences and tuberculate stems with indumentum. Geographically, they are only distributed in the Xinjiang in Western China, while other sections are mainly distributed in SW China. In this study, species of those two sections displayed distinctive papillose seed surface ornamentation (Figure 6g1–h2), indicating a close relationship between them.

In comparison with seed size and surface ornamentation, seed color and shape have limited taxonomic value in *Hypericum*. In this study, seed color tends to be uniform although five subtypes can be recognized. In general, only species of *Hypericum* sect. *Trigynobrathys* can be easily distinguished by having brownish-yellow seeds (Figure 1, [33, 34]), and species in other sections usually have brown seeds. In addition, the color can vary among different individuals from the same species (Figure S1, A–F). Previous studies reported that European *Hypericum* species have elliptic, cylindric to prolonged cylindric seeds (Alonso et al., 2013; Núñez, 1982; Szkudlarz & Celka, 2016). Here, seeds of most species were cylindric (28 spp.), followed by cylindric-elliptic (7 spp.) and prolonged cylindric (5 spp.). In general, seed shapes

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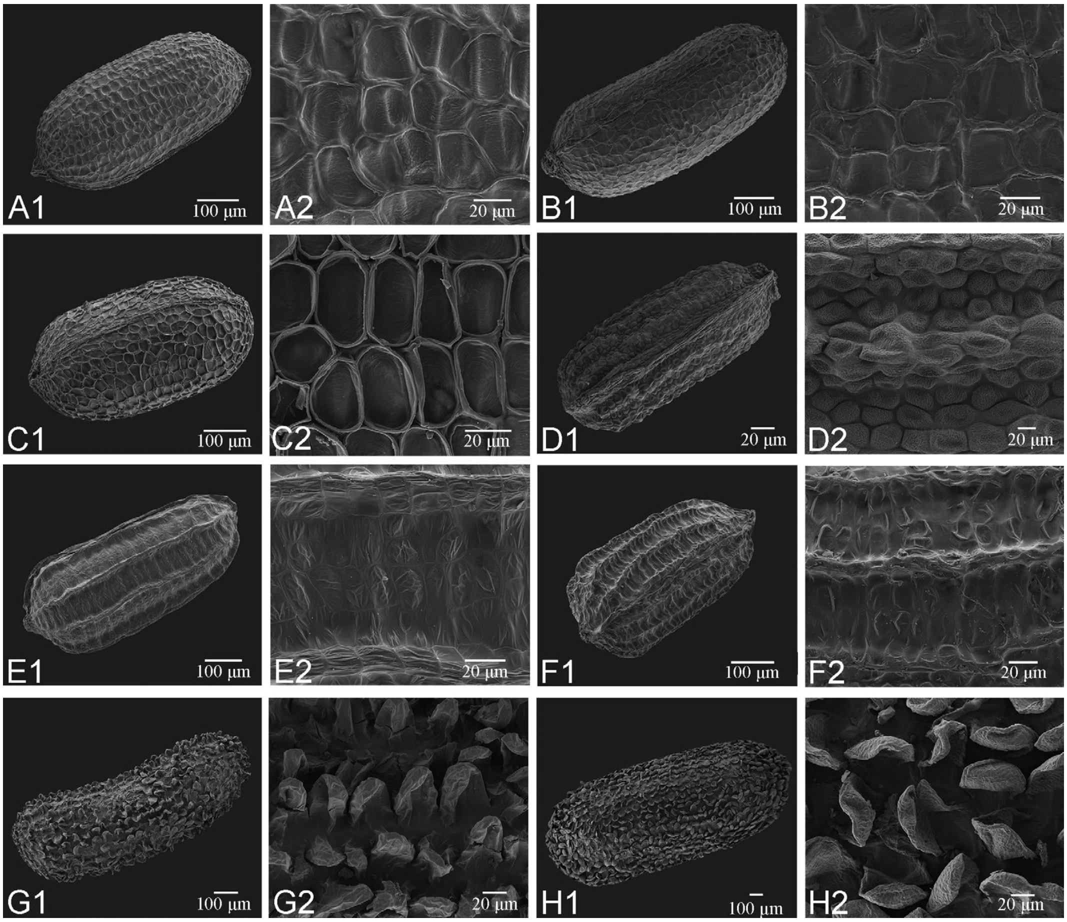

FIGURE 6 Seed micromorphology of *Hypericum* taxa under the scanning electron microscope (SEM). (a1, a2) *H. himalaicum*; (b1, b2) *H. monanthemum*; (c1, c2) *H. wightianum*; (d1, d2) *H. sampsonii*; (e1, e2) *H. japonicum*; (f1, f2) *H. gramineum*; (g1, g2) *H. hirsutum*; (h1, h2) *H. scabrum*.

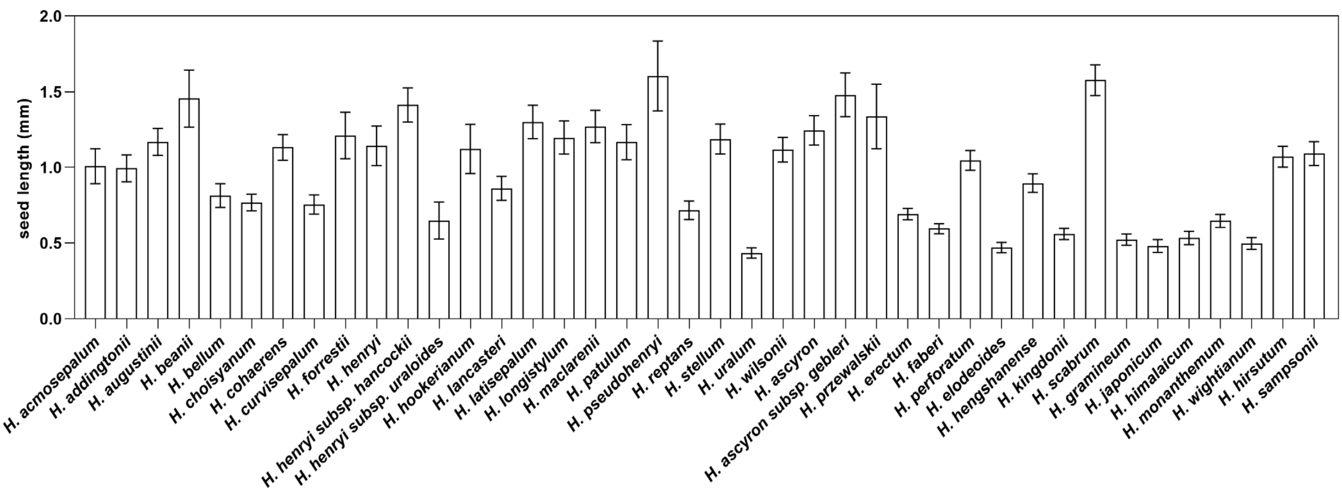

FIGURE 7 Variation in seed length of the studied *Hypericum* species (mean ± SD).

show inconsiderable variation and no pattern was discovered at the section or species level. This character apparently has limited taxonomic significance as suggested by previous studies (Alonso et al., 2013; Núñez, 1982; Szkudlarz & Celka, 2016).

5 | CONCLUSION

We provide the first comprehensive investigation of seed morphology for *Hypericum* from China, which is one of the most taxonomically

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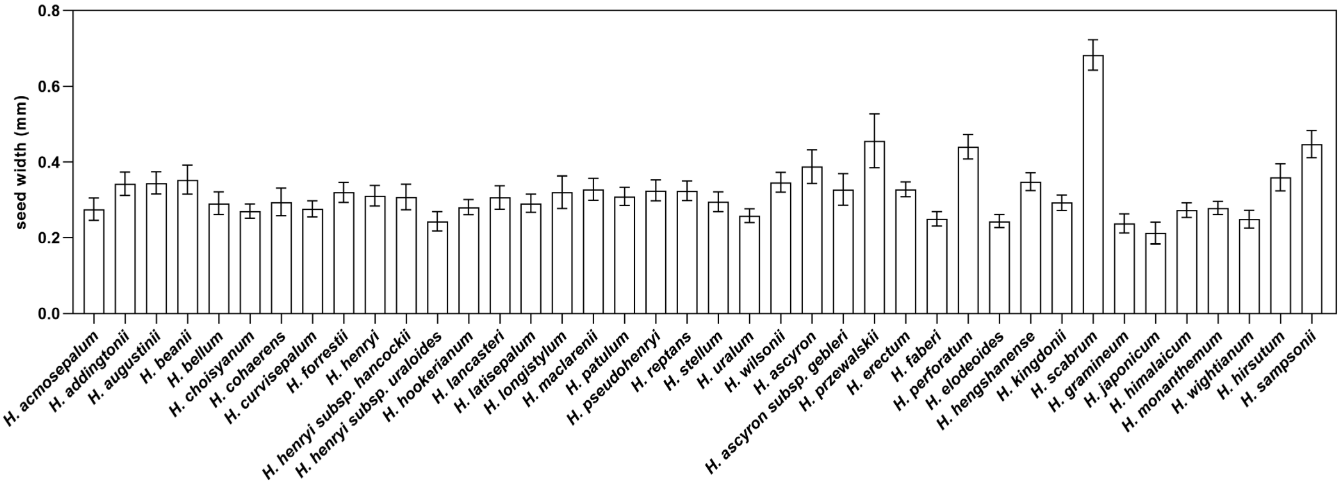

FIGURE 8 Variation in seed width of the studied *Hypericum* species (mean ± SD).

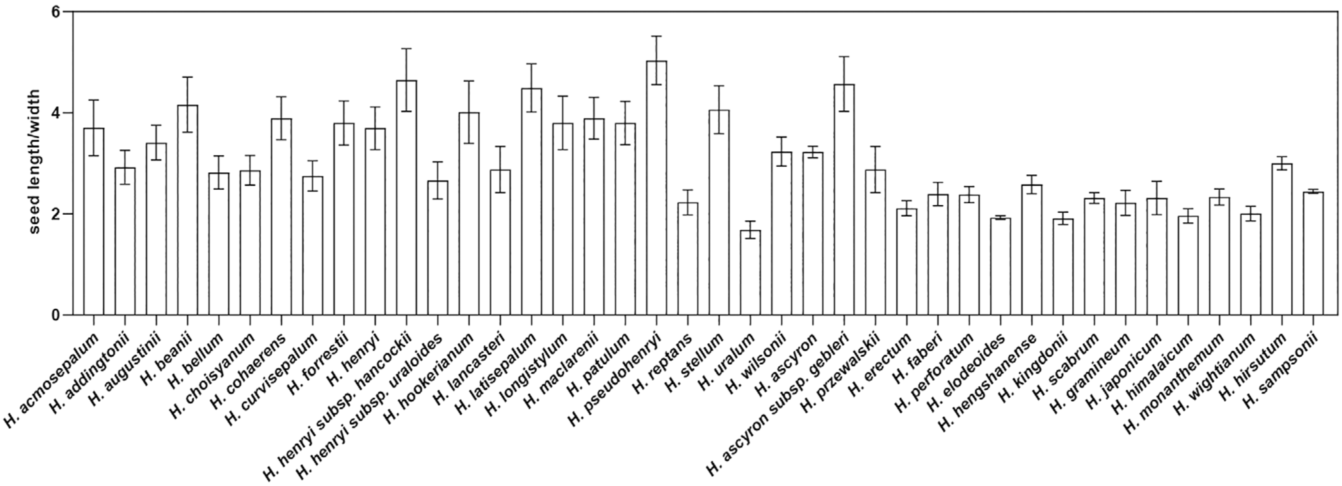

FIGURE 9 Variation in seed length/width of the examined *Hypericum* species (mean ± SD).

difficult plant groups. In total, macro- and micro-morphological characters of seeds from 40 taxa representing 9 sections were examined. We found that seed size, appendages, and surface ornamentation have significant taxonomic value at the sectional and species levels. On the contrary, seed color and shape are generally less important for the taxonomy of Chinese *Hypericum* species. Our study illustrated that the use of SEM can illuminate inconspicuous morphological affinities of seeds, suggesting that detailed investigation of seed morphology in the future can broaden our understanding of the taxonomy and character evolution of this medically important but taxonomically challenging group.

AUTHOR CONTRIBUTIONS

Rui-Zhu Bai: Methodology; software; data curation; writing – original draft; formal analysis; writing – review and editing. **Fei Zhao:** Resources; writing – original draft; funding acquisition. **Bryan T. Drew:** Methodology; writing – original draft; resources; writing – review and editing. **Gang Xu:** Funding acquisition; resources; supervision; writing – original draft; writing – review and editing. **Jie Cai:** Investigation; funding acquisition; writing – original draft; writing – review and editing; supervision; resources. **Shi-Kang Shen:** Investigation; writing – original draft; writing – review and editing; supervision; validation; resources. **Chun-Lei Xiang:** Methodology; investigation; funding acquisition; writing – original draft; writing – review and editing; supervision; formal analysis; resources; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCE

Alonso, M. Á., Agulló, J. C., Villar, J. L., Juan, A., & Crespo, M. B. (2013). Taxonomic relationships in the *Hypericum ericoides* aggregate (*H.* sect. *Coridium*, Hypericaceae). *Annales Botanici Fennici*, *50*, 195–207.

Amini, E., Zarre, S., & Assadi, M. (2011). Seed micro-morphology and its systematic significance in *Gypsophila* (Caryophyllaceae) and allied genera. *Nordic Journal of Botany*, *29*, 660–669.

Anjum, P. (2009). Seed morphology of the genus *Silene*: Caryophyllaceae from Pakistan and Kashmir. *International Journal of Biology and Biotechnology*, *6*, 219–227.

APG IV. (2016). An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, *181*, 1–20.

Arabi, Z., Ghahremaninejad, F., Rabeler, R. K., Heubl, G., & Zarre, S. (2017). Seed micromorphology and its systematic significance in tribe Alsineae (Caryophyllaceae). *Flora*, *234*, 41–59.

Bai, R. Z., Zhao, F., Cai, J., Shen, S. K., & Xiang, C. L. (2023). *Hypericum podocarpoides* N. Robson (Hypericaceae): A newly recorded species from China. *Plant Science Journal*, *41*, 1–6.

Beentje, H. J. (2010). *The Kew plant glossary: An illustrated dictionary of plant terms* (pp. 159–160). Kew.

Bojňanský, V., & Fargašová, A. (2007). *Atlas of seeds and fruits of central and east-European flora: The Carpathian Mountains region* (pp. 355–358). Springer Science & Business Media.

Butterweck, V. (2003). Mechanism of action of St John's wort in depression. *CNS Drugs*, *17*, 539–562.

Chernoff, M., Plitmann, U., & Kislev, M. E. (1992). Seed characters and testa texture in species of the Vicieae: Their taxonomic significance. *Israel Journal of Plant Sciences*, *41*, 167–186.

Corner, E. J. H. (1976). *The seed of dicotyledons* (p. 131). Cambridge University Press.

Crockett, S. L., Douglas, A. W., Scheffler, B. E., & Khan, I. A. (2004). Genetic profiling of *Hypericum* (St. John's Wort) species by nuclear ribosomal ITS sequence analysis. *Planta Medica*, *70*, 929–935.

Crockett, S. L., & Robson, N. K. (2011). Taxonomy and chemotaxonomy of the genus *Hypericum*. *Medicinal and Aromatic Plant Science and Biotechnology*, *5*, 1–13.

Crow, G. E. (1979). The systematic significance of seed morphology in *Sagina* (Caryophyllaceae) under scanning electron microscopy. *Brittonia*, *31*, 52–63.

Dahlgren, R. M. T. (1980). A revised system of classification of the angiosperms. *Botanical Journal of the Linnean Society*, *80*, 91–124.

Fawzi, N. M., Fawzy, A. M., & Mohamed, A. (2010). Seed morphological studies on some species of *Silene* L. (Caryophyllaceae). *International Journal of Botany*, *6*, 287–292.

Fedotova, T., & Ardjanova, R. (1992). Seed morphology in the genus *Gypsophila* (Caryophyllaceae). *Botanicheskii Zhurnal*, *77*, 1–16.

Gabr, D. G. (2014). Seed morphology and seed coat anatomy of some species of Apocynaceae and Asclepiadaceae. *Annals of Agricultural Sciences*, *59*, 229–238.

Galeotti, N. (2017). *Hypericum perforatum* (St John's wort) beyond depression: A therapeutic perspective for pain conditions. *Journal of Ethnopharmacology*, *200*, 136–146.

Ghimire, B., Jeong, M. J., Choi, G. E., Lee, H., Suh, G. U., Heo, K., & Ku, J. J. (2015). Seed morphology of the subfamily Helleboroideae (Ranunculaceae) and its systematic implication. *Flora*, *216*, 6–25.

Güneş, F. (2012). Seed morphology and their systematic importance of *Lathyrus* taxa belonging to *Platystylis* (= *Lathyrostylis*) section (Fabaceae) from Turkey. *African Journal of Agricultural Research*, *7*, 265–277.

Han, S., Sebastin, R., Lee, K. J., Wang, X., Shin, M. J., Kim, S. H., Lee, S., Lee, J. R., Cho, G. T., Hyun, D. Y., & Chung, J. W. (2021). Interspecific variation of seed morphological and micro-morphological traits in the genus *Vicia* (Fabaceae). *Microscopy Research and Technique*, *84*, 337–357.

Kanwal, K., Zafar, M., Khan, A. M., Mahmood, T., Abbas, Q., Ozdemir, F. A., Ahmad, M., Sultana, S., Rozina.Fatima, A., Aziz, A., & Niazi, A. (2022). Implication of scanning electron microscopy and light microscopy for oil content determination and seed morphology of Verbenaceae. *Microscopy Research and Technique*, *85*, 789–798.

Kramina, T. E., & Polevova, C. B. (2022). Taxonomic and ecological significance of seed morphology and ultrastructure in species of *lotus* formerly classified in *Dorycnium* (Leguminosae–Loteae). *Turczaninowia*, *25*, 175–189.

Li, X. W. (1990). Hypericum. In Z. Y. Wu (Ed.), *Flora Reipublicae Popularis Sinicae* (Vol. 50, pp. 2–112). Science Press.

Li, X. W., & Robson, N. K. B. (2007). Hypericum. In Z. Y. Wu & P. H. Raven (Eds.), *Flora of China* (Vol. 13, pp. 2–35). Science Press & Missouri Botanical Garden Press.

Luqman, M., Zafar, M., Ahmad, M., Ozturk, M., Sultana, S., Alam, F., & Ullah, F. (2019). Micromorphological observation of seed coat of *Eucalyptus* species (Myrtaceae) using scanning electron microscopy technique. *Microscopy Research and Technique*, *82*, 75–84.

Meseguer, A. S., Aldasoro, J. J., & Sanmartín, I. (2013). Bayesian inference of phylogeny, morphology and range evolution reveals a complex evolutionary history in St. John's wort (*Hypericum*). *Molecular Phylogenetics and Evolution*, *67*, 379–403.

Meseguer, A. S., & Sanmartín, I. (2012). Paleobiology of the genus *Hypericum* (Hypericaceae): A survey of the fossil record and its palaeogeographic implications. *Anales del Jardín Botánico de Madrid*, *69*, 97–106.

Mittermeier, R. A., Myers, N., Mittermeier, C. G., & Robles Gil, P. (1999). Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions. Cemex, Conservation International and Agrupacion Sierra Madre.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853–858.

Nardi, K. D. O., Oriani, A., & Scatena, V. L. (2016). Seed micromorphology and its taxonomic significance to *Xyris* (Xyridaceae, Poales). *Brazilian Journal of Botany*, *39*, 721–727.

Núñez, A. R. (1982). Estudio biosistemático del género *Hypericum* L. (Guttiferae) en la Península Ibérica e Islas Baleares. I. Caracteres semifinales. Trabajos del Departamento de Botanica, 12, 45–62.

Nürk, N. M., & Blattner, F. R. (2010). Cladistic analysis of morphological characters in *Hypericum* (Hypericaceae). *Taxon*, *59*, 1495–1507.

10970029, 2023, 11, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/jemt.24372 by KUNMING INSTITUTE OF BOTANY, Wiley Online Library on [09/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Nürk, N. M., Madriñán, S., Carine, M. A., Chase, M. W., & Blattner, F. R. (2013). Molecular phylogenetics and morphological evolution of St. John's wort (*Hypericum*; Hypericaceae). *Molecular Phylogenetics and Evolution*, *66*, 1–16.

Park, S. J., & Kim, K. J. (2004). Molecular phylogeny of the genus *Hypericum* (Hypericaceae) from Korea and Japan: Evidence from nuclear rDNA ITS sequence data. *Journal of Plant Biology*, *47*, 366–374.

Pilepić, K. H., Morović, M., Orač, F., Šantor, M., & Vejnović, V. (2010). RFLP analysis of cpDNA in the genus *Hypericum*. *Biologia*, *65*, 805–812.

Robson, N. K. B. (1977). Studies in the genus *Hypericum* L. (Guttiferae). 1. Infrageneric classification. *Bulletin of the British Museum (Natural History) Botany*, *5*, 293–355.

Robson, N. K. B. (1981). Studies in the genus *Hypericum* L. (Guttiferae). 2. Characters of the genus. *Bulletin of the British Museum (Natural History) Botany*, *8*, 55–226.

Robson, N. K. B. (1985). Studies in the genus *Hypericum* L. (Guttiferae) 3. Sections 1. *Campylosporus* to 6a. *Umbraculoides*. *Bulletin of the British Museum (Natural History) Botany*, *12*, 163–325.

Robson, N. K. B. (1987). Studies in the genus *Hypericum* L. (Guttiferae) 7. Section 29. *Brathys* (part 1). *Bulletin of the British Museum (Natural History) Botany*, *16*, 1–106.

Robson, N. K. B. (1990). Studies in the genus *Hypericum* L. (Guttiferae) 8. Sections 29. *Brathys* (part 2) and 30. *Trigynobrathys**. *Bulletin of the British Museum (Natural History) Botany*, *20*, 1–151.

Robson, N. K. B. (1996). Studies in the genus *Hypericum* L. (Guttiferae) 6. Sections 20. *Myriandra* to 28. *Elodes*. *Bulletin of the British Museum (Natural History) Botany*, *26*, 75–217.

Robson, N. K. B. (2001). Studies in the genus *Hypericum* L. (Guttiferae) 4 (1). Sections 7. *Roscyna* to 9. *Hypericum* sensu lato (part 1). *Bulletin of the British Museum (Natural History) Botany*, *31*, 37–88.

Robson, N. K. B. (2002). Studies in the genus *Hypericum* L. (Guttiferae) 4 (2). Section 9. *Hypericum* sensu lato (part 2): Subsection 1. *Hypericum* series 1. *Hypericum*. *Bulletin of the British Museum (Natural History) Botany*, *32*, 61–123.

Robson, N. K. B. (2006). Studies in the genus *Hypericum* L. (Clusiaceae). Section 9. *Hypericum* sensu lato (part 3): Subsection 1. *Hypericum* series 2. *Senanensia*, subsection 2. *Erecta* and section 9b. *Graveolentia*. *Systematics and Biodiversity*, *4*, 19–98.

Robson, N. K. B. (2010a). Studies in the genus *Hypericum* L. (Hypericaceae) 5 (1). Sections 10. *Olympia* to 15/16. *Crossophyllum*. *Phytotaxa*, *4*, 5–126.

Robson, N. K. B. (2010b). Studies in the genus *Hypericum* L. (Hypericaceae) 5 (2). Sections 17. *Hirtella* to 1.9 *Coridium*. *Phytotaxa*, *4*, 127–258.

Robson, N. K. B. (2012). Studies in the genus *Hypericum* L. (Hypericaceae) 9. Addenda, corrigenda, keys, lists and general discussion. *Phytotaxa*, *72*, 1–111.

Robson, N. K. B. (2016). And then came molecular phylogenetics—Reactions to a monographic study of *Hypericum* (Hypericaceae). *Phytotaxa*, *255*, 181–198.

Ruhfel, B. R., Bittrich, V., Bove, C. P., Gustafsson, M. H., Philbrick, C. T., Rutishauser, R., Xi, Z., & Davis, C. C. (2011). Phylogeny of the clusioid clade (Malpighiales): Evidence from the plastid and mitochondrial genomes. *American Journal of Botany*, *98*, 306–325.

Schindelin, J., Rueden, C. T., Hiner, M. C., & Eliceiri, K. W. (2015). The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular Reproduction and Development*, *82*, 518–529.

Stevens, P. F. (2007). Hypericaceae. In K. Kubitzki (Ed.), *The families and genera of vascular plants* (Vol. 9, pp. 194–201). Springer Verlag.

Sun, Q., Liu, P. L., Zhang, Q., Cao, L. X., & Jiang, Z. M. (2009). Seed morphology of nine species in the genus *Hypericum* L. and its systematical significance. *Acta Botanica Boreali-Occidentalia Sinica*, *32*, 276–280.

Szkudlarz, P. (2014). Seed morphology and anatomy of *Hypericum elegans* Steph. Ex Willd. *Biodiversity Research and Conservation*, *35*, 15–18.

Szkudlarz, P., & Celka, Z. (2016). Morphological characters of the seed coat in selected species of the genus *Hypericum* L. and their taxonomic value. *Biodiversity Research and Conservation*, *44*, 1–9.

Ullah, F., Papini, A., Shah, S. N., Zaman, W., Sohail, A., & Iqbal, M. (2019). Seed micromorphology and its taxonomic evidence in subfamily Alsinoideae (Caryophyllaceae). *Microscopy Research and Technique*, *82*, 250–259.

Veiskarami, G., Khodayari, H., Heubl, G., & Zarre, S. (2018). Seed surface ultrastructure as an efficient tool for species delimitation in the *Allium ampeloprasum* L. alliance (Amaryllidaceae, Allioideae). *Microscopy Research and Technique*, *81*, 1275–1285.

Waheed, A., Ahmad, M., Ghufran, M. A., Jabeen, A., Ozdemir, F. A., Zafar, M., Sultana, S., Shah, M. A., Majeed, S., & Khan, A. S. (2021). Implication of scanning electron microscopy in the seed morphology with special reference to three subfamilies of Fabaceae. *Microscopy Research and Technique*, *84*, 2176–2185.

Xiang, C. L., Funamoto, T., Evangelista, E. V., Zhang, Q., & Peng, H. (2013). Pollen morphology of the east Asiatic genus *Chelonopsis* (Lamioideae: Lamiaceae) and allied genera, with reference to taxonomic implications and potential pollination ecology. *Plant Biosystems*, *147*, 620–628.

Zhang, R., Ji, Y., Zhang, X., Kennelly, E. J., & Long, C. (2020). Ethnopharmacology of *Hypericum* species in China: A comprehensive review on ethnobotany, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, *254*, 112686.

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