



# Khoonmengia honbaensis, a new genus and species of temperate bamboo (Poaceae, Bambusoideae) from central-southern Vietnam

Yi-Hua Tong<sup>1,3</sup>, Xi-Rong Zheng<sup>1,2,6</sup>, You Yuan Zhang<sup>1,2</sup>, Qiao-Mei Qin<sup>1,2,7</sup>, Jing-Bo Ni<sup>1</sup>, Tien Chinh Vu<sup>4,5</sup>, Nian-He Xia<sup>1,3</sup>

1 Key Laboratory of Plant Resources Conservation & Sustainable Utilization/ Key Laboratory of Digital Botanical Garden of Guangdong Province, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China 2 University of Chinese Academy of Sciences, Beijing, 100049, China 3 Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar 4 Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, Hanoi, Vietnam 5 Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam 6 Department of Botany, Guangzhou Institute of Forestry and Landscape Architecture, Guangzhou 510540, China 7 Guangdong Eco-engineering Polytechnic, Guangzhou 510520, China

Corresponding author: Nian-He Xia (nhxia@scbg.ac.cn)

Academic editor: Yun-Hong Tan | Received 28 August 2019 | Accepted 16 November 2019 | Published 10 January 2020

**Citation:** Tong Y-H, Zheng X-R, Zhang YY, Qin Q-M, Ni J-B, Vu TC, Xia N-H (2020) *Khoonmengia honbaensis*, a new genus and species of temperate bamboo (Poaceae, Bambusoideae) from central-southern Vietnam. In: Jin X-H, Xia N-H, Tan Y-H (Eds) Plant diversity of Southeast Asia-II. PhytoKeys 138: 163–177. https://doi.org/10.3897/phytokeys.138.39512

#### **Abstract**

A new genus of Arundinarieae, *Khoonmengia*, is established to accommodate a unique new bamboo species, *K. honbaensis*, from central-southern Vietnam. The morphological features, habitats and distribution of *Khoonmengia* and related genera, i.e. *Ampelocalamus* and *Hsuehochloa*, are compared. The characters of its scrambling habit, internodes with brownish green dots, conspicuous nodes swollen at one side, elliptic buds wholly sunken into culm, extravaginal branching pattern, mid-culm branch complement with one central dominant branch elongating to reiterate the culm accompanied by several lateral slender branches, swollen culm sheath base with a distinctive zone of transverse wrinkles, synflorescence composed of only one spikelet, single or several to many synflorescences arranged into a raceme or panicle terminal on leafy branches, purple anthers and nut-like caryopsis with hardened pericarp and loosely adherent lemma and palea distinguish *K. honbaensis* from morphologically similar taxa. In order to investigate the phylogenetic position of this unknown bamboo, molecular phylogenetic analyses based on the nuclear gene GBSSI were also conducted, and the results proved that *K. honbaensis* is definitely a member of Arundinarieae with an isolated position, which also indicated that this species could not be assigned to any of the already described genera and supported the establishment of the new genus.

# **Keywords**

Arundinarieae, morphology, phylogeny, scrambling bamboos, synflorescence

### Introduction

Bamboos, including a single evolutionary radiation of 1,642 species in the grass family Poaceae, subfamily Bambusoideae, are important components in tropical to warm temperate forests (Vorontsova et al. 2016). Bambusoideae is classified into two tribes of woody bamboos (the tropical Bambuseae and the temperate Arundinarieae) and one tribe of herbaceous bamboos (the Olyreae) (Soreng et al. 2015). The Arundinarieae are the temperate woody bamboos, a diverse clade of 31 genera and ca. 546 species, with the center of diversity in East Asia (ca. 430 species), distributed primarily in forests of the northern temperate zone, but also in some high elevation tropical regions (BPG 2012; Clark et al. 2015). Arundinarieae is not only a taxonomically difficult group of bamboos, but also a troublesome one in molecular phylogenetics (Yang et al. 2013). Previous phylogenetic studies mainly based on plastid DNA divided Arundinarieae into twelve lineages, but the phylogenetic relationships among many clades were not well resolved (Zeng et al. 2010; Zhang et al. 2012; Ma et al. 2014; Attigala et al. 2016). Some analyses also revealed many inconsistencies between the plastid and the nuclear gene trees (Zhang et al. 2012; Yang et al. 2013). For example, Ampelocalamus actinotrichus had an affinity with individuals of Chimonocalamus in the plastid phylogeny (Zeng et al. 2010), rather than other taxa of Ampelocalamus. However, in the nuclear gene phylogenies, this species formed a clade with the congeneric taxa (Yang et al. 2013). These results implied that the nuclear genome and the plastid genome may have different evolutionary trajectories (Zhang et al. 2012; Yang et al. 2013). The most recent study based on phylogenetic analyses with RAD-seq data identified eight major lineages in Arundinarieae with strong support, which conflicts with earlier studies (Wang et al. 2017).

During an investigation of the bamboos in Hon Ba Nature Reserve, Khanh Hoa Province of central-southern Vietnam in October 2017, an unusual bamboo with unicaespitose habit, scandent stems, pachymorph rhizomes and semelauctant inflorescences on leafy flowering branches caught our attention. This species was misidentified as *Bambusa tulda* Roxb. during the background survey of Hon Ba Nature Reserve (Lee et al. 2014). The semelauctant inflorescence is a relatively rare condition in the Bambuseae. After closer examination, we found that it has three stamens and two stigmas. By its habit and floral characters, it should be a member of the tribe Arundinarieae. In Vietnam, only five clambering genera, i.e. *Melocalamus* Benth., *Maclurochloa* K. M. Wong, *Nianhochloa* H. N. Nguyen & V. T. Tran, *Cochinchinochloa* H. N. Nguyen & V. T. Tran, are currently recognized, but all of these genera belong to Bambuseae (Wong 1993; Tran et al. 2013). In Southeast Asia, many other genera also have climbing or clambering culm

habits, such as *Holttumochloa* K. M. Wong, *Kinabaluchloa* K. M. Wong, *Dinochloa* Büse, *Racemobambos* Holttum, *Chloothamnus* Büse, etc. However, none of these genera belong to Arundinarieae either. There are only two genera belonging to Arundinarieae in subtropical Asia, i.e. *Ampelocalamus* S. L. Chen, T. H. Wen & G. Y. Sheng and *Hsuehochloa* D. Z. Li & Y. X. Zhang, which have a combination of morphological characters including scandent stems, semelauctant inflorescences, three stamens and two stigmas (Chen et al. 1981; Zhang et al. 2018). However, this unknown bamboo has some both reproductive and vegetative characters that are different from these two subtropical genera (see Table 1).

The nuclear gene GBSSI (granule-bound starch synthase I) occurs as a single copy in Poaceae and was often used in recent phylogenetic studies on woody bamboos (Zhang et al. 2012; Goh et al. 2013; Yang et al. 2013). Zhang et al. (2012) showed that the phylogeny based on GBSSI was better resolved at the generic level than the plastid phylogeny. Therefore, in order to investigate the phylogenetic position of this unknown bamboo, we conducted molecular phylogenetic analyses of Asian Arundinarieae based on GBSSI.

# Materials and methods

# Sampling and morphological study

Samples of this putative new species were collected for morphological and molecular phylogenetic studies from the only known population in Hon Ba Nature Reserve, Khanh Hoa Province, central-southern Vietnam during our field investigation in Oct. 2017. Photographs were taken with a CANON EOS 60D camera and dried flowers were dissected and examined under an Olympus SZX16 Microscope; line drawings and descriptions were made by reference to dried specimens.

# DNA amplification and sequencing

Total genomic DNA was isolated from silica gel-dried leaf material using the Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China), following the manufacturer's instructions. The nuclear GBSSI sequence was amplified following the protocol used in Zeng et al. (2010). All PCR were performed in 25  $\mu L$  volumes with a Senso-Quest Labcycler 48 Gradient. A fragment from this unknown species was successfully sequenced by the DNA sequencing facility at Sangon Biotech (China). Automated sequencing output was checked visually for correct automated base-calling. Sequences were aligned using Bioedit v7.2.0 (Hall 1999) and adjusted manually where necessary. The newly obtained sequence has been deposited in Genbank.

In addition, sequences from the other 42 taxon representing nearly all known genera of Arundinarieae and outgroups, mainly following prior studies (Zeng et al. 2010;

	Khoonmengia	Hsuehochloa	Ampelocalamus	
Habit	Scrambling	Pendulous or procumbent	Scrambling	
Branching pattern	Extravaginal	Extravaginal	Transferring	
Nodes	Swollen at one side	Nearly flat	Swollen at one side	
Mid-culm branch comple-	One central dominant branch	3–7 branches, subequal	Several to numerous branches,	
ment	accompanied by 1-4 lateral		subequal, or one or three domi-	
	slender ones		nant branches accompanied by	
			numerous slender ones	
Bud	Elliptic, wholly sunken into	Elliptic, wholly sunken into culm	Ovate to broad ovate, not sunken	
	culm		or only base sunken into culm	
Culm sheath base	Swollen, with a distinctive	Flat, without a distinctive zone of	Usually swollen, without a	
	zone of transverse wrinkles	transverse wrinkles	distinctive zone of transverse	
			wrinkles	
Culm sheath auricles	Absent	Present, falcate, amplexicaul	Absent or present	
Culm sheath oral setae	Absent	Present, radiate	Absent or present	
Presence of dots on culm	With brownish green dots	Without dots	Without dots Absent or present	
Leaf auricles and oral setae	Absent	Present		
Synflorescence	Composed of only one spike-	Racemose, composed of 1 or few	Paniculate, composed of many	
	let, single or several to many	spikelets, single synflorescence on	spikelets, on leafy or leafless flow-	
	synflorescences arranged into	leafy flowering branches	ering branches	
	a raceme or panicle on leafy			
	flowering branches			
Glumes	(0-)1-2	Unknown	2	
Number of florets per	7–9	5	2–7	
spikelet				
Anther color	Purple	Purple	Yellow	
Caryopsis	Nut-like, with hardened	Unknown	Grain-like, without hardened	
, ,	pericarp and loosely adherent		pericarp and with closely adherent	
	lemma and palea		lemma and palea	

**Table 1.** Comparison of morphological characters, distributions and habitats of *Khoonmengia*, *Hsuehochloa* and *Ampelocalamus*.

Zhang et al. 2012; Yang et al. 2013; Attigala et al. 2016), were downloaded from the NCBI Genbank database (http://www.ncbi.nlm.nih.gov/genbank/) (Table 2). *Bonia amplexicaulis* (L. C. Chia, H. L. Fung & Y. L. Yang) N. H. Xia, *Neomicrocalamus prainii* (Gamble) Keng f., and *Bambusa ventricosa* McClure of the tribe Bambuseae were chosen as outgroups based on prior studies (Zeng et al. 2010; Zhang et al. 2012).

Granite montane, alt. 1500 m Limestone montane, alt. 500–950 m

Southwest China (Guizhou)

South and Southwest China (Gansu, Chongqing, Sichuan, Guizhou, Yunnan, Hainan)

Limestone, granite or basalt montane, alt. 200–1800 m

Central-southern Vietnam

# Phylogenetic analyses

Distribution

Habitat

Gaps were coded as present or absent using the simple indel coding method (Simmons and Ochoterena 2000). The best-fitting models were selected using jModeltest v2.1.4 under the Akaike Information Criterion (AIC) (Darriba et al. 2012). The model used for the GBSSI in this study was TrNef+G.

**Table 2.** Voucher information and GenBank accession numbers for taxa used in this study.

Taxon	Voucher no.	Source	GenBank acces-
Asidosasa ahimomia C. D. Chu &z C. S. Chao ay Kono f	7hana 08025 (VI INI)	Guangdong, China	sion no. (GBSSI)
Acidosasa chinensis C. D. Chu & C. S. Chao ex Keng, f.  Acidosasa chienouensis (T. H. Wen) C. S. Chao & T. H.	Zhang 08035 (KUN) Zhang 08065 (KUN)	Fujian, China	JN132035 JN132043
Wen	Zhang 0000) (KCTV)	1 ujian, Ciina	J14132043
Ampelocalamus actinotrichus (Merr. & Chun) S. L. Chen,	Zeng and Zhang 06054 (KUN)	Hainan, China	KM264660
T. H. Wen & G. Y. Sheng	Zeng and Zhang 000) I (ROIV)	Tiaman, Ciima	1411201000
Arundinaria gigantea (Walter) Muhl.	Zhang US1025 (KUN)	Arkansas, United States	JN131985
Arundinaria tecta (Walter) Muhl.	Triplett 173 (ISC)	South Carolina, United States	JN131988
Bambusa ventricosa McClure	Zhang KMBG09 (KUN)	Yunnan, China	JN131925
Bashania abietina T. P. Yi & L. Yang	Zhang 07092 (KUN)	Sichuan, China	JN132004
Bonia amplexicaulis (L. C. Chia, H. L. Fung & Y. L. Yang) N. H. Xia	Zeng and Zhang SB5 (KUN)	Yunnan, China	JN131926
Brachystachyum densiflorum (Rendle) Keng	Zeng and Zhang 06174 (KUN)	Zhejiang, China	JN131957
Chimonobambusa macrophylla (Hsueh & T. P. Yi) T. H. Wen & Ohrnb	Zhang 07091 (KUN)	Sichuan, China	JN131980
Chimonocalamus montanus Hsueh & T. P. Yi	Zhang 07057 (KUN)	Yunnan, China	JN132029
Chimonocalamus pallens Hsueh & T. P. Yi	Zhang 07071 (KUN)	Yunnan, China	JN132060
Drepanostachyum ampullare (T. P. Yi) Demoly	GLM 081860 (KUN)	Xizang, China	JN132079
Drepanostachyum hookerianum (Munro) Keng f.	DZL 199903 (KUN)	Kew, Britain	AF445165
Fargesia decurvata J. L. Lu	Zhang 07087 (KUN)	Hubei, China	JN131937
Fargesia fungosa T. P. Yi	Zhang 07048 (KUN)	Yunnan, China	JN131982
Fargesia nitida (Mitford) Keng f. & T. P. Yi	Zhang KMBG10 (KUN)	Sichuan, China	JN131941
Ferrocalamus strictus Hsueh & Keng f.	Zeng and Zhang SB1 (KUN)	Yunnan, China	JN132090
Gaoligongshania megalothyrsa (HandMazz.) D. Z. Li, Hsueh & N. H. Xia	JRX 9401 (KUN)	Yunnan, China	JN131945
Gelidocalamus rutilans T. H. Wen	Zeng and Zhang 06183 (KUN)	Zhejiang, China	JN131967
Himalayacalamus falconeri (Munro) Keng f.	GLM 081524 (KUN)	Xizang, China	JN132078
Hsuehochloa calcarea (C. D. Chu & C. S. Chao) D. Z. Li & Y. X. Zhang	Zhen-Hua Guo 013 (KUN)	GenBank	KM264662
Indocalamus sinicus (Hance) Nakai	Zeng and Zhang 06081 (KUN)	GenBank	JN131939
Indocalamus wilsonii (Rendel) C. S. Chao & C. D. Chu	Zeng and SD Zhang 07119 (KUN)	GenBank	JN131928
Indosasa crassiflora McClure	Zhang 07014 (KUN)	GenBank	JN132069
Khoonmengia honbaensis N. H. Xia, Y. H. Tong & X. R.	BVN2017048 (IBSC)	Vietnam	MN521458
Zheng Neomicrocalamus prainii (Gamble) Keng f.	LL07236 (KUN)	Xizang, China	JN131921
Oldeania alpina (K. Schum.) Stapleton	Triplett and Clark (2010), ZHZ200101 (KUN)	Locality unkown	AF445171
Oligostachyum sulcatum Z. P. Wang & G. H. Ye	Zhang 07024 (KUN)	Guangxi, China	JN131987
Ampelocalamus loudianensis T. P. Yi & R. S. Wang	MPF10052 (KUN)	Guizhou, China	KM264663
Ampelocalamus melicoideus Keng f.	MPF10142 (KUN)	Chongqing, China	KM264667
Ampelocalamus microphyllus Hsueh & T. P. Yi	MPF10123 (KUN)	Chongqing, China	KM264665
Ampelocalamus patellaris (Gamble) Stapleton	Zhang 07075 (KUN)	Yunnan, China	AF445163
Ampelocalamus scandens Hsueh & W. D. Li	Zhen-Hua Guo 013 (KUN)	Yunnan, China	AF445164
Phyllostachys edulis (Carriere) Houzeau	Zhang KMBG04 (KUN)	Yunnan, China	JN132018
Pleioblastus gramineus (Bean) Nakai	Zhang and Zeng 06157 (KUN)	Zhejiang, China	JN131990
Pleioblastus juxianensis T. H. Wen, C. Y. Yao & S. Y. Chen	Zhang and Zeng 06136 (KUN)	Zhejiang, China	JN132037
Pseudosasa japonica (Sieb. & Zucc.) Makino	Zhang 07023 (KUN)	Guangxi, China	JN132010
Sasa senanensis Rehder	Triplett 146 (KUN)	Tennessee, United States	JN132068
Sinobambusa tootsik (Sieb.) Makino	Zhang and Zeng 06090 (KUN)	Guangdong, China	JN132015
Thamnocalamus spathiflorus (Trin.) Munro	GLM 081775 (KUN)	Xizang, China	JN132083
Yushania basihirsuta (McClure) Z. P. Wang & G. H. Ye	Zeng and Zhang 06108 (KUN)	Hunan, China	JN131961
Yushania brevipaniculata (HandMazz.) T. P. Yi	Zhang 08005 (KUN)	Sichuan, China	JN131933

Phylogenetic analyses were conducted with PAUP\*v.4.0b10, MrBayes 3.2.5 (Ronquist et al. 2012) and GARLI 2.0 (Zwickl 2006). MP analyses were conducted using PAUP\*v.4.0b10 (Swofford 2003). Heuristic searches were performed with 1000 homogeneity replicates, tree-bisection-reconnection (TBR) branch swapping, MULTREES option off, and random addition of sequences with 1000 replicates.

ML analyses were conducted using GARLI 2.0 (Zwickl 2006), with 1000 bootstrap replicates. 50% majority-rule consensus tree was constructed using PAUP\*4.0b10.

BI analyses were conducted using MrBayes 3.2.5 (Ronquist et al. 2012). The runs were conducted starting with random trees, consisting of a single cold chain and three heated chains, with the temperature set to 0.1. The Markov chain Monte Carlo (MCMC) chains were run for 10 million generations and sampled trees every 1000 generations for the GBSSI gene data set. A 50% majority-rule consensus tree was constructed from the remaining trees, yielding the posterior probability (PP) values for each clade.

#### Results

A total of 1414 characters were included in the maximum parsimony (MP) analyses matrix, of which 133 characters were parsimony-informative, 238 variable characters were parsimony-uninformative and 843 characters were constant. The strict consensus tree for the 234 most parsimonious trees (tree length = 531; CI = 0.787; RI = 0.675; RC = 0.532) is shown in Fig. 1. The results of the MP, BI and ML analyses were almost identical except for slight position changes of some species (not shown). PP (posterior probabilities), MPBS (maximum parsimony bootstrap support) and MLBS (maximum likelihood bootstrap support) were included on the strict consensus tree from MP analyses. PP< 0.95 and MPBS/MLBS < 70% were considered as lacking support for a clade.

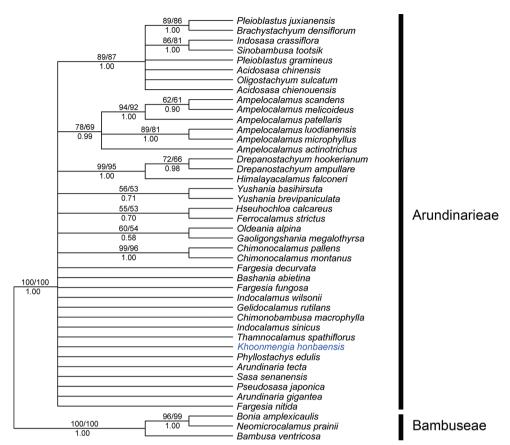
In the current study, the monophyly of the temperate woody bamboo clade was strongly supported, with 100% MPBS, 100% MLBS and 1.00 PP. Our putative new species was nested in the monophyletic clade of Arundinarieae. However, the phylogenetic relationships among groups of temperate woody bamboos were not resolved in this study.

#### Taxonomic treatment

*Khoonmengia* N.H.Xia, Y.H.Tong & X.R.Zheng, gen. nov. urn:lsid:ipni.org:names:77204206-1

Type. Khoonmengia honbaensis N.H.Xia, Y.H.Tong & X.R.Zheng.

**Diagnosis.** *Khoonmengia* resembles *Hsuehochloa* and *Ampelocalamus* in having pachymorph and short-necked rhizomes, florets with 3 stamens and 2 stigmas, but differs



**Figure 1.** The strict consensus of 234 equally most parsimonious trees based on the partial GBSSI gene. Numbers below branches indicate posterior probability of Bayesian Analysis, and numbers above branches indicate bootstrap values of MP and ML.

from the former by its scrambling habit, nodes swollen at one side, mid-culm branch complement with one central dominant branch elongating to reiterate the culm accompanied by 1–4 lateral slender branches, swollen culm sheath base with a distinctive zone of transverse wrinkles, culm and leaf sheaths without auricle or oral setae, and single or several to many synflorescences arranged into a raceme or panicle, and can be distinguished from the latter by extravaginal branching pattern, buds wholly sunken into culm, culm sheath base with a distinctive zone of transverse wrinkles, synflorescence composed of only one spikelet, purple anthers and nut-like caryopsis with hardened pericarp and loosely adherent lemma and palea.

**Description.** Shrubby bamboo. Rhizomes pachymorph, short-necked. Culms unicaespitose, erect at lower part, distally scrambling; internodes terete, with dense brownish green dots; nodes conspicuous, swollen at one side. Buds elliptic, wholly sunken into culm. Branches extravaginal, often solitary at lower part of culm, and usually with one central dominant branch elongating to reiterate the culm and 1–4

lateral slender ones in the middle part of culm. Culm sheaths persistent, basally swollen, with a distinctive zone of transverse wrinkles; auricles and oral setae absent; blade reflexed; ligule convex. Foliage leaves without auricles and oral setae; ligules convex. Synflorescence semelauctant, composed of only one spikelet subtended by one or several sheath-like bracts, single or several to many synflorescences arranged into a raceme or panicle which is terminal on leafy branches; spikelets with 8–9 florets. Glumes (0-)1–2. Palea slightly shorter than lemma. Lodicules 3. Stamens 3, filaments free, anthers purple. Styles 2, free, stigmas 2, plumose. Caryopsis nut-like, with a hardened pericarp and loosely adherent lemma and palea, apex with 2 persistent style bases.

# *Khoonmengia honbaensis* N.H.Xia, Y.H.Tong & X.R.Zheng, sp. nov. urn:lsid:ipni.org:names:77204207-1

Type. VIETNAM, Khanh Hoa, Hon Ba Nature Reserve, 1500 m, 17 October 2017, N. H. Xia et al. BVN2017048 (holotype, IBSC!; isotypes, SING!, VNM!).

**Description.** Culms erect at lower part, distally scrambling, 2–4(-10) m long; internodes terete, 20-32 cm long, 4-6 mm in diam., initially light purple, becoming gray-green, with dense brownish green dots turning black when dry; nodes conspicuous, swollen at one side, lower margin ciliate, supranodal ridge inconspicuous, intranodes glabrous. Buds elliptic, wholly sunken into culm. Branches extravaginal, often solitary at lower part of culm, and usually with one central dominant branch elongating to reiterate the culm and 1-4 lateral slender ones in the middle part of culm, lateral branches 10-25 cm long. Culm sheaths persistent, leathery, glossy, initially light purple, 8-9.5 cm long, abaxially with distinct veins, basally swollen, with a distinctive zone of transverse wrinkles; auricles and oral setae absent; blade reflexed, lanceolate, 6–9 cm long, glabrous, deciduous; ligule convex, ca. 2 mm high, glabrous. Leaves 3–8 per ultimate branch; leaf sheaths glabrous; auricles and oral setae absent; ligules convex, ca. 2 mm high; blades elliptic-lanceolate,  $10-20 \times 1-2.5$  cm, glabrous both sides except margin ciliate at the base when young, secondary veins 3-5 pairs, transverse veins distinct. Synflorescence semelauctant, composed of only one spikelet subtended by one or several sheath-like bracts, single or several to many synflorescences arranged into a raceme or panicle which is terminal on leafy branches; spikelets 4-7 cm long, florets 8-9. Glumes (0-)1-2, ovate, ca. 10 × 3 mm, apex acute, glabrous, 11-veined. Rachilla segments flat, ca. 6 mm, glabrous, apex inflated. Lemma ovate-lanceolate, 12–13 × 5 mm, glabrous, 13-veined, apex acute with a mucro; palea slightly shorter than lemma, 11–12 × 2–3 mm, 2-keeled, keels ciliolate, apex with excurrent keel vein, 3-veined between keels and 2-veined outside keels, veins inconspicuous; lodicules 3, ovate, membranous,  $3-5 \times 1.5-2$  mm, ciliolate, apex acuminate; stamens 3, filaments white, free, anthers tinged purplish when young, then becoming purple, ca. 7 mm long; ovary ovoid, 1 mm long, glabrous; styles 2, free, ca. 1 mm long, stigmas plumose,

ca. 3 mm long. Caryopsis nut-like, with a hardened pericarp and loosely adherent lemma and palea, dark brown, fusiform,  $8-9 \times ca$ . 3 mm, apex with 2 persistent style bases.

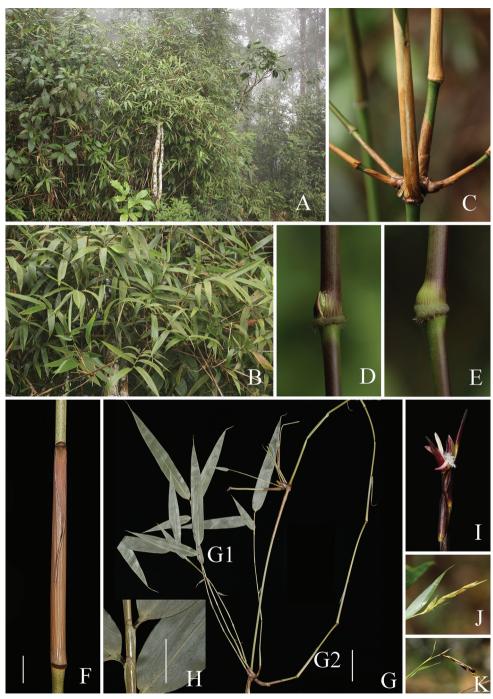
**Etymology.** *Khoonmengia* is named in honor of Dr. Khoon Meng Wong, a renowned botanist who has studied the bamboos and other plant groups of Southeast Asia for more than 35 years. The specific epithet is named after Hon Ba Nature Reserve, the type locality of this species.

**Distribution and habitat.** This species was only found in the type locality, i.e. Hon Ba Nature Reserve, Khanh Hoa Province of Vietnam. It occurs in high mountain broadleaved forests at an elevation of ca. 1500 m.

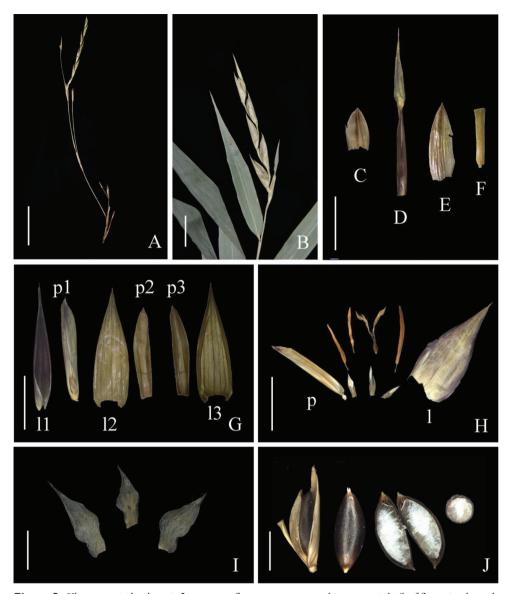
**Additional specimen examined** (paratype): VIETNAM, Khanh Hoa, Hon Ba Nature Reserve, 12°06'39.2"N, 108°56'47.2"E, C. Y. Lee et al. HIKK370 (HN!).

# **Discussion**

Morphological analysis (Table 1) revealed that this unknown bamboo owns several unique vegetative and reproductive characters that are different from the two closely related genera, i.e. Ampelocalamus and Hsuehochloa, such as culm with brownish green dots (Figs 2F, 5A), swollen culm sheath base with a distinctive zone of transverse wrinkles (Fig. 5A), synflorescence composed of solitary spikelet, single (Figs 2J-K, 3B, 4H) or several to many synflorescences arranged into a raceme or panicle (Figs 3A, 4G) terminal on leafy flowering branches, and nut-like caryopsis with loosely adherent lemma and palea (Fig. 3]). The nut-like bamboo caryopsis is different from the usual grain-like one by the hardened pericarp, and is reported only in some species of Bambuseae such as Cephalostachyum pallidum Munro, Dendrocalamus membranaceus Munro and D. strictus Nees before (Yu et al. 1993). Thus, the nut-like caryopsis type seems very rare in Arundinarieae. Our unknown bamboo species is also different from Ampelocalamus in the extravaginal branching pattern (vs. transferring), elliptic buds wholly sunken into culm (vs. ovate to broad ovate, not sunken or only base sunken into culm) (Fig. 6), and purple anthers (vs. yellow). For these three important generic characters, Hsuehochloa is the same as our unknown bamboo, which makes us infer that the closest genus to Khoonmengia may be Hsuehochloa. Although some important characters of Hsuehochloa such as number of glume, caryopsis type, are still unknown, besides the differences mentioned above, our unknown bamboo species can be further distinguished from Hsuehochloa by its scrambling habit (vs. pendulous or procumbent, not scrambling, according to the third author's field observation), nodes swollen at one side (vs. flat, Fig. 5B), mid-culm branch complement with one central dominant branch elongating to reiterate the culm accompanied by 1-4 lateral slender branches (vs. subequal 3–7 branches), and culm and leaf sheath auricle or oral setae absent (vs. present). Moreover, Hsuehochloa grows on the limestone mountain, while the unknown species grows in granitic montane broadleaved forests. More detailed comparisons of

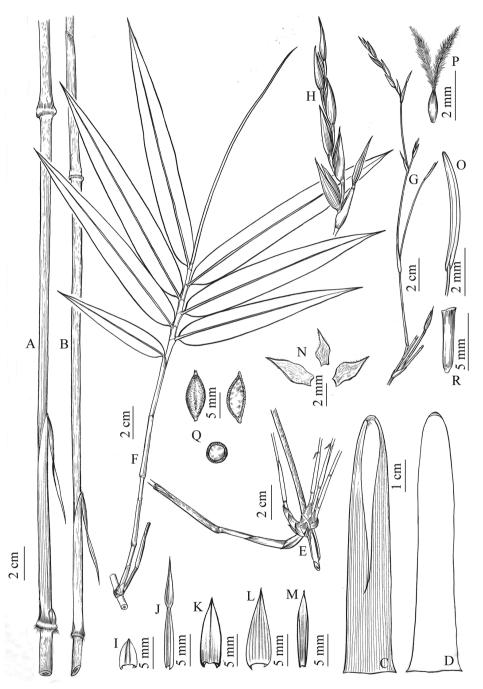


**Figure 2.** Khoonmengia honbaensis **A** habit **B** leafy branches **C** branch complement **D** node with buds breaking out of the culm sheath base **E** node with bud inside the intact culm sheath **F** culm sheath **G** leafy branches at culm apex (G1 Slender branches, G2 Dominant branch) **H** leaf sheath **I** florets **J** synflorescence composed of only one spikelet **K** infructescence (**F, G, H** from N. H. Xia et al. BVN2017048). Scale bars:  $1 \text{ cm}(\mathbf{F, H})$ ;  $5 \text{ cm}(\mathbf{G})$ .

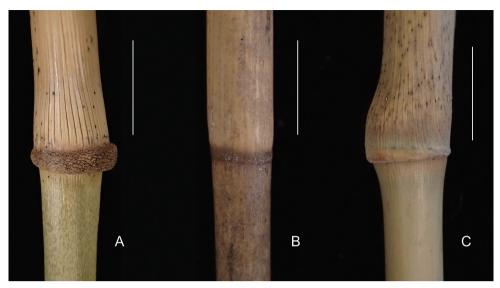


**Figure 3.** *Khoonmengia honbaensis* **A** many synflorescences arranged into a panicle (leaf flowering branch not shown) **B** single synflorescence composed of only one spikelet subtended by a sheath-like bract terminal on leaf flowering branch **C** prophyll **D** sheath-like bract **E** glume **F** rachilla segment **G** lemmas & paleas **H** dissection of one floret showing lemma, palea, 3 stamens, gynoecium with 2 stigmas and 3 lodicules **I** lodicules **J** nut-like caryopsis (leftmost, within its lemma and palea, and second from left, detached) and when sectioned vertically (third from left) and transversely (rightmost). (l = lemma, p = palea). Scale bars: 2 cm (**A**); 1 cm (**B**); 5 mm (**C-H, J**); 2 mm (**I**).

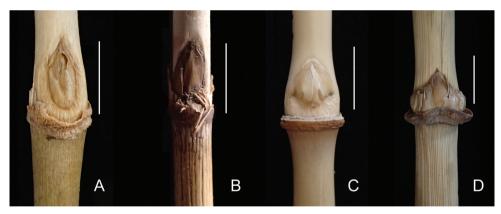
these genera are summarized in Table 1. The GBSSI phylogeny revealed that our putative new species is definitely a member of Arundinarieae with an isolated position, which indicated that this species could not be assigned to any of the already



**Figure 4.** *Khoonmengia honbaensis* **A, B** culm nodes and internodes with sheaths **C** culm sheath, abaxial view **D** culm sheath, adaxial view **E** branch complement **F** leafy branch **G** synflorescences arranged into a panicle **H** synflorescence composed of only one spikelet subtended by a sheath-like bract **I** prophyll **J** sheath-like bract **K** glume **L** lemma **M** palea **N** lodicules **O** stamen **P** pistil **Q** caryopsis and its vertical and cross sections **R** rachilla segment (From N. H. Xia et al. BVN2017048).



**Figure 5.** Comparison of culm leaf sheath bases **A** *Khoonmengia honbaensis*, showing swollen culm leaf sheath base with a distinctive zone of transverse wrinkles (From N. H. Xia et al. BVN2017048) **B** *Hsuehochloa calcarea*, showing flat and smooth culm leaf sheath base (From Y. Y. Zhang zyy-030, IBSC) **C** *Ampelocalamus actinotrichus*, showing slightly swollen and nearly smooth culm leaf sheath base (From N. H. Xia et al. HN-025, IBSC). Scale bars: 1 cm (**A, C**); 5 mm (**B**).



**Figure 6.** Comparison of buds **A** *Khoonmengia honbaensis*, showing elliptic bud wholly sunken into culm (From N. H. Xia et al. BVN2017048) **B** *Hsuehochloa calcarea*, showing elliptic bud wholly sunken into culm (From Y. Y. Zhang zyy-030, IBSC) **C** *Ampelocalamus actinotrichus*, showing ovate bud with the base sunken into culm (From N. H. Xia et al. HN-025, IBSC) **D** *Ampelocalamus melicoideus*, showing broad ovate bud not sunken into culm (From Y. Y. Zhang zyy-033, IBSC). Scale bars: 1 cm (**A, C, D**); 5 mm (**B**).

described genera. Based on the above analysis of morphology, molecular phylogenetic relationships and habitat, we propose to establish a new genus to accommodate this unknown bamboo.

# **Acknowledgments**

The authors are grateful to Mr. Ngyuen Van Dat and the staff in Hon Ba Nature Reserve for their help during the field trip. Our thanks also go to Dr. Joongku Lee for his help during locating the paratype specimen. This study was funded by the National Natural Science Foundation of China (grant nos. 31670196 & 31870180), Regional International Cooperation Project of Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (grant no. 2016CASSEABRIQG008) and Provincial Science and Technology Planning Project of Guangdong (grant no. 2017A030303061).

#### References

- Attigala L, Wysocki WP, Duvall MR, Clark LG (2016) Phylogenetic estimation and morphological evolution of Arundinarieae (Bambusoideae: Poaceae) based on plastome phylogenomic analysis. Molecular Phylogenetics and Evolution 101: 111–121. https://doi.org/10.1016/j.ympev.2016.05.008
- BPG [Bamboo Phylogeny Group] (2012) An updated tribal and subtribal classification of the bamboos (Poaceae: Bambusoideae). In: Gielis J, Potters G (Eds) Proceedings of the 9<sup>th</sup> World Bamboo Congress, 10–15 April 2012. World Bamboo Organization, Antwerp, Belgium, 3–27.
- Chen SL, Sheng GY, Wen TH (1981) *Ampelocalamus*, a new genus of Chinese bamboo. Acta Phytotaxomonica Sinica 19(3): 332–334.
- Clark LG, Londoño X, Ruiz-Sanchez E (2015) Bamboo taxonomy and habitat. In: Liese W, Köhl M (Eds) Bamboo-the Plant and its Uses. Springer. https://doi.org/10.1007/978-3-319-14133-6 1
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109
- Goh WL, Chandran S, Franklin DC, Isagi Y, Koshy KC, Sungkaew S, Yang HQ, Xia NH, Wong KM (2013) Multi-gene region phylogenetic analyses suggest reticulate evolution and a clade of Australian origin among paleotropical woody bamboos (Poaceae: Bambusoideae: Bambuseae). Plant Systematics and Evolution 299(1): 239–257. https://doi.org/10.1007/s00606-012-0718-1
- Hall TA (1999) Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Lee JK, Tran TB, Chang KS (2014) Floristic Diversity of Hon Ba Nature Reserve, Vietnam. Korea National Arboretum, Pocheon.
- Ma PF, Zhang YX, Zeng CX, Guo ZH, Li DZ (2014) Chloroplast phylogenomic analyses resolve deep-level relationships of an intractable bamboo tribe Arundinarieae (Poaceae). Systematic Biology 63(6): 933–950. https://doi.org/10.1093/sysbio/syu054
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

- Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49(2): 369–381. https://doi.org/10.1093/sysbio/49.2.369
- Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Zuloaga FO, Judziewicz EJ, Filgueiras TS, Davis JI, Morrone O (2015) A worldwide phylogenetic classification of the Poaceae (Gramineae). Journal of Systematics and Evolution 53(2): 117–137. https://doi.org/10.1111/jse.12150
- Swofford DL (2003) PAUP\*. Phylogenetic Analysis Using Parsimony (\*and other Methods) Version 4. Sinauer, Sunderland.
- Tran VT, Nguyen HN, Xia NH (2013) *Annamocalamus* H. N. Nguyen, N.-H. Xia & V. T. Tran, a new genus of bamboo (Poaceae) from Vietnam. Candollea 68(1): 159–165. https://doi.org/10.15553/c2013v681a23
- Vorontsova MS, Clark LG, Dransfield J, Govaerts R, Baker WJ (2016) World Checklist of Bamboos and Rattans. INBAR Technical Report No. 37.
- Wang XQ, Ye XY, Zhao L, Li DZ, Guo ZH, Zhuang HF (2017) Genome-wide RAD sequencing data provide unprecedented resolution of the phylogeny of temperate bamboos (Poaceae: Bambusoideae). Scientific Reports 7(1): 1–11. https://doi.org/10.1038/s41598-017-11367-x
- Wong KM (1993) Four new genera of bamboos (Gramineae: Bambusoideae) from Malesia. Kew Bulletin 48(3): 517–532. https://doi.org/10.2307/4118719
- Yang HM, Zhang YX, Yang JB, Li DZ (2013) The monophyly of *Chimonocalamus* and conflicting gene trees in Arundinarieae (Poaceae: Bambusoideae) inferred from four plastid and two nuclear markers. Molecular Phylogenetics and Evolution 68(2): 340–356. https://doi.org/10.1016/j.ympev.2013.04.002
- Yu FG, Hu CH, Chen L, Chen XY (1993) The morphological and anatomical characters of bamboo fruits with relation to systematics and evolution. Acta Botanica Sinica 35(10): 779–792.
- Zeng CX, Zhang YX, Triplett JK, Yang JB, Li DZ (2010) Large multi-locus plastid phylogeny of the tribe Arundinarieae (Poaceae: Bambusoideae) reveals ten major lineages and low rate of molecular divergence. Molecular Phylogenetics and Evolution 56(2): 821–839. https://doi.org/10.1016/j.ympev.2010.03.041
- Zhang YX, Zeng CX, Li DZ (2012) Complex evolution in Arundinarieae (Poaceae: Bambusoideae): incongruence between plastid and nuclear GBSSI gene phylogenies. Molecular Phylogenetics and Evolution 63(3): 777–797. https://doi.org/10.1016/j.ympev.2012.02.023
- Zhang YX, Ma PF, Li DZ (2018) A new genus of temperate woody bamboos (Poaceae, Bambusoideae, Arundinarieae) from a limestone montane area of China. PhytoKeys 109: 67–76. https://doi.org/10.3897/phytokeys.109.27566
- Zwickl DJ (2006) Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion. Ph.D. Dissertation, The University of Texas at Austin.