

The identity of *Sasa oblongula* C.H.Hu (Poaceae, Bambusoideae, Arundinarieae): evidence from morphology and molecular data

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Abstract

Sasa oblongula was described in 1987 based on a cultivated plant at the bamboo garden of Sun Yat-sen University. This species has two or three branches at the upper nodes, which differ from the rest of *Sasa* species that have a single branch per node. During the field trip to Baishi Town, Yunfu City, Guangdong Province in July 2021, one bamboo species with oblong foliage leaves was collected and matches the isotype. Then, our question was to test the identity of *S. oblongula* concerning other *Sasa* species based on morphology and molecular data. To do that, we sequenced the whole chloroplast genome of *S. oblongula* and did a phylogenetic analysis. Our morphological results indicate that the new collection is *S. oblongula*. The phylogenetic tree showed that *S. oblongula* is close to *Pseudosasa*, instead of *Sasa* species. Therefore, we transferred it to the genus *Pseudosasa*, and a revised description of *P. oblongula* is provided here.

Keywords

bamboo, China, phylogeny, *Pseudosasa*, taxonomy

Introduction

Sasa oblongula C.H.Hu (1987) was described based on two collections, i.e., *Y. L. Yang & C. H. Hu 198001* (Type) and *T. H. Wen & G. Y. Sheng 79413*, from the bamboo garden of Sun Yat-sen University, Guangdong Province. According to its protologue,

it was transplanted from somewhere in Guangdong with lack of a detailed address and could be distinguished by having small-medium-sized and oblong foliage leaves. It was well recognized and accepted as a distinctive species in the floras (Hu 1996; Wang and Stapleton 2006; Yi et al. 2008; Xia and Lin 2009; Vorontsova et al. 2016; Shi et al. 2022) and websites like GrassBase-The Online World Grass Flora (Clayton et al. 2016), Tropicos (www.tropicos.org), IPNI (www.ipni.org), POWO (powo.science.kew.org), The Plant List (www.theplantlist.org), GBIF (www.gbif.org). After examining paratype specimen *T. H. Wen & G. Y. Sheng 79413* in N and a failed attempt of searching for it in the bamboo garden of Sun Yat-sen University during the revision of *Sasinae* Keng f. (Keng 1982), Li (2009) treated it as a suspicious species. Significantly, the type specimens and protologue all demonstrated that this bamboo species possessed two or three branches at upper culm nodes, which conflicted with the strictly solitary branch of *Sasa* at each node (Makino and Shibata 1901; Suzuki 1978; Kobayashi 2017; Qin 2019). Thus, *S. oblongula* should not belong to the genus *Sasa* and may be a member of *Pseudosasa* based on the evidence available.

However, the previous molecular phylogenetic analysis (Zeng et al. 2010) showed a surprising result, namely that *S. oblongula*, eight Japanese *Sasa* species (including generic type), and one *Sasaella* Makino (1929) species, formed a subclade within the Arundinaria clade. To date, most Chinese *Sasa* species except *Sasa* subg. *Sasamorpha* have been transferred to *Sinosasa* L.C.Chia ex N.H.Xia, Q.M.Qin & Y.H.Tong and *Yushania* Keng f. (Keng 1957; Qin 2019; Qin et al. 2021; Li et al. 2023). We think that the voucher specimen *Zeng & Zhang 06055* of *S. oblongula* used by Zeng et al. for the molecular analysis is probably misidentified. Thus, the phylogenetic position of *S. oblongula* needs to be further studied with correct samples.

During the field trip to Baishi Town, Yunfu City, Guangdong Province in July 2021, one bamboo species with oblong foliage leaves was found. It matched the isotype very well and shares the same key characters, such as the slightly prominent culm supranodal ridge, the white powdery infranodal region, the glabrous internodes with three branches at an upper node, the solitary secondary branch, three to six foliage leaves clustered at the top of ultimate branches, the small-medium-sized and oblong foliage leaves with glabrous blades and conspicuous transverse veins. Therefore, we are certain that the specimens we collected are *S. oblongula*. Then, our question was to test the identity of *Sasa oblongula* concerning other *Sasa* species based on morphology and molecular data.

Materials and methods

Morphology

The sample of *Sasa oblongula* was collected from Hengjing Villiage, Baishi Town, Yunfu City, Guangdong, China during a field trip in July 2021. Observations and measurements were taken using a magnifier (SZ-6) and a ruler with a scale of 0.5 mm.

Some minor characters such as indumentum on ligules of both culm leaves and foliage leaves were observed with a stereomicroscope (Mshot MZ101). The description was made based on both living and dried material as well as relevant literature (e.g. Hu 1987, 1996; Wang and Stapleton 2006; Xia and Lin 2009). Comparisons between *S. oblongula* and *Pseudosasa cantorii* were conducted based on protologue and type specimens, and relevant specimens involved in the protologue of *Arundinaria cantorii* (\equiv *Pseudosasa cantorii*). The descriptive terms follow Beentje (2016) and herbaria acronyms follow Thiers (2021).

Sampling

For obtaining reliable results, a reasonable proof strategy with two steps was designed to identify the systematic position of *S. oblongula*. The first step is to test whether *S. oblongula* belongs to *Sasa* based on our plastid tree. The second step is to identify which genus *S. oblongula* belongs to based on SNP tree, mainly due to low discrimination rates for those 'three-branched' genera in plastid results (Guo et al. 2021). For the plastid tree, a total of 24 species from 11 genera were sampled. *Bambusa multiplex* and *Dendrocalamus strictus* were set as the outgroups. All accession numbers and voucher information are listed in Table 1. For the SNP tree, a total of 14 species from seven genera belonging to subtribe Arundinarieae were included. *Chimonobambusa sangzhiensis* was set as outgroup. Particular emphasis in our taxon sampling was placed on the inclusion that several key generic types were all involved in this study, including *Acidosasa*, *Indosasa*, *Oligostachyum*, *Pseudosasa*, and *Sasa*.

DNA extraction and sequencing

Young leaves at the vegetative growth stage were collected in the field. Total genomic DNA was isolated from silica-dried leaves following the manufacturer's specifications TIANGEN Genomic DNA Extraction Kit (TIANGEN, Beijing, China). DNA samples of concentration up to standard ($\geq 1 \mu\text{g}$) were sheared into fragments using Covaris M220 (Covaris, Woburn, MA). Insert size of 350 bp fragments were enriched by PCR, and the paired-end (2×150 bp) libraries were constructed on NovaSeq 6000 platform. About 20G deep genome skimming (DGS) data were generated. Finally, adapters and low-quality reads were filtered from raw data using Fastp v 0.23.1 (Chen et al. 2018) software.

Plastome assembly and chloroplast DNA regions mapping

The filtered clean reads were utilized to de novo assemble complete chloroplast (cp) genomes using GetOrganelle v 1.6.2 pipeline (Jin et al. 2018). Six k-mer values, including 21, 45, 65, 85, 105, 125, were set for plastid contigs connection. Subsequently, the filtered plastid reads were transferred to Bandage (Wick et al. 2015) software for visualization processing. Two opposite plastid sequences exported from Bandage were aligned with the reference sequence *Phyllostachys edulis* (GenBank accession No. HQ337796),

and one that matched the genomic direction of the reference was retained. The final cp genomes were manually corrected in Geneious 9.1.4 (Kearse et al. 2012).

After referring to previous plastid phylogeny studies of Arundinarieae (Zeng et al. 2010; Zhang et al. 2012), eight plastid DNA regions (*atpI-atpH*, *psaA-ORF170*, *rpl32-trnL*, *rpoB-trnC*, *rps16-trnQ*, *trnD-trnT*, *trnS-trnG* and *trnT-trnL*) were selected to reconstruct plastid phylogenetic tree. Our cp genomes were annotated from eight DNA regions of *Acidosasa purpurea* with $\geq 70\%$ sequence similarity in Geneious. Then, all the annotated plastid DNA regions were extracted from whole cp genomes. Sequence directions were visualized and adjusted using Mauve v 2.4.0 (Darling et al. 2004).

SNP calling

The latest high-quality genome sequence of moso bamboo (*Phyllostachys edulis*) (Zhao et al. 2018) was selected as the chromosome-level reference genome to build an index using the software SAMtools v 1.9 (Danecek et al. 2021) and Picard v 2.27.3 (Broad Institute 2019). After filtration of low-quality data, our clean reads were processed in removal of duplicates using Fastuniq v 1.1 (Xu et al. 2012). New filtered paired reads were aligned to the reference genome by Bowtie2 v 2.4.4 (Langmead and Salzberg 2012) with the parameter of minimum acceptable alignment score for L, 0.3, 0.3. After that, SAMtools was further employed to sort alignment (BAM files). Picard was utilized to remove duplicates again with the parameter “MarkDuplicates”. GATK v 4.2.2.0 (Van der Auwera and O’Connor 2020) was performed to anchor variant calling including SNP and InDel using the joint calling method “HaplotypeCaller” in the genomic variant call format (GVCF). Each sample based on reads with mapping quality was set as at least 10 and the kmer size was set as 10 to 25. After completion of variants calling, the tool “CombineGVCFs” in GATK was carried out to combine all the GVCF files. The tool “GenotypeGVCFs” was then utilized to identify joint-called variants. Subsequently, the tool “SelectVariants” was implemented to select single nucleotide polymorphic sites (SNPs). Filtration of SNPs of low quality was then conducted in the tool “VariantFiltration” with the parameter “QD < 2.0, MQ < 40.0, FS > 60.0, SOR > 3.0, MQRankSum < -12.5 and ReadPosRankSum < -8.0”. Finally, the tool “SelectVariants” was run to extract filtered SNPs.

For a reliable phylogenetic tree based on SNP dataset, we considered that filtered raw SNPs with high missing genotype rates and low minor allele frequency will affect the accuracy of the phylogenetic trees and thus should be removed. Therefore, plink v 1.90b4.6 (Purcell et al. 2007) was operated to filter those low-quality SNPs with parameter “geno” set as 0.1 and “maf” set as 0.01. Filtered variants were then pruned with the parameter “indep-pairwise” set as 50, 10, 0.2, representing its window size, a variant count to shift the window and pairwise r^2 threshold for SNPs, respectively. Finally, new clean SNP dataset was generated, and the GVCF format was transferred to PHYLIP format for phylogenetic analysis using the python script “vcf2phylip.py” (Ortiz 2019).

Alignments construction and phylogenetic trees inference

Chloroplast DNA regions and SNP dataset were utilized to reconstruct the phylogenetic tree, respectively. Eight plastid matrices were aligned using MAFFT v 7.450 (Katoh and Standley 2013) and concatenated as a super matrix. Maximum likelihood (ML) tree was inferred for plastid and SNP datasets using IQTREE v 1.6 in SH-aLRT test and ultrafast bootstrap (UFBoot) value (Nguyen et al. 2015). Node supports rates with SH-aLRT $\geq 80\%$ and UFboot $\geq 95\%$ were reliable and shown on each node. The final results were visualized with Figtree 1.4.4 (Rambaut 2018).

Result

Morphological comparison

Sasa oblongula has leptomorph rhizome, glabrous culm internodes, white powdery infranodal region, flat or slightly prominent nodes and culm supranodal ridge, mostly solitary branch at lower culm nodes and two to three (Fig. 3E, if three branches, central slightly dominant than lateral) branches at mid and upper culm nodes, glabrous culm leaf sheath (Fig. 3G) with erect and lanceolate blades, falcate auricles and ligules with ciliolate margin, glabrous foliage leaves blades and conspicuous transverse veins. These vegetative characters mentioned above make it fit well with the circumscription of *Pseudosasa* Makino ex Nakai (1925), rather *Sasa*. After examining the specimens of similar species and referring to the related literature (Munro 1868; Chia et al. 1983), we found that *S. oblongula* is most similar to *P. cantorii* (Munro) P. C. Keng ex S. L. Chen et al. (Zhu et al. 2006) by sharing one to three branches per nodes, glabrous internodes, the white powdery infranodal region, slightly prominent supranodal ridge, culm leaf sheath with falcate auricles, erect and lanceolate blades with serrulate margin, foliage leaf sheath with ciliate margin and truncate ligules, glabrous foliage leaf blades with conspicuous transverse veins, but differs by having nearly solid (vs. hollow) culm internode with appressed (vs. patent) branches, intravaginal (vs. transferred) and glabrous (vs. setose) abaxially culm leaf sheath with ciliate upper (vs. wholly) margin and arched (vs. truncate) ligules with ciliolate (vs. glabrous) margin, 3–6 foliage leaves with irregular (vs. coplanar) arrangement clustered at the top ultimate branch, glabrous (vs. hirsute) abaxially foliage leaf sheath with 1–4 mm (vs. 5–13 mm) long length per adjacent sheath apex, small-medium-sized (7–10 × 1.5–2.6 cm vs. 12.5–25 × 2.5–3.2 cm) foliage leaf blades with 6–7-paired (vs. 7–9-paired) secondary veins. A more detailed comparison between the two species is provided in Table 2.

Phylogenetic analysis

To make clear the position of *S. oblongula* and its relationship with *P. cantorii*, phylogenetic Maximum likelihood analysis was conducted based on plastid, and SNP dataset was shown with SH-aLRT and UFboot values noted at each node. Our plastid phylo-

Table 2. Comparison of *Pseudosasa oblongula* and *Pseudosasa cantorii*.

Morphology	<i>Pseudosasa oblongula</i>	<i>Pseudosasa cantorii</i>
Culm internode	Nearly solid, with appressed branches	Hollow, with patent branches
Culm leaves	Intravaginal, glabrous abaxially	Transferred, setose abaxially and readily deciduous when old
Margin	Ciliate on the upper	Ciliate wholly
Ligule	Arched, with ciliate margin	Truncate, with glabrous margin
Foliage leaves	3–6, with irregular arrangement clustered at the top ultimate branch	4–7, with a coplanar arrangement at the top ultimate branch
Sheath	Glabrous abaxially, 1–4 mm long per adjacent sheath apex	Hirsute abaxially, 5–13 mm long per adjacent sheath apex
Blades	7–10 × 1.5–2.6 cm, with 6–7-paired secondary veins	12.5–25 × 2.5–3.2 cm, with 7–9-paired secondary veins

genetic tree indicated that *S. oblongula* was distantly related to those Japanese *Sasa* species (including generic type) (Fig. 4, SH-aLRT=99.5% & UFboot=100%) and those *Sinosasa* species (SH-aLRT=100% & UFboot=100%) previously assigned to *Sasa* from China (Qin et al. 2021). The SNP phylogenetic tree suggested that *S. oblongula* was sister to *P. cantorii* with strong support (Fig. 5, SH-aLRT=100% & UFboot=100%), supporting our morphological study well.

Taxonomic treatment

Pseudosasa oblongula (C.H.Hu) N.H.Xia & X.Li, comb. nov.

urn:lsid:ipni.org:names:77317186-1

Figs 1–3

Sasa oblongula C. H. Hu, J. Bamboo Res., 6(4):18 (1987). Basionym.

Type. CHINA. Guangdong: type locality unknown, cultivated in Bamboo Garden, Sun Yat-sen University, 5 April 1980, *Y. L. Yang* & *C. H. Hu 198001* (holotype: N, photo!; isotypes: N019023154, Fig. 1A; N019023155, image!; N019023156, Fig. 1B)

Description. Shrubby bamboo, rhizomes leptomorph. Culm erect, 1–1.5 m tall, 2–7 cm in diameter; branches appressed, usually 1 branch at the lower culm nodes and 2–3 at the mid or upper nodes of culm (if 3 branches, central slightly dominant than lateral); internodes terete or slightly flattened above branches, 5–27 cm long, glabrous, nearly solid; nodes flat or slightly prominent, white powdery under nodes; supranodal ridge flat or slightly prominent; intranodes 5–8 mm high, glabrous; culm buds solitary, ovate to elliptic, light yellow, puberulent abaxially at upper part, ciliate on the upper margin, apex obtuse. Culm leaf sheath persistent or deciduous later, intravaginal, thinly leathery, 1/3–2/3 as long as internodes, glabrous abaxially, ciliate on the upper margin and sometimes glabrescent, longitudinal ribs conspicuous; sheath scar with remains of sheath base; auricles falcate to long-elliptic, obliquely ascending, 2–4 × 1–2 mm; oral setae erect or slightly curved, 3–10 mm long; ligule entire, 0.5–1 mm high, asperous abaxially, ciliate on the margin, apex arched; blades narrowly lanceolate to lanceolate, erect, glabrous abaxially, margin sparsely serrate. Foliage leaves 3–6



Figure 1. Isotypes of *Pseudosasa oblongula*, Y.L. Yang & C.H. Hu 198001 (A N019023154 B N019023156). Photos downloaded from Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>).



Figure 2. *Pseudosasa oblongula* (C.H.Hu) N. H. Xia & X. Li, *N. H. Xia XNH-187* (IBSC). Photo by Xing Li.

clustered at the top of ultimate branches, with irregular arrangement; sheath thinly leathery, glabrous abaxially, margins densely ciliate, sometimes glabrous, thinly white-powdery, longitudinal ribs conspicuous, length per adjacent sheath apex very short, 1–4 mm; auricles undeveloped, ovate to falcate or absent, 1–3 × 1–1.5 mm; oral setae erect or curved, 5–10 mm long, usually deciduous when old; inner ligule 0.5–1 mm high, densely ciliolate on margin, apex truncate; outer ligule ca. 0.5 mm high, ciliolate on margin; blades oblong to oblong-lanceolate, papyraceous, 7–10 × 1.5–2.6 cm,

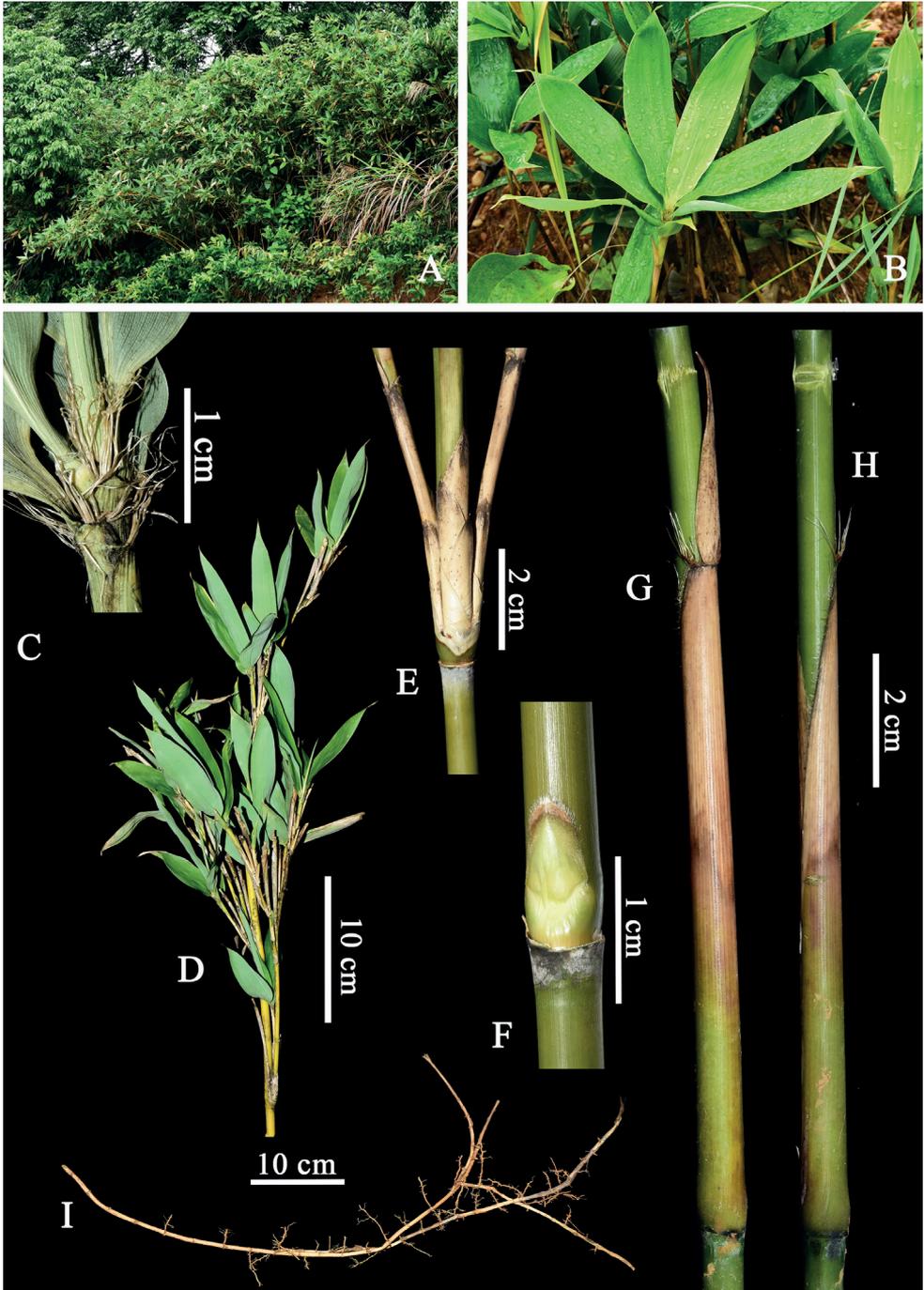


Figure 3. *Pseudosasa oblongula* (C.H.Hu) N. H. Xia & X. Li **A** habit **B** foliage leaf, showing oblong blade **C** part of terminal branch, showing sheath and ligule **D** partial culm and branches **E** nodes of the upper culm, showing three branches complement **F** culm bud **G, H** culm leaf, showing lanceolate blades, falcate auricles and glabrous sheath **I** leptomorph rhizomes. Photos **E, F** by Zhuo-yu Cai, others by Xing Li.

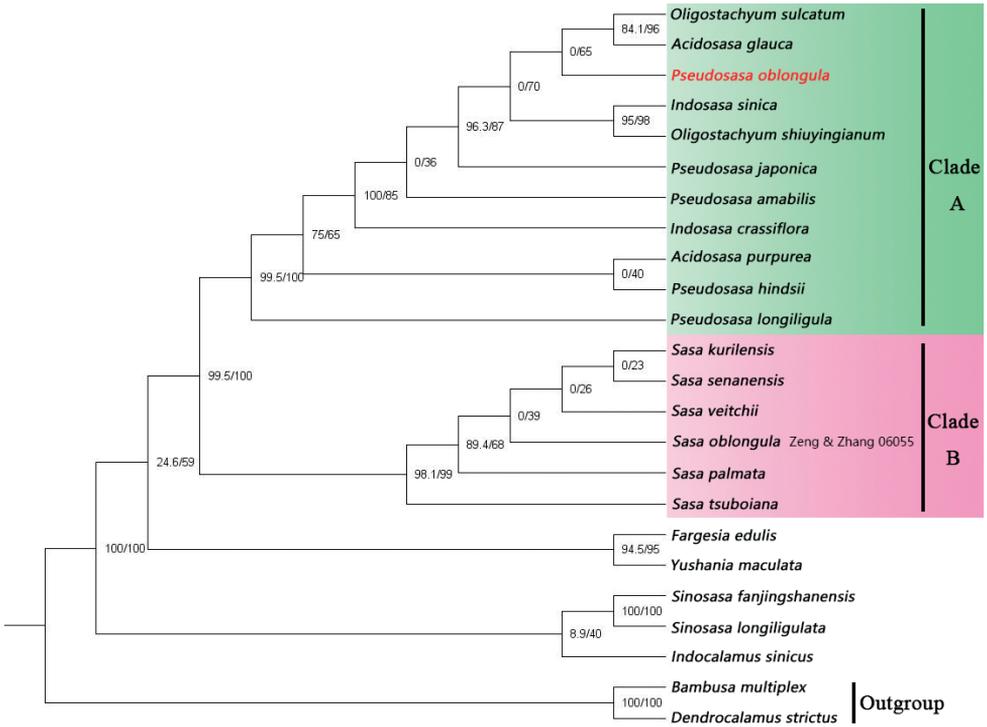


Figure 4. Phylogenetic relationships of *Pseudosasa oblongula* and other 23 species of which most taxa belong to the tribe Arundinarieae based on eight combined plastid sequences. The value of the SH-aLRT test (left) and ultrafast bootstrap (right) are indicated on each node.

glabrous adaxially and abaxially, apex acute to attenuate, base obtuse to unequal rounded, margin serrate; secondary veins 6–7 pairs, tertiary veins 6–7 pairs, transverse veins conspicuous; petioles 2–4 mm long, glabrous; Inflorescence unknown.

Phenology. New shoots were produced from March to July.

Vernacular names. Jǔ Yè Shǐ Zhú (Chinese pronunciation); 矩叶矢竹 (Chinese name).

Additional specimens examined. *Pseudosasa oblongula*: CHINA. Guangdong: type locality unknown, cultivated in Bamboo Garden, Sun Yat-sen University, 12 April 1979, *T. H. Wen & G. Y. Sheng 79413* (JSB518673 image!); Yunfu City, Yunan County, Baishi Town, Hengjing village, 4 July 2021, *N. H. Xia XNH-187* (IBSC!); *ibid.* 22°52'8"N, 111°51'59"E, elev. 206 m, 11 June 2022, *J. B. Ni & X. Li LX142* (IBSC!). *Pseudosasa cantorii*: CHINA. Hong Kong: Lantau Island, *Cantor s.n.*, quoad foliage leaf (K000876243, image!); Green Island, 1 May 1981, *L. C. Chia et al. Nan-zhu 2875* (US 00031256, image!); Shatin, Siu Lek Yuen, 18 Oct. 1980, *L. C. Chia et al. Nan-zhu 2823* (US00031257, image!); *ibid.* *L. C. Chia et al. Nan-zhu 2830* (US00031259, image!); Xinjie, Jiadaoli Farm, 22 April 1981, *Nan-zhu 2867* (IBSC!); *ibid.* 15 October 1980, *Nan-zhu 2810* (IBSC!).

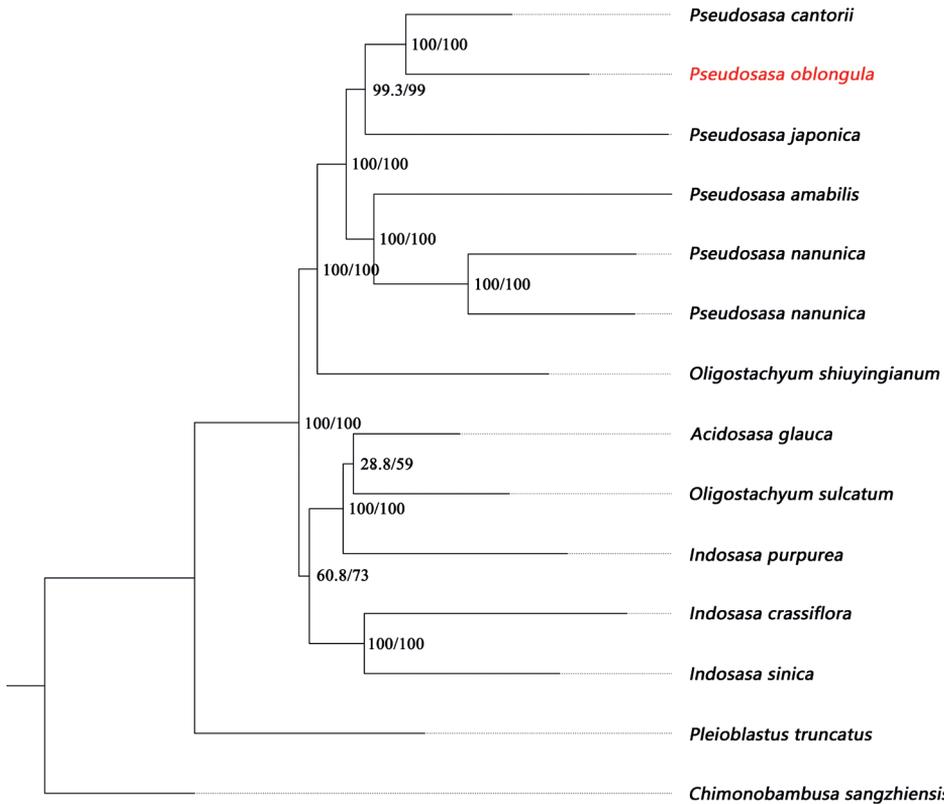


Figure 5. Phylogenetic relationships of *Pseudosasa oblongula* and other 13 species belong to the tribe Arundinarieae based on single nucleotide polymorphism dataset. The value of SH-aLRT test (left) and ultrafast bootstrap (right) are indicated on each node.

Discussion

Sasa oblongula, mainly characterized by its oblong foliage leaves, was published based on sterile materials introduced in the bamboo garden of Sun Yat-sen University. It differed from Japanese *Sasa* species by having 1–3 branches per node (vs. 1 branch) and remote geographic distribution, indicating that it was not obviously the member of *Sasa*. After the examination of the voucher specimen *Zeng & Zhang 06055* from Zeng et al. (2010), we were certain that this specimen does not represent *S. oblongula* since it possesses solitary branch at upper culm nodes, undeveloped or absent culm leaf auricles, and long-lanceolate foliage leaf blades. Our phylogenetic study revealed that the actual *S. oblongula* and those Japanese *Sasa* species are dispersed in two different clades (Fig. 4, Clade A & B). Furthermore, it and *P. cantorii* form a well-supported clade with two different branch lengths based on SNP phylogenetic tree (Fig. 5), supporting the result of morphology.

However, previous studies (Zhang et al. 2012; Guo et al. 2021) showed that *Pseudosasa* is polyphyletic, and the phylogenetic relationships between *Pseudosasa* and several other genera of subtribe Arundinariinae (Zhang et al. 2020), such as *Pleioblastus*, *Oligostachyum*, *Indosasa*, etc., have not been resolved. *Sasa oblongula* was closely related

to Chinese *Pseudosasa* species in morphology and phylogeny, and thus was congruently assigned to the genus *Pseudosasa* here. Accordingly, a new combination *Pseudosasa oblongula* (C. H. Hu) N. H. Xia & X. Li was proposed.

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Supplementary material I

SNP matrix

Authors: Xing Li

Data type: phylogenetic

Explanation note: This SNP matrix contains 14 species with 36490 bp.

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