Exploring phylogenetic relationships within the subgenera of *Bambusa* based on DNA barcodes and morphological characteristics

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**The genus Bambusa belongs to the subtribe Bambusinae and the subfamily Bambusoideae. The subgenera of Bambusa has not been satisfactorily circumscribed, and this remains a major taxonomic issue. Simultaneously, genera such as Dendrocalamus and Gigantochloa have not been confidently assigned to Bambusa. Here, the phylogenetic relationships among subgenera were investigated using five chloroplast DNA markers (rpl32-trnL, rpl16, matK, rbcL, and trnH-psbA) for a sample of 50 ingroup and 16 outgroup species. A total of 186 key morphological descriptors were studied for the 50 ingroup species. The results indicated that five chloroplast DNA markers were possible to distinguish Bambusa species from other species and divide them into several clusters. Phylogenetic analyses conducted using morphological descriptors and a combined marker (rpl32-trnL+rpl16) revealed three and five distinct lineages, respectively, among the currently recognized Bambusa species. The branching pattern of the dendrogram was not completely consistent with the classical taxonomic classification of Bambusa. In addition, not all varieties and cultivars were clustered with McClure classifications. As the maximum parsimony topology and morphological analyses were inconsistent, some clustering results overlapped. Overall, the results obtained here do not support the current classification of the Bambusa subgenera.**

The genus *Bambusa*, belonging to the subtribe Bambusinae and the subfamily Bambusoideae, is among the largest woody bamboo genera, comprising over 100 species⁴. The classical botanical classification of bamboo species is based on the morphological characteristics of their culms, branches, and sheaths, owing to the infrequency of their blossoming. The *Flora of China*⁵ (FOC), the newest authoritative botany book in China, divides *Bambusa* into four subgenera based on the morphological characteristics of the culm, branch, and sheath: *Bambusa*, *Leleba*, *Lingnania*, and *Dendrocalamopsis*. The subgenus *Dendrocalamopsis* was not included in previous versions of the *Flora Reipublicae Popularis Sinicae*⁶ (FRPS). The FOC has been updated to reflect new findings in bamboo research; however, the definitions of some species remain controversial. Like *Bambusa oldhamii*, some studies continue to use the former name *Dendrocalamopsis oldhamii*⁶.

The distinctive life pattern, such as infrequent flowering and predominance of asexual reproduction render the taxonomic classification of bamboo difficult⁷. Consequently, there have been many misnamed species. For example, the *Bambusa* species *B. chungii*, *B. guangxiensis*, and *B. cerosissima* have been misnamed *Lingnania chungii*, *L. funghomii*, and *L. cerosissima*, respectively⁸. With the addition of flowering materials, Chinese species were hitherto placed in the *Sasa* subgenus *Sasa*. Qin⁹ et al. strongly argued that, considering the monophyly of the Chinese representatives of the *Sasa* subgenus *Sasa*, a new genus, *Sinosasa*, should be erected.

Both morphological and molecular systematics have been utilized to solve the problems of bamboo classification. Among the various approaches used in molecular systematics, DNA sequencing has become one of the most
widely used methods applied in bamboo classification\(^7^,\)\(^8\), especially at the genus level\(^9^,\)\(^10\). Recently, substantial progress has been made towards understanding the evolutionary relationships of *Bambusa* and its allies (*Bambusa*, *Dendrocalamus*, *Gigantochloa*, and *Melocalamus*) as a close group, in particular based on their shared characteristic of a solid, thickened, and hairy ovary stalk (using molecular data\(^11\)). Yang\(^11\) et al. used nuclear gene (GBSSI) and plastid DNA sequences (psbA-trnH, rpl32-trnL, and rps16), which allowed *Bambusa* and *Dendrocalamopsis* to be classified into one of two clades with reasonable support. Through this approach, 17 *Bambusa* samples were classified into three clades, and this result supported the present subgeneric classification of *Bambusa*. However, other studies have not supported this classification. The phylogeny of bamboo species has also been analyzed using only internal transcribed spacer (ITS) sequences. In this group, each branch was composed of several species of three subgenera (not including the subgenus *Dendrocalamopsis*), and the *Bambusa* and *Dendrocalamus* species formed a group with a bootstrap value of 100\(^12\). Goh\(^13^,\)\(^14\) et al. used chloroplast DNA markers (rps16-trnQ, trnC-rpoB, and trnD-T) and a nuclear DNA marker (GBSSI) to classify *Bambusa*, *Dendrocalamus*, and *Gigantochloa* as distinct lineages. This approach identified four *Bambusa* subgenera, which differed from the subgeneric classification. Chloroplast DNA sequences have been extensively used to infer plant phylogeny for uniparental inheritance through comparison with nuclear DNA sequences\(^15\).

Several DNA markers have been used as core plant barcodes, such as the plastid (chloroplast) markers rbcL, matK, and trnH-psbA. Nuclear ribosomal ITSs have also been used\(^16\). Statistical results revealed that these three plastid markers showed high levels of universality (87.1–92.7\%) and that the combination of ITS and any of the plastid DNA markers was able to discriminate 69.9–79.1\% of species\(^17\). In this study, many DNA barcoding primers (trnL-trnF, trnS-trnG, psbB-psbF, rpl16, rpl32-trnL, rbcL, matK, trnH-psbA, and ITS) were utilized with the aim of amplifying the DNA sequences of bamboo samples. Unfortunately, the plastid DNA markers trnL-trnF, trnS-trnG, and psbB-psbF failed to amplify most specimens, as did the nuclear marker ITS.

In addition to DNA barcoding, researchers have attempted to arrange morphological characteristics into a data matrix using cladistic analysis\(^18\). DAS\(^19\) et al. scored 32 key morphological descriptors for 15 bamboo species and standardized them as qualitative and quantitative interval data to construct a tree graph, using the unweighted pair-group method of arithmetic averages. For other plants, Tilney\(^20\) et al. used morphological and anatomical characteristics as scoring feature matrices for the cladistic analysis of *Lichtensteinia* (Apiaceae).

Based on morphological data, Kim\(^21\) et al. conducted principal component analysis and cluster analysis on native chrysanthenum in South Korea. In the present study, the phylogenetic relationships among the four subgenera of *Bambusa* were investigated (50 samples) using DNA sequence data and morphological characteristics, employing a much larger taxon sample than has been previously available. This included representatives from all subgenera of *Bambusa* that have previously been described. DNA sequence data were derived from the plastid markers.

**Materials and methods**

**Materials.** A total of 66 taxa from *Bambusa* and some other bamboo species (Table 1, all Latin names were obtained from the FOC) representing ten genera were sampled for molecular phylogenetic analysis. There were 50 species from *Bambusa* belonging to the four subgenera described in the FOC\(^2\), including *D. oldhamii* and *Neosinocalamus affinis*, which were not accepted as *Bambusa* in the FRPS, but were moved to *Bambusa* in 2007\(^2\) and named *B. oldhamii* and *B. eimeinis*, respectively. The outgroup taxa included *Dendrocalamus*, *Drepanostachyum*, *Indosasa*, *Melocanna*, *Neosinocalamus*, *Oligostachyum*, *Phyllostachys*, *Pseudosasa*, *Pleioblastus*, *Shibataea*, and *Sinobambusa*. Fifty taxa from *Bambusa* were collected and analyzed for morphological phylogeny.

**DNA isolation, amplification, cloning, and sequencing.** Leaves were collected from the Hu’an Bamboo Garden (Fujian Province, China) and Lin’an Taihu Lake Source Bamboo Garden (Zhejiang Province, China). Total DNA was extracted from silica-gel-dried young leaves, using a modification of the method described by Fulton\(^23\) et al. Polymerase chain reaction (PCR) amplification, cloning, and the sequencing of rpl16 were performed according to the forward\(^24\) and reverse primers\(^24\), following the protocol of Cornelia\(^25\) et al. For rpl32-trnL, the primers rpl32-F and trnL were used, following the protocol of Shaw\(^25\) et al.; for rbcL, the primers rbcL-1F and rbcL-724R were used, following the protocol of Fay\(^26\) et al.; for matK, the primers matK-ML and matK-MU were used, following the protocol of Zhu\(^27\) et al.; and for the psbA-trnH region, the primers psbA\(^28\) and trnH2\(^29\) were used, following the protocol of Tate and Simpson\(^30\). All the primer sequences are shown in Table 2.

PCR was conducted using the TaKaRa Ex™ kit (Takara Biomedical Technology Co., Ltd., Beijing, China) with the following program settings: 5 min at 95.0 °C; 35 cycles of 30 s at 95.0 °C, 30 s at annealing temperature, 40 s at 72.0 °C; 7 min at 72.0 °C; and then holding at 4.0 °C. The annealing temperatures used here were 51.0–56.0 °C. The PCR reaction mixture contained 10 ng of DNA samples, 0.5 μL (10 μM) each of forward and reverse primers, 0.5 μL of deoxyribonucleotide triphosphate (dNTP), 2.5 μL of 10 × buffer, and 0.5 μL of deoxyribonucleoside (DNase); double distilled water (ddH2O) was added to make the volume up to 25 μL. PCR products were purified using Promega Wizard^®^ PCR Clean-up System kits (Promega Biotech Co., Ltd., Beijing, China) following the manufacturer’s instructions. DNA sequencing was performed commercially by Shanghai Sunny Biotechnology Co., Ltd. (Shanghai, China).

**DNA sequence alignment and phylogenetic analyses.** DNA sequences were edited using CHROMAS v2.6.5 and aligned by MUSCLE (embedded in MEGAX), with default parameters. They were adjusted manually where necessary. All sequence data were uploaded to the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA706162&o=acc_s%3Aa). Maximum
parsimony (MP) analysis was conducted based on the separate rpl32-trnL, rpl16, matK, rbcL, and trnH-psbA datasets and with a combined rpl32-trnL+rpl16 dataset.

MP analysis was performed with MEGAX (https://www.megasoftware.net/); all characteristics were equally weighted, and gaps were coded as missing data. Heuristic searches of 1,000 random addition replicates were conducted using subtree-pruning-regrafting (SPR) branch swapping. This was done to obtain the most parsimonious trees, and ten trees from each random sequence were saved. Estimates of clade robustness were obtained through bootstrap values (BV) calculated from 1000 replicate analyses, conducted using the heuristic search strategy and through a simple addition sequence of the taxa. The incongruence length difference (ILD) test of Farris et al. was used to evaluate the statistical significance of character incongruence among the rpl32-trnL and rpl16 intron datasets before their combined analysis.

### Table 1.

| Name of bamboo        | Missing data       | Name of bamboo        | Missing data       |
|-----------------------|--------------------|-----------------------|--------------------|
| Bambusa albolineata   | Bambusa pachinensis| Bambusa arundinaea    | Bambusa pachinensis var. hirsutissima |
| Bambusa blumeana      | Bambusa perversibulis| Bambusa bonoposis     | Bambusa prominens |
| Bambusa cerosissima   | Bambusa sinospinosa| Bambusa changii       | Bambusa sancta |
| Bambusa changii var. velutina | Bambusa tereis    | Bambusa cornigera     | Bambusa textilis |
| Bambusa contracta     | Bambusa textilis var. gracilis | Bambusa corniculata | Bambusa textilis var. Purpurascens |
| Bambusa distegia      | Bambusa tuloides   | Bambusa dolichocladia | trnH-psbA          |
| Bambusa duriauscula   | rpl16              | Bambusa emeiensis / N. affinis | Bambusa vulgaris |
| Bambusa eutuldoides   | Bambusa vulgaris cv. Vittata | Bambusa eutuldoides var. basistriata | Bambusa vulgaris var. Wamin |
| Bambusa eutuldoides var. viridi-vittata | Bambusa vulgarensis | Bambusa flexuosa      | Dendrocalamus membranaceus |
| Bambusa gibba         | Dendrocalamus minor | rpl16                 | Dendrocalamus minor var. amoenus |
| Bambusa gibbowoides   | rpl32-trnL, rpl16  | Bambusa indica        | Drespanostachyum scandens |
| Bambusa lenta         | Indosasa shibataeoides | matK                 | |
| Bambusa longisiculata | Melocanna baccifera | Bambusa macrotis      | Neosinocalamus affinis cv. Viridiflavus |
| Bambusa multiplex     | Oligostachyum fabrici | Bambusa multiplex | Phyllostachys heterocladia |
| Bambusa multiplex cv. Alphonse-Kart | matK                | Bambusa multiplex cv. Fernleal | Phyllostachys heterocycla |
| Bambusa multiplex cv. Silverstripe | matK                | Bambusa multiplex cv. Stripsest Fernleal | Phyllostachys violascens |
| Bambusa multiplex cv. Striped Fernleal | Pseudosasa amabilis | Bambusa multiplex var. riviereorum | Pseudosasa japonica var. tsutsmaniana |
| Bambusa multiplex var. shimadae | Pleioblastus viridistriata | Bambusa multiplex | |
| Bambusa mutabilis     | Shibataeae chinensis var. Aureo-strata | Bambusa oldhamii / D. oldhamii | Binobamnusa tootsik var. luteol-albo-strata |

Table 1. Sixty-six taxa from Bambusa and the outgroup. Marker name in the missing data column indicates that there was an amplification or sequencing failure; * indicates missing morphological characteristic data.

### Table 2.

| Mark      | Prime-F           | Prime-R           |
|-----------|-------------------|-------------------|
| rpl32-trnL | CTGTCTCTGTTGACGCACTG | CAGTCCAAAAACACGACCTTC |
| rpl16     | CTATGGCTTATGGTGAGTATTC | TCTTCTGCTATGGTGCTTACG |
| matK      | AAGAGGGAATCTCTGCTCAA | AGGGTGTCACAGGGTCATT |
| rbcL      | ATGTCACCCAAAGAGAGAAAGAC | TCCGATGTACCTGCAGTAGC |
| trnH-psbA | CGGCATGTCGTTGAGATTCACAA | GTTATGCAACGTAATGCTT |

Table 2. Sequences of the five primers used in this study.
Bambusa species according to the diversity information to be reasonable barcoding candidates for identifying Bambusa available. While matK, rbcL, and trnH-psbA could separate Bambusa 16, and matK at 0.00458 and 0.00406, respectively (Table 3). In contrast, rpl32-trnL, rp16, and matK appeared by any of the authors.

Herbarium vouchers. Hua’an Bamboo Botanical Garden and Hangzhou Lin’an Táihúyuan Ornamental Bamboo Planting Garden supported the research work of this article. The Materials were collected by Y.G.Z and L.J.G in accordance with related management rules without damaging the growth of bamboo, and the relevant herbarium vouchers were kept in Hua’an botanical garden and Hangzhou Lin’an Táihúyuan Ornamental Bamboo Planting Garden. The list of specific species is attached in the annex (Table A2).

Results
Phylogenetic analyses. In this study, all five markers, rpl32-trnL, rpl16, matK, rbcL, and trnH-psbA, were independently detected by MP. Based on the five MP trees and supplemented by information on diversity acquired using DnaSP v5\(^32\), phylogenetic analyses of Bambusa were performed using combined DNA barcoding (rpl32-trnL+rpl16).

The nucleotide (Pi) and haplotype (Hd) diversities of these five DNA barcodes indicated that rbcL and trnH-psbA were not suitable for identifying Bambusa as their Pi values were much lower than those of rpl 32-trnL, rpl 16, and matK at 0.00458 and 0.00406, respectively (Table 3). In contrast, rpl32-trnL, rp16, and matK appeared to be reasonable barcoding candidates for identifying Bambusa species according to the diversity information available. While matK, rbcL, and trnH-psbA could separate Bambusa from other genera, they grouped >70% of the sampled Bambusa into one cluster. In comparison, rpl32-trnL and rp16 both divided Bambusa into several clusters.

A combined barcode (rpl 32-trnL+rpl 16) was also used to analyze the phylogeny of 66 taxa after the ILD was tested. The \(p\)-value of the ILD was 0.05 and the combined marker successfully divided bamboo into several clusters, as shown in Fig. 1 (left). The tree length, consistency index (CI), and retention index (RI) of the MP analyses for rpl32-trnL+rpl16 were 2392, 0.79, and 0.91, respectively. The BVs were mapped onto the MP topologies and tested. The Bambusa taxa formed two major clusters, B and C, and cluster C was further divided into four clusters (A [100 BV], B [100 BV], C [61 BV]), with cluster A as an outgroup (Fig. 1, left), constituting members of the Shibataeae, Drepanostachyum, Phyllostachys, Pseudosasa, Oligostachyum, Sino-bambusa, Indosasa, and Pleioblastus. However, three species, Dendrocalamus minor var. amoenus, Dendrocalamus membranaceus, and Melocanna baccifera (cluster C) were considered as an outgroup and were therefore not included. Bambusa taxa formed two major clusters, B and C, and cluster C was further divided into four sub-clusters (C1 [54 BV], C2 [64 BV], C3 [66 BV], and C4 [52 BV]) and several monotypic and small clades.

The branching pattern of the dendrogram was not completely consistent with the classical taxonomic classification of Bambusa proposed by the FOC, especially at the subgenus level. The subgenus Lingnania (blue strip in Fig. 1) contained the greatest number of species sampled in this study, while members of the subgenus Bambusa (pink strip) were scattered among all the Bambusa clusters. The subgenus Lelebu (green strip) did not appear

| Mark          | Number of the sites | Nucleotide diversity, Pi | Haplotype diversity, Hd |
|---------------|---------------------|--------------------------|-------------------------|
| rpl32-trnL    | 1109                | 0.07872                  | 0.903                   |
| rpl16         | 1283                | 0.07573                  | 0.771                   |
| matK          | 1611                | 0.06624                  | 0.803                   |
| rbcL          | 698                 | 0.00458                  | 0.587                   |
| trnH-psbA     | 636                 | 0.00406                  | 0.581                   |
| rpl16 + rpl32-trnL | 2392          | 0.08462                  | 0.961                   |

Table 3. Diversity information of DNA sites based on 66 bamboo species.

Morphological characteristic analyses. Based on the China Industry Standard Guidelines for conducting distinctness, uniformity, and stability tests, 186 key morphological descriptors were used to assess Bambusa members. Morphological descriptors were scored as follows: each species was considered as a separate independent operational taxonomic unit (OTU). One hundred and eighty-six key morphological descriptors were used (one root descriptor about aerial root; 22 culm descriptors about powder ring, hair ring, surface cover, color, internode length, diameter, shape, and sheath-node bulge; nine branch descriptors about branch thorn, lowest branch height, and leaf number; six leaf descriptors for length, hair, and base shape; 18 culm descriptors for sheaths about surface cover, hair ring, brim hair, length and color streak; 50 descriptors for sheath auricles about length, the length ratio value of the two auricles, corrugated fold, shape, oral setae length, root location, and extension condition; 54 sheath blade descriptors about shape, reflex, corrugation, hairy, color, tip shape, base length, and length; and 26 sheath ligule descriptors about length, shape, eyelash, and eyelash length). The specific morphological characteristics that were selected are listed in Table A1, which were assessed from each of the 50 OTUs (five replications per OTU) studied in the field. Mean values obtained from five independent replications were used as representative OTU data for each quantitative morphological descriptor. If the sample characteristics conformed to descriptors, they were marked as “0”; if not, they were marked as “1.” The scored qualitative and quantitative interval data were standardized to construct a dendrogram using neighbor-joining (NJ) performed via PowerMarker V3.25.

Ethical approval. This article does not contain any studies with human participants or animals performed by any of the authors.

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in the four sub-clusters of cluster C, and essentially was found in several monotypic and small clades of cluster C, except for B. chungii in cluster B. B. oldhamii (orange stripe) belonged to the subgenus *Dendrocalamopsis* in cluster C3. Meanwhile, several species belonging to the same subgenus were also clustered with a high degree of confidence in our MP analysis. For example, in cluster C2, most of the species belonged to the subgenus *Leleba*, except for *B. contracta*, *B. ventricosa* cv. Nana, and *B. indigena*, while in cluster C1, most species belonged to the subgenus *Leleba*, except for *B. macrotis* and *D. membranaceus*.

Varieties and cultivars did not always stay with their McClure classifications. For example, *B. chungii* and *B. chungii* var. *velutina* were separated into two clusters and two varieties of *B. eutuldoides* were assigned to cluster C1 but were separated from *B. eutuldoides* (in B). Meanwhile, cultivars of *B. textilis* were assigned to cluster C2 but were separated from *B. textilis* (in B), and *B. vulgaris* and *B. vulgaris* cv. Vittata formed a small cluster.

Figure 1. Strict consensus of the most parsimonious trees based on two cpDNA datasets (left), dendrogram derived from NJ cluster analysis based on 186 morphological descriptors of 50 bamboo species (right). Strips with different colors indicate subgenera: *Lingnania* (blue), *Bambusa* (pink), *Leleba* (green), *Dendrocalamopsis* (orange), and the outgroup (yellow).
Analyses of morphological characteristics.  In the absence of flower or fruit characteristics, culm sheaths and characteristics were treated as two taxonomic features for classifying Bambusa. According to 186 key morphological descriptors, the entire dendrogram (Fig. 1, right) was split into three clusters (H, I, and J). One main cluster (H) was divided into two sub-clusters (K and L) and four clusters (K, L, I, and J) did not completely conform to the existing classification. For instance, B. textilis and B. teres were totally isolated in a small cluster (J) belonging to the subgenus Leleba and species in the subgenus Lingnania were all in one subclade of cluster L. Critically, the subgenera Bambusa and Leleba were not separated from one another. Meanwhile, varieties and cultivars, such as B. vulgaris and B. multiplex, were more likely to stay with their McClure classifications. Bambusa vulgaris and its two cultivars formed a small clade in cluster I and B. changii and B. changii var. velutina were grouped into cluster L. The varieties and cultivars of B. multiplex were placed into cluster K; B. textilis, B. textilis cv. Purpurascens, and B. textilis var. gracilis were split into clusters J, L, and K, respectively, and B. tuldoides cv. Swolleninternode and B. tuldoides were split into clusters I and L.

Topological congruences.  The MP topology analyses were largely inconsistent with the morphological analysis; but cluster C2 in the MP analysis was largely consistent with cluster K in the morphological analysis. This highly consistent cluster included B. textilis var. gracilis, B. indigena, B. pachinensis var. hirsutissima, B. ventricosa cv. Nana, B. corniculata, B. multiplex, B. multiplex cv. Alphone-Karr, B. multiplex cv. Silverstripe, B. multiplex var. shimadai, and B. multiplex var. rivierorum. These species share some of the same characteristics: no aerial root, wedge-shaped leaf base, sheath cladethickness/ culm sheathlength <1, sheath blade erect, and hairy ventral.

Discussion
Bambusa and its allies.  In the present study, the DNA barcode rpl32-trnL-rpl16 identified Bambusa from other genera that are close to Bambusa, although it struggled to distinguish Bambusa from Dendrocalamus. Previous molecular studies have not convincingly shown that Bambusa is a monophyletic genus when related genera have also been considered. Sun et al. used ITS and random amplified polymorphic DNA (RAPD) and found that three Dendrocalamus species (D. latiflorus, D. membranaceus, and D. strictus) were nested among the Bambusa taxa. Yang et al. used the combined ITS+GBSSI+trnL-F combinatorial regions to show that eight Bambusa taxa (including B. oldhamii) were resolved as a monotypic clade in a phylogenetic tree supported by the posterior probability of Bayesian analysis. However, the sister grouping of Dendrocalamus has been strongly supported; Goh et al. used the combined plastid DNA rps16-trnQ-trnC-rpoB-trnD-T and sampled 53 kinds of bamboo. They determined that Dendrocalamus and Gigantochloa were embedded in Bambusa taxa; however, the nuclear DNA marker (GBSSI) indicated that Dendrocalamus may exist as a subclade departed from Bambusa, but can still be considered its sister. DAS et al. attempted to construct a phylogenetic tree using 32 morphological characteristics for 15 bamboo species, but failed to separate Bambusa, Dendrocalamus, and Gigantochloa successfully. Here, Dendrocalamus was completely embedded in Bambusa taxa, B. emeiensis and B. oldhamii were also intermixed with Bambusa; they were classified as new members of Bambusa, having previously been named N. affinis and D. oldhamii, respectively.

Morphological characteristics analyses and subgeneric classification.  According to the FOC, the genus Bambusa has four subgenera: Lingnania, Dendrocalamopsis, Bambusa, and Leleba. The subgenus Lingnania was found to share the following typical characteristics: a culm sheath with a narrow blade, a base only one-third of the width of the sheath apex; culm internodes that are usually longer than 30 cm, and thin walls (often < 8 mm). Three other subgenera shared the following characteristics: a culm sheath with a broad blade, a base 1/2–3/4 of the width of the sheath apex; culm internodes shorter than 30 cm, and thick walls (up to 2 cm). Meanwhile, the subgenus Dendrocalamopsis shared the following typical characteristics: culm sheath auricles that are large, rounded, irregular, or absent and spikelets that are loose at maturity, with broad florets on short rachilla segments. Otherwise, the characteristics of the subgenus Bambusa were found to be branchlets of lower branches specialized into tough or weak leafless thorns, and with culm sheaths with persistent blades. The subgenus Leleba had branchlets in their lower branches that were normal and leafy; and their culm sheath blade was deciduous.

To the best of our knowledge, this study represents the first attempt to distinguish Bambusa subgenera by using 186 morphological descriptors to sample more than 50 Bambusa taxa. As mentioned above, the traditional classification uses eight to 14 morphological characteristics to identify a subgenus, which are fewer than the number of morphological characters used in this study. Therefore, it is not surprising that the morphological phylogenetic tree generated here did not coincide exactly with the existing Bambusa subgenus classification. Establishing a phylogenetic tree based on morphological characteristics is a novel way to explore bamboo classification. According to the findings of this approach, we described more than 39 morphological features as 186 key morphological descriptors. Thus, the results were focused more on the overall characteristics of each species, rather than on one or several obvious or easily identifiable features.

Controversial bamboo species.  The FRPS classified B. arundinacea as a member of the subgenus Bambusa. However, Xia et al. pointed out that B. vulgaris was incorrectly named by Aiton as B. arundinacea and that B. auriculata and B. striata were also the same species as B. vulgaris. DAS et al. did not support this point
Based on morphological characters and molecular analysis. Instead, they found that, from a morphological perspective, these four bamboo species (B. arundinacea, B. vulgaris, B. auriculata, and B. striata) differed from each other, and B. striata and B. vulgaris showed greater similarity to each other than the others in RADP analysis. Here, B. auriculata and B. striata were not sampled, and the data of morphological characteristics and DNA sequences between B. arundinacea and B. vulgaris were different in this study.

**Application of the codes.** Compared with flowering plants, the classification of bamboos is more challenging for researchers and workers that are not engaged in examining phylogenetic relationships. Using DNA barcodes to classify or identify species will be more widely applied with the growth of molecular biology technology because of its easy operability, even though it may not align perfectly with traditional botanical classification. The codes rpl32-trnL and rpl16 are two loci on plastid DNA. Phylogenetic analyses that are based on whole chloroplast genomes have been used to resolve relationships within the subfamily Bambusoideae. Wang et al. suggested the use of a larger dataset, indicating that insufficient parsimony information characters were the main cause for poor resolution in temperate bamboos.

Based on morphological features, morphological codes were used as a classification method to evaluate whether they could be a match for traditional classification. However, following statistical analysis, results showed that it could not be appropriately explained in the context of morphological classification. A new operating model for morphological codes needs to be developed for the application of this technique in botanical classification.
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**Author contributions**

Y.F.B. conceived and designed the study. Y.F.B. and A.K.W. carried out the experiments and wrote the manuscript. Z.X.Z. exulted the experiment outdoor. H.Z. and Q.F.L. analyzed the data and prepared the tables and figures, H.J.C., S.H.L, and X.H.D. proofread the manuscript. Z.Z.X. revised the article to make the language more accurate. Y.G.Z and L.J.G collected the samples needed for the research.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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