

Morphological and Molecular Identification of a *Schizostachyum* Bamboo (Poaceae: Bambusoideae) from Manado City, North Sulawesi, Indonesia

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ABSTRACT

Bamboo (Poaceae: Bambusoideae) is a group of woody grasses with high ecological and economic value that is widely distributed across the Indonesian archipelago, including North Sulawesi. Identifying bamboo to species level using morphology alone is difficult because the reproductive organs that serve as the primary taxonomic determinants appear only during flowering cycles that may span several decades. This study aimed to identify a bamboo specimen collected in Manado City by integrating morphological characterisation with DNA barcoding based on the chloroplast *matK* gene. A specimen coded CP3 was collected purposively in Taas Sub-district, Tikala District, Manado City, and its vegetative characters were documented following the bamboo identification key for Sulawesi. Genomic DNA was extracted using a Genomic DNA Mini Kit (Plant), the *matK* region was amplified by PCR using the *matK*-3Fr and *matK*-1Rf primer pair, and the amplicon was verified on a 1.2% agarose gel before bidirectional Sanger sequencing. Chromatograms were edited in Geneious Prime, assembled into a consensus sequence, and queried against the GenBank database using BLASTn. Morphologically,

the specimen was identified as *Schizostachyum brachycladum* based on subequal branching, nodes lacking aerial roots and a nodal knee, and persistent golden-brown culm sheaths bearing an erect triangular blade. PCR produced a single band of approximately 800 to 900 bp and the consensus sequence was 837 bp long. BLAST analysis returned 100% query cover for all hits, with percent identity ranging from 99.52% to 100%. The two highest matches, *Cephalostachyum pergracile* and *Schizostachyum zollingeri*, both belong to *Schizostachyum* under currently accepted nomenclature, so the *matK* barcode resolved the specimen to genus level but not to species level. The gradation of identity values followed the phylogenetic structure of the subtribe Melocanninae. These findings indicate that *matK* is informative for genus-level assignment in *Schizostachyum* while species-level discrimination requires a multi-locus approach, and they provide the first *matK* reference sequence for bamboo from Manado City.

Keywords: bamboo, DNA barcoding, *matK*, *Schizostachyum*, Manado City.

INTRODUCTION

Bamboo is a group of woody grasses belonging to the family Poaceae, subfamily Bambusoideae, that is distributed widely across tropical and subtropical regions. Approximately 1,670 bamboo species classified into 125 genera have been recorded worldwide, with Asia serving as the centre of diversity.²² Indonesia alone hosts around 176 bamboo species, of which 105 are endemic, making the archipelago one of the most important reservoirs of bamboo diversity in the world.²⁶ The genus *Schizostachyum* Nees is one of the dominant elements of this flora and belongs to the subtribe Melocanninae within the tribe Bambuseae.⁵ Beyond its taxonomic significance, bamboo underpins rural livelihoods through construction, handicraft, culinary and ceremonial uses, and it contributes to soil stabilisation, watershed protection and carbon sequestration in tropical landscapes. Reliable species identification is therefore a prerequisite for any inventory, conservation programme or sustainable utilisation scheme built on this resource.

Conventional bamboo taxonomy relies heavily on generative characters such as spikelets and floral structures. This dependence creates a practical bottleneck because many bamboo species flower only once in several decades, so specimens encountered in the field are almost always in the vegetative stage.⁵ Vegetative characters that are used as substitutes, including culm colour, internode length, branching pattern and culm sheath morphology, are informative but also plastic, since they respond to light intensity, soil type, humidity and culm age.²¹ The consequence is that morphologically similar taxa are frequently confused, and identification outcomes may differ between observers working on the same clump.

DNA barcoding was introduced as a standardised molecular approach that uses a short, agreed-upon region of the genome to assign an unknown specimen to a known species.⁹ For land plants, the Consortium for

the Barcode of Life recommended *matK* and *rbcL* as the core barcode pair, with *matK* providing the higher discriminatory power of the two.⁴ The *matK* gene is located within the *trnK* intron of the chloroplast genome and exhibits a comparatively high nucleotide substitution rate, which in principle allows closely related plant species to be separated.³ The marker has been applied successfully to a range of taxa in North Sulawesi, including *Liliopsida* species catalogued through BOLD Systems,¹² *Sangihe* nutmeg,²³ and ornamental aroids,¹³ and it remains widely used in Indonesian plant barcoding studies because of its stable amplification and the extensive reference data available for it.²⁵

The performance of *matK* in bamboo, however, is not uniform. A study of 15 bamboo taxa in Sabah, Malaysia reported 100% identification accuracy at genus level but only about 60% at species level using *matK* and *rbcL*.²⁷ Other work found that most standard plastid markers are nearly invariant across *Bambusa*, *Dendrocalamus*, *Ochlandra* and *Melocanna*, so that even generic boundaries become blurred,⁷ and sequences of *Bambusa vulgaris* have been reported to match *Gigantochloa* and *Dendrocalamus* entries at complete identity, a pattern attributed to interspecific hybridisation and polyploidy in the group.¹⁵ In contrast, *matK* showed high discriminatory potential among Himalayan temperate bamboos, although that resolution rested on a small number of fixed diagnostic nucleotides rather than on variation distributed along the entire locus.¹⁷ Taken together, these findings indicate that the usefulness of *matK* in bamboo is taxon dependent and must be evaluated separately for each lineage, and the slow molecular evolution characteristic of Bambusoideae is the underlying reason for this limitation.²⁰ Sulawesi is recognised as a centre of bamboo diversity, with 39 species in 12 genera recorded for the island, yet that inventory rests entirely on morphological examination.⁸ A subsequent survey in Kuwil Village, North Sulawesi likewise identified

its material morphologically and reported *Bambusa blumeana* as a new record for the region without molecular verification.¹⁰ To date, no published study has applied DNA barcoding to bamboo from North Sulawesi, and no *matK* reference sequence originating from bamboo in Manado City is deposited in public databases. This study therefore aimed to identify a bamboo specimen collected in Manado City by combining morphological characterisation with *matK*-based DNA barcoding, and to evaluate the taxonomic resolution that *matK* can deliver for a member of the genus *Schizostachyum*.

MATERIALS & METHODS

Study Area and Sampling

This study was conducted from December 2025 to May 2026. The specimen, coded CP3, was collected purposively from a naturally established bamboo clump growing among shrubs and trees near a residential area in Taas Sub-district, Tikala District, Manado City, North Sulawesi (1.462630° N, 124.863185° E). Geographic coordinates were recorded using the Google Maps application. Vegetative characters were documented in the field, comprising rhizome and clump habit, culm height, culm circumference, internode length, culm surface and colour, presence or absence of aerial roots and a nodal knee, branching pattern, culm sheath persistence and ornamentation, and leaf blade dimensions. Culm circumference and internode length were measured at five points along the culm, and leaf blade length and width were measured on five blades. Culm diameter was derived from mean circumference. Morphological identification followed the bamboo identification key for Sulawesi.⁸ Young leaf tips were then collected, placed in sterile ziplock bags and labelled before transport to the laboratory. All laboratory work was carried out at the Laboratory Advanced Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado.

DNA Extraction

Total genomic DNA was extracted using a Genomic DNA Mini Kit (Plant) (Geneaid) following the manufacturer protocol. Approximately 3 mm x 3 mm of young leaf tissue was placed in a 1.5 mL microtube and lysed in 400 µL Buffer GP1 supplemented with 5 µL RNase A, then homogenised manually with a micropestle and incubated at 60°C for 10 minutes. Following incubation, 100 µL Buffer GP2 was added, mixed by inversion, chilled for 2 minutes and centrifuged at 10,000 rpm for 1 minute. The supernatant was transferred to a fresh tube and combined with 750 µL Buffer GP3 containing isopropanol, then loaded onto a GD Column seated in a 2 mL collection tube and centrifuged at 10,000 rpm for 1 minute. The membrane was washed with 400 µL Buffer W1 and subsequently with 600 µL Wash Buffer containing absolute ethanol, each followed by centrifugation at 10,000 rpm (30 seconds) and dried by an additional 3 minutes of spin. DNA was eluted with 100 µL pre-warmed Elution Buffer (60°C) applied to the centre of the membrane, held for 3 to 5 minutes and recovered by centrifugation at 10,000 rpm for 30 seconds. The eluate was stored at –20°C until amplification.

PCR Amplification

The *matK* region was amplified using the primer pair *matK*-3Fr (5'-CGT ACA GTA CTT TTG TGT TTA CGA G-3') and *matK*-1Rf (5'-ACC CAG TCC ATC TGG AAA TCT TGG TTC-3').¹³ Each 40 µL reaction contained 20 µL MyTaq HS Red Mix (Bioline), 1.5 µL of each primer, 17 µL sterile Milli-Q water and 2 µL DNA template. Thermal cycling comprised initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 40 seconds and extension at 72°C for 50 seconds, with a final extension at 72°C for 1 minute and holding at 4°C.²³

Gel Electrophoresis

Amplification products were verified on a 1.2% agarose gel prepared in 1X TBE buffer. Approximately 5 to 10 µL of PCR product was loaded alongside a 1 kb DNA ladder, electrophoresis was run at 100 V for approximately 30 minutes, and DNA bands were visualised under a UV transilluminator. Band size, sharpness and the absence of smearing or non-specific products were assessed as indicators of amplification quality prior to sequencing.¹

DNA Sequencing and Bioinformatics Analysis

The verified PCR product was sent to 1st BASE (Malaysia) for bidirectional Sanger sequencing using the same forward and reverse primers employed in amplification. The resulting chromatograms were edited and trimmed in Geneious Prime v2025.2.2 to remove low-quality reads at both ends of the sequence. Forward and reverse reads were aligned using the MUSCLE algorithm and assembled into a consensus sequence.²⁵ The consensus was exported in FASTA format and compared against the GenBank nucleotide database using BLASTn (<https://blast.ncbi.nlm.nih.gov>). Scientific name, query cover, percent identity, and accession number were recorded for the ten highest-scoring hits. A percent identity of 98% or greater against a reference sequence was adopted as the threshold for a valid molecular identification.¹⁸ Because BLAST returns the taxon labels attached to database entries rather than currently accepted names, the nomenclature of every top hit was subsequently reconciled against Plants of the World Online¹⁹ and the World Checklist of Bamboos and Rattans²⁴ before the identification was interpreted.

RESULT

Morphological Characterisation of Specimen CP3

Specimen CP3 formed a dense clump supported by a short-necked sympodial rhizome system. The culms were erect and straight, reaching approximately 8 to 9 m in height, with a mean circumference of 15.54 cm corresponding to a mean diameter of 4.95 cm, and a mean internode length of 51.4 cm. The culm surface was covered by a thin white waxy layer on young culms, and culm colour was uniformly dark green without any shift toward purple or black. No spines were observed at the base of the clump. Branching arose from the middle to upper portion of the culm and consisted of subequal lateral branches, none of which was enlarged into a single dominant branch. The nodes bore neither aerial roots nor a nodal knee. Culm sheaths were of medium size and persistent, remaining attached to mature culms, golden brown in colour with a finely hairy outer surface, and carried rounded auricles with bristles and a broadly triangular, erect and tapering blade. Leaf blades were long-lanceolate, with a mean length of 32.5 cm and a mean width of 4.3 cm, uniformly green and smooth on the upper surface under macroscopic examination. The full set of characters is summarised in Table 1 and illustrated in Figure 1.

Two of these characters carry the greatest diagnostic weight. The subequal branching pattern is the key character separating *Schizostachyum* from *Bambusa*, *Dendrocalamus* and *Gigantochloa*, all of which develop a single dominant lateral branch, while the complete absence of aerial roots at the nodes further excludes *Dendrocalamus* and *Gigantochloa*.⁸ Within *Schizostachyum*, the erect triangular sheath blade distinguishes the specimen from *S. lima* and *S. latifolium*, in which the sheath blade is reflexed.⁸ On this basis the specimen was identified morphologically as *Schizostachyum brachycladum* (Kurz ex Munro) Kurz.

Table 1. Diagnostic vegetative characters of bamboo specimen CP3 collected in Manado City.

Character	Observation on specimen CP3
Clump and rhizome	Dense clump, sympodial rhizome with short neck
Culm height	Approximately 8 to 9 m, erect and straight
Culm circumference and diameter	Mean 15.54 cm, mean diameter 4.95 cm (n = 5)
Internode length	Mean 51.4 cm (n = 5)
Culm surface and colour	Thin white waxy layer on young culms, uniformly dark green
Node	Without aerial roots and without a nodal knee
Branching	From mid to upper culm, subequal lateral branches without a dominant branch
Culm sheath	Medium sized, persistent on mature culms, golden brown, finely hairy
Sheath auricle and blade	Rounded auricles with bristles, broadly triangular erect blade with tapering apex
Leaf blade	Long-lanceolate, mean 32.5 cm long and 4.3 cm wide (n = 5)
Spines at clump base	Absent

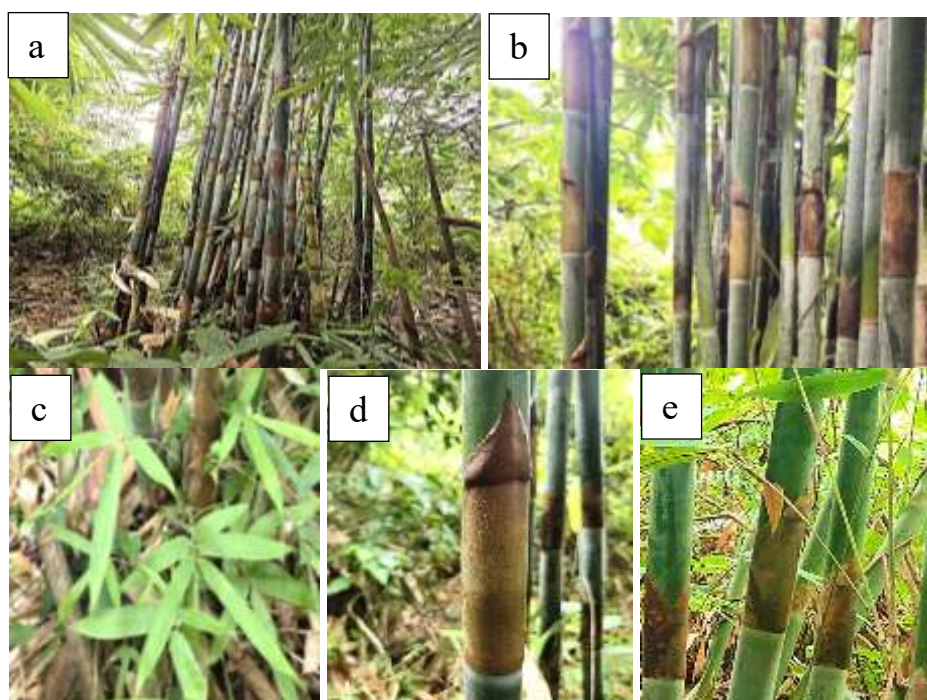


Figure 1. Bamboo specimen CP3: (a) clump, (b) culm, (c) leaf, (d) culm sheath, and (e) branching pattern.

PCR Amplification and Gel Electrophoresis

Amplification of the *matK* region from specimen CP3 was successful. Electrophoresis on a 1.2% agarose gel produced a single band located between the 750 bp and 1000 bp markers of the 1 kb ladder, corresponding to an amplicon of approximately 800 to 900 bp (Figure 2). No smearing, double bands or primer dimers

were observed, indicating that the primer pair annealed specifically and that the extracted DNA served as a suitable template. The band appeared slightly diffuse rather than sharply defined, which is consistent with the biochemical character of bamboo tissue, since polysaccharides and polyphenols that are not fully removed by silica column extraction migrate together with the template DNA and blur the band

edges.² A high template concentration applied without dilution can produce the same effect through overloading.¹ Neither observation compromised the suitability of

the product for sequencing, since the amplicon size matched expectation, only one product was present, and there was no evidence of extensive template degradation.

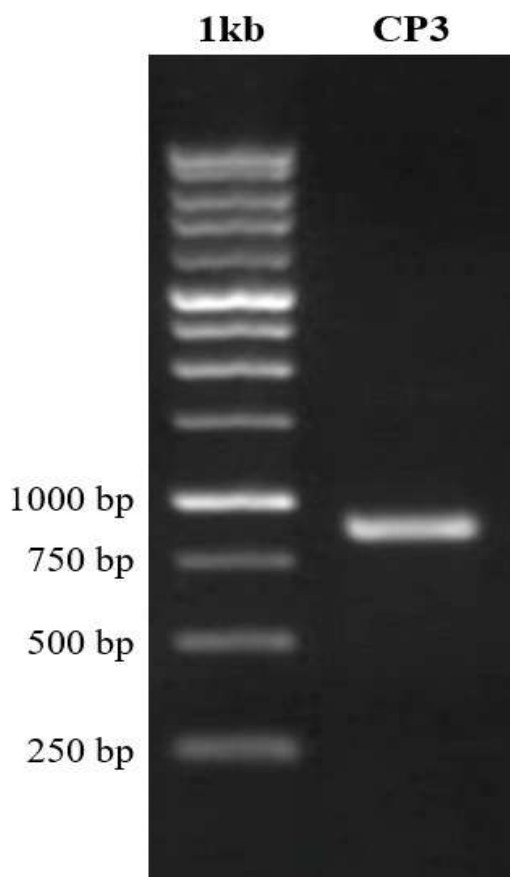


Figure 2. Agarose gel electrophoresis (1.2%) of the *matK* PCR product of specimen CP3 showing a single band of approximately 800 to 900 bp against a 1 kb DNA ladder.

Sequence Quality and Consensus Assembly

The chromatograms obtained from both sequencing directions showed sharp, single and well-separated peaks along most of the read, with adenine, thymine, guanine and cytosine represented by red, green, yellow and blue peaks respectively. The identity bar of the assembly was continuously green across almost the entire length, indicating that the forward and reverse reads agreed at nearly every base position. The absence of

overlapping peaks confirmed that a single target fragment had been amplified without interfering contamination. A minor decline in peak quality occurred at the very beginning and the very end of each read, as is typical of Sanger sequencing, and those regions were trimmed before assembly.⁶ The resulting consensus sequence was 837 bp in length, which is consistent with the band size observed by electrophoresis and confirms that the amplified *matK* fragment was read in full (Figure 3).

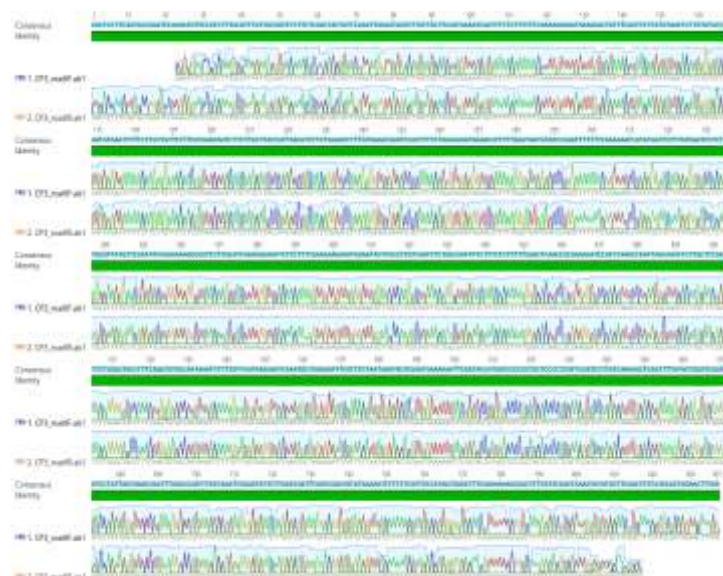


Figure 3. Representative chromatogram of the *matK* sequence of specimen CP3 showing sharp, well-separated nucleotide peaks.

BLAST-Based Molecular Identification

The 837 bp consensus sequence was compared against the GenBank database using BLASTn. All ten highest-scoring hits returned a query cover of 100%, with percent identity ranging from 99.52% to 100% (Table 2). The two highest matches, *Cephalostachyum pergracile* (EU434274.1) and *Schizostachyum zollingeri* (EU434280.1), both reached 100% identity.

They were followed by *Neohouzeaua kerriana*, *Schizostachyum dumetorum* var. *xinwuense*, *S. diffusum* and *S. auriculatum* at 99.88%, then *Thyrsostachys siamensis* and *S. funghomii* at 99.76%, and finally two accessions of *Melocanna baccifera* at 99.52%. All hits therefore exceeded the 98% threshold adopted for a valid molecular identification,¹⁸ but the identity values did not converge on a single species.

Table 2. Ten highest-scoring BLAST hits for the *matK* consensus sequence of specimen CP3 (search performed 14 June 2026).

Scientific Name	Query Cover (%)	Percent Identity (%)	Accession
<i>Cephalostachyum pergracile</i>	100	100.00	EU434274.1
<i>Schizostachyum zollingeri</i>	100	100.00	EU434280.1
<i>Neohouzeaua kerriana</i>	100	99.88	EU434276.1
<i>Schizostachyum dumetorum</i> var. <i>xinwuense</i>	100	99.88	LC590825.1
<i>Schizostachyum diffusum</i>	100	99.88	NC_063135.1
<i>Schizostachyum auriculatum</i>	100	99.88	NC_068071.1
<i>Thyrsostachys siamensis</i>	100	99.76	EF137444.1
<i>Schizostachyum funghomii</i>	100	99.76	EF137440.1
<i>Melocanna baccifera</i>	100	99.52	KU569971.1
<i>Melocanna baccifera</i>	100	99.52	EF125174.1

Reconciling the labels of the two highest hits against current nomenclature clarified this pattern. *Cephalostachyum pergracile* Munro and *Schizostachyum pergracile*

(Munro) R.B.Majumdar are homotypic synonyms referring to a single taxon, and the accepted name is placed in *Schizostachyum*.¹⁹ Both top hits therefore

point to the same genus, and the *matK* barcode assigned specimen CP3 to *Schizostachyum* with confidence while leaving the species undetermined.

DISCUSSION

The 837 bp consensus obtained in this study falls within the amplicon range normally reported for *matK* in woody bamboos, where the locus amplifies consistently at approximately 800 bp.¹⁷ The agreement between the electrophoretic band size and the assembled consensus length, combined with clean bidirectional chromatograms, indicates that the sequence used for identification is complete and reliable. This matters because *matK* is not always straightforward to amplify in plants. A comparative barcoding survey of tropical cloud forest trees recorded a PCR success rate of only 57.24% for *matK*, well below the 79.28% obtained with *trnH-psbA*.¹¹ Bamboo tissue compounds the difficulty because it is rich in secondary metabolites that inhibit polymerase activity, so successful single-band amplification from a silica column extract is itself a useful methodological outcome for future work on bamboo in this region.²

The central result of this study is the mismatch between the taxonomic depth of the morphological identification and that of the molecular identification. Morphology placed the specimen at *Schizostachyum brachycladum*, whereas *matK* resolved it only to *Schizostachyum*. This is not a contradiction between the two lines of evidence but a difference in resolution, and the two are in fact congruent at the level where they overlap. Both approaches agree that the specimen belongs to *Schizostachyum* rather than to *Bambusa*, *Dendrocalamus* or *Gigantochloa*, which are the genera most commonly reported from Sulawesi.⁸ Integrative agreement of this kind, where an independent molecular marker corroborates a morphological placement at generic level, is the outcome that DNA barcoding is best positioned to deliver in taxonomically difficult groups.²⁰

The identity of the second-ranked hit deserves closer attention. *Schizostachyum brachycladum* (Kurz ex Munro) Kurz has as its basionym *Melocanna zollingeri* var. *brachyclada* Kurz ex Munro, meaning that the taxon was originally described as an infraspecific entity within the *zollingeri* complex before being raised to species rank and transferred to *Schizostachyum*.¹⁹ A complete *matK* match between specimen CP3 and *Schizostachyum zollingeri* is therefore consistent with the specimen belonging to *S. brachycladum*, because the two taxa were historically treated as a single species and are nomenclaturally linked. It should be stressed that this is supporting context rather than phylogenetic proof, since no phylogenetic reconstruction was performed in this study and a sister relationship between the two taxa has not been demonstrated here. What the molecular data establish is that the barcode does not contradict the morphological determination and that the closest available references fall within the expected part of the genus. Distribution data point in the same direction, since Sulawesi is included in the native range recorded for *S. brachycladum* whereas it is not listed among the territories recorded for *S. zollingeri*.¹⁹

Three factors explain why *matK* stopped short of species-level assignment. The first is intrinsic to the subfamily. Bambusoideae exhibit a slow rate of molecular evolution, so conservative coding regions such as *matK* accumulate too few substitutions to separate recently diverged species.²⁰ The second is the mode of inheritance of the marker. Because *matK* resides in the chloroplast genome, it is maternally inherited and cannot capture the reticulate signal produced by interspecific hybridisation and polyploidy, both of which are well documented in woody bamboos and have been invoked to explain identical plastid sequences across nominally distinct bamboo genera.¹⁵ The third is the state of the reference database. BLAST can only return the taxa that are represented in GenBank, and no *matK* entry attributed to *S.*

brachycladum appeared among the retrieved hits. When a reference sequence for the target species is absent, even a perfectly sequenced query is forced to match the closest available relative, and the resulting identification is bounded by the database rather than by the marker.¹⁴

The distribution of identity values across the ten hits is nonetheless informative. Rather than being uniform, the values declined in a stepwise manner that tracks phylogenetic distance: 100% for *Schizostachyum* in the strict sense, 99.88% for *Neohouzeaua* and additional *Schizostachyum* species, 99.76% for *Thyrsostachys*, and 99.52% for *Melocanna*. All of these genera belong to the subtribe Melocanninae,⁵ and the gradient mirrors the accepted internal structure of that group. This behaviour contrasts sharply with the pattern reported for the *Bambusa*–*Dendrocalamus*–*Gigantochloa* complex, in which plastid markers are so uniform that a single query returns full identity to several genera simultaneously and no phylogenetic gradient is discernible.⁷ The presence of a clear gradient in *Schizostachyum* indicates that *matK* retains genuine phylogenetic signal in Melocanninae even though that signal is insufficient for species delimitation.

These findings align with, and help refine, the results reported from Sabah, where *matK* and *rbcL* identified bamboo with 100% accuracy at genus level but only about 60% accuracy at species level.²⁷ The present study reproduces the genus-level success for a member of *Schizostachyum* and likewise falls short at species level, which suggests that the Sabah figures are transferable to Melocanninae in Sulawesi. It also indicates that adding a second plastid marker is unlikely to solve the problem, since *rbcL* is more conserved than *matK* and both markers plateau at a similar level of species-level success.²⁷ A comparative evaluation of barcoding markers for Indonesian bamboo reached the same conclusion, reporting that *matK* is dependable for generic classification while the best species-level resolution was

achieved by the nuclear marker *ITS2*.² Work on *Fargesia*, a taxonomically difficult bamboo genus of temperate China, points in the same direction, showing that nuclear ribosomal data and complete plastomes outperform standard barcodes when species boundaries are shallow.¹⁶ The practical implication for bamboo research in North Sulawesi is that future studies should adopt a multi-locus design combining *matK* with *ITS2*, ideally supported by phylogenetic reconstruction and by sequencing morphologically vouchered reference material from the region.

Beyond the methodological findings, this study delivers a concrete data contribution. It provides the first *matK* sequence generated from bamboo collected in Manado City, adding molecular data to a regional flora that has until now been documented only through morphological survey.^{8,10} The sequence can serve as a comparative reference for subsequent barcoding work in North Sulawesi, and the integrative protocol applied here, in which BLAST output is reconciled against accepted nomenclature before interpretation, offers a template for avoiding the misidentification that can arise when database labels are accepted uncritically. The main limitation of the study is that it is based on a single specimen and a single locus, so the species-level identity of the specimen remains a morphological hypothesis awaiting molecular confirmation.

CONCLUSION

DNA barcoding based on the chloroplast *matK* gene was applied to a bamboo specimen collected in Taas Sub-district, Tikala District, Manado City. Morphological characterisation identified the specimen as *Schizostachyum brachycladum* on the basis of subequal branching, nodes without aerial roots or a nodal knee, and persistent golden-brown culm sheaths bearing an erect triangular blade. PCR produced a single *matK* band of approximately 800 to 900 bp, and bidirectional Sanger sequencing yielded a

clean consensus sequence of 837 bp. BLAST analysis returned 100% query cover for all hits with percent identity between 99.52% and 100%, and the two highest matches, *Cephalostachyum pergracile* and *Schizostachyum zollingeri*, both resolve to the genus *Schizostachyum* under currently accepted nomenclature. The *matK* marker therefore confirmed the generic placement of the specimen and corroborated the morphological identification at that level, but it could not discriminate among species within the genus. The stepwise decline of identity values across Melocanninae indicates that *matK* retains phylogenetic signal in this lineage even where species-level resolution is lacking. Species-level identification of bamboo in North Sulawesi will require a multi-locus approach combining *matK* with a nuclear marker such as *ITS2*, together with the deposition of vouchered reference sequences for locally occurring taxa.

Declaration by Authors

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