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Morphological, phytochemical, and molecular profiling of bamboo species growing in various ecosystems of Cagayan Province, Luzon, Philippines

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Abstract. *Opeña JM, Bumanglag RA, Cabang VMT. 2023. Morphological, phytochemical, and molecular profiling of bamboo species growing in various ecosystems of Cagayan Province, Luzon, Philippines. Biodiversitas 24: 4342-4358.* The characterization of bamboo species posed advantages to the bamboo industry's development in the Philippines. This study aimed to provide baseline information on the environmental and biological profiles of bamboo species in Cagayan Province, Philippines. Study sites were visited for bamboo morphological characterization using character states. Leaves were collected for qualitative phytochemical screening of 10 secondary metabolites and DNA sequencing using the *rbcL* plastid gene. The 28 accessions of *Bambusa*, *Dendrocalamus*, *Dinochloa*, *Gigantochloa*, *Guadua*, *Melocanna*, *Phyllostachys*, *Schizostachyum*, and *Thyrsostachys* were characterized and these bamboos were growing in forests, coastal or sparsely vegetated lands, urban, freshwater, grasslands, and agricultural ecosystems. Bamboo species distinctiveness' was described through identification keys on growth habit, culm internode, nodal structure, young shoot, and flowering incidence. The bamboos contain coumarins, saponins, steroids, and terpenoids, while quinones are absent. DNA sequencing using *rbcL* was effective in the identification of *Bambusa vulgaris* species and unknown bamboo at the species level. *Dendrocalamus*, *Guadua*, *Melocanna*, and *Schizostachyum* species were successfully identified by *rbcL* at the genus level. Most of the species were identified as *Dendrocalamus* species with *rbcL*. The findings provide valuable information on species identification and certification for asexual propagation, genetic conservation, phytochemical extraction, and utilization for health.

Keywords: Arundinarieae, Bambuseae, DNA barcoding using *rbcL*, morphology, secondary metabolites

INTRODUCTION

Bamboo belongs to the subfamily Bambusoideae of the grass family Poaceae, and was classified into 3 groups, namely: Arundinarieae (temperate woody bamboo such as *Phyllostachys*); Bambuseae (tropical woody bamboo such as *Bambusa, Gigantochloa, Dendrocalamus, Dinochloa*, *Thyrsostachys, Guadua, Melocanna, Pseudostachyum*, and *Schizostachyum*); and Olyreae (herbaceous bamboo) (Clark et al. 2015). There are 1,250 woody bamboo species in the world (Benton 2015) and 700 species are found in South and Southeast Asia (Uchimura 1980). The Philippines has 62 bamboo species, with 21 endemic species and 41 introduced species (Rojo 1999; Virtucio 2009). Bamboos such as *Bambusa blumeana*, *B. merrillii*, *B. oldhamii*, *B. philippinensis*, *B. vulgaris*, *Cyrtochloa puser*, *Dendrocalamus asper*, *D. latiflorus*, *Gigantochloa atter*, *G. levis*, *Schizostachyum lima*, and *S. lumampao* are identified as commercially important species in the Philippines (DENR-ERDB 2016). These bamboos were distributed throughout the Philippines. However, the ecosystems where the species grew were the least reported. Moreover, the kind of ecosystems or habitats in the northernmost part of the Philippines, like the Cagayan Province, where bamboos grow, is a limiting knowledge that needs to be established, hence this study. Only reports on the habitats of *B. blumeana*, *B. merrillii*, *B. philippinensis*, *B. vulgaris*, *D. asper*, *D. latiflorus*, *G. levis*, and *S. lumampao* in the Philippines were known (Gonzales et al. 1992; Caasi-Lit et al. 2010; Aguinsatan et al. 2019).

Bamboo is considered one of the most useful plants in the Philippines due to its various benefits. The species were utilized in the Cagayan Valley region as food (Caasi-Lit et al. 2010), medicine (Baddu and Ouano 2018), housing, handicrafts, and furniture materials (DTI 2020; Mabborang et al. 2022). Due to these advantages, the region has existing bamboo stands for production and utilization. The Cagayan Valley region has 2,209 ha of bamboo plantation stands and 2,456.10 ha of bamboo natural stands, which comprise 16.88% of the plantation share in the Philippines (Caasi-Lit et al. 2010). Despite that, what is known about the biological characteristics of bamboo growing in the region is limited. The morphology of the culm, culm-sheath, branching, node nature, leaves, and rhizome system was used to identify bamboo botanically (Banik 2015). Clark et al. (2015) described the woody bamboos growing in Southeast Asia, where *Bambusa*, *Dendrocalamus*, *Guadua*, and *Gigantochloa* species have thick clumps (pachymorph type), hollow woody culms, complicated aerial vegetative branching, and acropetal or bidirectional branch growth, while *Melocanna*, *Pseudostachyum*, and *Schizostachyum* species have long, hollow aerial culm internodes with thin walls, rhizomes with short or elongated necks, and non-thorny culms. Meanwhile, phytochemical

characterization of *Scizostachyum*, *Gigantochloa*, *Bambusa*, *Dendrocalamus*, and *Phyllostachys* species was done in various studies (Jovale et al. 2014; Tongco et al. 2016; Wani et al. 2019; Karawak et al. 2020), which can be used as the basis for extraction of secondary metabolites for the production of pharmaceuticals, food, and industrial products.

Species characterization through DNA barcoding is a more precise method for identifying and certifying bamboo species (Dev et al. 2020) using specific molecular markers or barcode regions such as *matK*, *rbcL*, *rpoC1*, *psbA-trnH*, and *ITS2* (Sawarkar et al. 2021). This study utilized the ribulose-1,5-bisphosphate carboxylase (*rbcL*) barcode region in DNA sequencing of bamboo because of its slow evolution rate characteristics (Wolfe et al. 1987). The *rbcL* was reported in Bambussoideae inter-specific and intra-specific groups (Sosa et al. 2013), and phylogenetic relationships between *Bambusa* subgenera (Wang et al. 2022). Therefore, this study aims to describe the ecosystems and morphological traits of various bamboo species growing in Cagayan Province, Philippines, determine their secondary metabolite profiles, and compare the species morphological identities to their molecular identities through DNA sequencing using the *rbcL* barcode region.

MATERIALS AND METHODS

Species sampling

Municipalities of Cagayan, Philippines, namely: Gonzaga, Santa Ana, Aparri, Buguey, Allacapan, Lal-lo, Gattaran, and Tuguegarao City, were visited for the bamboo species morphological characterization and sample collection (Figure 1). The conduct of the study at various research sites was communicated to the Local Government Units (LGUs) of Cagayan through the Office of the Municipal Mayor. To comply with the legal requirements of EO 247 (Bioprospecting) and RA 9147 (Wildlife Resources Conservation and Protection Act), gratuitous permits and Protected Area Management Board (PAMB) resolutions were obtained from the Department of Environment and Natural Resources (DENR) Region 2, Tuguegarao City, Cagayan for conducting the research in protected areas of Cagayan such as the Palaui Island Protected Landscape and Seascape in Santa Ana, Mt. Cagua (Baua-Wangag Watershed and Forest Reserve, Sierra Madre Mountain Range) in Gonzaga, and the Magapit Protected Landscape in Lal-lo and Gattaran. A dry season from June to July and a wet season from September to December were experienced at the study sites from June to December 2022.

Ecosystem and species identification

The type of ecosystems such as woodland and forest, coastal (sparsely vegetated land), freshwater (wetlands, rivers, lakes, etc.), grassland, agricultural or cropland, and urban ecosystems where bamboo species grew were assessed using the descriptions of Maes et al. (2015). A combination of walk/vehicular ride, visual encounter, and photo documentation was carried out during the conduct. Bamboo species identification was done using the descriptions and illustrations of Roxas (2012). Scientific names and species origin in the Philippines were verified through "Plants of the World Online" (https://powo.science.kew.org/). The accession code follows the place of collection and the collection number.

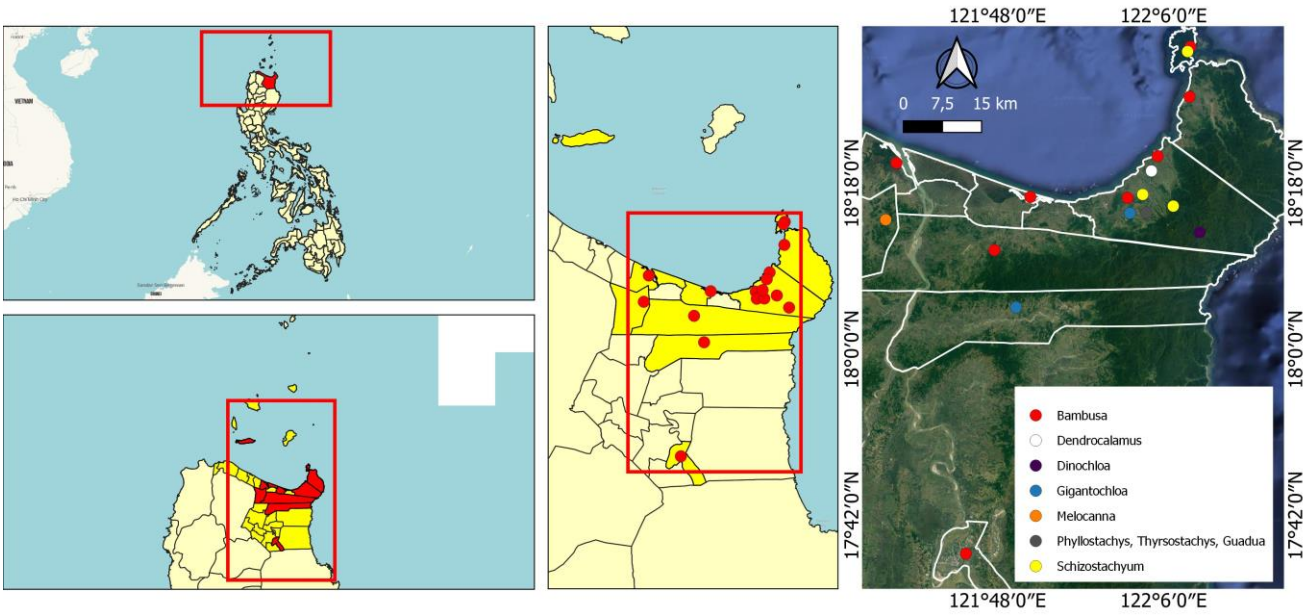

Figure 1. Species sampling of bamboo at various research sites in Cagayan, Philippines

Morphological characterization

The morphological characterization of the bamboo species was carried out following the character states on culm base and culm-node-internode (type of rhizomes, tillering/branching, growth habit, primary buds per mid-culm, and nodal line position) provided by Clark (2005), and young shoot (culm sheath color, location of trichomes in the culm sheath, and concentration of trichomes in the culm sheath) by Ricohermoso et al. (2015). Moreover, internode color and measurements, such as internode length, internode diameter, internode color, and appearance, were also taken. The internode length and diameter were taken on mature culms at the 4th to 8th internode from the ground (Sharma and Nirmala 2018).

Phytochemical screening

Fresh and healthy leaves were gathered from bamboo mother plants at various research sites in Cagayan Province, Philippines. The qualitative phytochemical screening was done at the Cagayan State University (CSU)-Andrews Campus Central Analytical Laboratory, Tuguegarao City, Cagayan, Philippines. Plant sample preparation and ethanolic extraction follow the modified procedure of Muñoz et al. (2021). The freshly harvested young and mature bamboo leaves were washed with running tap water, rinsed with distilled water once, and air-dried for 2 weeks. The collected bamboo leaves were oven-dried at 70°C for 5 days. Dried bamboo leaves were pulverized using a grinder, and 10 g of the leaf powder was extracted with 30 mL of 70% laboratory-grade ethanol (Sigma-Aldrich Pte. Ltd., Philippines). The mixture was stirred at high temperature, stored in the refrigerator overnight, then removed from the refrigerator, and agitated at room temperature prior to filtration using Whatman filter paper #1 (Sigma-Aldrich Co.). The filtrate was concentrated using a rotary evaporator (Fisher Scientific) at 50°C, then the residue was resuspended in distilled water, placed in a container, and stored in the refrigerator before testing.

Qualitative tests (colorimetric analysis) for the presence of various phytochemicals using the procedures of Guevarra (2005) and Muñoz et al. (2021) were performed. The ethanolic leaf extracts were subjected to qualitative phytochemical tests to detect the presence of anthocyanin (sodium hydroxide test), carotenoids (carbon tetrachloride-sulfuric acid test), coumarins (sodium hydroxide test), flavonoids (Shinoda test), phenols (ferric chloride test), quinones (potassium hydroxide test), saponins (Froth test), steroids (Liebermann-Buchard Reaction), tannins (lead acetate test), and terpenoids (Salkowski test).

Molecular characterization

Genomic DNA extraction

Fresh and healthy young leaves were collected and air-dried for 3 days. About 1 g of leaves were weighed, placed on a transparent plastic sealer with 1 g of silica gel, and properly labeled. The bamboo leaf samples were sent to the DNA Sequencing Core Facility of the Philippine Genome Center (PGC), University of the Philippines Diliman (UPD), Quezon City, Philippines, for DNA extraction. Isolation of bamboo DNA was performed using the procedures of the PGC (2022). The leaf samples were prepared by adding liquid nitrogen and pulverized using a mortar and pestle. The genomic DNA was extracted from the leaves of bamboo species using the cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). Extraction was done by adding 500 µL of CTAB solution to 10 mg of dried leaf samples. The samples were vortexed for 10 min, heated in a dry bath for 30 min at 60°C, and centrifuged at 13000 rpm for 5 min. About 250 µL of chloroform:isoamyl alcohol was added to the solution, centrifuged for 1 min at 13000 rpm, and the upper aqueous solution containing plant DNA was transferred to a new microcentrifuge tube. Moreover, the samples were added with 50 µL 3 M NaOAC solution followed by 500 µL ice-cold 100% ethanol, incubated at -20°C for 1 h, centrifuged for 1 min at 13000 rpm, discarded the supernatant, and added with 500 µL 75% ice-cold absolute ethanol. The samples were centrifuged for 1 min at 13000 rpm and repeated the steps of discarding the supernatant and adding ice-cold absolute ethanol. The supernatant was discarded, and the precipitate was air-dried for 15 min at room temperature to remove residual ethanol. The DNA precipitate was then reconstituted by adding 30-50 µL nuclease-free suspension water. The quality of the DNA precipitate was checked using the NanoDrop TM 8000 Spectrophotometer (Thermo Scientific, USA). Upon obtaining the 1.8-2.0 results under 260/280 nm, the genomic DNAs were electrophoresed in a 1.2% agarose gel marked using 1 kb DNA ladder (Invitrogen) and examined on an AlphaImager Mini (Protein Simple, USA).

DNA amplification and capillary sequencing

The extracted genomic DNAs were subjected to polymerase chain reaction (PCR) for gene amplification and then capillary sequencing in the DNA Sequencing Core Facility of the PGC-UPD. PCR gene amplification and capillary sequencing were done using the procedures of the PGC (2022). The PCR mixtures were performed in a volume of 35 µL of containing 0.5 µL (1x) *rbcL-F* [5'-ATGTCACCACAAACAGAGACTAAAGC-3'] and *rbcL-R* [5'-GTAAAATCAAGTCCACCRCG-3'] primers (Costion et al. 2011), 1 µL PCR buffer, 0.05 µL Taq polymerase, 0.5 µL $MgCl_2$, and 0.5 µL dNTP mix. Cycling parameters on the thermal cycler are as follows: 95°C for 5 min for predenaturation; 35 cycles of 95°C for 1 min; 52°C for 30 s; 70°C for 1 min; 72°C for 10 min for extension; and hold at 4°C. Capillary sequencing involves the incorporation of fluorescently labeled chain terminator ddNTPs; the reaction components include amplicons, primers, and the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit. Cycling parameters on the Bio-Rad T100 Thermal Cycler are as follows: pre-hold at 4°C; 96°C for 1 min; 25 cycles of 96°C for 10 s; 50°C for 5 s; 62°C for 4 min; and hold at 4°C. Ethanol precipitation was carried out to remove unincorporated ddNTPs, excess primers, and primer dimers. Capillary electrophoresis on the ABI 3730xl DNA Analyzer using a 50 cm 96-capillary array, POP7TM polymer, and 3730xl Data Collection Software v3.1. Base calling was done on Sequencing Analysis Software v5.4.

Data analysis

The types of ecosystems where the bamboo species grow were determined at the research sites (Maes et al. 2015). The morphology of the bamboo was described through qualitative or visual characterization of the culm base, culm internode, nodal structure, and young shoot appearance (Clark 2005; Ricohermoso et al. 2015). The presence of various phytochemicals in the bamboo leaves was determined through colorimetric analysis (Guevarra 2005; Muñoz et al. 2021), where a positive symbol (+) corresponds to the present secondary metabolite, while a negative symbol (-) corresponds to the absent secondary metabolite in the ethanolic extracts.

The chromatograms of the sequenced bamboo species were edited and trimmed using the BioEdit 7.2 program (Hall 1999). The Basic Local Alignment Search Tool (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to submit the DNA sequences for homology searches. In the NCBI-BLAST, the query bamboo species DNA sequences were compared to the known bamboo species DNA sequences. The percentage of species identification, DNA query length, and DNA identities were noted. To verify the species identification, the morphological identities of the bamboo species growing in Cagayan were matched from the BLAST GenBank.

RESULTS AND DISCUSSION

Ecosystems of bamboo species

This study adds information on the ecosystems or habitats where bamboo species grow in the Philippines. The bamboo species growing in various ecosystems of Cagayan Province, Philippines, is presented in Table 1. *Bambusa* species grew in primary and secondary forests, urban or residential areas, wetlands, creeks, rivers, lakes, irrigation canals, grasslands, agricultural fields, and plantations. *Dendrocalamus* species grew in a residential area and secondary forest in Gonzaga, while *Dinochloa* species grew in the primary forests of Mt. Cagua (an active volcano in Gonzaga). Thin-walled, hollow bamboo species, such as *Gigantochlo*a and *Schizostachyum* grew in primary and secondary forests of Magapit Protected Landscape in Gattaran, Mt. Tabungao in Gonzaga, and Palaui Island Protected Landscape and Seascape in Santa Ana. Moreover, the bamboo species grew in grasslands, agricultural fields, and plantations. Species variants of *Bambusa vulgaris* var. *striata* [sp.2] and *Schizostachyum* sp. [sp.2] with green culms and yellow vertical linings in the internodes grew in a coastal area or sparsely vegetated land and a forest creek, respectively. *Phyllostachys* species grew in a secondary forest, grassland, and agricultural plantation. *Thyrsostachys*, *Melocanna*, and *Guadua* species also grew in grasslands and agricultural plantations. Habitat of *Bambusa*, *Gigantochloa*, and *Schizostachyum* were in freshwater, forest, grassland, and agricultural ecosystems of Cagayan (Table 1; Figure 2).

Bamboo species growing in Cagayan, such as *Bambusa*, *Dinochloa*, *Dendrocalamus*, *Gigantochloa*, *Schizostachyum*, *Thyrsostachys*, *Guadua*, and *Phyllostachys* grew in forests, grasslands, and agricultural ecosystems. Previous reports revealed that bamboos like *Bambusa*, *Dendrocalamus*, *Dinochloa*, *Gigantochloa*, *Guadua*, and *Schizostachyum* grew in habitats like grassy habitats, lowland moist tropical forests or lower montane forests (Judziewicz et al. 1999; Ahmad et al. 2021), valleys, along rivers or streams, and secondary forests (Dransfield 1994). Previous reports in the Philippines mentioned that *D. asper*, *B. vulgaris*, *G. levis*, *B. blumeana*, and *B. merrillii* grew in freshwater ecosystems like riverbanks, creeks, and streams (Gonzales et al. 1992; Caasi-Lit et al. 2010; Aguinsatan et al. 2019), while *S. lumampao*, *B. vulgaris* var. *vittata*, and *Dinochloa* sp. grew in bamboo groves and bridges (Caasi-Lit et al. 2010). Also, *G. levis* and *D. asper* grew in hilly, mountainous, and upland areas (Caasi-Lit et al. 2010; Aguinsatan et al. 2019). In this study, *D. asper*, *G. levis*, *B. blumeana*, and *B. merrillii* grew in similar ecosystems in the Philippines, which other researchers observed.

Morphological characterization

The morphological characteristics of bamboo species growing in various ecosystems of Cagayan Province, Luzon, Philippines, are presented in Table 2. Roxas (2012) described the culm height, culm color, culm diameter, culm and leaf sheaths, branch per node, branch and branchlets, nodes, and leaf blades of various bamboo species in the Philippines. Ricohermoso et al. (2015) also described the young shoots (culm sheath striations; culm sheath color, shape, and size; trichome concentration and appearance; blade color, shape, size, and appearance; and auricle and ligule appearance) of *G. levis*, *D. latiflorus*, *D. asper*, *B. philippinensis*, *B. vulgaris* var. *striata*, *B. vulgaris*, *B. blumeana*, and *B. merrillii* in the Philippines. Morphological characterization of bamboo by field identification keys focused mainly on vegetative characters, such as height, growth habit, branching, node appearance, culm internode appearance, leaves, leaf sheath, and culm-sheath, and generative characters, such as panicle, number of stamens, presence of lodicules, spikelets, caryopsis, and filaments (Kumar et al. 2001). Morphological characterization of bamboo in this study was done on 25 bamboo species (10 species of *Bambusa*, 5 species of *Schizostachyum*, 3 species of *Gigantochloa*, 2 species of *Phyllostachys*, and 1 species each for *Dendrocalamus*, *Dinochloa*, *Melocanna*, *Thyrsostachys*, and *Guadua*) which are specifically growing in the Cagayan Province, Philippines, which consisted of 28 bamboo accessions. This study contributes information on the morphology of bamboo species growing in Cagayan ecosystems, specifically on the culm internode measurements, growth habit, culm internode color and appearance, number of primary buds per mid-culm, nodal line position, rhizome system in the tropics, number of tillers in the rhizomes, and occurrence of flowering in Cagayan. Moreover, this study provides additional information on the young shoot morphology (culm sheath color, trichome location, and trichome concentration in the culm sheath) of other bamboos not reported by Ricohermoso et al. (2015). Hence, this study reports field identification keys to the different bamboo genera in Cagayan, Philippines.

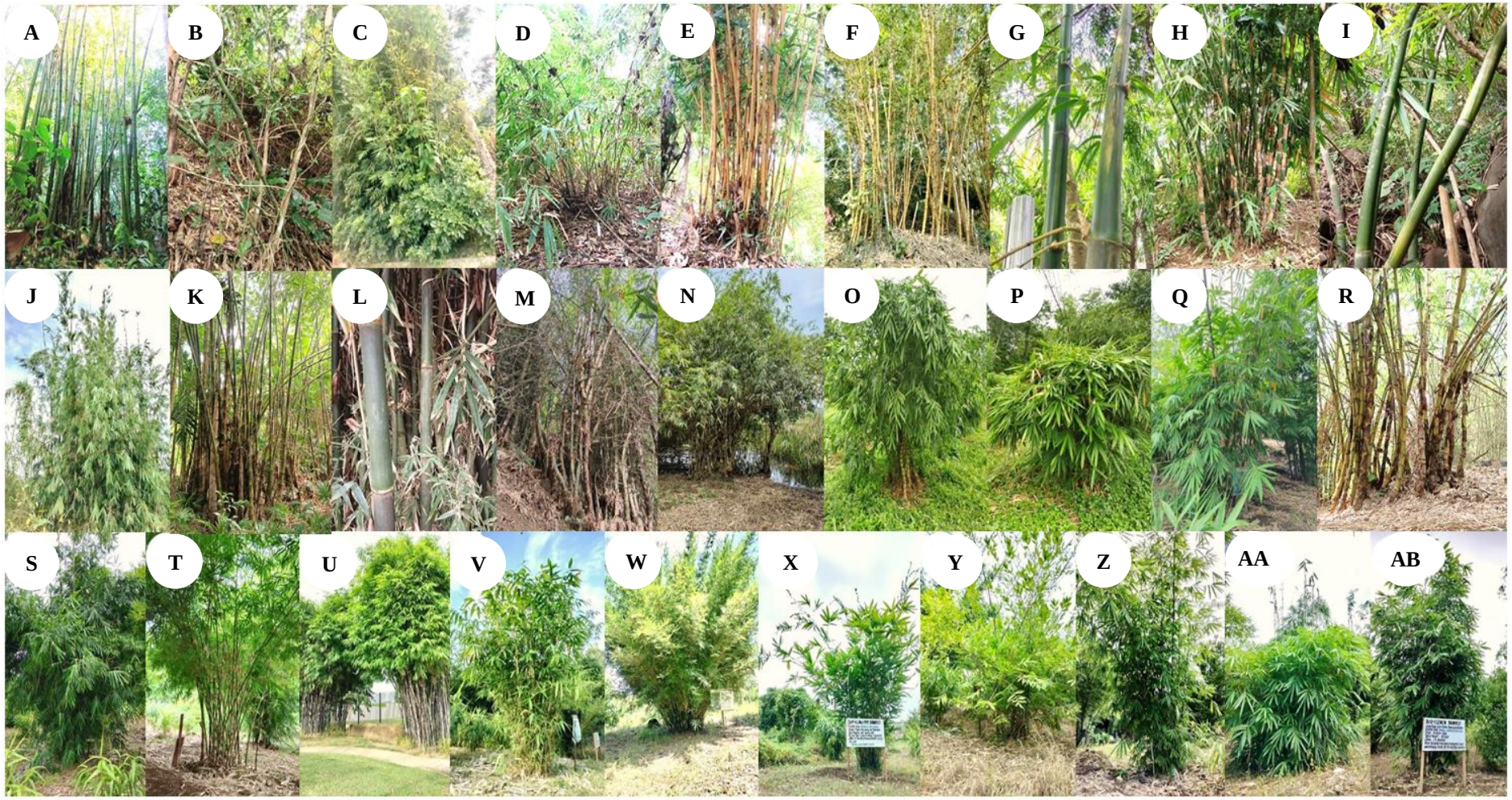

Figure 2. Bamboo species at the research sites: Forest ecosystems: A. SAP-036, B. GON-005, D. SAP-040, E. GON-006, F. GON-160, H. GAT-229, P. APR-237, R. GON-007. Urban ecosystems: C. SAM-141, K. GON-060, U: TUG-152. Sparsely vegetated land or coastal ecosystem: G. GON-084. Freshwater ecosystems: I. GON-026, L. LAL-214, M. APR-234, N. BUG-154, O. GON-013. Grassland and agricultural ecosystems: J. ALC-228, Q. GON-099, S. GON-098, T. GON-100, V. GON-112, W. GON-104, X. GON-106, Y. GON-107, Z. GON-108, AA. GON-109, AB. GON-105

Table 1. Details of bamboo species in the present study

Accession code	Species identification based on morphology		Place of collection	Ecosystems: habitats	Population-specific characters	Species origin in the Philippines
	Common name	Scientific name				
SAP-036	Kawayang kiling	*Bambusa vulgaris* Schrad. ex J.C. Wendl.	San Vicente, Santa Ana, Cagayan	Forest ecosystem: Palaui Island Protected Landscape and Seascape primary forest	Shiny and glossy dark green culms	Introduced
GON-005	Mangnaw bamboo	*Dinochloa* sp.	Santa Clara, Gonzaga, Cagayan	Forest ecosystem: Mt. Cagua (Baua-Wangag Watershed and Forest Reserves) primary forest	Large, rough-textured culms with several large prominent lenticel-like structure in the internodes	Native
SAM-141	Unknown sp1	*Bambusa* sp. [sp.1]	Diora Zinungan, Sta Ana, Cagayan	Urban ecosystem: residential area	Small, shiny green culms; smooth-textured leaves (abaxial)	
SAP-040	Unknown sp2	*Schizostachyum* sp. [sp.1]	San Vicente, Santa Ana, Cagayan	Forest ecosystem: Palaui Island Protected Landscape and Seascape primary forest	Very fragile, rough-textured culms with abundant trichomes; intravaginal branching pattern (new branch)	Introduced
GON-006	Buhong dilaw	*Schizostachyum brachycladum* (Kurz ex Munro) Kurz	Flourishing, Gonzaga, Cagayan	Forest ecosystem: secondary forest of Mt. Tabungao		Introduced
GON-160	Green stripe/golden bamboo sp1	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.1]	Flourishing, Gonzaga, Cagayan	Forest ecosystem: bamboo plantation, secondary forest	Bright yellow culms with unequal width of vertical green stripes	Introduced
GON-084	Green stripe/ golden bamboo sp2	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.2]	Amunitan, Gonzaga, Cagayan	Coastal ecosystem: coastal line or sparsely vegetated land	Dark green culms with a single vertical yellow line in the internodes	Introduced
GAT-229	Bolo	*Gigantochloa levis* (Blanco) Merr.	Magapit-Capissayan Rd, Gattaran, Cagayan	Forest ecosystem: Magapit Protected Landscape primary growth forest		Native
GON-026	Unknown sp3	*Schizostachyum* sp. [sp.2]	Santa Clara, Gonzaga, Cagayan	Freshwater ecosystem: creek	Dark green culms while other culms have vertical yellow stripes or linings in the internodes	Introduced
ALC-228	Unknown sp4	*Melocanna* sp.	Dagupan, Allacapan, Cagayan	Grassland ecosystem: hills Agroecosystem: rice field	Glossy light green culms with long branches and small leaves that are grouped or clustered	Introduced
GON-060	Giant bamboo	*Dendrocalamus asper* (Schult. & Schult.f.) Backer	Tapel, Gonzaga, Cagayan	Urban ecosystem: residential area Forest ecosystem: secondary forest	Culms have brown velvety hairs near the nodes	Native
LAL-214	Unknown sp5	*Bambusa* sp. [sp.2]	San Mariano, Lal-lo, Cagayan	Freshwater ecosystem: wetland, creek Urban ecosystem: residential area	Green culms with numerous dark trichomes	-
APR-234	Spiny bamboo /Kawayang tinik	*Bambusa blumeana* Schult.f. (=*Bambusa spinosa* Roxb.)	Zinarag, Aparri, Cagayan	Freshwater ecosystem: Linao river	Culms have numerous thorns	Native
BUG-154	Bayog	*Bambusa merrillii* Gamble	Santa Isabel, Buguey, Cagayan	Freshwater ecosystem: lake	Culms with less thorns	Native

GON-013	Wamin bamboo	*Bambusa vulgaris* f. *waminii* T.H. Wen (=*Bambusa vulgaris* Schrad. ex J.C.Wendl.)	Flourishing, Gonzaga, Cagayan	Freshwater ecosystem: irrigation canal	Culms with larger bulging internodes	Introduced
APR-237	Buddha belly bamboo	*Bambusa ventricosa* McClure	Zinarag, Aparri, Cagayan	Forest ecosystem: secondary forest	Culms with smaller bulging internodes	Introduced
GON-099	Black bamboo	*Gigantochloa atroviolacea* Widjaja	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations	Green culms with black colorations in the internodes	Introduced
GON-007	Green stripe vivax bamboo	*Phyllostachys vivax* f. *aureocaulis* N.X. Ma (=*Phyllostachys vivax* McClure)	Flourishing, Gonzaga, Cagayan	Forest ecosystem: secondary forest	Yellow culms with random green stripes. Young culms and branches have white linings	Introduced
GON-098	Tiger bamboo	*Bambusa maculata* Widjaja	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations		Introduced
GON-100	Moso bamboo	*Phyllostachys edulis* (Carrière) J.Houz.	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations		Introduced
TUG-152	Timor black bamboo	*Bambusa lako* Widjaja	Carig Sur, Tuguegarao City, Cagayan	Urban ecosystem: residential area	Black culms (some culms have green colorations)	Introduced
GON-112	Buho bamboo	*Schizostachyum lumampao (Blanco) Merr.*	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations		Native
GON-104	Thailand pole/ Monastery bamboo	*Thyrsostachys siamensis* Gamble	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations		Introduced
GON-106	Long pipe bamboo	*Bambusa atra Lindl.*	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations		Native
GON-107	Iron bamboo	*Guadua angustifolia Kunth*	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations	Green culms with white bands around the nodes	Introduced
GON-108	Pink bamboo	*Gigantochloa kuring Widjaja*	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations	Green culms with pink colorations in the internodes	Introduced
GON-109	Anos bamboo	*Schizostachyum lima (Blanco) Merr.*	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations		Native
GON-105	Asian Lemon bamboo	*Bambusa eutuldoides McClure*	Flourishing, Gonzaga, Cagayan	Grassland ecosystem: grassland Agroecosystem: dragonfruit and tiger grass plantations		Introduced

Table 2. Morphological profile of bamboo species growing in various ecosystems of Cagayan Province, Luzon, Philippines

Accession code	Species identification based on morphology	Culm-node-internode morphology					
		Growth habit	Internode length (cm)	Internode diameter (cm)	Culm internodes	culm color and appearance	Primary buds per mid-culm
SAP-036	*Bambusa vulgaris* Schrad. ex J.C. Wendl.	Erect	23.68	9.52	Hollow	Dark green	1
GON-005	*Dinochloa* sp.	Twining	33.96	4.37	Hollow	Dark green	1
SAM-141	*Bambusa* sp. [sp.1]	Erect	27.40	1.76	Hollow	Light green	1
SAP-040	*Schizostachyum* sp. [sp.1]	Erect	26.28	1.72	Hollow	Dark green with numerous trichomes	1
GON-006	*Schizostachyum brachycladum* (Kurz ex Munro) Kurz	Erect	58.28	4.26	Hollow	Golden yellow	1
GON-160	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.1]	Apically arching or pendulous	25.82	7.56	Hollow	Golden yellow with vertical green stripes	1
GON-084	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.2]	Apically arching or pendulous	26.48	5.70	Hollow	Green with single vertical yellow lining	1
GAT-229	*Gigantochloa levis* (Blanco) Merr.	Erect	32.82	4.70	Hollow	Dark green	1
GON-026	*Schizostachyum* sp. [sp.2]	Apically arching or pendulous	42.66	6.80	Hollow	Dark green with vertical yellow stripes	1
ALC-228	*Melocanna* sp.	Erect	42.18	3.92	Hollow	Glossy light green	1
GON-060	*Dendrocalamus asper* (Schult. & Schult. f.) Backer	Apically arching or pendulous	27.20	9.56	Hollow	Dark green with brown trichomes near the nodes	1
LAL-214	*Bambusa* sp. [sp.2]	Erect	31.34	5.46	Hollow	Dark green with dark trichomes	1
APR-234	*Bambusa blumeana* Schult.f. (= *Bambusa spinosa* Roxb.)	Apically arching or pendulous	22.90	9.22	Hollow	Dark green	1
BUG-154	*Bambusa merrillii* Gamble	Apically arching or pendulous	17.28	6.06	Solid	Dark green	1
GON-013	*Bambusa vulgaris* f. *waminii* T.H. Wen (=*Bambusa vulgaris* Schrad. ex J.C.Wendl.)	Apically arching or pendulous	9.10	5.94	Solid	Dark green	1
APR-237	*Bambusa ventricosa* McClure	Erect	5.83	7.32	Solid	Dark green	1
GON-099	*Gigantochloa atroviolacea* Widjaja	Apically arching or pendulous	26.36	4.60	Hollow	Dark green with black coloration in the internodes	1
GON-007	*Phyllostachys vivax* f. *aureocaulis* N.X. Ma (= *Phyllostachys vivax* McClure)	Erect	25.78	7.10	Hollow	Golden yellow with vertical green stripes (white stripes appears in young culms and branches)	1
GON-098	*Bambusa maculata* Widjaja	Apically arching or pendulous	19.46	3.80	Solid	Dark green with yellow stripes	1
GON-100	*Phyllostachys edulis* (Carrière) J.Houz.	Apically arching or pendulous	34.0	2.90	Solid	Dark green	1
TUG-152	*Bambusa lako* Widjaja	Erect	28.20	4.0	Hollow	Black/purple black	1
GON-112	*Schizostachyum lumampao* (Blanco) Merr.	Erect	39.44	8.40	Hollow	Light green	1
GON-104	*Thyrsostachys siamensis* Gamble	Apically arching or pendulous	29.64	1.50	Hollow	Dark green	1
GON-106	*Bambusa atra* Lindl.	Apically arching or pendulous	54.60	1.66	Hollow	Light green	1
GON-107	*Guadua angustifolia* Kunth	Apically arching or pendulous	18.30	2.80	Solid	Light green with white bands around the nodes	1
GON-108	*Gigantochloa kuring* Widjaja	Apically arching or pendulous	33.76	3.56	Hollow	Dark green with pink stripes in the internodes	1
GON-109	*Schizostachyum lima* (Blanco) Merr.	Erect	39.40	3.40	Hollow	Light green with trichomes	1
GON-105	*Bambusa eutuldoides* McClure	Apically arching or pendulous	40.70	3.46	Hollow	Dark green	1

Table 2. Morphological profile of bamboo species growing in various ecosystems of Cagayan Province, Luzon, Philippines (*continued*)

Accession code	Culm-node-internode morphology	Culm base morphology		Young shoot morphology			Incidence of flowering*
	Nodal line position	Type of rhizomes	Tillering/ branching	Location of trichomes in culm sheath	Concentration of trichomes in culm sheath	Culm sheath color	
SAP-036	Dipping slightly below the bud	Pachymorph	Two or more tillers	Upper half	Abundant	Green	Not flowering
GON-005	Dipping slightly below the bud	Pachymorph	Two or more tillers	Entire	Abundant	Whitish brown	Not flowering
SAM-141	Dipping slightly below the bud	Pachymorph	Two or more tillers	Upper half	Abundant	Green	Not flowering
SAP-040	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Dark green	Not flowering
GON-006	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Yellow	Flowering
GON-160	Dipping slightly below the bud	Pachymorph	Two or more tillers	Upper half	Moderate	Green	Not flowering
GON-084	Dipping slightly below the bud	Pachymorph	Two or more tillers	Upper half	Moderate	Green	Not flowering
GAT-229	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Reddish brown	Not flowering
GON-026	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Green tinged with purple	Not flowering
ALC-228	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Yellowish brown	Flowering
GON-060	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Reddish brown to purple brown	Not flowering
LAL-214	Dipping slightly below the bud	Pachymorph	Two or more tillers	Upper half	Abundant	Green	Not flowering
APR-234	Dipping slightly below the bud	Pachymorph	Two or more tillers	Upper half	Very few	Green to grayish green	Not flowering
BUG-154	Dipping markedly below the bud	Pachymorph	Two or more tillers	Entire	Very few	Green to grayish green	Not flowering
GON-013	Dipping markedly below the bud	Pachymorph	Two or more tillers	Upper half	Very few	Green to grayish green	Not flowering
APR-237	Dipping slightly below the bud	Pachymorph	Two or more tillers	Medial	Moderate	Green to grayish green	Not flowering
GON-099	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Green	Not flowering
GON-007	Dipping slightly below the bud	Pachymorph	Two or more tillers	Upper half	Moderate	Green	Not flowering
GON-098	Dipping markedly below the bud	Pachymorph	Two or more tillers	Absent	Absent	Green	Flowering
GON-100	Dipping slightly below the bud	Pachymorph	Two or more tillers	Entire	Moderate	Brown	Not flowering
TUG-152	Horizontal	Pachymorph	Two or more tillers	Absent	Absent	Green tinged with purple	Not flowering
GON-112	Horizontal	Pachymorph	Two or more tillers	Entire	Very few	Green	Not flowering
GON-104	Horizontal	Pachymorph	Two or more tillers	Absent	Absent	Green	Not flowering
GON-106	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Green	Flowering
GON-107	Dipping slightly below the bud	Pachymorph	Two or more tillers	Absent	Absent	Green tinged with purple	Flowering
GON-108	Horizontal	Pachymorph	Two or more tillers	Entire	Moderate	Yellow green	Not flowering
GON-109	Horizontal	Pachymorph	Two or more tillers	Lower part	Very few	Yellow	Not flowering
GON-105	Horizontal	Pachymorph	Two or more tillers	Upper half	Moderate	Yellow green	Flowering

Note: *Observed during the study period from June to December 2022

1. Key to the species of *Bambusa* Schreb.
1a. Erect growth; widest culm internodes among the genus; young shoots with abundant trichomes located in the upper half of the green culm sheaths ..... *B. vulgaris*
1b. Golden yellow culms with an unequal width of vertical green stripes; other species have dark green culms with a single line of vertical yellow stripe; young shoots with moderate trichomes located in the upper half of the green culm sheaths…… *B. vulgaris* var. *striata*
1c. Culms with abundant thorns …….. *B. blumeana*
1d. Partially-thorny culms ……. *B. merrillii*
1e. Culms with bulging internodes and nodal lines that dip markedly below the bud ..... *B. vulgaris* f. *waminii*
1f. Erect growth; culms with bulging internodes and nodal lines that dip slightly below the bud; shortest culm internodes among the genus; young shoots with moderate trichomes located in the middle of the green to grayish green culm sheaths ..... *B. ventricosa*
1g. Dark green culms with yellow stripes; young shoots with green culm sheaths without trichomes.*B. maculata*
1h. Light green culms; longest and narrowest culm internodes among the genus; flowering species from June to December……..... *B. atra*
1i. Erect growth; black or purple-black mature culms; young shoots have green tinged with purple culm sheaths without trichomes …… *B. lako*
1j. Young shoots with moderate trichomes located in the upper half of the yellow-green culm sheaths; flowering species from June to December …… *B. eutuldoides*

2. Key to the species of *Dendrocalamus* Nees
2a. Dark green culms with brown velvety hairs near the nodes; young shoots with moderate trichomes located entirely within the red or purple-brown culm sheaths …… *D. asper*

3. Key to the species of *Dinochloa* Büse
3a. Twining growth; culms are rough-textured with several large, prominent lenticel-like structures in the internodes; young shoots with abundant trichomes located entirely within the whitish brown culm sheaths ….... *Dinochloa* sp.

4. Key to the species of *Gigantochloa* Kurz ex Munro
4a. Erect growth; dark to light green culms; reddish or brick brown young shoots…….. *G. levis*
4b. Dark green culms with black coloration in the internodes; shortest culm internodes among the genus; green young shoots…… *G. atroviolacea*
4c. Dark green culms with pink stripes in the internodes; narrowest culm internodes among the genus; yellow-green young shoots …… *G. kuring*

5. Key to the species of *Guadua* Kunth
5a. Green culms with white bands around the nodes ……..... *G. angustifolia*

6. Key to the species of *Melocanna* Trin.
6a. Glossy light green culms; yellowish brown young shoots; flowering species from June to December ….... *Melocanna* sp.

7. Key to the species of *Phyllostachys* Siebold & Zucc.
7a. Erect growth; pachymorph rhizome system; golden yellow culms with random green stripes; young shoots with moderate trichomes located in the upper half of the green culm sheaths…….... *P. vivax* f. *aureocaulis*
7b. Apically arching growth; pachymorph rhizome system; dark green culms; young shoots with moderate trichomes located entirely within the brown culm sheaths ……..... *P. edulis*

8. Key to the species of *Schizostachyum* Nees
8a. Dark green culms with numerous trichomes; culms are very fragile and rough-textured; shortest and narrowest culm internodes among the genus; young shoots with moderate trichomes located entirely within the dark green culm sheaths …… *Schizostachyum* sp. [sp1]
8b. Pendulous growth; dark green culms with vertical yellow stripes in the internodes ……..... ……..……. *Schizostachyum* sp. [sp2]
8c. Golden yellow culms; longest culm internodes; young shoots with moderate trichomes located entirely within the yellow culm sheaths; flowering species from June to December…… *S. brachycladum*
8d. Light green culms; widest culm internodes; young shoots with very few trichomes located entirely within the green culm sheaths …… *S. lumampao*
8e. Light green culms with trichomes; young shoots with very few trichomes located in the lower part of the yellow culm sheaths …… *S. lima*

9. Key to the species of *Thyrsostachys* Gamble
9a. Young shoots have green culm sheaths without trichomes ……. *T. siamensis*

All the bamboo species growing in Cagayan ecosystems have pachymorph rhizomes. Makita (1998) and Uchimura (1980) disclosed that tropical bamboos, such as *Schizostachyum*, *Bambusa*, and *Dendrocalamus* have clump-forming or pachymorph rhizome systems, while temperate bamboos like *Phyllostachys* have leptomorph rhizome systems. In this study, *Phyllostachys vivax* f. *aureocaulis* and *P. edulis* were observed to have pachymorph rhizomes. The growth change of the rhizome system from leptomorph to pachymorph was attributed to the tropical environment and ecosystems in Cagayan where these species grew. Pachymorph rhizomes might more easily adapt to dry soils and climates (Chen 2021).

Culm internode length variations may be related to the culm growth process (Chen 2021). Tandug and Torres (1985) measured the culm internodes of *B. vulgaris* and *S. lumampao* in Cagayan Province. In their study, *B. vulgaris* growing in Ballesteros, Cagayan has 29.02 cm long and 6.73 cm wide culm internodes, while *S. lumampao* growing in Allacapan, Cagayan has 45.25 cm long and 4.96 cm wide culm internodes. To compare the bamboo species, this study reported that *B. vulgaris* growing in Palaui Island Protected Landscape and Seascape in Santa Ana, Cagayan, has 23.68 cm long and 9.52 cm wide culm internodes. Moreover, *S. lumampao* growing in Gonzaga, Cagayan, has 39.44 cm long and 8.40 cm wide culm internodes. Culm internodes of *B. vulgaris* growing in Santa Ana, Cagayan, are 5.34 cm shorter and 2.79 cm wider than those species growing in Ballesteros, Cagayan, while culm internodes of *S. lumampao* growing in Gonzaga, Cagayan, are 5.81 cm shorter and 3.44 cm wider than those species growing in Allacapan, Cagayan, hence, bamboo species growing in northeastern Cagayan (Santa Ana and Gonzaga) have shorter and wider culm internodes as compared to bamboo species growing in northwestern Cagayan (Allacapan and Ballesteros). The growth variations in the culm internode length and diameter of the bamboo species were affected by the ecosystems where the species grew. In this study, *B. vulgaris* grew in primary forests where the light was limited, while *S. lumampao* grew in grassland and agricultural ecosystems where the light was abundant. Culm elongation can be influenced by light conditions and shading in the growing environment (Banik 2015).

The culm color and appearance of bamboo species in Cagayan were also described in this study. A color variation in the culm internodes of *B. vulgaris* var. *striata* [sp.2] and *Schizostachyum* sp. [sp.2] was observed. Vertical yellow stripes have developed in the culm internodes of *Schizostachyum* sp. growing in a forest creek (GON-084), while other species, except *Schizostachyum brachycladum* have no yellow coloration in the culm. Meanwhile, the culm color was interchanged in *B. vulgaris* var. *striata* where species growing in a secondary growth forest (GON-160) has bright yellow culms with vertical green stripes (which is the common culm color), while a species variant growing in a coastal area or sparsely vegetated land (GON-084) has dark green culms with a single vertical yellow lining in the internode. *B. vulgaris* var. *striata* [sp.2] has several green culms in the clump, but most of the culms present in the clump are bright yellow. The color variation of the bamboo culms was affected by several genes that can influence the pigmentation process of the culms, thus resulting in a phenotypic variation (Xia et al. 2015). Meanwhile, the flowering of the bamboo species in Cagayan was observed from June to December. Sinohin (1990) disclosed that bamboo species in the Philippines form flower buds starting from October to November and bloom throughout the year.

Phytochemical screening of bamboo species

The phytochemical profile of bamboo species found in various ecosystems of Cagayan is presented in Table 3. *Bambusa* species contain 2-8 phytochemicals; *Schizostachyum* and *Gigantochloa* species contain 4-6 phytochemicals; *Phyllostachys* species contain 5-9 phytochemicals; *Dinochloa* sp. contains 6 phytochemicals; and *Dendrocalamus*, *Guadua*, *Thyrsostachys*, and *Melocanna* species contain 5 phytochemicals present in the ethanolic leaf extracts. All of the bamboos contain coumarins, while quinones are not detected. Saponins are detected in all tested bamboo species except *Dinochloa* sp. Steroids are also present in the bamboos, except in *Bambusa* sp. [sp.2], *Bambusa ventricosa*, and *B. lako*. In terms of the phytochemicals present in each bamboo species, *Bambusa* sp. [sp.1], *Melocanna* sp., *Thyrsostachys siamensis*, *Bambusa atra*, *Guadua angustifolia*, and *Schizostachyum lima* were found to contain coumarins, phenols, saponins, steroids, and terpenoids. The same secondary metabolites, except phenols, were present in *B. vulgaris*, *S. lumampao*, *Bambusa eutuldoides*, *B. vulgaris* var. *striata* [sp.1], and *G. levis*. *Gigantochloa atroviolacea* and *Bambusa maculata* both contain similar secondary metabolites, but tannins were added to the phytochemical content. Coumarins, flavonoids, saponins, steroids, and terpenoids were detected in *B. vulgaris* var. *striata* [sp.2] and *D. asper*. Meanwhile, all of the secondary metabolites tested except quinones are present in *P. vivax* f. *aureocaulis*, while *Bambusa vulgaris* f. *waminii* contains the same secondary metabolites except for quinones and terpenoids. The fewest secondary metabolites (coumarins and saponins) were noted in *B. ventricosa.* Also, few secondary metabolites were detected in *B. lako* (containing only coumarins, flavonoids, and saponins) and *B. merrillii* (containing only coumarins, saponins, and steroids).

It was noted that most of the bamboo species contain coumarins, saponins, steroids, and terpenoids. The aforementioned phytochemicals have therapeutic advantages and have been used as treatments for several diseases. Coumarins have been found to exert anti-inflammatory, anti-HIV (Liu et al. 2019), antioxidant, antitumor (Khalil and Mustafa 2020), antifungal, and anti-aflatoxigenic activities (Ali et al. 2021). Saponins were reported to have antidiabetic (Guzmán et al. 2020), immunostimulatory, hypocholesterolemic, antifungal, antitumor, antibacterial, antiviral, anti-inflammatory and antiparasitic activities (Oleszek and Oleszek 2020). Steroids have the potential for many key physiological processes and serve as systemic endocrine regulators. Steroids are beneficial for cell and organ growth, reproduction, and cardiovascular health (Cole et al. 2019), while terpenoids have been found to have anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic activities (Masyita et al. 2022).

Commonly-grown bamboo species in Cagayan Province, Philippines are *B. vulgaris*, *B. vulgaris* var. *striata*, *B. blumeana*, *B. merrillii*, *G. levis*, and *D. asper*. Caasi-Lit et al. (2010) reported that *Bambusa* species, such as *B. merrillii*, *B. blumeana*, and *B. vulgaris* were present in Cagayan. These bamboos were grown by bamboo growers in their backyards, on bamboo plantations, and in different habitats in the province. The phytochemical content findings of *B. vulgaris*, *D. asper*, and *B. blumeana* were different from the findings of Dionglay et al. (2018), who claimed the presence of tannins and flavonoids in *B.*

vulgaris, tannins in *D. asper*, and terpenoids in *B. blumeana* ethanolic leaf extracts. Our report has found the presence of coumarins and steroids in *B. vulgaris*; coumarins, flavonoids, and saponins in *D. asper*; and coumarins, and phenols in *B. blumeana* ethanolic leaf extracts. The presence of these secondary metabolites was not reported in the previous study. This study also reports the phytochemical profile of *B. merrillii* where coumarins, saponins, and steroids are detected in the species.

Tongco et al. (2016) reported the presence of saponins in *G. levis* ethanolic leaf extracts. Aside from saponins, this study has found the presence of coumarins, steroids, and terpenoids in *G. levis*. The findings on *B. vulgaris* var. *striata* were parallel with those of Baguistan et al. (2017), who claimed the presence of flavonoids, saponins, steroids, and terpenoids in the species. Furthermore, the presence of coumarins in *B. vulgaris* var. *striata* ethanolic leaf extracts was evident in this study. The variation in the phytochemical contents of the bamboo species could have been caused by ecological factors such as climatic conditions and the soil properties of the ecosystems or natural habitats where they grow. Environmental factors could influence the synthesis, subsequent accumulation, enhancement, or reduction of secondary metabolites in plants when they are grown in specific environments (Ashraf et al. 2018; Tavakoli et al. 2022).

Molecular characterization

The specific chloroplast barcode region utilized in the study is *rbcL*, which was one of the barcode regions used for the identification of some commercially important Indian bamboo species (Dev et al. 2020). The *rbcL* primer was chosen due to its specificity and efficiency in amplifying the desired regions of the samples. Using the morphological identities of the bamboo species, the gel electrophoresis showed that *B. ventricosa* (APR-237), *B. merrillii* (BUG-154), *P. vivax* f. *aureocaulis* (GON-007), *B. vulgaris* f. *waminii* (GON-013), *Schizostachyum* sp. [sp.2] (GON-026), *B. maculata* (GON-098), *G. atroviolacea* (GON-099), *B. eutuldoides* (GON-105), *B. vulgaris* var. *striata* [sp.1] (GON-160) and [sp.2] (GON-084), *B. vulgaris* (SAP-036), *D. asper* (GON-060), and *B. lako* (TUG-152) produced brighter and more intense bands. A less bright band was noted in *Melocanna* sp. (ALC-228). The gel image shows that all the bamboo leaf samples tested exhibited single and intense bands with a ~700 bp size target (Figure 3).

Table 3. Phytochemical profile of bamboo species growing in various ecosystems of Cagayan Province, Luzon, Philippines

Accession code	Species identification based on morphology	Secondary metabolites*									
		Ant	Car	Cou	Fla	Phe	Qui	Sap	Ste	Tan	Ter
SAP-036	*Bambusa vulgaris* Schrad. ex J.C. Wendl.	-	-	+	-	-	-	+	+	-	+
GON-005	*Dinochloa* sp.	-	-	+	+	+	-	-	+	+	+
SAM-141	*Bambusa* sp. [sp.1]	-	-	+	-	+	-	+	+	-	+
SAP-040	*Schizostachyum* sp. [sp.1]	-	+	+	-	-	-	+	+	+	+
GON-006	*Schizostachyum brachycladum* (Kurz ex Munro) Kurz	+	-	+	+	-	-	+	+	-	+
GON-160	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.1]	-	-	+	-	-	-	+	+	-	+
GON-084	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.2]	-	-	+	+	-	-	+	+	-	+
GAT-229	*Gigantochloa levis* (Blanco) Merr.	-	-	+	-	-	-	+	+	-	+
GON-026	*Schizostachyum* sp. [sp.2]	-	-	+	+	+	-	+	+	-	+
ALC-228	*Melocanna* sp.	-	-	+	-	+	-	+	+	-	+
GON-060	*Dendrocalamus asper* (Schult. & Schult.f.) Backer	-	-	+	+	-	-	+	+	-	+
LAL-214	*Bambusa* sp. [sp.2]	-	-	+	+	-	-	+	-	-	+
APR-234	*Bambusa blumeana* Schult.f. (= *Bambusa spinosa* Roxb.)	-	-	+	-	+	-	+	+	-	-
BUG-154	*Bambusa merrillii* Gamble	-	-	+	-	-	-	+	+	-	-
GON-013	*Bambusa vulgaris* f. *waminii* T.H. Wen (=*Bambusa vulgaris* Schrad. ex J.C.Wendl.)	+	+	+	+	+	-	+	+	+	-
APR-237	*Bambusa ventricosa* McClure	-	-	+	-	-	-	+	-	-	-
GON-099	*Gigantochloa atroviolacea* Widjaja	-	-	+	-	+	-	+	+	+	+
GON-007	*Phyllostachys vivax* f. *aureocaulis* N.X. Ma (= *Phyllostachys vivax* McClure)	+	+	+	+	+	-	+	+	+	+
GON-098	*Bambusa maculata* Widjaja	-	-	+	-	+	-	+	+	+	+
GON-100	*Phyllostachys edulis* (Carrière) J.Houz.	-	-	+	-	-	-	+	+	+	+
TUG-152	*Bambusa lako* Widjaja	-	-	+	+	-	-	+	-	-	-
GON-112	*Schizostachyum lumampao* (Blanco) Merr.	-	-	+	-	-	-	+	+	-	+
GON-104	*Thyrsostachys siamensis* Gamble	-	-	+	-	+	-	+	+	-	+
GON-106	*Bambusa atra* Lindl.	-	-	+	-	+	-	+	+	-	+
GON-107	*Guadua angustifolia* Kunth	-	-	+	-	+	-	+	+	-	+
GON-108	*Gigantochloa kuring* Widjaja	-	+	+	-	+	-	+	+	-	+
GON-109	*Schizostachyum lima* (Blanco) Merr.	-	-	+	-	+	-	+	+	-	+
GON-105	*Bambusa eutuldoides* McClure	-	-	+	-	-	-	+	+	-	+

Note: Ant: anthocyanin, Car: carotenoids, Cou: coumarins, Fla: flavonoids, Phe: phenols, Qui: quinones, Sap: saponins, Ste: steroids, Tan: tannins, Ter: terpenoids. (+) present; (-) absent

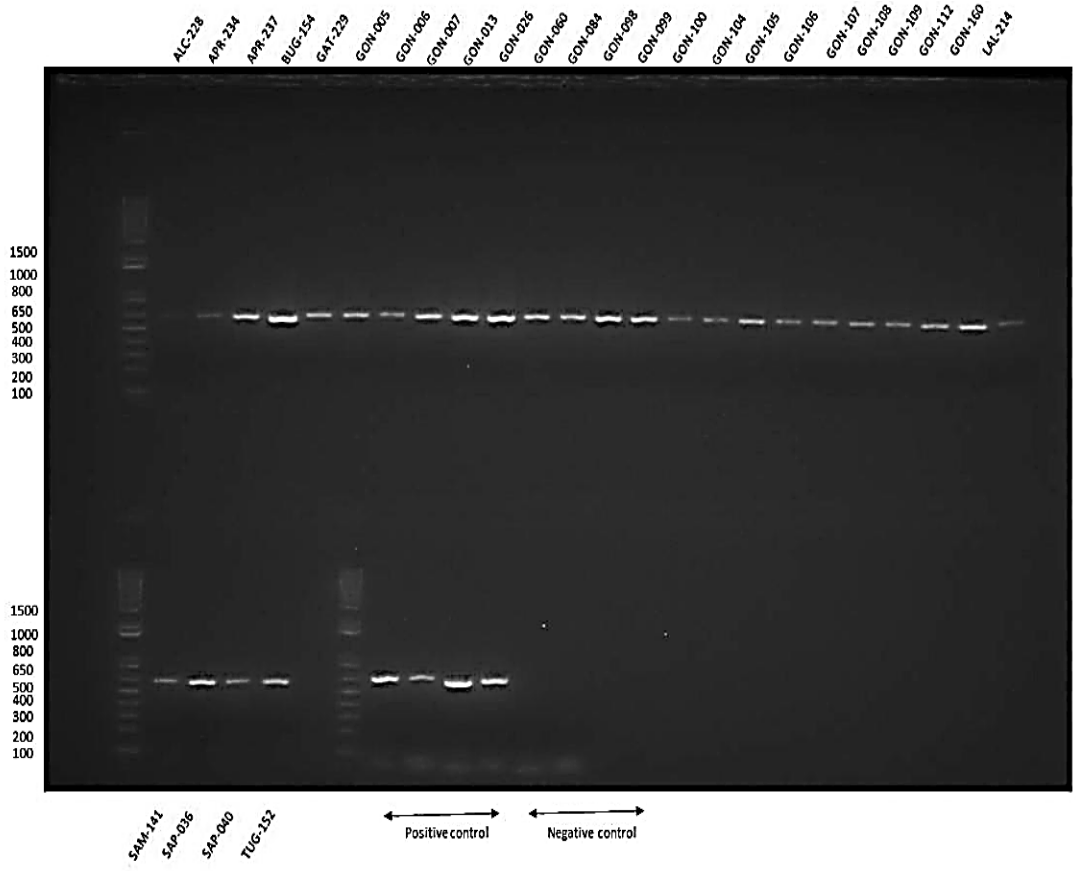

Figure 3. Gel electrophoresis of an amplified gene with a ~1500 bp size. The post-PCR product was loaded onto 1.2% agarose and ran at 100 V for 45 min for DNA marker 1kb plus ladder (Invitrogen)

DNA sequencing using the *rbcL* gene aims to validate the bamboo species identification and compare the species morphological and molecular identities. The morphological and molecular identity comparisons of bamboo species are presented in Table 4. Based on the morphology of the bamboo species, 13 accessions are *Bambusa*, 3 accessions are *Gigantochloa*, 5 accessions are *Schizostachyum*, 2 accessions are *Phyllostachys*, and 1 accession each for *Dendrocalamus*, *Dinochloa*, *Melocanna*, *Thyrsostachys*, and *Guadua*. Upon subjecting the bamboo species to DNA sequencing using *rbcL*, the molecular identification of many bamboo species was different from their morphological identification. Molecular characterization revealed that 5 accessions are *Bambusa*, 15 accessions are *Dendrocalamus*, 5 accessions are *Pseudostachyum*/ *Schizostachyum*, 2 accessions are *Melocanna*, and 1 accession is *Guadua*. The percentage identification for the DNA sequencing of the query sequences was recorded as 97.67% to 99.82% homology from the established accessions in the BLAST GenBank, with DNA query lengths ranging from 555 bp to 575 bp.

According to BLAST GenBank, the sequences of *B. vulgaris*, *B. vulgaris* var. *striata* [sp.1], *Bambusa* sp. [sp.2], *B. lako*, and *P. vivax* f. *aureocaulis* were all identified as *Bambusa* species. *D. asper*, *Dinochloa* sp., *Bambusa* sp. [sp.1], *B. vulgaris* var. *striata* [sp.2], *B. blumeana*, *B. merrillii*, *B. vulgaris* f. *waminii*, *B. ventricosa*, *B. maculata*, *B. atra*, *G. atroviolacea*, *Gigantochloa kuring*, *P. edulis*, *T. siamensis*, and *S. lima* were all identified as *Dendrocalamus* species. *Schizostachyum* and *Gigantochloa* species, such as *Schizostachyum* sp. [sp.1], *Schizostachyum* sp. [sp.2], *S. lumampao*, *S. brachycladum*, and *G. levis* were all molecularly identified as *Pseudostachyum* or *Schizostachyum* species. Meanwhile, *Melocanna* sp. and *B. eutuldoides* were both identified as *Melocanna* species, while *G. angustifolia* remained a species of *Guadua*.

It seems that the *rbcL* can successfully identify species of *Bambusa*, such as *B. vulgaris* and *B. vulgaris* var. *striata* [sp.1] at the species level, while the identification of *Schizostachyum*, *Melocanna*, *Dendrocalamus*, and *Guadua* using *rbcL* was possible but only at the genus level. Also, DNA barcoding using the *rbcL* may specifically identify the unknown bamboo species in Cagayan at the species level, which poses an advantage in the identification and certification of unknown bamboo for botanical, agricultural, medicinal, and industrial purposes. Identities of unknown bamboos in Cagayan, such as *Schizostachyum* sp. [sp.1 and sp.2], *Melocanna* sp., and *Bambusa* sp. [sp.2] were revealed as *Pseudostachyum polymorphum* or *Schizostachyum polymorphum*, *Melocanna baccifera*, and *B. vulgaris*, respectively. DNA barcoding using *rbcL* was reported in the identification and certification of *B. vulgaris* and *M. baccifera* (Dev et al. 2020).

Table 4. Identification of bamboo species growing in various ecosystems of Cagayan Province, Luzon, Philippines based on morphological and molecular characters

Accession code	Species identification based on morphology	Species identification based on the *rbcL* gene	Percentage identification (%)	DNA query length (bp)	Identities (bp/bp)	Sequence ID on GenBank BLAST
SAP-036	*Bambusa vulgaris* Schrad. ex J.C. Wendl.	*Bambusa vulgaris* Schrad. ex J.C. Wendl.	99.46	560	557/560	MH560435.1
GON-005	*Dinochloa* sp.	*Dendrocalamus hookeri* Munro	99.29	566	562/566	MT113210.1
SAM-141	*Bambusa* sp. [sp.1]	*Dendrocalamus longispathus* (Kurz) Kurz	99.82	559	558/559	MH560433.1
SAP-040	*Schizostachyum* sp. [sp.1]	*Pseudostachyum polymorphum* Munro (=*Schizostachyum polymorphum* (Munro) R.B.Majumdar)	99.46	559	556/559	KR529912.1
GON-006	*Schizostachyum brachycladum* (Kurz ex Munro) Kurz	*Schizostachyum funghomii* McClure	99.11	564	559/564	EF125084.1
GON-160	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.1]	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble	99.46	560	557/560	KU647278.1
GON-084	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble [sp.2]	*Dendrocalamus strictus* (Roxb.) Nees	99.12	570	565/570	MH560434.1
GAT-229	*Gigantochloa levis* (Blanco) Merr.	*Pseudostachyum polymorphum* Munro (=*Schizostachyum polymorphum* (Munro) R.B.Majumdar)	99.64	555	553/555	KR529912.1
GON-026	*Schizostachyum* sp. [sp.2]	*Pseudostachyum polymorphum* Munro (=*Schizostachyum polymorphum* (Munro) R.B.Majumdar)	99.46	560	557/560	KR529912.1
ALC-228	*Melocanna* sp.	*Melocanna baccifera* (Roxb.) Kurz	99.12	570	565/570	EF125082.1
GON-060	*Dendrocalamus asper* (Schult. & Schult. f.) Backer	*Dendrocalamus sikkimensis* Gamble ex Oliv.	99.28	556	552/556	MT113221.1
LAL-214	*Bambusa* sp. [sp.2]	*Bambusa vulgaris* Schrad. ex J.C. Wendl.	99.29	561	557/561	MH560435.1
APR-234	*Bambusa blumeana* Schult. f. (= *Bambusa spinosa* Roxb.)	*Dendrocalamus strictus* (Roxb.) Nees	99.47	566	563/566	MH560434.1
BUG-154	*Bambusa merrillii* Gamble	*Dendrocalamus strictus* (Roxb.) Nees	98.77	569	562/569	MH560434.1
GON-013	*Bambusa vulgaris* f. *waminii* T.H. Wen (=*Bambusa vulgaris* Schrad. ex J.C.Wendl.)	*Dendrocalamus strictus* (Roxb.) Nees	98.41	565	556/565	MH560434.1
APR-237	*Bambusa ventricosa* McClure	*Dendrocalamus strictus* (Roxb.) Nees	98.95	571	565/571	MH560434.1
GON-099	*Gigantochloa atroviolacea* Widjaja	*Dendrocalamus sikkimensis* Gamble ex Oliv.	99.29	567	563/567	MT113221.1
GON-007	*Phyllostachys vivax* f. *aureocaulis* N.X. Ma *Phyllostachys vivax* McClure	*Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble	99.11	560	555/560	KU647278.1
GON-098	*Bambusa maculata* Widjaja	*Dendrocalamus strictus* (Roxb.) Nees	98.95	573	567/573	MH560434.1
GON-100	*Phyllostachys edulis* (Carrière) J.Houz.	*Dendrocalamus strictus* (Roxb.) Nees	98.94	567	561/567	MH560434.1
TUG-152	*Bambusa lako* Widjaja	*Bambusa vulgaris* Schrad. ex J.C. Wendl.	97.67	559	546/559	MH560435.1
GON-112	*Schizostachyum lumampao* (Blanco) Merr.	*Pseudostachyum polymorphum* Munro (=*Schizostachyum polymorphum* (Munro) R.B.Majumdar)	99.64	562	560/562	KR529912.1
GON-104	*Thyrsostachys siamensis* Gamble	*Dendrocalamus sikkimensis* Gamble ex Oliv.	98.23	566	556/566	MT113221.1
GON-106	*Bambusa atra* Lindl.	*Dendrocalamus sikkimensis* Gamble ex Oliv.	98.95	570	564/570	MT113221.1
GON-107	*Guadua angustifolia* Kunth	*Guadua paniculata* Munro	99.29	565	561/565	HQ847278.1
GON-108	*Gigantochloa kuring* Widjaja	*Dendrocalamus strictus* (Roxb.) Nees	99.47	566	563/566	MH560434.1
GON-109	*Schizostachyum lima* (Blanco) Merr.	*Dendrocalamus hookeri* Munro	99.13	575	570/575	MT113210.1
GON-105	*Bambusa eutuldoides* McClure	*Melocanna baccifera* (Roxb.) Kurz	99.13	572	567/572	EF125082.1

Furthermore, the molecular identification of *Dinochloa*, *Gigantochloa*, *Phyllostachys*, *Thysostachys*, and other species of *Bambusa* (*B. blumeana*, *B. merrillii*, *B. vulgaris* var. *striata* [sp.2], *B. vulgaris* f. *waminii*, *B. ventricosa*, *B. maculata*, *B. atra*, and *B. eutuldoides*) do not match with the expected identification based on morphology, possibly due to the low discriminatory power of the *rbcL* barcode region against these mentioned bamboo genera and species. The low discriminating power of *rbcL* was also reported in the identification of various tropical Indian bamboo (Dev et al. 2020).

Meanwhile, *Bambusa* species such as *B. vulgaris* var. *striata* [sp.2], *B. blumeana*, *B. merrillii*, *B. vulgaris* f. *waminii*, *B. ventricosa*, and *B. maculata* were identified as *Dendrocalamus strictus* based on *rbcL*. *D. strictus* has culms that are thick-walled or almost solid, glabrous when young, and turn dull green when mature (Roxas 2012). It was indicated that most of the *Bambusa* species tested in this study were identified as *Dendrocalamus* species. A higher similarity was reported between *Dendrocalamus* and *Bambusa* (Konzen et al. 2017), where *Dendrocalamus* belongs to the same tribe as *Bambusa* (Kelchner and Bamboo Phylogeny Group 2013), and both genera have similar chromosome numbers (Thakur et al. 2015). According to Sun et al. (2005), bamboos were subjected to identification using internal transcribed spacers (ITS) and random amplified polymorphic DNA (RAPD). It was found that *D. strictus* was embedded among the *Bambusa* taxa.

Gigantochloa species, such as *G. atroviolacea* and *G. kuring* were molecularly identified as *Dendrocalamus* species (*Dendrocalamus sikkimensis* and *D. strictus*) with *rbcL*. Results considered the possibility of a close relationship between *Gigantochloa* and *Dendrocalamus* due to equal ploidy levels. The same reason was noted for *B. eutuldoides*, which was identified as *M. baccifera*. Thakur et al. (2015) reported that tropical bamboos like *Bambusa*, *Dendrocalamus*, *Gigantochloa*, and *Melocanna* have chromosome number 72 (2n), while temperate bamboos like *Phyllostachys* have chromosome number 48 (2n). Based on the results of this study, *Phyllostachys* species were identified as *Bambusa* and *Dendrocalamus* species, which are distant genera in terms of ploidy level. *Phyllostachys* species, being temperate bamboos, were grown in tropical environments in Cagayan. With the adaptation of the species to the tropics, genetic variation may have occurred. The chromosome number of bamboo species may appear to vary with variation in native climatic conditions from temperate to tropical zones (Thakur et al. 2015). Thus, this phenomenon in *Phyllostachys* must be explored in the future. The probability of genetic variation in the species played a role in the molecular identification of bamboo. As a result, *P. vivax* f. *aureocaulis* was identified as *B. vulgaris* var. *striata* with *rbcL*. The similarity can be linked to the species' resemblance in culm color and appearance, nodal characteristics, young shoot appearance, and rhizome system with *B. vulgaris* var. *striata*.

Another possible factor that can affect the identification of bamboo is the genetic variability in the morphological growth forms, which can cause variation in the culm, clump, and growth characteristics of bamboo if they are grown in different environments or ecosystems. Das et al. (2017) disclosed that *D. strictus* undergoes different growth forms due to edaphic factors (soil phosphorus and organic material) and climatic conditions (rainfall). These factors played a major role in shaping the morphology of *D. strictus* growth forms such as intertwined, closed clumps, and solid culms due to low rainfall and low soil phosphorus environments, while the species produced open clumps, straight, taller, and hollow culms due to high rainfall and high soil organic matter environments.

The Cagayan Valley region has its existing bamboo stands as potential sources of clonal materials for propagation in bamboo nurseries. However, difficulty in the precise identification of bamboo species is a challenge in the mass propagation of quality planting materials. Proper identification of bamboo for certification of planting materials is one of the major advantages of molecular characterization through DNA barcoding (Dev et al. 2020). Precise identification of bamboos can benefit bamboo growers through asexual multiplication for mass planting in rehabilitation areas such as denuded forest lands (Chokkalingam et al. 2006) and utilization in government activities such as the DENR National Greening Program (Dolom et al. 2019). Molecular characterization paves the way for successful mass propagation, especially in planting material selection, since bamboos are species-specific when it comes to asexual propagation methods (Banik 1995).

In conclusion, there are about 25 bamboo species identified in the Cagayan Province, Luzon, Philippines, which are growing in various ecosystems, such as forests and woodlands, residential areas, sparsely vegetated lands or coastal areas, waterways, hills, agricultural fields, and grasslands. These bamboo species are composed of 13 accessions of *Bambusa* (with 10 species), 5 accessions of *Schizostachyum*, 3 accessions of *Gigantochloa*, 2 accessions of *Phyllostachys*, and 1 accession of *Dendrocalamus*, *Dinochloa*, *Melocanna*, *Thyrsostachys*, and *Guadua*, which consisted of 28 bamboo accessions. The morphological characters presented the uniqueness of bamboo species Cagayan ecosystems in terms of growth habit; culm internode size, color, and appearance; trichome location, concentration, and culm sheath color of young shoots; nodal characteristics; and flowering incidence. Phytochemical screening showed that most of the bamboo species tested have coumarins, saponins, steroids, and terpenoid contents. *P. vivax* f. *aureocaulis* and *B. vulgaris* f. *waminii* have more phytochemical contents than the other bamboo species. Both species contain anthocyanin and carotenoids, which are absent in most bamboo species. *B. lako*, *B. merrillii*, and *B. ventricosa* have the fewest phytochemicals. All bamboo species do not contain quinones. Molecular identification through DNA barcoding using *rbcL* revealed that from the 9 bamboo genera morphologically identified in this study, consisting of 28 bamboo accessions, it was reduced to only 5 bamboo genera which consisted of 5 accessions of *Bambusa*, 15 accessions of *Dendrocalamus*, 5 accessions of *Pseudostachyum*/ *Scizostachyum*, 2 accessions of

Melocanna, and 1 *Guadua* accession. The molecular identities of the bamboo were not parallel to the expected morphological identities except for *B. vulgaris* and *B. vulgaris* var. *striata*. Moreover, DNA barcoding using the *rbcL* can somehow verify the identities of the unknown bamboo species in Cagayan and can identify bamboo genera, such as *Schizostachyum*, *Melocanna*, *Dendrocalamus*, and *Guadua* at the genus level.

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