CMU Journal of Science
The Official Scientific Journal of Central Mindanao University
ISSN Print: 0116-7847 js.cmu.edu.ph ISSN Online: 2704-3703

Research Article

Multi-locus Barcoding and Phylogeny of *Coelogyne* and *Dendrochilum* Species in Mt. Talomo, Philippines

Aljan C. Espinas[1*], Reggie Y. Dela Cruz[2], Eliazar A. Peniton Jr.[3], Jennifer G. Opiso[4], Fulgent P. Coritico[5], Kier C. Agad[6], Glenda Z. Doblas[7]

[1,3,7.] Molecular Biology & Biotechnology, Genetics, & Microbiology Division, Institute of Biological Sciences, Central Mindanao University, Musuan, Maramag, Bukidnon 8714, Philippines

[2.] Molecular Biology and Biotechnology, Genetics, and Microbiology Division, Institute of Biological Sciences, College of Arts and Sciences, Central Mindanao University, Musuan, Maramag, Bukidnon 8710, Philippines, Tel. No.: +63(88)3561910 2 Tuklas Lunas Development Center, Central Mindanao University, Musuan, Maramag, Bukidnon, 8710, Philippines, Tel. No.: +63(88)3561910

[4.] Plant Biology Division, Institute of Biological Sciences, College of Arts and Sciences, Central Mindanao University, Musuan, Bukidnon, Philippines, 8714, and University of the Philippines, Los Baños, Laguna

[5.] Plant Biology Division, Institute of Biological Sciences, College of Arts and Sciences, and Center for Biodiversity Research and Extension in Mindanao, Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines, 8714

[6.] Natural Science Department, College of Arts and Sciences, University of Southeastern Philippines, Inigo St., Davao City, Philippines, 8000

Citation: Espinas, A.C., Dela Cruz, R.Y., Peniton, E.A. Jr., Opiso, J.G., Coritico, F.P., Agad, K.C., & Doblas, G.Z. (2025). "Multi-locus Barcoding and Phylogeny of *Coelogyne* and *Dencrochilum* Species in Mt. Talomo, Philippines." CMU Journal of Science. 29(1), 05

Academic Editor: Dr. Mark Lloyd G. Dapar

Received: June 10, 2025
Revised: July 03, 2025
Accepted: July 04, 2025
Published: July 25, 2025

ABSTRACT

DNA barcoding is a vital tool for species identification, effectively complementing traditional morphological approaches, particularly within complex and diverse plant families like Orchidaceae. This study focused on the ten orchid accessions collected from Mt. Talomo, Mindanao, to evaluate the efficacy of employing four barcode loci (*rbcL*, *matK*, *ITS*, *trnH-psbA*) for precise molecular and taxonomic resolution. DNA extraction, PCR amplification, and Sanger sequencing were performed, with sequences assembled and analyzed using BioEdit, Geneious®, and MEGA12. BLAST comparisons against NCBI confirmed species identities. Results showed that while molecular barcoding generally aligns with morphological classification, the combined matK gene and the nuclear ribosomal ITS region significantly resolved species- and genus-level distinctions. Specifically, matK provided high resolution with several matches showing 100% identity in GenBank, while the ITS region demonstrated superior discriminatory power due to its greater sequence variability, proving highly effective in distinguishing closely related species. Out of ten, nine gave DNA sequences that identified them as *Coelogyne* or *Dendrochilum* species. Only three were identified at the species level: This synergistic effect of matK and ITS was instrumental in resolving the identities of *Coelogyne* species, including the Philippine-endemic *Coelogyne coccinea* and *Coelogyne apoensis*, and the native *Coelogyne glumacea*, all of which face threats from habitat loss and overcollection.

Phylogenetic analysis, particularly using combined matK and ITS sequences, revealed clear barcoding gaps and well-resolved monophyletic clusters for conspecifics. These results underscore the critical role of these combined markers for accurate species identification and advocate for an expanded DNA barcode library to support targeted conservation efforts for Mindanao's unique and threatened orchid flora.

Keywords: *Coelogyne*, DNA Barcoding, ITS, matK, Mindanao Endemic.

1. INTRODUCTION

Orchidaceae represents one of the most diverse and ecologically significant plant families, comprising over 28,000 species with remarkable adaptations as epiphytes, lithophytes, and terrestrial plants (Zhang et al., 2015; Chase et al., 2015). In the Philippines, this family exhibits exceptional endemism, with approximately 1,100 species, of which 70% are found nowhere else on Earth (Cootes, 2011; Pelser et al., 2011). The island of Mindanao serves as a critical biodiversity hotspot for endemic orchids, including the Mt. Apo-endemic *Coelogyne apoensis* and the critically endangered *Dendrobium schuetzei* (Naive et al., 2017). These species face unprecedented threats from deforestation, with Mindanao losing 32% of its forest cover between 2000 and 2020 (Global Forest Watch, 2021). Compounding this decline is the rampant illegal collection of ornamental plants in the trade (Barcelona et al., 2013). Their restricted distributions and specialized habitat requirements make Philippine orchids particularly vulnerable to extinction, underscoring the need for urgent conservation interventions.

In addition to their ecological importance, Philippine orchids possess significant ethnobotanical value, as many species are utilized in traditional medicine due to their bioactive compounds (Rosa, 2010; Castillo-Pérez et al., 2024). Polysaccharides and alkaloids derived from endemic Dendrobium and *Coelogyne* species have been shown to possess immunomodulatory and anticancer properties (Shukla et al., 2022 & Wati et al., 2021). However, the sustainable utilization of these genetic resources remains hampered by taxonomic uncertainties, as many Philippine orchids exhibit cryptic morphological variation that challenges traditional identification methods (Pelser et al., 2011). This problem is exacerbated by the declining number of expert taxonomists capable of accurate morphological identification (Hebert & Gregory, 2005), creating a critical need for reliable molecular identification tools.

DNA barcoding has emerged as a transformative approach for orchid conservation, yet significant gaps persist in its application to Philippine taxa. While the rbcL+matK combination has been recommended as the standard plant barcode (CBOL, 2009), recent studies suggest these loci may lack sufficient resolution for species-level identification in tropical orchids (Li et al., 2016; Kim et al., 2014). The nuclear ribosomal *ITS* region and chloroplast *trnH-psbA* spacer have shown promise in distinguishing closely related orchid species (Xiang et al., 2011). However, their efficacy remains untested for Mindanao's unique flora. This study represents the first comprehensive evaluation of a four-locus barcoding system (rbcL, matK, ITS, and trnH-psbA) specifically designed to address the taxonomic challenges of Philippine orchids.

The current research focuses on Mt. Talomo's orchid populations, which remain understudied despite their ecological significance. Preliminary surveys indicate the presence of several endemic species with highly restricted distributions, including potential new taxa awaiting formal description (Amoroso et al., 2016). The combination of morphological characterization with multi-locus DNA barcoding would establish a reliable identification system for Mindanao's orchids and clarify phylogenetic relationships among endemic taxa. The inclusion of the ITS region is particularly innovative, as it provides complementary nuclear data to overcome the limitations of purely chloroplast-based barcodes in recently diverged lineages (Inda et al., 2012).

This research carries significant implications for both science and conservation policy. The generated DNA barcode library will serve as a critical resource for monitoring illegal orchid trade, with potential applications in forensic botany (Lahaye et al., 2008). Phylogenetic results will inform protected area management in Mindanao, particularly for lesser-known species that are currently outside existing conservation programs. By merging molecular systematics with conservation biology, we can better preserve the Philippines' endangered orchid flora, which faces escalating risks from habitat loss and climate change.

2. METHODOLOGY

Sample Collection and Morphological Identification

Before sample collection and field interviews, necessary regulatory and ethical approvals were secured, including the DENR Gratuitous Permit (WGP No XI-2023) through the Protected Area Management Board (PAMB), and Prior Informed Consent (PIC) from barangay officials and local cultural communities of Mount Talomo, Sitio Mamaon, Sibulan, Davao City. Mount Talomo (7°02'38"N, 125°20'17"E). Field surveys and systematic sampling were conducted across designated sites to document and collect all observable orchid species. For each species, 3 to 5 individuals, spaced at least 5 meters apart, were collected. Young, healthy leaves were silica-dried on-site for DNA extraction. All voucher specimens have been deposited at the Central Mindanao University Herbarium (CMUH). Each orchid individual was examined for its growth habit (monopodial or sympodial), vegetation type, and habitat preference (terrestrial, epiphytic, or lithophytic). Researchers documented the morphological features of the inflorescence during collection, including the color, shape, size, and other distinguishing characteristics of the petals, sepals, and labellum.

Morphological identification was initially based on the available references and officially confirmed by the pool of experts of the University Museum.

Molecular Identification and Phylogenetic Analysis

Approximately 40 to 50 mg of silica-dried tissue was homogenized with a tungsten carbide bead in 2 mL tubes using a Mixer Mill MM 400 set to 30 Hz for 1.5 minutes after being flash-frozen in liquid nitrogen. DNA extraction was conducted using the Qiagen DNeasy Plant Mini Kit, with slight modifications. Lysis occurred with 400 µL of Buffer AP1 and 8 µL of RNase A at 65 °C for 1 hour. After adding 130 µL of Buffer P3, the mixture was cooled on ice for 5 minutes. The lysates were then centrifuged at 20,000 × g for 5 minutes, passed through QIAshredder columns, and re-centrifuged. The flow-through was mixed with 1.5 volumes of Buffer AW1 and loaded onto DNeasy columns, followed by washes with Buffer AW2. DNA was eluted twice with 50 µL Buffer AE. DNA quality and integrity were assessed via gel electrophoresis. The concentration and purity of undiluted genomic DNA were measured using DNA that was eluted twice with 50 µL of Buffer AE. The quality and integrity of the DNA were assessed through gel electrophoresis. The concentration and purity of the undiluted genomic DNA were measured using a MicroDrop plate and a Microplate Reader (MultiSKAN Go, Thermo Scientific) by recording the UV absorbance at 230, 260, and 280 nm. DNA purity was evaluated using the A260/A280 ratio. Polymerase chain reaction (PCR) was used to amplify four genetic markers: one intergenic spacer (*trnH–psbA*), two plastid genes (rbcL, matK), and one nuclear region (ITS) (Table 1). PCR reactions (25 µL) contained ~10 ng template DNA, 1.5 µL 1× PCR buffer with $MgCl_2$, 0.5 µL 25 mM $MgCl_2$, 0.2 µL 0.3 mM dNTP mix, 0.35 µL each of 0.3 mM forward and reverse primers, 0.2 µL 0.5 U Taq polymerase (KAPA BIOSYSTEMS), and Ultrapure Water. Amplification was performed in an Applied Biosystems Veriti or BIO-RAD T100 thermal cycler under the following profile: initial denaturation at 94 °C for 4 min; 35 cycles of 94–95 °C for 30 s, 49–56 °C for 30 s, and 72 °C for 1 min; final extension at 72 °C for 10 min; hold at 4 °C. PCR products were verified via gel electrophoresis and visualized using a Bio-Rad Gel Doc™ EZ Imager. PCR products were purified and subjected to Sanger sequencing using the ABI 3730xl DNA Analyzer, following Applied Biosystems protocols of Macrogen, Seoul, South Korea. Chromatograms out of ten morphospecies were processed using BioEdit (Hall, 1999), FinchTV (version 1.4.0; Geospiza Inc., 2006), and Geneious® Prime (version 2025.1.2) for trimming, editing, and consensus assembly. Sequence identity was verified using NCBI BLAST (Sayers et al., 2025; Altschul et al., 1990). Phylogenetic analysis was performed in RAxML v8 (Stamatakis, 2014) using the maximum likelihood method with 1,000 bootstrap replicates in Geneious®, and the resulting tree was visualized in FigTree v1.4.4.

3. RESULTS AND DISCUSSION

Morphological Description

Sample code: *Dendrochilum*_sp_DET001
Scientific name*: Coelogyne apoensis* (T.Hashim.) M.W.Chase & Schuit. (Figure 1)

A relatively small, tufted epiphytic herb. Roots arise from the rhizome, moderately thick and branched. Pseudobulbs are clustered on a short rhizome, obpyriform, 1.7–2.2 cm long and 0.7–1.1 cm in diameter, each bearing a single leaf. They are initially enclosed by ~3 imperfectly tubular cataphylls that are acute to obtuse, strongly setose, and disintegrate into persistent fibrous remnants. Leaves are petiolate with channeled petioles (~4 cm long). Blades are dorsiventrally flattened, leathery, shiny dark green adaxially and dull light green abaxially, lanceolate, obtuse at the apex, entire, and ~15.3 × 2.1 cm. The lower surface shows 3–7 prominent veins with numerous indistinct secondaries. Both surfaces, particularly the abaxial surface, are covered with numerous minute dark setae.

Figure 1. In situ habitat and growth habit of *Coelogyne apoensis* (T.Hashim.) M.W.Chase & Schuit, **(A)** Flowering individual showing an erect inflorescence. **(B)** Lateral view of the flower of Coelogyne apoensis showing the morphological characteristics of the perianth segments and column structure.

Sample code: *Dendrochilum*_sp_DET004
Scientific name: *Dendrochilum* sp.1 (Figure 2)

It is a relatively small, tufted, upright, sympodial epiphytic herb. Roots: Arising from the rhizome, 0.1–0.15 cm in diameter, unbranched. Rhizomes are very short, up to 0.3 cm long and 0.5 cm in diameter. Pseudobulbs are fusiform, clustered on a short rhizome, measuring 10.0–21.0 mm long and 4.0–5.0 mm in diameter; unifoliate; covered with 1–4 tubular, attenuate to acuminate papery cataphylls that soon disintegrate into persistent fibers.

Figure 2. In situ photograph of *Dendrochilum* sp.1 showing the natural growth habit. The individual is observed growing epiphytically on a *Myrtaceae* branch within an evergreen broad-leaved forest at an elevation of 1745 m.

Sample code: *Dendrochilum*_sp_DET005
Scientific name: *Dendrochilum* sp.2 (Figure 3)

A small, glabrous epiphytic herb. Roots arise from the rhizome and pseudobulbs, thin and unbranched. Pseudobulbs are clustered on a short rhizome, sub-globose to broadly ellipsoid (0.5–0.8 × 0.4–0.5 cm), occasionally brownish to maroon at the tips, each bearing a single leaf. Initially enclosed by one tubular, acute to acuminate, glabrous cataphyll that disintegrates into persistent fibers. Leaves conduplicate, petiolate; petiole channeled (0.5–0.6 cm); blade linear-lanceolate, dorsiventrally complanate, pale green, sometimes leathery, acute to obtuse (1.8–3.5 × 0.15–0.25 cm), crenulate, with 3 distinct and 6 indistinct veins, the outer veins ~0.05 cm from the margin.

Figure 3. *Dendrochilum* sp.2 *in its natural habitat*. The plant is shown growing epiphytically in a primary montane forest at approximately 1500 m elevation, illustrating its growth habit and ecological preference.

Sample code: *Dendrochilum*_sp_DET007
Scientific name: *Coelogyne coccinea* (H.A.Pedersen & Gravend.) M.W.Chase & Schuit. (Figure 4)

This species is a compact epiphytic orchid that thrives under warm to cool environmental conditions. The plant bears pear-shaped pseudobulbs, each giving rise to a single, leathery, lanceolate leaf. The pseudobulbs are tightly clustered along a short rhizome and function as the structural base for the leaves. The roots, which emerge from the rhizome, are moderately thick, branched, and contribute to the plant's firm attachment to its substrate. The leaf is petiolate, featuring a channeled petiole and a coriaceous (leathery) texture. It has many-flowered, synanthous, and racemose inflorescence that emerges alongside new vegetative growth. This inflorescence reaches a length of approximately 0.15 meters, arising on a very slender, cylindrical peduncle that is approximately 148 mm long and finely setose. The rachis is slightly drooping, bearing flowers arranged distichously in an alternate pattern. The floral bracts are persistent and glumaceous, broadly ovate-triangular to almost hemi-circular when flattened. Flowers exhibit a deep crimson hue. The perianth segments, sepals, and petals are somewhat spreading, with entire, inwardly curved margins. Each segment is smooth and three-veined. The dorsal sepal is linear-oblong, rounded to obtuse at the apex, and measures 0.59–0.64 cm in length and 0.18–0.2 cm in width. The lateral sepals are obliquely oblong and obtuse to rounded (occasionally minutely mucronate), with dimensions of 0.6–0.65 cm by 0.22–0.23 cm. Petals are oblanceolate-oblong, obtuse, and measure 0.55–0.65 cm in length and 0.22–0.23 cm in width. The labellum is sessile, firmly attached to the column, somewhat fleshy, and elliptic-oblong in shape. Its margins curve inward near the base, and the apex is sub-acuminate. The surface is smooth and glabrous. The labellum measures 0.54–0.56 cm in length and 0.24–0.27 cm in width, and is three-veined. Two prominent lateral ridges extend from near the base to near the apex, tapering beyond the midpoint, while a much smaller, often faint, central ridge runs from the mid-region to the tip.

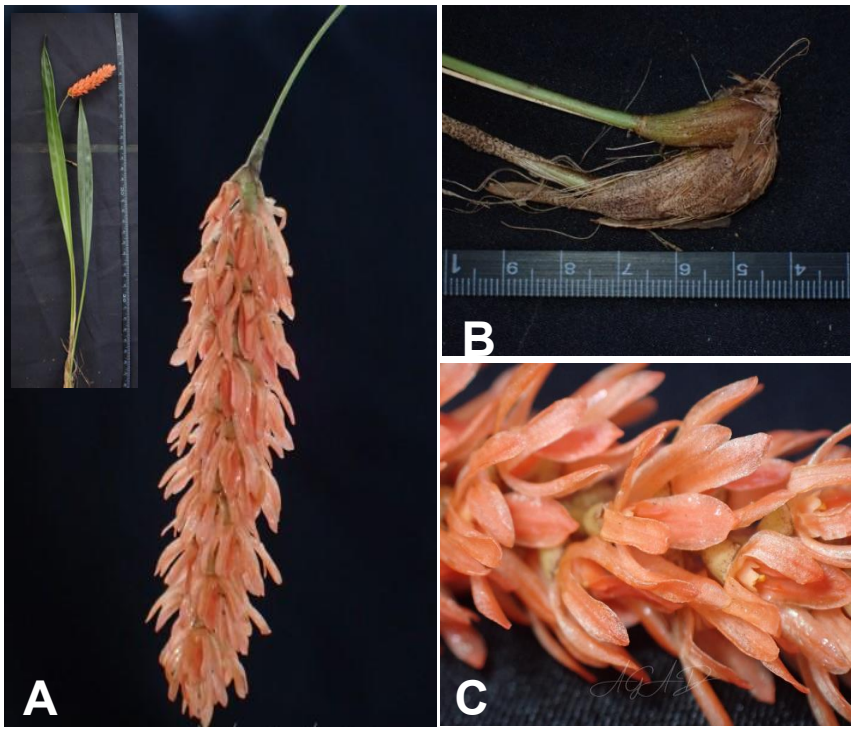

Figure 4. Vegetative and floral morphology of *Coelogyne coccinea* (H.A.Pedersen & Gravend.) M.W.Chase & Schuit. **(A)** Habit: compact, epiphytic growth with clustered, pear-shaped pseudobulbs and moderately thick, branched roots arising from a short rhizome. Leaf: single, lanceolate, leathery leaf per pseudobulb with a channeled petiole. Inflorescence: synanthous, racemose, many-flowered, with a slender, setose peduncle and slightly drooping rachis bearing distichously arranged deep crimson flowers. **(B)** Pseudobulb: pear-shaped, closely spaced, each bearing a single leaf; surface smooth and green, emerging from a compact rhizome. **(C)** Flower detail: sepals and petals spreading with entire, incurved margins; labellum elliptic-oblong, three-veined, with two prominent lateral ridges and a faint central ridge; robust, winged column with erect stelidia and apical anther cap.

Sample code: *Dendrochilum*_sp_DET008
Scientific name: *Dendrochilum* sp.3 (Figure 5)

A sympodial, tufted epiphytic herb. Roots thin, branched (~0.1 cm diameter), arising from a creeping, branching rhizome (2–4 cm long, ~0.5 cm diameter). Pseudobulbs clustered, pyriform, slightly curved, up to 3 × 0.6 cm, each with a single leaf. Young pseudobulbs are enclosed by tubular, acute cataphylls that disintegrate into persistent papery fibers. Leaves linear, leathery, conduplicate at the base, up to 25.5 × 0.5 cm, with a channeled petiole to 4.7 × 0.1 cm. Three distinct adaxial veins and five indistinct abaxial veins are visible.

Figure 5. Habitat and growth habit of *Dendrochilum* sp.3**.** *Dendrochilum* sp.3 grows epiphytically in situ within a primary montane forest at approximately 1,280 m above sea level. The image illustrates the natural growth habit and microhabitat preference of the species, highlighting its adaptation to high-elevation, humid forest environments.

Sample code: *Dendrochilum*_sp_DET010
Scientific name: *Dendrochilum* sp.4 (Figure 6)

A small, glabrous epiphytic herb. Roots arise from the rhizome and pseudobulbs, slender and unbranched. Pseudobulbs are clustered on a short rhizome, occasionally brownish to reddish near the tips, sub-globose to broadly ellipsoid, 0.5–0.8 × 0.4–0.5 cm, and unifoliate. Each is enclosed by a single tubular, acute to acuminate, glabrous cataphyll that disintegrates into persistent fibers. Leaves are conduplicate and petiolate, with distinctly channeled petioles 0.5–0.6 cm long. The blade is dorsiventrally flattened, pale green on both surfaces, sometimes coriaceous, linear-lanceolate, acute to obtuse, 1.8–3.5 × 0.15–0.25 cm, with crenulate margins. Three prominent veins are visible adaxially; six indistinct veins occur abaxially, with outermost veins ~0.05 cm from the margin.

Figure 6. Habitat and growth habit of *Dendrochilum sp. DET010*, growing epiphytically in situ within a primary montane forest at 1,745 m elevation. The image highlights the species' natural habitat and microhabitat preference on exposed ridges and mossy forest summits, indicating its adaptation to high-elevation environments.

Morphological Description:

The species exhibits an upright, sympodial growth habit. Pseudobulbs are unifoliate, ovoid, up to 30 mm high and 15 mm in diameter, each bearing a single leathery, linear to lanceolate leaf measuring up to 300 × 40 mm.

Sample code: *Dendrochilum*_sp_DET013

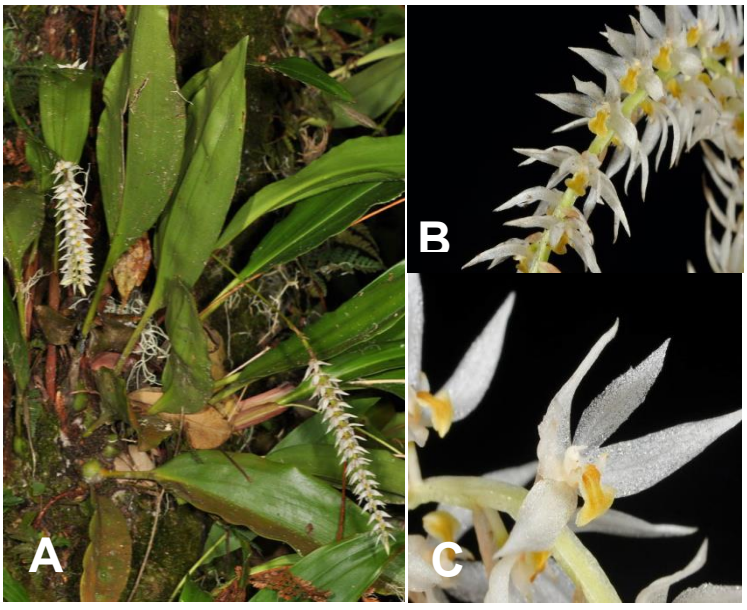

Figure 7. *Coelogyne glumacea* (Lindl.) M.W.Chase & Schuit. A. Habit showing upright, sympodial growth with unifoliate pseudobulbs. B. Arching inflorescence emerging concurrently with new vegetative growth. C. Close-up of flower showing white sepals, petals, and a vividly colored three-lobed labellum.

Sample code: *Dendrochilum*_sp_DET015
Scientific name: *Dendrochilum* sp.5 (Figure 8)

It is a relatively small, tufted epiphyte. Roots are thin, arising from a short rhizome. Pseudobulbs are fusiform, 3–4 × 0.3 cm, 1-leaved, clustered, initially enclosed by 4 imperfectly tubular, acute to obtuse, setose cataphylls that degrade into persistent fibers. The leaf is petiolate, with a channeled petiole measuring 7.7–8 cm in length. The blade is linear-lanceolate, acute, entire, dorsiventrally flattened, and herbaceous, measuring 22–23 × 1 cm, with seven prominent and several indistinct veins.

Figure 8. *Dendrochilum* sp.5. Habitat and growth habit of *Dendrochilum* sp.5 growing epiphytically in situ within a primary montane forest at 1,616 m.a.s.l. The image highlights the species' natural growth habit and microhabitat preference, demonstrating its adaptation to high elevations.

Sample code: *Dendrochilum*_sp_DET016
Scientific name: *Dendrochilum* sp.6 (Figure 9)

The species exhibits an upright, sympodial growth habit. Pseudobulbs are clustered, conical-oblong, wrinkled, unifoliate, measuring 1.5–4 cm × 4–7 mm, and covered with cataphylls extending to the petiole base. Leaves are petiolate with grooved petioles (1.5–4 cm × 1–2 mm). Blades are lanceolate, leathery, glabrous, 7–11 × 1–2 cm, with a rounded apex and cuneate base; margins entire. Lateral veins run parallel, with three distinct nerves visible on both surfaces, though less pronounced adaxially. Bracts are elliptic-ovate, 11-nerved, persistent, involute, measuring 4.5 × 2.3 mm; floral bracts are ~3.5 × 1.7 mm with a single basal bract.

Figure 9. *Dendrochilum* sp.6. Habit showing upright, sympodial growth with clustered, conical-oblong pseudobulbs.
Sample code: *Dendrochilum*_sp_DET018
Scientific name: *Dendrochilum* sp.7 (Figure 10)

The species exhibits an upright, sympodial growth habit. Pseudobulbs are clustered and conical-oblong in shape, measuring approximately 1.66 cm in length and 2.2 mm in diameter. Each pseudobulb is unifoliate and covered by three cataphylls that extend to the base of the petiole, reaching about 25mm in length. Internodes are sessile. Leaves are sessile and leathery, with a linear blade measuring between 6.4 and 10.2 cm in length and 1.5 to 2 mm in width. The leaf apex is acute-acuminate, while the base is acute and slightly plicate. Both adaxial and abaxial surfaces are glabrous, with thickened margins and two distinct nerves visible on each side. Roots emerge from the rhizome and are thin and unbranched. The pseudobulbs are clustered on a short rhizome and appear fusiform, ranging from 1.7 to 1.9 cm in length and approximately 0.4 cm in diameter. Each pseudobulb bears a single leaf and is initially covered by three to four tubulars, acute to acuminate cataphylls that are setose and soon disintegrate into persistent fibers. Leaves are petiolate, with a distinctly channeled petiole measuring 0.8 to 1.1 cm long. The leaf blade is dorsiventrally complanate, leathery, narrowly linear, acute at the tip, and entire, measuring 9.5–10.5 cm in length and approximately 0.1 cm in width.

Figure 10. *Dendrochilum* sp.7 showing the growth habit of the plant in situ, exhibiting a mycorrhizal association with a Myrtaceae tree at an elevation of 1500 meters above sea level (masl).

DNA Barcode Analysis

The CBOL Plant Working Group recommends the five loci rpoB, rpoC1, rbcL, matK, and ITS for plant DNA barcoding. Although rpoB, rpoC1, and rbcL are known to exhibit limited species-level resolution, they remain commonly used in barcode studies (Chase et al., 2005; Kress & Erickson, 2007; Lahaye et al., 2008; CBOL Plant Working Group, 2009). In this study, four loci (*matK*, *rbcL*, *ITS*, and trnH-psbA) were selected and assessed for their effectiveness in orchid species identification. Amplification of the target loci (*matK*, *rbcL*, *ITS*, and *trnH-psbA*) presented several challenges. The amplification success rates for the four loci (*rbcL*, *matK*, *ITS*, and *trnH-psbA*), which produced an amplicon size of 800-950 bp (*matK*), 1000-1500 bp (*rbcL*), and 700-750 bp (*ITS*), as depicted in Figure 11. Despite literature recommendations suggesting annealing temperatures between 50–64°C (Lau et al., 2001 & CBOL, 2009), extensive gradient PCR optimization was necessary. Successful amplification of 10 isolates was achieved for *matK*, *rbcL*, and *ITS* primers (Figure 11). However, amplification using *trnH-psbA* primers yielded inconsistent results, including faint and multiple non-

specific bands, suggesting the need for further optimization or alternative primer sets for this locus (Figure 11). Edited DNA sequences were analyzed using BLASTn (NCBI GenBank) and BOLD databases to assess species identity based on sequence similarity (Table 2). Among the nine analyzed orchid samples across four loci, *matK* provided the highest resolution, with several matches showing 100% identity in GenBank. BLAST identity scores ranged from 97.90% to 100%, while BOLD scores varied between 89.66% and 99.89%. The ITS region demonstrated the highest discriminatory power due to greater sequence variability, effectively distinguishing closely related species. In contrast, rbcL showed the lowest resolution, likely due to its conserved nature. These findings align with previous studies (Parveen et al., 2017; Asahina et al., 2010), which reported higher species identification success with matK and ITS compared to rbcL. ITS has also been highlighted in studies as a robust marker for orchid barcoding, achieving up to 100% amplification and sequencing success, particularly in Dendrobium (Feng et al., 2015). These results confirm that while rbcL is useful at higher taxonomic levels, matK and ITS are more effective for species-level resolution in Orchidaceae.

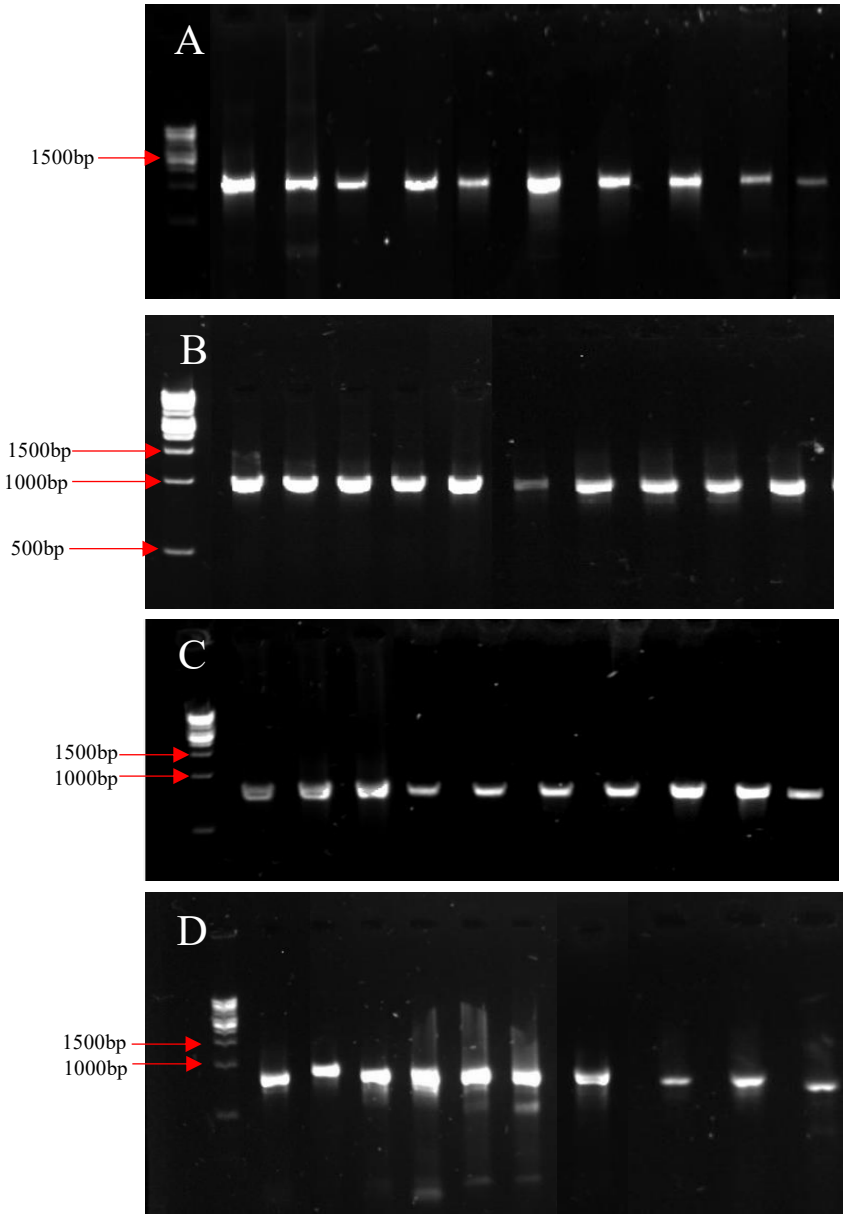

Figure 11. Agarose Gel Electrophoresis Profiles of PCR Amplification Products from Ten Orchid Samples Using Four DNA Barcode Regions (*rbcL*, *matK*, *ITS*, and *trnH–psbA*). Lane L contains the 1 kb DNA ladder (Vivantis) as a molecular size reference. (A) PCR amplification of the *rbcL* region (~850–1000 bp) from 10 orchid samples. (B) PCR amplification of the *matK* region (~800–950 bp) from 10 orchid samples. (C) PCR amplification of the *ITS* region (~700–750 bp) from 10 orchid samples. (D) PCR amplification of the *trnH–psbA* region (~350–950 bp) from 10 orchid samples. Successful amplifications are indicated by distinct bands at expected molecular sizes.

Molecular Identification

DNA barcoding studies have demonstrated the utility of combining molecular and morphological data in species identification and phylogenetic resolution of orchids (Table 4). For *Dendrochilum* specimens (DET004, DET008, DET016, DET015, and DET018), nucleotide sequences from four markers (*rbcL*, *matK*, *ITS,* and *TrnH-psbA*) successfully identified the four samples at the genus level as *Dendrochilum*. Specimens DET004, DET008, DET016, DET015, and DET018 formed a clade closely related to *Coelogyne glumacea* (Lindl.) (Figures 15 and 16), with 99–100% identity supported by both BLASTn and BOLD results. Although flowering specimens were not available during the sampling period, preliminary taxonomic identification was conducted based on vegetative morphology. The individuals exhibited a combination of diagnostic characters consistent with members of the genus *Dendrochilum* Blume (Orchidaceae), including narrowly lanceolate leaves with slender petioles, sharply reduced and inconspicuous pseudobulbs, and the presence of a distinctive pink sheath enveloping the basal portion of the petiole. These vegetative traits are known to occur among several *Dendrochilum* species, which are often characterized by their tufted epiphytic habit, narrowly elliptic to lanceolate leaves, and sheathing cataphylls that may be pigmented (Seidenfaden, 1992; Cootes, 2011; Gravendeel et al., 2001). Given the observed morphological features and supported by preliminary molecular data, it is hypothesized that the samples belong to the same *Dendrochilum* species, pending confirmation through floral examination and comprehensive phylogenetic analysis. These findings are consistent with previous research by Tsaballa et al. (2023), which emphasized the efficacy of matK and ITS in discriminating orchid species. Ho et al. (2021) also showed that rbcL had strong discriminatory power among 21 jewel orchid accessions, surpassing matK alone or in combination. Similarly, Parveen et al. (2017) confirmed that the ITS and matK marker combination was species-specific in 94 Indian orchid species, supporting their use in precise molecular identification. Notably, samples DET001, DET007, and DET013 were successfully identified at the species level as *Coelogyne apoensis*, *Coelogyne coccinea*, and *Coelogyne glumacea*, respectively, based on the combined resolution provided by the chloroplast *rbcL* and *matK* markers, as well as the nuclear ITS region (Figures 12–16). These specimens were classified under the genus *Coelogyne*, following the most recent taxonomic updates recognized by the World Flora Online and supported by the phylogenetic framework proposed by Chase et al. (2021), which consolidated several formerly distinct genera, including *Dendrochilum*, under *Coelogyne* in light of molecular evidence.

Genetic Distance Analysis

Pairwise genetic distances were computed using the concatenated *rbcL*, *matK*, and *ITS* regions for our nine orchid sequences in MEGA12, under the Kimura 2-parameter model with pairwise deletion (Table 3, Figure 12). The final alignment comprised 5,652 positions, yielding an overall mean genetic distance of 0.12 substitutions per site. A recent analysis of medicinal orchids reported much broader interspecific distances for *ITS* (0–0.64) and ITS2 (0–0.60), while *rbcL* remained highly conserved (0–0.02). The average interspecific distances for *ITS* (~0.34) and ITS2 (~0.32) significantly exceed those of *matK* (~0.06) and rbcL (~0.01) (Raskoti & Ale, 2021). In a Vietnamese study of jewel orchids, *matK* showed mean intraspecific and interspecific divergences of 0.025 and 0.034, respectively; *rbcL* exhibited much lower divergence (0.0025 vs. 0.051) (Ho et al., 2021). When benchmarks are compared, the combined mean distance of 0.12 is consistent with the high divergence expected when *ITS* is included. This value indicates moderate to high-resolution power suitable for species discrimination and aligns with interspecific divergence reported in other orchid families.

Phylogenetic analysis

Maximum likelihood (ML) phylogenetic analysis based on aligned chloroplast and nuclear DNA sequences using MAFFT and constructed through Neighbor-Joining (NJ), showing the monophyletic relationship of *Dendrochilum* samples to the *Coelogyne* species in combined nucleotide sequences of *matK*+ITS (Figure 13) and RAxML (Figure 14) has shown congruent topologies. The resulting ML trees displayed well-resolved clades, with most branch nodes supported by high bootstrap values ranging from 80% to 100%, indicating strong statistical confidence in the inferred phylogenetic relationships.

In this study, the phylogeny of *Coelogyne* in the Philippines was evaluated using combined analyses of *matK+ITS* sequences from 25 *Coelogyne* species, including 9 unidentified *Dendrochilum* samples, conducted under maximum likelihood (ML) methods with *Vanilla planifolia* as the outgroup. One sample (DET010) failed to produce high-quality sequence data across all targeted barcode regions and was therefore excluded from subsequent phylogenetic analyses. The resulting consensus trees, supported by 1,000 bootstrap replications, revealed two main clades within Coelogyninae, indicating both monophyletic and polyphyletic groupings. These findings align with previous research by Gravendeel et al. (2001), which showed that certain morphological traits, such as ovary indumentum and flower number, correspond well with molecular clades, while others, like lip base and petal

shape, were found to be homoplasious. This highlights the importance of integrating molecular data to achieve accurate phylogenetic reconstruction and classification within the subtribe Coelogyninae. In a study on morphologically similar *Coelogyne* species endemic to Peninsular Malaysia, Kok Hon et al. (2020) the researchers successfully developed RAPD-based SCAR (Sequence Characterized Amplified Region) markers to genetically differentiate *C. tiomanensis, C. stenochila*, and *C. kaliana*. Due to their close morphological resemblance, traditional identification methods were insufficient. However, SCAR markers enabled species-specific differentiation through distinct amplification patterns and fragment sizes, highlighting their effectiveness in resolving closely related taxa where morphological traits alone were inadequate.

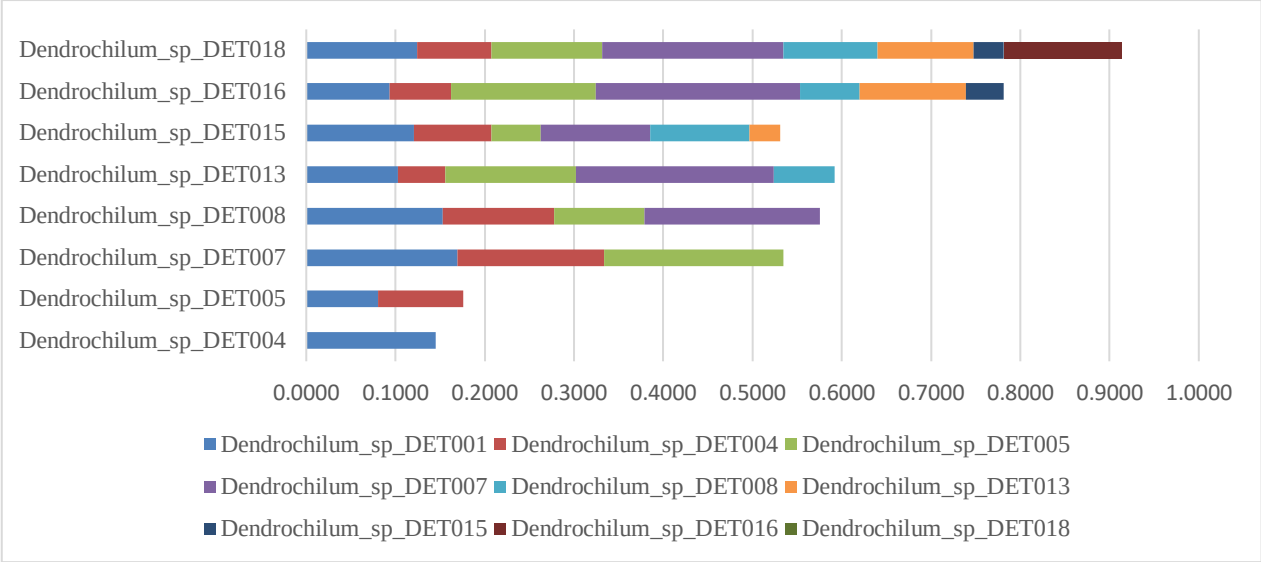

Figure 12. Pairwise Evolutionary Divergence Estimates Among Sampled Taxa Using the Maximum Composite Likelihood Method.

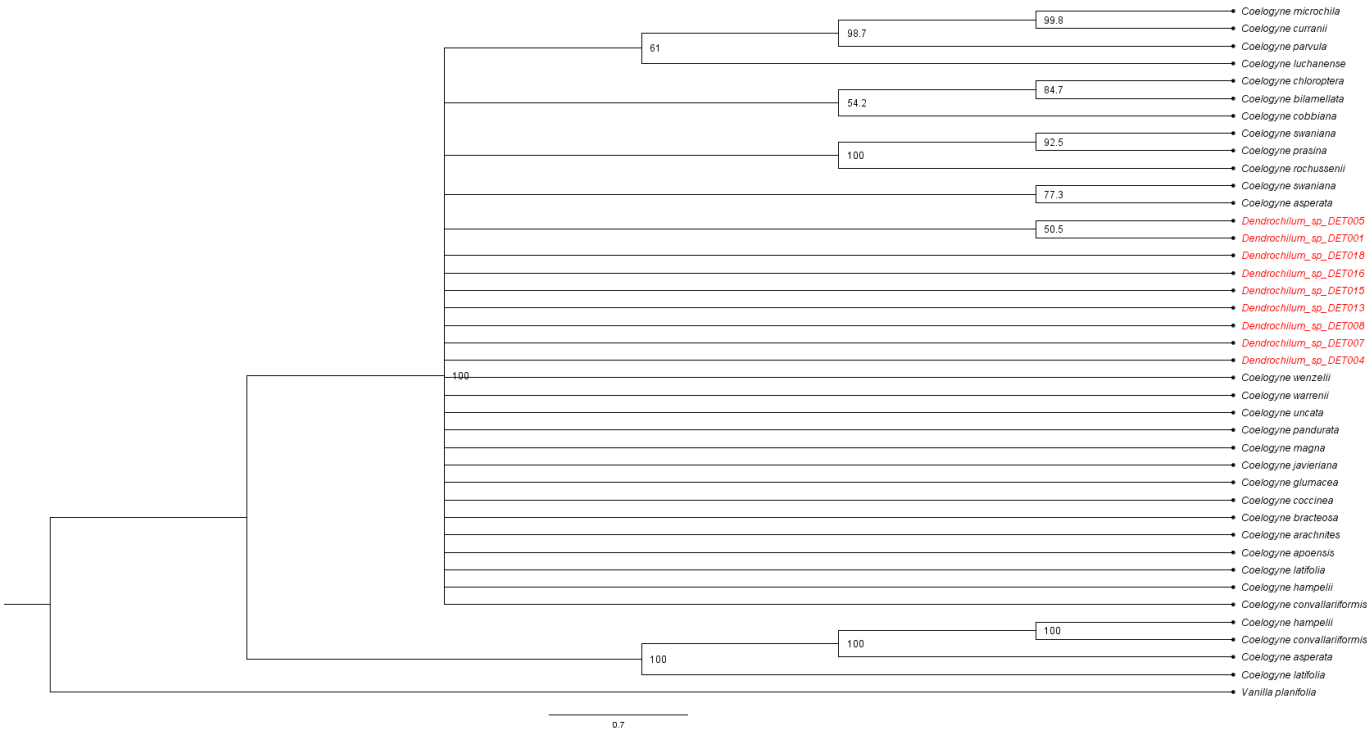

Figure 13. Neighbor-Joining (NJ) phylogenetic tree based on combined *matK* and *ITS* sequences of *Coelogyne* species from Mindanao, Philippines. The tree was generated in Geneious® Prime 2025.1.2 using the Tamura-Nei model with 1,000 bootstrap replicates. Bootstrap values are shown at the nodes. Unknown samples sequenced in this study are highlighted in red. *Vanilla planifolia* was used as the outgroup.

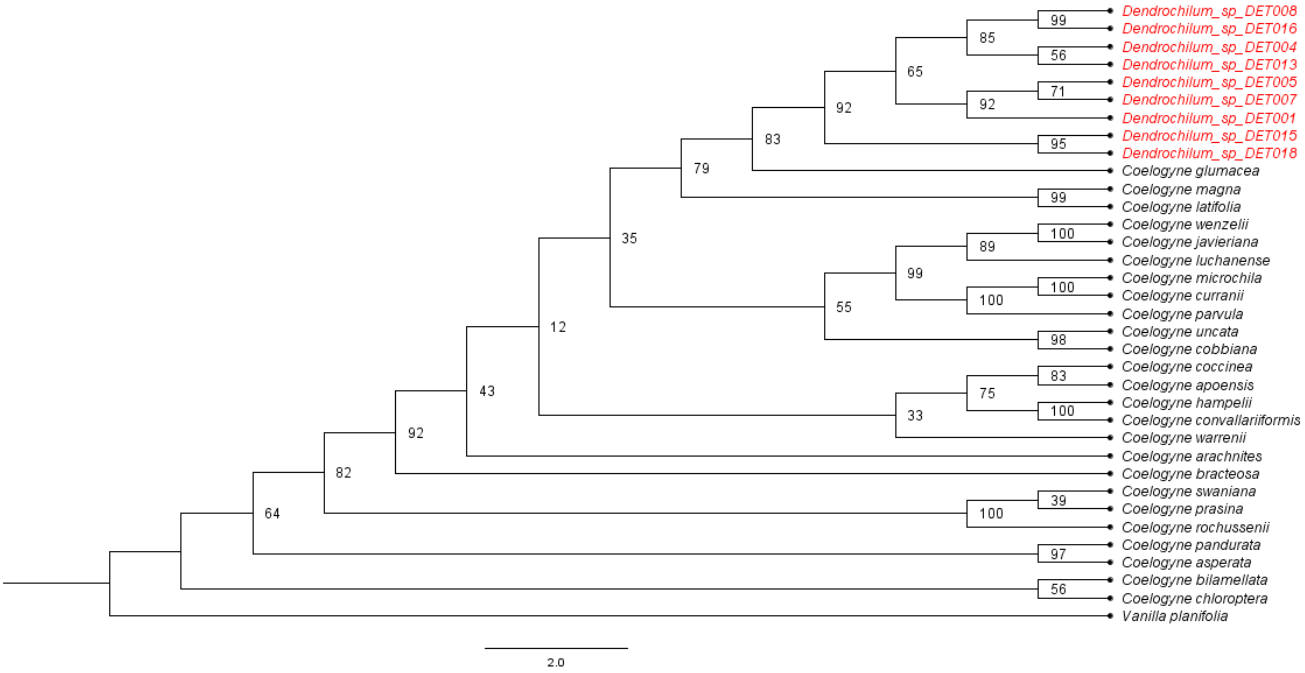

Figure 14. Maximum likelihood (ML) cladogram based on combined *rbcL*, *matK*, & *ITS* sequence data. The tree was generated in Geneious® Prime 2025.1.2 using RAxML v8 with the Tamura-Nei model and 1,000 bootstrap replicates. Bootstrap support values are shown at the nodes. Unknown samples sequenced in this study are highlighted in red. *Vanilla planifolia* was used as the outgroup.

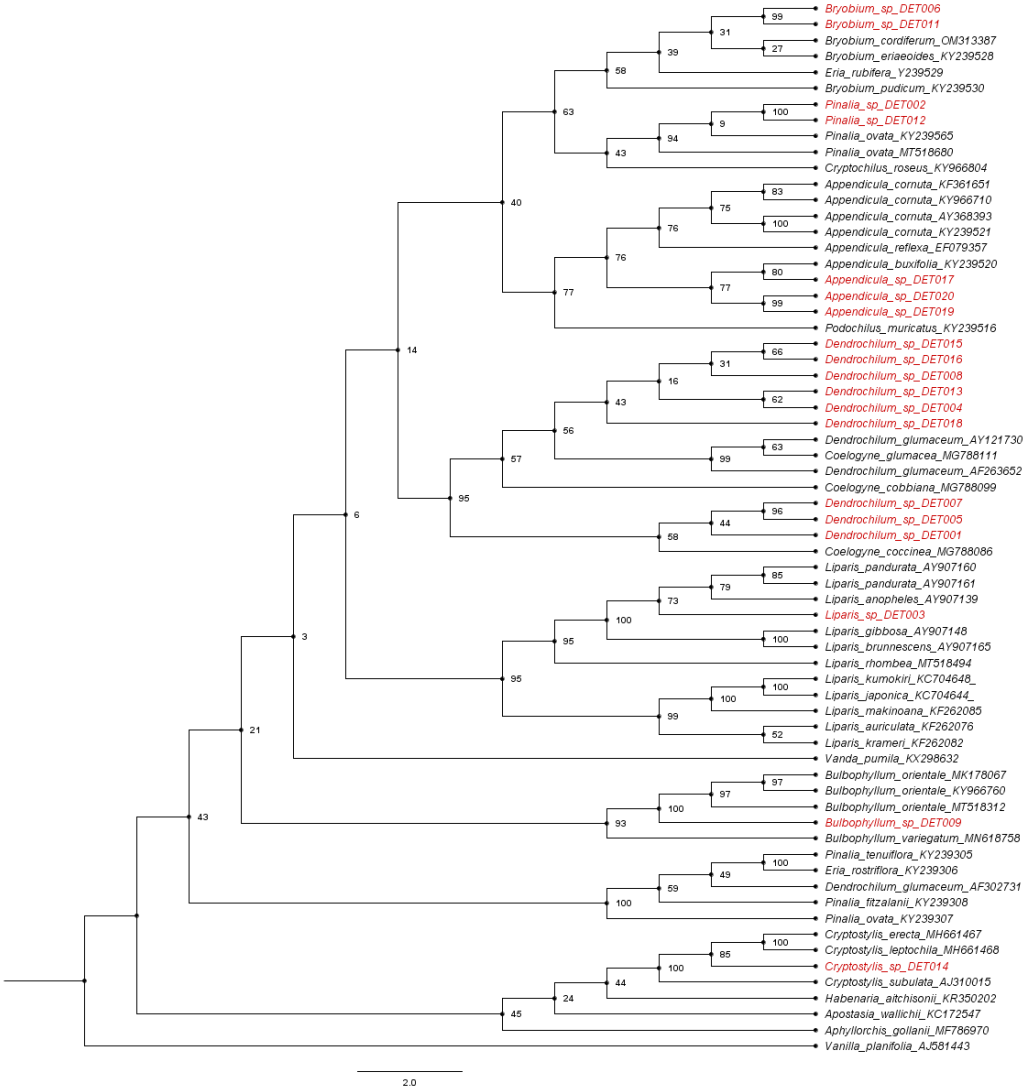

Figure 15. Maximum likelihood phylogenetic tree of orchid samples based on the chloroplast matK gene. The tree was constructed using RAxML version 8 (Stamatakis, 2014) implemented in Geneious® Prime 2025.1.2, employing the maximum likelihood method with 1,000 bootstrap replicates. Bootstrap support values are indicated at the corresponding nodes. Unknown samples sequenced in this study are highlighted in red. Vanilla planifolia was used as the outgroup to root the phylogenetic tree.

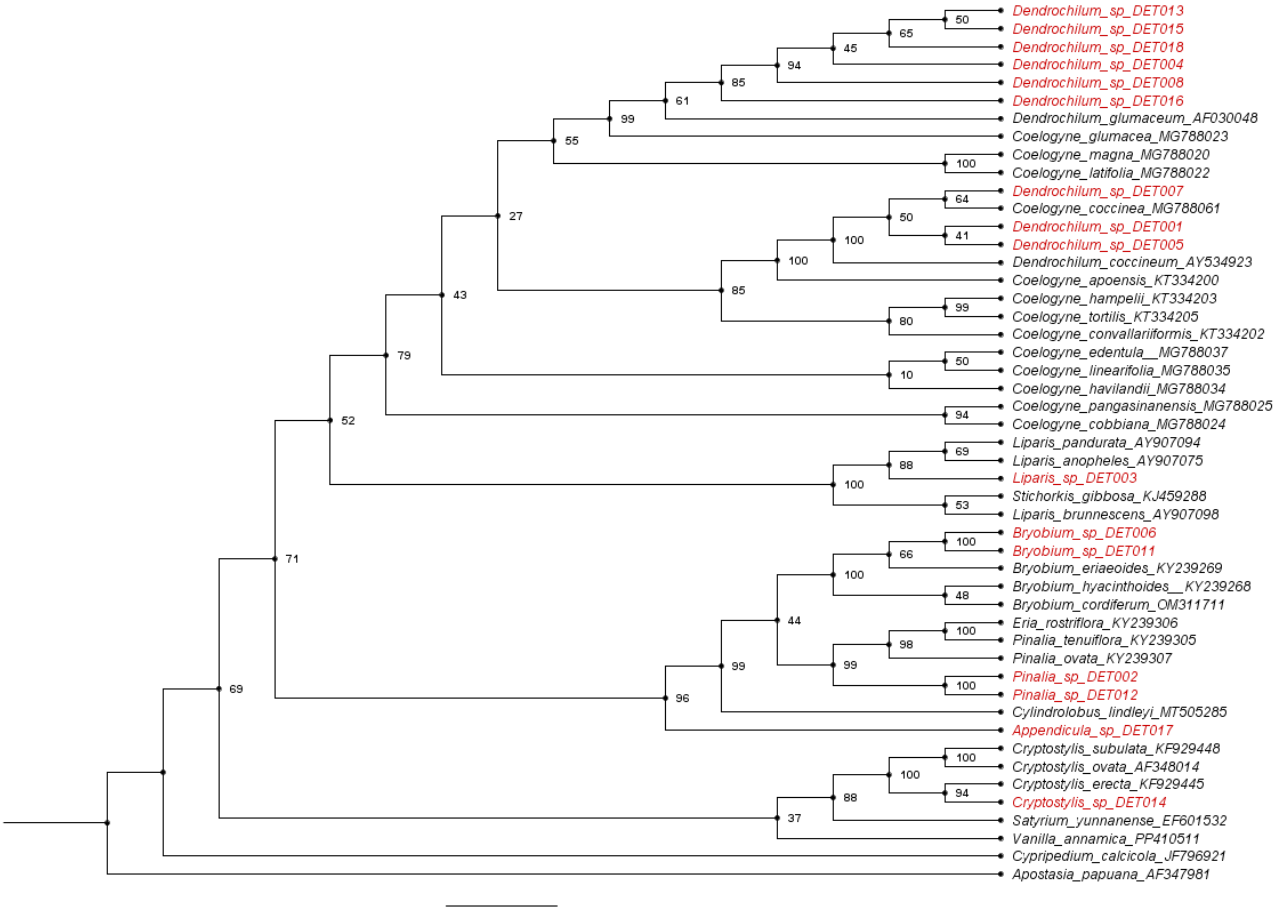

Figure 16. Maximum likelihood phylogenetic tree of orchid samples based on the nuclear ribosomal ITS region. The phylogenetic tree was inferred using the maximum likelihood method with RAxML version 8 (Stamatakis, 2014), implemented in Geneious® Prime 2025.1.2. Bootstrap support values, based on 1,000 replicates, are shown at the nodes. Samples newly sequenced in this study are highlighted in red. Cypripedium calcicole and Apostasia papuana were included as outgroup taxa to root the tree.

4. CONCLUSION

This study evaluated the effectiveness of four DNA barcode loci: *rbcL*, *matK*, *ITS*, and trnH-psbA in identifying nine orchid species from Mt. Talomo. The *matK* + *ITS* combination conclusively demonstrates the superior accuracy for identifying the *Coelogyne* species and *Dendrochilum* species. The molecular analyses resolved species-level distinctions for *Coelogyne coccinea*, *Coelogyne apoensis*, and *Coelogyne glumacea*. This approach effectively overcame the ambiguities of traditional morphological identification, which can be challenging due to factors like phenotypic plasticity or cryptic species. However, molecular data alone may be insufficient due to low interspecific divergence or incomplete reference databases. Integrating morphological traits remains essential. These results affirm the value of combining loci for enhanced taxonomic resolution, particularly among closely related taxa.

Author Contributions: Conceptualization and study design: A.C.E., G.Z.D., and R.Y.D.C.; Data acquisition, writing – original draft preparation, and reference organization: E.A.P.J., J.G.O., F.P.C., and K.C.A.; Writing – review and editing: A.C.E. and G.Z.D. All authors have read and approved the final version of the manuscript.

Funding: This research was primarily supported by the Department of Science and Technology – Science Education Institute (DOST-SEI) through the STRAND scholarship grant.

Data Availability Statement: The data supporting the findings of this study are included within the article. Additional raw data are available from the corresponding author upon reasonable request.

Acknowledgments: The author gratefully acknowledges the financial support provided by the Department of Science and Technology – Science Education Institute (DOST-SEI) through the STRAND scholarship. Special appreciation goes to Aurfeli Nietes, Guiller Opiso, and Daryl Salas for their valuable assistance. The author also acknowledges the Natural Science Research Center – Tuklas Lunas Development Center (NSRC-TLDC) for providing laboratory facilities and technical resources. The author extends sincere gratitude to Dr. Geonyzl L. Alviola, Dr. Elsa May D. Baron, Sir Rene Alfred Anton Bustamante, and Prof. Rene Louis Bonifacio for their critical insights and guidance.

Conflicts of Interest: The authors have no competing interests to declare that are relevant to the content of this article.

Appendix A

Table 1. Barcoding loci and corresponding primers used for PCR amplification of orchid DNA, including target regions from chloroplast and nuclear genomes. Primer sequences and annealing conditions were adapted from Xu et al. (2015)

Locus	Primer name	Primer Sequence (5′-3′)	Annealing Temperature	Target Amplicon Length	Reference
rbcl	1F	ATGTCACCACAAACAGAAAC	52–55°C	850–1,000 bp	Goldman et al. 2001
	1360R	CTTCACAAGCAGCAGCTAGTTC			
matk	1F	ATGTCACCACAAACAGAAAC	48–52°C	850–1,000 bp	Cuénoud et al. (2002)
	1326R	TCT AGC ACA CGA AAG TCG AAG T			
ITS	ITS1	TCCGTAGGTGAACCTGCGG	50–55°C	600–750 bp	White et al., 1990, Gardes & Bruns, 1993
	ITS4	TCCTCCGCTTATTGATATGC			
trnH-psbA	trnH	CGC GCA TGG TGG ATT CAC AAT CC	52–55°C	350–500 bp	White et al., 1990, Shaw et al., 2005
	psbA	GTT ATG CAT GAACGT AAT GCT C			

Table 2. Summary of BLASTn Results for 10 Orchid Samples Across Four Loci (*rbcL*, *matK*, *ITS*, *trnH-psbA*) with Match Statistics and Accession Data

Sample Code:	***Dendrochilum*_sp_DET001**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Coelogyne apoensis*	97.28%	0	2068	1320	43.6	OR687508.1
ITS	*Coelogyne apoensis*	96.47%	0	1303	1,119	53.4	KT334200.1
matK	*Coelogyne apoensis*	99.22%	0	1590	1219	34.8	MG788147.1
trnH-psbA	*Coelogyne apoensis*	98.86%	0	2511	1269	35.2	OR687508.1
Sample Code:	***Dendrochilum*_sp_DET004**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum glumaceum*	99.92	0	2240	1246	44.3	AF264164.1
ITS	*Coelogyne glumacea*	98.89	0	1100	651	55.3	MG788023.1
matK	*Dendrochilum glumaceum*	99.89	0	1650	896	31.9	AF302696.1

trnH-psbA	*Coelogyne xyrekes*	94.74	0	1170	778	34.4	KP694322.1
Sample Code:	***Dendrochilum*_sp_DET005**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum glumaceum*	99.68	0	2259	1262	43.8	AF264164.1
ITS	*Coelogyne coccinea*	99.69	0	1155	645	52.7	MG788061.1
matK	*Coelogyne coccinea*	100	0	1635	889	31.8	MG788086.1
trnH-psbA	*Coelogyne trinervis*	93.13	0	1274	889	38.2	KP694317.1
Sample Code:	***Dendrochilum*_sp_DET007**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum glumaceum*	96.89	0	1004	641	41.5	AF264164.1
ITS	*Coelogyne coccinea*	95.91	0	986	644	54.2	MG788061.1
matK	*Coelogyne coccinea*	100	0	1550	839	32.4	MG788086.1
trnH-psbA	*Coelogyne trinervis*	93.13	0	1274	889	38.2	KP694317.1

Sample Code:	***Dendrochilum*_sp_DET008**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum cobbianum*	99.81	0	1934	1079	42.4	AY381119.1
ITS	*Coelogyne glumacea*	98.91	0	1120	650	55.1	MG788023.1
matK	*Dendrochilum glumaceum*	100	0	1611	872	31.9	AF302696.1
trnH-psbA	*Coelogyne xyrekes*	94.13	0	1077	727	36.2	KP694322.1
Sample Code:	***Dendrochilum*_sp_DET013**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum glumaceum*	100	0	2305	1278	43.7	AF264164.1
ITS	*Coelogyne glumacea*	99.38	0	1135	652	54.1	MG788023.1
matK	*Dendrochilum glumaceum*	100	0	1629	884	32	AF302696.1
trnH-psbA	*Coelogyne xyrekes*	94.24	0	1099	728	34.9	KP694322.1
Sample Code:	***Dendrochilum*_sp_DET015**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum glumaceum*	100	0	2226	1234	44	AF264164.1
ITS	*Coelogyne glumacea*	99.36	0	1103	648	55.7	MG788023.1
matK	*Dendrochilum glumaceum*	99.89	0	1640	892	31.7	AF302696.1

trnH-psbA	*Coelogyne xyrekes*	90.54	0	922	701	33.4	KP694322.1
Sample Code:	***Dendrochilum*_sp_DET016**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum glumaceum*	99.92	0	2269	1262	44	AF264164.1
ITS	*Coelogyne glumacea*	98.77	0	1151	650	54.3	MG788023.1
matK	*Dendrochilum glumaceum*	99.66	0	1615	884	31.7	AF302696.1
trnH-psbA	*Coelogyne xyrekes*	94.34	0	1072	699	33.6	KP694322.1

Sample Code:	***Dendrochilum*_sp_DET018**						
DNA Region	**BLAST Search Match**	**% Identity**	**E value**	**Total Score**	**Aligned Length (bp)**	**% GC**	**Accession Number**
rbcL	*Dendrochilum glumaceum*	100	0	2280	1264	44	AF264164.1
ITS	*Coelogyne glumacea*	99.22	0	1127	649	55	MG788023.1
matK	*Dendrochilum glumaceum*	99.77	0	1628	889	31.8	AF302696.1
trnH-psbA	*Coelogyne xyrekes*	94.04	0	1079	727	35.2	KP694322.1

Table 3. Pairwise genetic distance matrix of the 9 orchid species based on the concatenated *rbcL*, *matK*, and *ITS* markers

Sample code	*Dendrochilum* sp DET001	*Dendrochilum* sp DET004	*Dendrochilum* sp DET005	*Dendrochilum* sp DET007	*Dendrochilum* sp DET008	*Dendrochilum* sp DET013	*Dendrochilum* sp DET015	*Dendrochilum* sp DET016	*Dendrochilum* sp DET018
Dendrochilum sp DET001	0								
Dendrochilum sp DET004	0.1447								
Dendrochilum sp DET005	0.0806	0.0951							
Dendrochilum sp DET007	0.1695	0.1639	0.2013						
Dendrochilum sp DET008	0.1529	0.1247	0.1011	0.1965					
Dendrochilum sp DET013	0.1025	0.0534	0.1462	0.2215	0.0686				

Dendrochilum sp DET015	0.1209	0.0862	0.0558	0.1224	0.1109	0.0346			
Dendrochilum sp DET016	0.0937	0.0683	0.1623	0.2289	0.0669	0.119	0.0425		
Dendrochilum sp DET018	0.1241	0.0834	0.1242	0.2026	0.1054	0.1081	0.0338	0.1318	0

Table 4. Taxonomic Classification and Final Identification of Orchid Species Based on Morphological Traits and BLAST Analysis Using *rbcL*, *matk*, *ITS*, rbcL, and *trnH-psbA* Barcodes, with Corresponding GenBank Accession Numbers

		DNA Barcoding Results				
		rbcL	**matK**	**ITS**	**TrnH-psbA**	
Sequence ID	**Taxonomic Genus/Species**	**Species name**				**Final ID**
*Dendrochilum*_sp_DET001	*Dendrochilum* sp.1	*Coelogyne apoensis* (OR687508.1)	*Coelogyne apoensis* (KT334200.1)	*Coelogyne apoensis* (KT334200.1)	*Coelogyne apoensis* (KT334200.1)	*Coelogyne apoensis* (T.Hashim.) M.W.Chase & Schuit.
*Dendrochilum*_sp_DET004	*Dendrochilum* sp.2	*Dendrochilum glumaceum* (AF264164.1)	*Coelogyne glumacea* (MG788023.1)	*Dendrochilum glumaceum* (AF302696.1)	*Coelogyne xyrekes* (KP694322.1)	*Dendrochilum* sp.1
*Dendrochilum*_sp_DET005	*Dendrochilum* sp.3	*Dendrochilum glumaceum* (AF264164.1)	*Coelogyne coccinea* (MG788061.1)	*Coelogyne coccinea* (MG788061.1)	*Coelogyne trinervis* (KP694317.1)	*Dendrochilum* sp.2
*Dendrochilum*_sp_DET007	*Dendrochilum* sp.4	*Dendrochilum glumaceum* (AF264164.1)	*Coelogyne coccinea* (MG788061.1)	*Coelogyne coccinea* (MG788061.1)	*Discarded (not used due to low sequence quality)*	*Coelogyne coccinea* (H.A.Pedersen & Gravend.) M.W.Chase & Schuit.
*Dendrochilum*_sp_DET008	*Dendrochilum* sp.5	*Dendrochilum cobbianum* (AY381119.1)	*Coelogyne glumacea* (MG788023.1)	*Dendrochilum glumaceum* (AF302696.1)	*Coelogyne xyrekes* (KP694322.1	*Dendrochilum* sp.3
*Dendrochilum*_sp_DET013	*Dendrochilum* sp.7	*Dendrochilum glumaceum* (AF264164.1)	*Coelogyne glumacea* (MG788023.1)	*Dendrochilum glumaceum* (AF302696.1)	*Coelogyne xyrekes* (KP694322.1	*Coelogyne glumacea* (Lindl.) M.W.Chase & Schuit.
*Dendrochilum*_sp_DET015	*Dendrochilum* sp.8	*Dendrochilum cobbianum* (AY381119.1)	*Coelogyne glumacea* (MG788023.1)	*Dendrochilum glumaceum* (AF302696.1)	*Coelogyne xyrekes* (KP694322.1	*Dendrochilum* sp.5
*Dendrochilum*_sp_DET016	*Dendrochilum* sp.9	*Dendrochilum cobbianum* (AY381119.1)	*Coelogyne glumacea* (MG788023.1)	*Dendrochilum glumaceum* (AF302696.1)	*Coelogyne xyrekes* (KP694322.1	*Dendrochilum* sp.6
*Dendrochilum*_sp_DET018	*Dendrochilum* sp.10	*Dendrochilum cobbianum* (AY381119.1)	*Coelogyne glumacea* (MG788023.1)	*Dendrochilum glumaceum* (AF302696.1)	*Coelogyne xyrekes* (KP694322.1	*Dendrochilum* sp.7

5. REFERENCES

Zhang S-B, Chen W-Y, Huang J-L, Bi Y-F, Yang X-F (2015) Orchid Species Richness along Elevational and Environmental Gradients in Yunnan, China. PLoS ONE 10(11): e0142621. https://doi.org/10.1371/journal.pone.

Chase, M. W., Cameron, K. M., Freudenstein, J. V.,

Pridgeon, A. M., Salazar, G., van den Berg, C., & Schuiteman, A. (2015). An updated classification of Orchidaceae, Botanical Journal of the Linnean Society, Volume 177, Issue 2, February 2015, Pages 151–174, https://doi.org/10.1111/boj.12234

Cootes, J. (2011). Philippine Native Orchid Species Katha Publishing, Philippines.

Pelser, P.B., Barcelona, J.F. & Nickrent, D.L. (2011 onwards) Co's Digital Flora of the Philippines. Available from: http://www.philippineplants.org (Accessed 25 Aug. 2022).

Naive, M. A., Boos, R., De Leon, M. D. & Cootes, J. (2017). Two new *Dendrochilum* (Orchidaceae) species from Mindanao, Philippines. Orchideen Journal, 24, 56-60.

Global Forest Watch (2021) Global Deforestation Rates & Statistics by Country. GFW. https://www.globalforestwatch.org/dashboards/country/KEN

Barcelona, J.F, Nickrent, D.L., Callado J. R, & Pelser, P. (2013). Co's Digital Flora of the Philippines: plant identification and conservation through cybertaxonomy. Philippine Journal of Science. 142. 57-67.

Gutierrez, Rosa. (2010). Orchids: A review of uses in traditional medicine, their phytochemistry and pharmacology. Journal of Medicinal Plant Research. 4. 592-638.

Castillo-Pérez, L. J., Ponce-Hernández, A., Alonso-Castro, A. J., Solano, R., Fortanelli-Martínez, J., Lagunez-Rivera, L., & Carranza-Álvarez, C. (2024). Medicinal Orchids of Mexico: A Review. Pharmaceuticals (Basel, Switzerland), 17(7), 907. https://doi.org/10.3390/ph17070907

Shukla, M. K., Monika, Thakur, A., Verma, R., Lalhlenmawia, H., Bhattacharyya, S., Bisht, D., Singh, A., Parcha, V., & Kumar, D. (2022). Unravelling the therapeutic potential of the orchid plant against cancer. South African Journal of Botany, 150, 69–79. https://doi.org/10.1016/j.sajb.2022.07.005

Wati, R. K., de Graaf, E. F., Bogarín, D., Heijungs, R., van Vugt, R., Smets, E. F., & Gravendeel, B. (2021). Antimicrobial Activity of Necklace Orchids is Phylogenetically Clustered and can be Predicted with a Biological Response Method. Frontiers in Pharmacology, 11, 586345. https://doi.org/10.3389/fphar.2020.586345

Hebert, P. D., & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. Systematic biology, 54(5), 852–859. https://doi.org/10.1080/10635150500354886

CBOL Plant Working Group (2009). A DNA barcode for land plants. Proceedings of the National Academy of Sciences of the United States of America, 106(31), 12794–12797. https://doi.org/10.1073/pnas.0905845106

Li, Y., Tong, Y., & Xing, F. (2016). DNA Barcoding Evaluation and Its Taxonomic Implications in the Recently Evolved Genus Oberonia Lindl. (Orchidaceae) in China. Frontiers in Plant Science, 7, 1791. https://doi.org/10.3389/fpls.2016.01791

Kim, H. M., Oh, S. H., Bhandari, G. S., Kim, C. S., & Park, C. W. (2014). DNA barcoding of Orchidaceae in Korea. Molecular ecology resources, 14(3), 499–507. https://doi.org/10.1111/1755-0998.12207

Xiang, X.G.; Zhang, J.B.; Lu, A.M.; Li, R.Q. (2011). Molecular identification of species in Juglandaceae: A tiered method. J. Syst. Evol. 49, 252–260. https://doi.org/10.1111/j.1759-6831.2011.00116.x

Amoroso, V. B., Coritico, F. P., & Fritsch, P. W. (2016). Species richness and conservation status of ferns and lycophytes in Mt. Hamiguitan Range Wildlife Sanctuary, Davao Oriental, Philippines. *Philippine Journal of Science, 142*(2), 127–137.

Inda, L. A., Pimentel, M., & Chase, M. W. (2012). Phylogenetics of tribe Orchideae (Orchidaceae: Orchidoideae) based on combined DNA matrices: inferences regarding timing of diversification and evolution of pollination syndromes. Annals of botany, 110(1), 71–90. https://doi.org/10.1093/aob/mcs083

Lahaye, R., van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T. G., & Savolainen, V. (2008). DNA barcoding the floras of biodiversity hotspots. Proceedings of the National Academy of Sciences of the United States of America, 105(8), 2923–2928. https://doi.org/10.1073/pnas.0709936105

Hall, T.A. (1999) BioEdit: A User-Friendly Biological

Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98.

FinchTV V1.4, 2006. A Brilliant Trace Viewer. Geospiza, Inc. http://www.geospiza.co m/Products/finchtv.shtml

Geneious® Prime (2025.1.2). http://www.geneious.com

Sayers, E. W., Beck, J., Bolton, E. E., Brister, J. R., Chan, J., Connor, R., Feldgarden, M., Fine, A. M., Funk, K., Hoffman, J., Kannan, S., Kelly, C., Klimke, W., Kim, S., Lathrop, S., Marchler-Bauer, A., Murphy, T. D., O'Sullivan, C., Schmieder, E., Skripchenko, Y., … Pruitt, K. D. (2025). Database resources of the National Center for Biotechnology Information in 2025. Nucleic Acids Research, 53(D1), D20–D29. https://doi.org/10.1093/nar/gkae979

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of molecular biology, 215(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Stamatakis A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics (Oxford, England), 30(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Chase, M. W., Salamin, N., Wilkinson, M., Dunwell, J. M., Kesanakurthi, R. P., Haider, N., & Savolainen, V. (2005). Land plants and DNA barcodes: short-term and long-term goals. Philosophical Transactions of the Royal Society of London. Series B, Biological sciences, 360(1462), 1889–1895. https://doi.org/10.1098/rstb.2005.1720

Kress, W. J., & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. PloS one, 2(6), e508. https://doi.org/10.1371/journal.pone.0000508

Lau, D. T., Shaw, P. C., Wang, J., & But, P. P. (2001). Authentication of medicinal Dendrobium species by the internal transcribed spacer of ribosomal DNA. Planta medica, 67(5), 456–460. https://doi.org/10.1055/s-2001-15818

Parveen, I., Singh, H. K., Malik, S., Raghuvanshi, S., & Babbar, S. B. (2017). Evaluating five different loci (rbcL, rpoB, rpoC1, matK, and ITS) for DNA barcoding of Indian orchids. Genome, 60(8), 665–671. https://doi.org/10.1139/gen-2016-0215

Asahina, H., Shinozaki, J., Masuda, K., Morimitsu, Y., & Satake, M. (2010). Identification of medicinal *Dendrobium* species by phylogenetic analyses using matK and rbcL sequences. Journal of natural medicines, 64(2), 133–138. https://doi.org/10.1007/s11418-009-0379-8

Feng, S., Jiang, Y., Wang, S., Jiang, M., Chen, Z., Ying, Q., & Wang, H. (2015). Molecular Identification of Dendrobium Species (Orchidaceae) Based on the DNA Barcode ITS2 Region and Its Application for Phylogenetic Study. International Journal of Molecular Sciences, 16(9), 21975-21988. https://doi.org/10.3390/ijms160921975

Raskoti, B. B., & Ale, R. (2021). DNA barcoding of medicinal orchids in Asia. Scientific Reports, 11(1). PMID: 34880330; PMCID: PMC8654824. https://doi.org/10.1038/s41598-021-03025-0

Ho, V. T., Tran, T. K. P., Vu, T. T. T., & Widiarsih, S. (2021). Comparison of matK and rbcL DNA barcodes for genetic classification of jewel orchid accessions in Vietnam. Journal, genetic engineering & biotechnology, 19(1), 93. https://doi.org/10.1186/s43141-021-00188-1.

Seidenfaden, G. (1992). The Orchids of Indochina. *Opera Botanica*, (114), 502.

World Flora Online. (n.d.). *World Flora Online*. Retrieved July 3, 2025, from https://www.worldfloraonline.org/

Chase, M. W., Gravendeel, B., Sulistyo, B. P., Wati, R. K., & Schuiteman, A. (2021). Expansion of the orchid genus Coelogyne (Arethuseae; Epidendroideae) to include Bracisepalum, Bulleyia, Chelonistele, Dendrochilum, Dickasonia, Entomophobia, Geesinkorchis, Gynoglottis, Ischnogyne, Nabaluia, Neogyna, Otochilus, Panisea, and Pholidota. Phytotaxa, 510(2), 101–148. https://doi.org/10.11646/phytotaxa.510.2.

Tsaballa, A., Kelesidis, G., Krigas, N., Sarropoulou, V., Bagatzounis, P., & Grigoriadou, K. (2023). Taxonomic Identification and Molecular DNA Barcoding of Collected Wild-Growing Orchids Used Traditionally for Salep Production. Plants (Basel, Switzerland), 12(17), 3038. https://doi.org/10.3390/plants12173038

Gravendeel, B., Chase, M. W., de Vogel, E. F., Roos, M. C., Mes, T. H., & Bachmann, K. (2001). Molecular phylogeny of Coelogyne (Epidendroideae; Orchidaceae) based on plastid RFLPS, matK, and nuclear ribosomal ITS sequences: evidence for polyphyly. American journal of botany, 88(10), 1915–1927. https://doi.org/10.2307/3558367

Kok Hon, Y., Yong, C. S., Abdullah, J. O., & Go, R. (2020). Development of species-specific SCAR markers for identification and authentication of three rare Peninsular Malaysian endemic Coelogyne (Orchidaceae) orchids. F1000Research, 9, 1161. https://doi.org/10.12688/f1000research.26170.2