



Research Article

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

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### 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.

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## 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; Pelsner 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 (Pelsner 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  $\mu$ L of Buffer AP1 and 8  $\mu$ L of RNase A at 65 °C for 1 hour. After adding 130  $\mu$ L of Buffer P3, the mixture was cooled on ice for 5 minutes. The lysates were then centrifuged at 20,000  $\times$  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  $\mu$ L 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  $\mu$ L 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  $\mu$ L) contained ~10 ng template DNA, 1.5  $\mu$ L 1 $\times$  PCR buffer with MgCl<sub>2</sub>, 0.5  $\mu$ L 25 mM MgCl<sub>2</sub>, 0.2  $\mu$ L 0.3 mM dNTP mix, 0.35  $\mu$ L each of 0.3 mM forward and reverse primers, 0.2  $\mu$ L 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  $\times$  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.



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\_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.

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, subglobose 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.



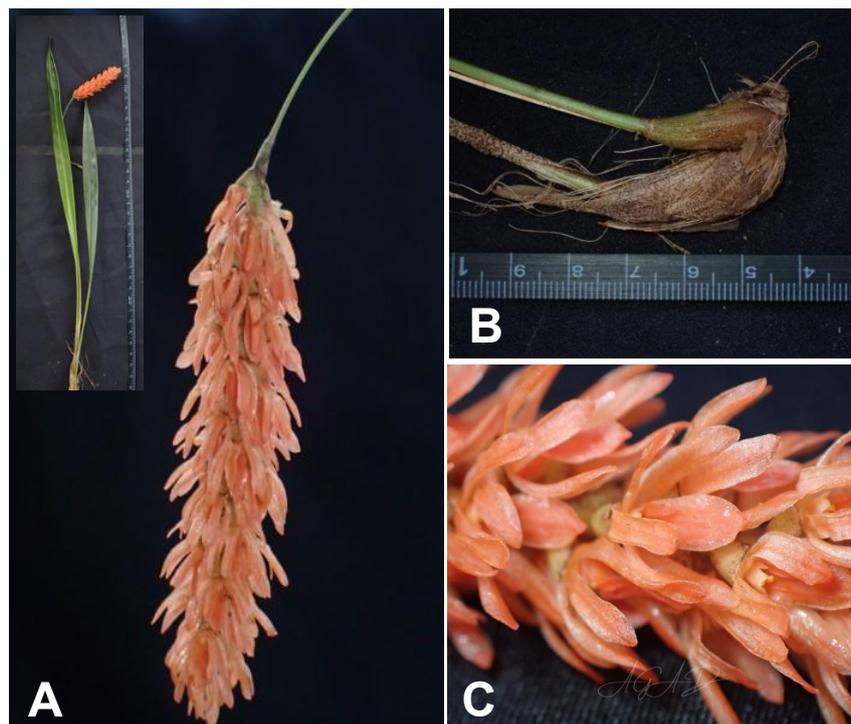
Figure 3. *Dendrochilum* sp.2 in its natural habitat. The plant is shown growing epiphytically in a primary montane forest at approximately 1500 m elevation, illustrating its growth habit and ecological preference.

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 stielidia and apical anther cap.



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\_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.

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.

Sample code: *Dendrochilum*\_sp\_DET013

**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\_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.

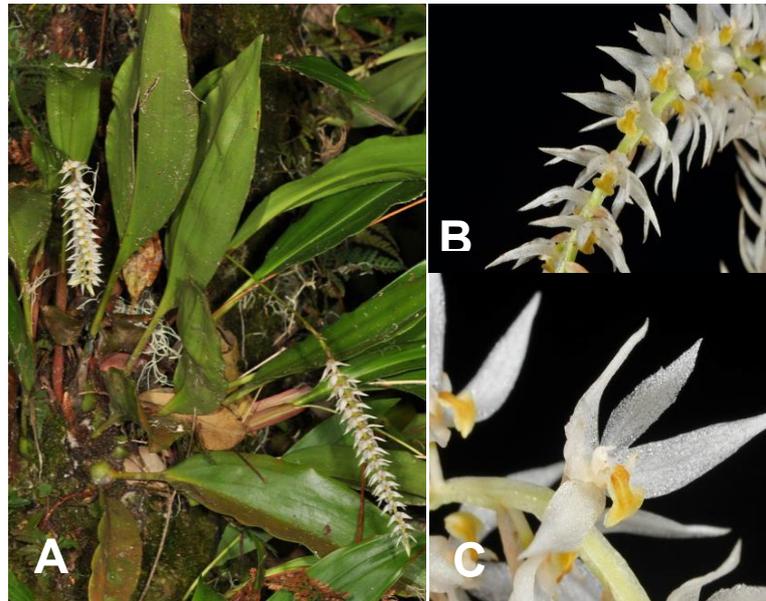


Figure 7. *Coelogyne glumacea* (Lindl.) M.W.Chase & Schuit. A. Habit showing upright, sympodial growth with unifoliate pseudobulbs. B. Arching inflorescence emerging concurrently with new vegetative growth. C. Close-up of flower showing white sepals, petals, and a vividly colored three-lobed labelium.



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.

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 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)



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.

### 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.

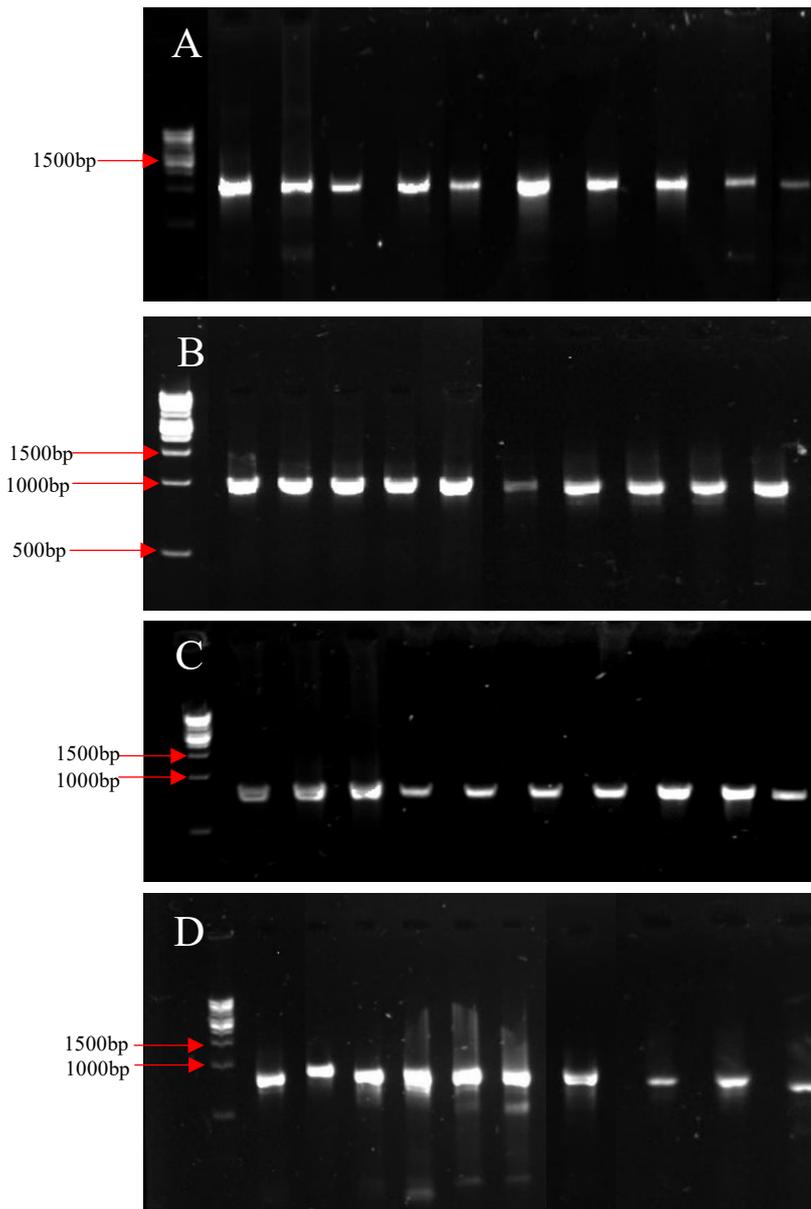


Figure 11. Agarose Gel Electrophoresis Profiles of PCR Amplification Products from Ten Orchid Samples Using Four DNA Barcode Regions (*rbcL*, *matK*, *ITS*, and *trnH-psbA*). Lane L contains the 1 kb DNA ladder (Vivantis) as a molecular size reference. (A) PCR amplification of the *rbcL* region (~850–1000 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.

### 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.

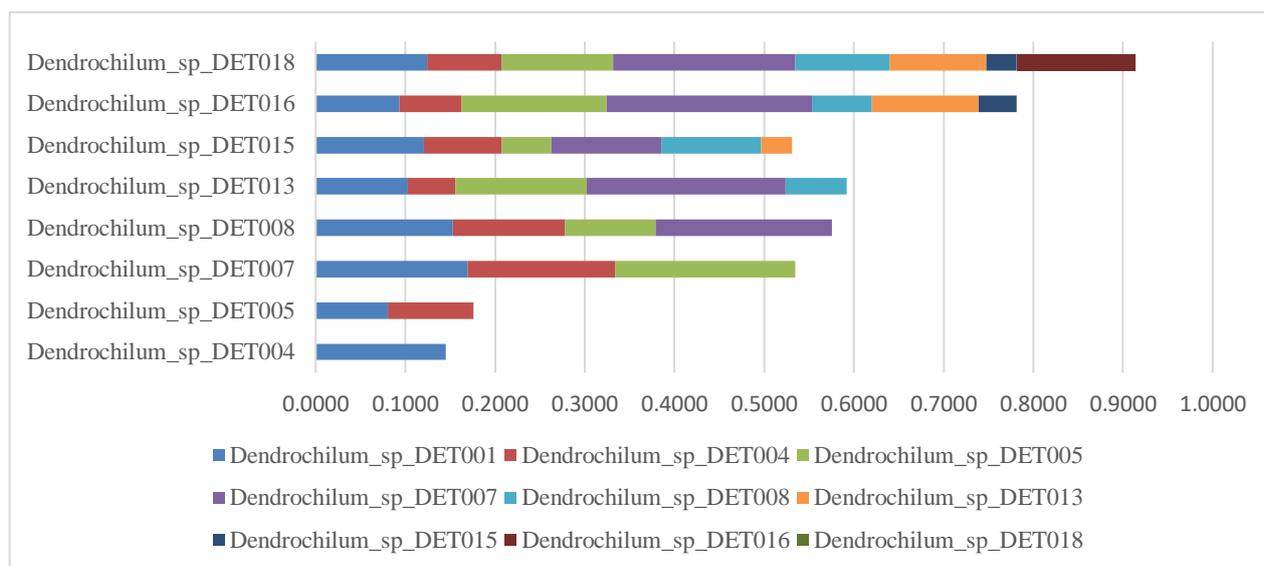


Figure 12. Pairwise Evolutionary Divergence Estimates Among Sampled Taxa Using the Maximum Composite Likelihood Method.





#### 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.

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**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.

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## 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       | CTTACAAGCAGCAGCTAGTTC          |                       |                        |  |
| 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

| <b>Sample Code:</b>           |                               |                   |                |                    |                            |             |                         |
|-------------------------------|-------------------------------|-------------------|----------------|--------------------|----------------------------|-------------|-------------------------|
| <b>Dendrochilum_sp_DET001</b> |                               |                   |                |                    |                            |             |                         |
| <b>DNA Region</b>             | <b>BLAST Search Match</b>     | <b>% Identity</b> | <b>E value</b> | <b>Total Score</b> | <b>Aligned Length (bp)</b> | <b>% GC</b> | <b>Accession Number</b> |
| <b>rbcL</b>                   | <i>Coelogyne apoensis</i>     | 97.28%            | 0              | 2068               | 1320                       | 43.6        | OR687508.1              |
| <b>ITS</b>                    | <i>Coelogyne apoensis</i>     | 96.47%            | 0              | 1303               | 1,119                      | 53.4        | KT334200.1              |
| <b>matK</b>                   | <i>Coelogyne apoensis</i>     | 99.22%            | 0              | 1590               | 1219                       | 34.8        | MG788147.1              |
| <b>trnH-psbA</b>              | <i>Coelogyne apoensis</i>     | 98.86%            | 0              | 2511               | 1269                       | 35.2        | OR687508.1              |
| <b>Sample Code:</b>           |                               |                   |                |                    |                            |             |                         |
| <b>Dendrochilum_sp_DET004</b> |                               |                   |                |                    |                            |             |                         |
| <b>DNA Region</b>             | <b>BLAST Search Match</b>     | <b>% Identity</b> | <b>E value</b> | <b>Total Score</b> | <b>Aligned Length (bp)</b> | <b>% GC</b> | <b>Accession Number</b> |
| <b>rbcL</b>                   | <i>Dendrochilum glumaceum</i> | 99.92             | 0              | 2240               | 1246                       | 44.3        | AF264164.1              |
| <b>ITS</b>                    | <i>Coelogyne glumacea</i>     | 98.89             | 0              | 1100               | 651                        | 55.3        | MG788023.1              |
| <b>matK</b>                   | <i>Dendrochilum glumaceum</i> | 99.89             | 0              | 1650               | 896                        | 31.9        | AF302696.1              |
| <b>trnH-psbA</b>              | <i>Coelogyne xyrekes</i>      | 94.74             | 0              | 1170               | 778                        | 34.4        | KP694322.1              |
| <b>Sample Code:</b>           |                               |                   |                |                    |                            |             |                         |
| <b>Dendrochilum_sp_DET005</b> |                               |                   |                |                    |                            |             |                         |
| <b>DNA Region</b>             | <b>BLAST Search Match</b>     | <b>% Identity</b> | <b>E value</b> | <b>Total Score</b> | <b>Aligned Length (bp)</b> | <b>% GC</b> | <b>Accession Number</b> |
| <b>rbcL</b>                   | <i>Dendrochilum glumaceum</i> | 99.68             | 0              | 2259               | 1262                       | 43.8        | AF264164.1              |
| <b>ITS</b>                    | <i>Coelogyne coccinea</i>     | 99.69             | 0              | 1155               | 645                        | 52.7        | MG788061.1              |
| <b>matK</b>                   | <i>Coelogyne coccinea</i>     | 100               | 0              | 1635               | 889                        | 31.8        | MG788086.1              |
| <b>trnH-psbA</b>              | <i>Coelogyne trinervis</i>    | 93.13             | 0              | 1274               | 889                        | 38.2        | KP694317.1              |
| <b>Sample Code:</b>           |                               |                   |                |                    |                            |             |                         |
| <b>Dendrochilum_sp_DET007</b> |                               |                   |                |                    |                            |             |                         |
| <b>DNA Region</b>             | <b>BLAST Search Match</b>     | <b>% Identity</b> | <b>E value</b> | <b>Total Score</b> | <b>Aligned Length (bp)</b> | <b>% GC</b> | <b>Accession Number</b> |
| <b>rbcL</b>                   | <i>Dendrochilum glumaceum</i> | 96.89             | 0              | 1004               | 641                        | 41.5        | AF264164.1              |
| <b>ITS</b>                    | <i>Coelogyne coccinea</i>     | 95.91             | 0              | 986                | 644                        | 54.2        | MG788061.1              |
| <b>matK</b>                   | <i>Coelogyne coccinea</i>     | 100               | 0              | 1550               | 839                        | 32.4        | MG788086.1              |
| <b>trnH-psbA</b>              | <i>Coelogyne trinervis</i>    | 93.13             | 0              | 1274               | 889                        | 38.2        | KP694317.1              |

| Sample Code: <i>Dendrochilum_sp_DET008</i> |                               |            |         |             |                     |      |                  |
|--|-------------------------------|------------|---------|-------------|---------------------|------|------------------|
| DNA Region                                 | BLAST Search Match            | % Identity | E value | Total Score | Aligned Length (bp) | % GC | Accession Number |
| rbcl                                       | <i>Dendrochilum cobbianum</i> | 99.81      | 0       | 1934        | 1079                | 42.4 | AY381119.1       |
| ITS  | <i>Coelogyne glumacea</i>     | 98.91      | 0       | 1120        | 650                 | 55.1 | MG788023.1       |
| matK                                       | <i>Dendrochilum glumaceum</i> | 100        | 0       | 1611        | 872                 | 31.9 | AF302696.1       |
| trnH-psbA                                  | <i>Coelogyne xyrekes</i>      | 94.13      | 0       | 1077        | 727                 | 36.2 | KP694322.1       |
| Sample Code: <i>Dendrochilum_sp_DET013</i> |                               |            |         |             |                     |      |                  |
| DNA Region                                 | BLAST Search Match            | % Identity | E value | Total Score | Aligned Length (bp) | % GC | Accession Number |
| rbcl                                       | <i>Dendrochilum glumaceum</i> | 100        | 0       | 2305        | 1278                | 43.7 | AF264164.1       |
| ITS  | <i>Coelogyne glumacea</i>     | 99.38      | 0       | 1135        | 652                 | 54.1 | MG788023.1       |
| matK                                       | <i>Dendrochilum glumaceum</i> | 100        | 0       | 1629        | 884                 | 32   | AF302696.1       |
| trnH-psbA                                  | <i>Coelogyne xyrekes</i>      | 94.24      | 0       | 1099        | 728                 | 34.9 | KP694322.1       |
| Sample Code: <i>Dendrochilum_sp_DET015</i> |                               |            |         |             |                     |      |                  |
| DNA Region                                 | BLAST Search Match            | % Identity | E value | Total Score | Aligned Length (bp) | % GC | Accession Number |
| rbcl                                       | <i>Dendrochilum glumaceum</i> | 100        | 0       | 2226        | 1234                | 44   | AF264164.1       |
| ITS  | <i>Coelogyne glumacea</i>     | 99.36      | 0       | 1103        | 648                 | 55.7 | MG788023.1       |
| matK                                       | <i>Dendrochilum glumaceum</i> | 99.89      | 0       | 1640        | 892                 | 31.7 | AF302696.1       |
| trnH-psbA                                  | <i>Coelogyne xyrekes</i>      | 90.54      | 0       | 922         | 701                 | 33.4 | KP694322.1       |
| Sample Code: <i>Dendrochilum_sp_DET016</i> |                               |            |         |             |                     |      |                  |
| DNA Region                                 | BLAST Search Match            | % Identity | E value | Total Score | Aligned Length (bp) | % GC | Accession Number |
| rbcl                                       | <i>Dendrochilum glumaceum</i> | 99.92      | 0       | 2269        | 1262                | 44   | AF264164.1       |
| ITS  | <i>Coelogyne glumacea</i>     | 98.77      | 0       | 1151        | 650                 | 54.3 | MG788023.1       |
| matK                                       | <i>Dendrochilum glumaceum</i> | 99.66      | 0       | 1615        | 884                 | 31.7 | AF302696.1       |
| trnH-psbA                                  | <i>Coelogyne xyrekes</i>      | 94.34      | 0       | 1072        | 699                 | 33.6 | KP694322.1       |
| Sample Code: <i>Dendrochilum_sp_DET018</i> |                               |            |         |             |                     |      |                  |
| DNA Region                                 | BLAST Search Match            | % Identity | E value | Total Score | Aligned Length (bp) | % GC | Accession Number |
| rbcl                                       | <i>Dendrochilum glumaceum</i> | 100        | 0       | 2280        | 1264                | 44   | AF264164.1       |
| ITS  | <i>Coelogyne glumacea</i>     | 99.22      | 0       | 1127        | 649                 | 55   | MG788023.1       |
| matK                                       | <i>Dendrochilum glumaceum</i> | 99.77      | 0       | 1628        | 889                 | 31.8 | AF302696.1       |
| trnH-psbA                                  | <i>Coelogyne xyrekes</i>      | 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                       | <i>Dendroch ilum sp</i><br>DET001 | <i>Dendroch ilum sp</i><br>DET004 | <i>Dendroch ilum sp</i><br>DET005 | <i>Dendroch ilum sp</i><br>DET007 | <i>Dendroch ilum sp</i><br>DET008 | <i>Dendroch ilum sp</i><br>DET013 | <i>Dendroch ilum sp</i><br>DET015 | <i>Dendroch ilum sp</i><br>DET016 | <i>Dendroch ilum sp</i><br>DET018 |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| <i>Dendroch ilum sp</i><br>DET001 | 0                                 |                                   |                                   |                                   |                                   |                                   |                                   |                                   |                                   |
| <i>Dendroch ilum sp</i><br>DET004 | 0.1447                            |                                   |                                   |                                   |                                   |                                   |                                   |                                   |                                   |
| <i>Dendroch ilum sp</i><br>DET005 | 0.0806                            | 0.0951                            |                                   |                                   |                                   |                                   |                                   |                                   |                                   |
| <i>Dendroch ilum sp</i><br>DET007 | 0.1695                            | 0.1639                            | 0.2013                            |                                   |                                   |                                   |                                   |                                   |                                   |
| <i>Dendroch ilum sp</i><br>DET008 | 0.1529                            | 0.1247                            | 0.1011                            | 0.1965                            |                                   |                                   |                                   |                                   |                                   |
| <i>Dendroch ilum sp</i><br>DET013 | 0.1025                            | 0.0534                            | 0.1462                            | 0.2215                            | 0.0686                            |                                   |                                   |                                   |                                   |
| <i>Dendroch ilum sp</i><br>DET015 | 0.1209                            | 0.0862                            | 0.0558                            | 0.1224                            | 0.1109                            | 0.0346                            |                                   |                                   |                                   |
| <i>Dendroch ilum sp</i><br>DET016 | 0.0937                            | 0.0683                            | 0.1623                            | 0.2289                            | 0.0669                            | 0.119                             | 0.0425                            |                                   |                                   |
| <i>Dendroch ilum sp</i><br>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

| Sequence ID                        | Taxonomic Genus/Species      | DNA Barcoding Results                           |   |   |   | Final ID   |
|------------------------------------|------------------------------|---|---|---|---|--|
|                                    |                              | <i>rbcl</i>                                     | <i>matK</i>                               | <i>ITS</i>                                      | <i>TrnH-psbA</i>                                    |  |
| <i>Dendrochilum</i> _sp_DET00<br>1 | <i>Dendrochilum</i><br>sp.1  | <i>Coelogyne apoensis</i><br>(OR687508.1)       | <i>Coelogyne apoensis</i><br>(KT334200.1) | <i>Coelogyne apoensis</i><br>(KT334200.1)       | <i>Coelogyne apoensis</i><br>(KT334200.1)           | <i>Coelogyne apoensis</i><br>(T.Hashim.)<br>M.W.Chase & Schuit.                |
| <i>Dendrochilum</i> _sp_DET00<br>4 | <i>Dendrochilum</i><br>sp.2  | <i>Dendrochilum glumaceum</i><br>(AF264164.1)   | <i>Coelogyne glumacea</i><br>(MG788023.1) | <i>Dendrochilum glumaceum</i><br>(AF302696.1)   | <i>Coelogyne xyrekes</i><br>(KP694322.1)            | <i>Dendrochilum m</i> sp.1   |
| <i>Dendrochilum</i> _sp_DET00<br>5 | <i>Dendrochilum</i><br>sp.3  | <i>Dendrochilum glumaceum</i><br>(AF264164.1)   | <i>Coelogyne coccinea</i><br>(MG788061.1) | <i>Coelogyne coccinea</i><br>(MG788061.1)       | <i>Coelogyne trinervis</i><br>(KP694317.1)          | <i>Dendrochilum m</i> sp.2   |
| <i>Dendrochilum</i> _sp_DET00<br>7 | <i>Dendrochilum</i><br>sp.4  | <i>Dendrochilum glumaceum</i><br>(AF264164.1)   | <i>Coelogyne coccinea</i><br>(MG788061.1) | <i>Coelogyne coccinea</i><br>(MG788061.1)       | Discarded<br>(not used due to low sequence quality) | <i>Coelogyne coccinea</i><br>(H.A.Pederse n & Gravend.)<br>M.W.Chase & Schuit. |
| <i>Dendrochilum</i> _sp_DET00<br>8 | <i>Dendrochilum</i><br>sp.5  | <i>Dendrochilum m cobbianum</i><br>(AY381119.1) | <i>Coelogyne glumacea</i><br>(MG788023.1) | <i>Dendrochilum m glumaceum</i><br>(AF302696.1) | <i>Coelogyne xyrekes</i><br>(KP694322.1)            | <i>Dendrochilum m</i> sp.3   |
| <i>Dendrochilum</i> _sp_DET01<br>3 | <i>Dendrochilum</i><br>sp.7  | <i>Dendrochilum glumaceum</i><br>(AF264164.1)   | <i>Coelogyne glumacea</i><br>(MG788023.1) | <i>Dendrochilum m glumaceum</i><br>(AF302696.1) | <i>Coelogyne xyrekes</i><br>(KP694322.1)            | <i>Coelogyne glumacea</i><br>(Lindl.)<br>M.W.Chase & Schuit.                   |
| <i>Dendrochilum</i> _sp_DET01<br>5 | <i>Dendrochilum</i><br>sp.8  | <i>Dendrochilum m cobbianum</i><br>(AY381119.1) | <i>Coelogyne glumacea</i><br>(MG788023.1) | <i>Dendrochilum m glumaceum</i><br>(AF302696.1) | <i>Coelogyne xyrekes</i><br>(KP694322.1)            | <i>Dendrochilum m</i> sp.5   |
| <i>Dendrochilum</i> _sp_DET01<br>6 | <i>Dendrochilum</i><br>sp.9  | <i>Dendrochilum m cobbianum</i><br>(AY381119.1) | <i>Coelogyne glumacea</i><br>(MG788023.1) | <i>Dendrochilum m glumaceum</i><br>(AF302696.1) | <i>Coelogyne xyrekes</i><br>(KP694322.1)            | <i>Dendrochilum m</i> sp.6   |
| <i>Dendrochilum</i> _sp_DET01<br>8 | <i>Dendrochilum</i><br>sp.10 | <i>Dendrochilum m cobbianum</i><br>(AY381119.1) | <i>Coelogyne glumacea</i><br>(MG788023.1) | <i>Dendrochilum m glumaceum</i><br>(AF302696.1) | <i>Coelogyne xyrekes</i><br>(KP694322.1)            | <i>Dendrochilum m</i> sp.7   |

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