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# Molecular Characteristics of Lopang (*Gymnopetalum cochinchinense*) Originating from Riau Based on *matK* and *trnL-trnF Intergenic Spacer*

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**Abstract.** Lopang (*Gymnopetalum cochinchinense*) is an herbaceous plant traditionally consumed as a vegetable by communities in Riau Province, Indonesia. However, DNA barcode sequence data for this species remain limited. These findings underscore the importance of analyzing DNA barcode sequences of *matK* and *trnL-trnF intergenic spacer* (IGS) lopang from Riau. This study highlights the analysis of DNA barcode sequences *matK* and *trnL-trnF* IGS in lopang from Riau province. This study applies sampling, DNA extraction, Polymerase Chain Reaction (PCR), electrophoresis, sequencing, and bioinformatics data analysis using BioEdit 7, BLASTn (Basic Local Alignment Search Tool) to find sequence similarities with the GenBank database, Management and analyzed DNA sequences with MESQUITE, Multiple alignments using ClustalW and MEGA11 to create dendrograms**.** Fresh leaves were collected from Rokan Hulu Regency in Riau Province. The results showed that *matK* and *trnL-trnF* IGS DNA sequences of lopang measured 752 bp and 410 bp, respectively. BLASTn analysis revealed that lopang has 99.73% similarity with *G. chinense* based on the *matK* sequence and 99.76% similarity based on the *trnL-trnF* IGS sequence. The analysis revealed a variation of 13 nucleotides, 1 critical nucleotide and no indels in the *matK* sequence, while in the *trnL-trnF* IGS sequence there were 19 nucleotide variations, 1 critical nucleotide and 5 indels. As a final point, lopang from Riau is closely related to *G. chinense.* The findings of this research contribute to the molecular identification of this species and benefit science, such as providing an understanding of plant evolution, species identification, genetic analysis, and the development of molecular markers in the Cucurbitaceae family.

**Keywords**: *Gymnopetalum cochinchinense*; lopang; *matK*; Riau; *trnL-trnF intergenic spacer*.

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## INTRODUCTION

*Gymnopetalum cochinchinense* (Lour.) Kurz. is a fruit-bearing vegetable plant belonging to the family Cucurbitaceae, which includes 101 genera and around 1,000 species widely distributed in tropical regions (Turhadi et al., 2024). This plant is known by various local names, such as lopang in Sungai Bakah Village, Melawi Regency (Niconaus et al., 2023) and Riau, and Taw-Kin-Mon in Vietnam (Tin et al., 2020). According to Tin et al. (2020), *G. cochinchinense* is an annual, monoecious plant with a simple tendril plant. The leaves are five lobed with the blades changing shape into broad lines. Staminate and pistillate flowers are located in leaf axils, solitary. Epigynous flowers, white in color, approximately 5.0 cm in diameter when fully bloomed. The flower calyx is 5 lobed. The inner flower corolla is also 5 lobed. Three stamens, go deep into the swollen part of the calyx tube; 3 anthers are double, 1 monothecous, the other two dithecous. Inferior ovary, oval, 8-10 longitudinal stripes; long pistil stalk, three stigmas, each bifid. The

pepo fruit is ovate to oval, pointed at the top, with about 10 long ribs, and turns red when ripe, cracked. There are many seeds, oval shaped. Flowering and fruiting occur between July and December.

Lopang produces secondary metabolites known as cucurbitacins, which serve as a natural defense mechanism against pathogens. Lopang also exhibits significant bioactivities, including anti-cancer, anti-inflammatory, and anti-diabetic properties (Almeida *et al.* 2022). Lopang fruit has been utilized by local people in various ways, such as a vegetable valued for its high nutritional content, which amounts to 343.01 grams per 100 grams of fruit (Niconaus *et al.* 2023; Kumar *et al.* 2023).

The *Gymnopetalum* genus, found in the Malesia region, comprises three species, including *G. chinense* (Lour.) Merr., *G. orientale* W.J.de Wilde & Duyfjes, and *G. scabrum* (Lour.) W.J.de Wilde & Duyfjes, along with two varieties *G. scabrum* var. *scabrum* and *G. scabrum* var. *pectinatum*. However, de Wilde *et al.* (2015) reclassified *G. scabrum* var. *pectinatum* as a distinct species, thereby expanding the genus in the Malesia region to four species, including *G. chinense, G. oriantale, G. scabrum,* and *G. Pectinatum*. This genus is closely related to *Trichosanthes*, a distinguishing feature between these two genera is the presence of prominent probracts at the nodes, whereas the primary distinction between the two genera is found in the floral characteristics, specifically the presence or absence of a tuft on the flower calyx. *Gymnopetalum* genus flowers lack these tassels, and the overall shape of the folded petals in mature shoots differs as well. The *Trichosanthes* genus has short and round petals, while the *Gymnopetalum* genus has longer and wider petals. Molecular evidence shows that the *Gymnopetalum* genus is monophyletic with the *Trichosanthes* genus. Furthermore, phylogenetic data indicate that a character has evolved independently in genus *Hodgsonia* and genus *Trichosanthes* or *Gymnopetalum*, specifically the long tuft flower calyx (Pratami *et al.* 2019).

One of the molecular studies for identification and phylogenetic analysis is the DNA barcoding technique. This technique was developed to overcome the problem of species identification based on morphological characters. In plants, there is a high plasticity in their morphological characters to respond to environmental factors. This makes it difficult to draw clear boundaries between species within the same genus or family. In addition, this technique was developed to help and facilitate the identification of organisms for people who are not experts in the field of taxonomy, such as animal and plant quarantine employees, geneticists, ecologists, etc., as well as the limitations of morphological identification when the specimen is damaged or lacking some parts (Jannah *et al.* 2021).

Some DNA barcodes that can be used in plants are *internal transcribed spacer* (ITS), protein coding genes (*matK, rbcL, rpoC1*, and *rpoB*) and non-coding regions in the chloroplast genome (*trnL-trnF IGS*, *psbK-psbI IGS*, *trnH-psbA IGS*, and *rpl16 intron*). The maturase K (*matK*) and ribulose 1,5-biphosphate carboxylase/oxygenase large subunit *(rbcL)* genes are DNA barcodes that have been developed and standardized for DNA barcoding in seed plants (Candramila *et al.* 2023).

The *matK* gene encodes the K sub-unit maturase enzyme, which plays a role in cutting introns from RNA transcripts of several genes in the plant chloroplast genome (Barthet *et al*., 2020). Generally, the length of the nucleotide sequence of the *matK* gene is approximately 1500 bp (Sundari *et al.* 2022). This gene has high resolution and more varied sequences, so that is considered better and more accurate in identifying plants and can distinguish at the species level (Antil *et al.* 2023). The *matK* gene has been used as a DNA barcode in Metroxylon sago and other palms (Abbas *et al.* 2020), Durio species on Ternate Island (Sundari *et al.* 2022), and phylogenetic analysis of *Baccaurea* spp. In West Sumatera (Saswita *et al.* 2023) and *Coelogyne* spp. (Pratiwi *et al.* 2023), classification of jewel orchid accessions in Vietnam (Ho *et al.* 2021), distinguishing species from the families of Solanaceae and Fabaceae (Herman *et al.* 2023), and identification of *Momordica* (Cucurbitaceae) species from the Indian subcontinent (Ramesh *et al.* 2022).

Another DNA region that has also been widely used in plants is *trnL-trnF* IGS. The region does not encode protein, and there is a space between two genes, namely *trnL* (UAA) and *trnF* (GAA) genes. Compared with *matK* and *rbcL,* this gene is highly mutated with significant variation among plants, making it better for distinguishing species across several plant genera, and it is easier and more preferred for identifying and discovering new species. The *trnL-trnF* region of IGS has been widely applied to several plants, such as *Scopellaria marginata* from East Java, Indonesia

(Turhadi *et al.* 2024), Tunisian date palm (*Phoenix dactylifera* L.) (Soumaya *et al.* 2023), and Turkish sweet corn (*Zea mays* var. *saccharata*) (Filiz *et al.* 2024). This study aims to characterize lopang from Riau based on the *matK* and *trnL-trnF IGS* sequences, which are expected to provide benefits in science, such as providing an understanding of plant evolution, species identification, genetic analysis, and the development of molecular markers in the Cucurbitaceae family. In addition, it is expected to provide benefits to the community, such as plant breeding, biodiversity conservation, sustainable agriculture, and identification of plant products.

## METHOD

### DNA Extraction

Fresh leaves of lopang (*Gymnopetalum cochinchinense*) were collected from Rokan Hulu Regency, Riau Province, Indonesia. For DNA amplification, the following primer pairs were used: matK-413F-1 (5'-TAA TTT ACR ATC AAT TCA TTC AAT ATT TCC-3') and matK-1227R-3 (5'-GAR GAT CCR CTR TRA TAA TGA AAA AGA TTT-3') for *matK* amplification, and B49317_F2 (5'-CGA AAT CGG TAG ACG CTA CG-3') and A50272_R3 (5'-ATT TGA ACT GGT GAC ACG AG-3') for *trnL-trnF* IGS amplification (Roslim et al., 2023).

Approximately 0.5 g of fresh lopang leaves was cut with sterilized scissors and ground using a mortar and pestle in liquid nitrogen until a fine powder was obtained. The powder was transferred to a 1.5 mL microtube, and DNA extraction was performed using the Genomic DNA Mini Kit for Plants (Geneaid), following the manufacturer's protocol. DNA quality was checked using electrophoresis on a 1% agarose gel in 1× TBE buffer at 50 volts for 45 minutes.

### Polymerase Chain Reaction

PCR reactions were prepared in a 50 µL total volume containing: 1× PCR buffer with $Mg^{2+}$, 0.2 mM dNTPs, 2.4 µM of each primer, 2 U of DreamTaq DNA polymerase (Thermo Scientific), 1 ng of total DNA, and dd$H_2O$ to adjust the final volume. PCR was conducted with the following thermal profile: initial denaturation at 95°C for 3 minutes, then 35 cycles of: denaturation at 95°C for 45 seconds, annealing at 49.2°C for *trnL-trnF IGS* and 47.5°C for *matK* for 45 seconds, extension at 72°C for 90 seconds, then final extension at 72°C for 10 minutes (Roslim et al., 2023).

### Electrophoresis

PCR products were analyzed by electrophoresis on a 1% agarose gel in 1× TBE buffer containing 5 µg/mL ethidium bromide, run at 50 volts for 45 minutes. DNA bands were visualized under UV light using a UV transilluminator (WiseUV WUV-M20, Daihan Scientific) and photographed with a digital camera (Olympus SP-500 UZ). PCR products were then purified and sent to PT Genetika Science (Jakarta, Indonesia) for sequencing at 1st BASE (Malaysia).

### Data analysis

Sequence data were analyzed following the protocol by Herman et al. (2023). Forward and reverse reads were aligned using BioEdit v7.0.0 (Hall, 1999). The sequences were compared to the GenBank database using BLASTn (https://blast.ncbi.nlm.nih.gov). The top 10 matching sequences were selected for comparison. Multiple sequence alignment was conducted using ClustalW (Thompson *et al*., 1994). Phylogenetic analysis was performed using the Neighbor-Joining (NJ) method in MEGA11 (Tamura et al., 2021). Bootstrap analysis with 1,000 replicates (Felsenstein, 1985) was used to evaluate the confidence level of the resulting tree branches.

## RESULTS

### *Gymnopetalum cochinchinense* description

Characteristics of *G. cochinchinense* according to Tin *et al.* (2020) are an annual, monoecious, simple tendril plant (Figure 1a). The leaves are five lobed with the blades changing shape into broad lines (Figure 1b). Staminate (Figure 1e) and pistillate (Figure 1d) flowers are located in leaf axils, solitary. The pepo fruit is ovate to oval, pointed at the top, with about 10 long ribs, and turns red when ripe, cracked (Figure 1g). There are many seeds, oval shaped (Figure 1j). Flowering and fruiting occur between July and December.

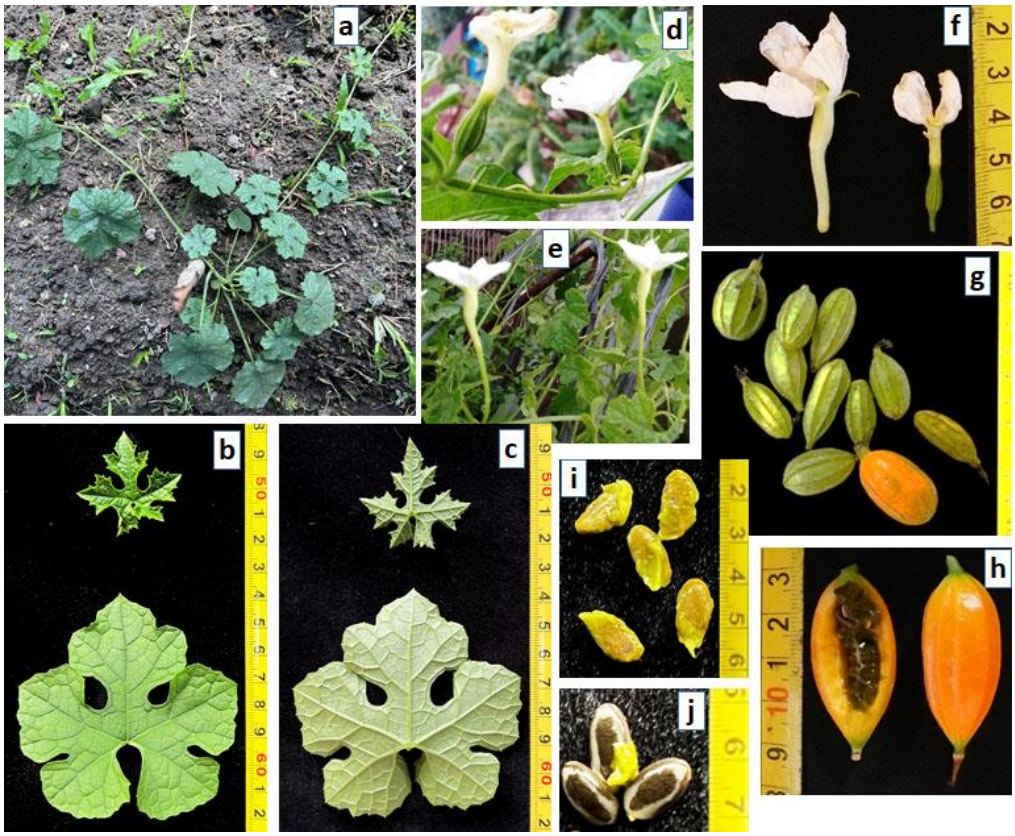


**Figure 1.** Lopang (*Gymnopetalum cochinchinense* (Lour.) Kurz.) from Riau. (a) Habitus, (b) upper and (c) lower leaf surfaces, (d) pistillate flowers, (e) staminate flowers, (f) staminate and pistillate flowers, (g) young fruit (green) and mature or ripe fruit (orange), (h) longitudinal section of ripe fruit with black seeds, (i) seeds from young fruit, and (j) seeds from ripe fruit (personal documentation).

The genus *Gymnopetalum* is closely related to *Trichosanthes*, with an intermediate trait between the two genera being the presence of conspicuous probracts at the nodes. The key distinction lies in their floral morphology, particularly the presence or absence of tufts on the flower calyx. *Gymnopetalum* flowers lack these tufts and have longer, wider petals, while *Trichosanthes* flowers are short and rounded (de Boer et al., 2012). Phylogenetic analysis based on the *matK* gene showed that *G. integrifolium* and four *Trichosanthes* species are grouped together, supported by a 71% bootstrap value, reinforcing the genetic relationship between the two genera. Notably, *G. integrifolium* is also referred to as *Trichosanthes integrifolia* and *Trichosanthes scabra*.

*Trichosanthes* species are widely distributed across southern, southeastern, and eastern Asia, as well as Australia and the Pacific Islands. It is the largest genus in the Cucurbitaceae family, consisting of over 100 species. Its typical features include being perennial, dioecious, and climbing with tendrils, and having bright red, ovate fruits. *G. orientale* resembles *Trichosanthes* due to the occasional presence of ridges on the nodes. Molecular data indicate that 60% of *Trichosanthes* species analyzed are monophyletic with *Gymnopetalum* (Liu et al., 2021). Moreover, the long-tasseled corolla evolved independently in *Hodgsonia* and *Trichosanthes*, and was lost in several *Gymnopetalum* species, coinciding with a shift from nocturnal to diurnal flowering in *G. scabrum*, *G. chinense*, and *G. tubiflorum* (Boer et al., 2012).

### Sequence Analysis of *matK* of Lopang (*Gymnopetalum cochinchinense*) from Riau

Two sequences were obtained (Figure 2) and submitted to the GenBank Nucleotide database with accession numbers OQ174523 and OQ174524. All of the *matK* gene products are about 752 bp of the chloroplast plastid region of the *G. cochinchinense* species, without any differences in nucleotides obtained.

>OQ174523 | Gymnopetalum cochinchinense DIR099
maturase K (matK) gene, partial cds; chloroplast

ATGTGTCAGATGTATTAATACCCTATCCCCTCCATCTGGAAATTTTAG
TTCAAATCCTTCGCTCCTGGGTGAAAGATGCCTCTTCTTTTCATTTAT
TACGGTTCTTTTTTCACGAGTATTGTAATTGGAATAGTCTTAGTACTT
CAAAAAAATTGATTTCTTTTTTTTCAAAAAGAAATCGAAGATTAGTCT
TGTTCCTATATAATTCTTATGTATGTGAATACGAATCCATTTTCCTTT
TTCTACGTAACCAATCTTCTCATATACGATTAACTTCTTATAGGGGCC
TTTTTGAGCGAATATATTTCTATGGAAAAATCGAACATCTTGTCAAAG
TGTTTGCTAATTATTTTTCGGCTATCTTACGGGTCTTCAAGGATCCTT
TCATGCATTATGTTAGATATCAAGGAAAATCAATTCTGGTTTCAAAAG
ATACGCCACTTCTGATGAATAAGTGGAAATATTACCTTGTCAATTTAT
GGCAATGTCATTTTTATGTGTGGTCACAACCAGAAAGGATCTATATAA
ACCAATTATCCAAGCGTTCTCTTTACTTTTTGGGCTATATTTCAAGTG
TGCGACTAAATACTTCAGTGGTATGGAGTCAGATGCTAGAAAATTCAT
TTCTAATAGATAATGCTACGAAGAAACTCGATACACTAGTTCCTATTA
TTACTCTGCTTGGATCATTGGCTAAAGCGAAATTTTGTAACGTGTTAG
GGCATCCCATTAGTAAGACGACCTGGATCGAT

>OQ174524 | Gymnopetalum cochinchinense DIR100
maturase K (matK) gene, partial cds; chloroplast

ATGTGTCAGATGTATTAATACCCTATCCCCTCCATCTGGAAATTTTAG
TTCAAATCCTTCGCTCCTGGGTGAAAGATGCCTCTTCTTTTCATTTAT
TACGGTTCTTTTTTCACGAGTATTGTAATTGGAATAGTCTTAGTACTT
CAAAAAAATTGATTTCTTTTTTTTCAAAAAGAAATCGAAGATTAGTCT
TGTTCCTATATAATTCTTATGTATGTGAATACGAATCCATTTTCCTTT
TTCTACGTAACCAATCTTCTCATATACGATTAACTTCTTATAGGGGCC
TTTTTGAGCGAATATATTTCTATGGAAAAATCGAACATCTTGTCAAAG
TGTTTGCTAATTATTTTTCGGCTATCTTACGGGTCTTCAAGGATCCTT
TCATGCATTATGTTAGATATCAAGGAAAATCAATTCTGGTTTCAAAAG
ATACGCCACTTCTGATGAATAAGTGGAAATATTACCTTGTCAATTTAT
GGCAATGTCATTTTTATGTGTGGTCACAACCAGAAAGGATCTATATAA
ACCAATTATCCAAGCGTTCTCTTTACTTTTTGGGCTATATTTCAAGTG
TGCGACTAAATACTTCAGTGGTATGGAGTCAGATGCTAGAAAATTCAT
TTCTAATAGATAATGCTACGAAGAAACTCGATACACTAGTTCCTATTA
TTACTCTGCTTGGATCATTGGCTAAAGCGAAATTTTGTAACGTGTTAG
GGCATCCCATTAGTAAGACGACCTGGATCGAT

**Figure 2.** Sequence of *matK* lopang (*Gymnopetalum cochinchinense*) from Riau

BLASTn analysis shows that *matK* lopang sequence has high similarity to *G. chinense* species with 99.73% similarity, 100% query cover value, and zero E-value, meanwhile the lowest similarity, 99.20%, was observed between two species of the genus *Trichosanthes*, namely *T. dunniana* and *T. rosthornii,* with 100% query cover value and zero *E-value* (Table 1).

**Table 1.** BLASTn analysis of *matK* sequence of lopang (*Gymnopetalum cochinchinense*) from Riau

| Description | Family | Query Cover (%) | E-value | Per. Ident (%) | Accession |
|---|---|---|---|---|---|
| *Gymnopetalum chinense* | Cucurbitaceae | 100 | 0.0 | 99.73 | NC_072506.1 |
| *Trichosanthes nervifolia* | Cucurbitaceae | 100 | 0.0 | 99.34 | NC_046883.1 |
| *Trichosanthes dafangensis* | Cucurbitaceae | 100 | 0.0 | 99.34 | NC_072515.1 |
| *Trichosanthes pubera subsp. rubriflos* | Cucurbitaceae | 100 | 0.0 | 99.34 | NC_072514.1 |
| *Trichosanthes pedata* | Cucurbitaceae | 100 | 0.0 | 99.34 | NC_072509.1 |
| *Trichosanthes subrosea* | Cucurbitaceae | 100 | 0.0 | 99.34 | NC_072508.1 |
| *Trichosanthes cordata* | Cucurbitaceae | 100 | 0.0 | 99.34 | MZ395840.1 |
| *Gymnopetalum integrifolium* | Cucurbitaceae | 100 | 0.0 | 99.34 | DQ536683.1 |
| *Trichosanthes dunniana* | Cucurbitaceae | 100 | 0.0 | 99.20 | MZ427970.1 |
| *Trichosanthes rosthornii* | Cucurbitaceae | 100 | 0.0 | 99.20 | MT211650.1 |

**Table 2.** Nucleotide differences in the *matK* sequence from the Cucurbitaceae family

| Accessions | Nucleotide number (vertically)* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 4 | 1 0 9 | 1 6 5 | 2 0 1 | 2 2 0 | 2 5 7 | 3 0 2 | 3 3 7 | 3 5 4 | 4 0 4 | 5 5 3 | 6 4 8 | 6 8 7 |
| *Gymnopetalum cochinchinense DIR099* | T | C | T | **A** | T | T | T | **T** | T | T | T | A | A |
| *Gymnopetalum cochinchinense DIR100* | . | . | . | **.** | . | . | . | **.** | . | . | . | . | . |
| *Gymnopetalum chinense* | . | . | . | **C** | . | . | . | **.** | . | . | G | . | . |
| *Trichosanthes nervifolia* | . | G | G | **C** | . | . | . | **C** | . | . | . | C | . |
| *Trichosanthes dafangensis* | C | . | G | **C** | C | . | . | **A** | . | . | . | . | . |
| *Trichosanthes pubera subsp. rubriflos* | . | G | G | **C** | . | . | . | **C** | . | . | . | C | . |
| *Trichosanthes pedata* | . | G | G | **C** | . | . | . | **C** | . | . | . | C | . |
| *Trichosanthes subrosea* | . | G | G | **C** | . | . | . | **C** | . | . | . | C | . |
| *Trichosanthes cordata* | C | . | G | **C** | . | . | G | **A** | . | . | . | . | . |
| *Gymnopetalum integrifolium* | . | . | G | **C** | . | . | . | **C** | G | G | . | . | . |
| *Trichosanthes dunniana* | C | . | G | **C** | . | . | G | **A** | . | . | . | . | . |
| *Trichosanthes rosthornii* | C | . | G | **C** | . | A | . | **A** | . | . | . | . | G |

(*) The nucleotide numbers arranged vertically represent the nucleotide positions referencing lopang from Riau (*Gymnopetalum cochinchinense DIR099*). A dot (.) indicates that the nucleotide at a specific position is identical to the nucleotide of lopang from Riau (*G. cochinchinense DIR099*). Nucleotides in boxes and bold are critical nucleotides for identification of lopang from Riau (*G. cochinchinense DIR099*).

There are 13 nucleotide variations in the *matK* sequence among the accessions studied. The nucleotide at position 201 is a critical nucleotide, and it's important for distinguishing lopang from other accessions studied. In this position, lopang has nucleotide A (adenine) while other accessions have nucleotide C (cytosine) (Table 2).

Dendogram based on *matK* sequence shows that two lopang individuals studied are in the same group with *G. chinense* with 89% bootstraps value and are separated from the other accessions. These results indicate that the lopang plant from Riau is more closely related to *G. chinense* than other species from the Cucurbitaceae family studied (Figure 3).

### Sequence Analysis of *trnL-trnF* Intergenic Spacer Lopang (*Gymnopetalum cochinchinense*) from Riau

The stranded *trnL-trnF* IGS sequence of lopang obtained in this study is 410 bp in length. This sequence has been registered in GenBank with accession number OQ174520 (Figure 4).

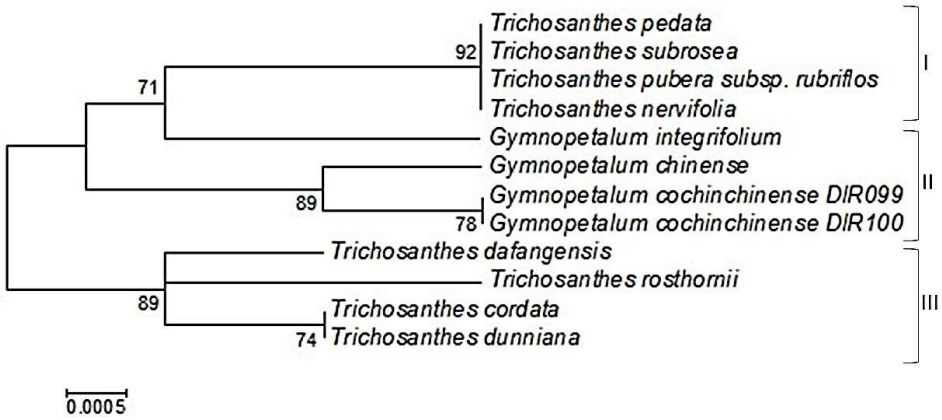


**Figure 3.** Dendogram based on *matK* sequences using the Neighbor Joining method with 1000 times bootstraps.

```
>OQ174520 | Gymnopetalum cochinchinense DIR118
tRNA-Leu (trnL) gene, partial sequence; trnL-
trnF intergenic spacer, complete sequence; and
tRNA-Phe (trnF) gene, partial sequence;
chloroplast
CAAGTCCCTCTATCCCCAAAACCCTAAAAAGGCCCGTTGGCCTCTTTA
ATTATTTCTCCTTTCATTAGCAATTCACAATTCGTTATGTTTCTCATT
CATTCGACTCTTTCACAAGCGTATCTTAGCGGAAATTTTATTTCTTAT
CACAAGACTTGTGATATATATTAGATATGATACACGTACAAACGAACA
TCCTTGCGCAAGGAATCCCCGTTGTTAAATTTGAATGATTAACAATAC
AATACTGTCTACTGTACTGAAACTTCCAAAGTCTTATCCAAGCCCTGA
AATTTCGTGGATCTTCAAAAAGAAGACTTTGGAATACCTTTTTTCTTA
TTTACAATTGACATAGACCAAAGTCATCTATTAAAATAAGGATAATGT
GTCGGAAATGGCCGGGATAGCTCAGT
```

**Figure 4.** Lopang *trnL-trnF IGS* sequence from Riau

BLASTn analysis shows that the *trnL-trnF* IGS lopang sequence has high similarity with *G. chinense* species with 99.76% similarity, 100% query cover value, and zero E-value meanwhile, the lowest similarity, 97.07%, was observed between two species of the genus *Trichosanthes*, namely *T. dafangensis and T. pedata,* with 100% query cover value and zero E-value (Table 3).

Analysis *trnL-trnF* IGS sequence shows that there are 19 nucleotide variations consisting of 14 substitutions and 5 indels. The nucleotide at position 56 is a critical nucleotide for distinguishing the Riau-origin lopang from the other accessions analyzed, where at this position, lopang from Riau has nucleotide C (cytosine) while other accessions have nucleotide A (adenine) (Table 4).

**Table 3.**BLASTn analysis of the *trnL-trnF* IGS sequence of lopang (*Gymnopetalum cochinchinense*) from Riau

| Description | Family | Query Cover (%) | E value | Per. Ident (%) | Accession Number |
|---|---|---|---|---|---|
| *Gymnopetalum chinense* | Cucurbitaceae | 100 | 0.0 | 99.76 | NC_072506.1 |
| *Trichosanthes reticulinervis* | Cucurbitaceae | 100 | 0.0 | 97.56 | NC_072512.1 |
| *Trichosanthes dunniana* | Cucurbitaceae | 100 | 0.0 | 97.56 | MZ427957.1 |
| *Nothoalsomitra suberosa* | Cucurbitaceae | 100 | 0.0 | 97.32 | NC_046876.1 |
| *Trichosanthes truncata* | Cucurbitaceae | 100 | 0.0 | 97.32 | NC_046875.1 |
| *Trichosanthes kirilowii* | Cucurbitaceae | 100 | 0.0 | 97.32 | NC_041088.1 |
| *Trichosanthes schlechteri* | Cucurbitaceae | 100 | 0.0 | 97.32 | NC_072511.1 |
| *Trichosanthes smilacifolia* | Cucurbitaceae | 100 | 0.0 | 97.32 | NC_072510.1 |
| *Trichosanthes dafangensis* | Cucurbitaceae | 100 | 0.0 | 97.07 | NC_072515.1 |
| *Trichosanthes pedata* | Cucurbitaceae | 100 | 0.0 | 97.07 | NC_072509.1 |

**Table 4.** Nucleotide variations in *trnL-trnF intergenic spacer* sequences

| | Nucleotide number (vertically)* | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Accessions** | **41** | **42** | **56** | **68** | **69** | **102** | **122** | **135** | **158** | **168** | **188** | **209** | **241** | **242** | **243** | **244** | **245** | **267** | **361** |
| *Gymnopetalum cochinchinense DIR118* | C | C | **C** | G | C | G | G | T | A | G | T | C | A | A | T | A | C | C | C |
| *Gymnopetalum chinense* | . | . | **A** | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *Trichosanthes reticulinervis* | . | . | **A** | . | A | T | T | . | . | T | . | . | - | - | - | - | - | . | . |
| *Trichosanthes dunniana* | . | . | **A** | . | A | T | T | . | . | T | . | . | - | - | - | - | - | . | . |
| *Nothoalsomitra suberosa* | G | . | **A** | . | . | T | T | . | . | T | . | A | - | - | - | - | - | . | . |
| *Trichosanthes truncata* | . | . | **A** | . | A | T | T | G | . | T | . | . | - | - | - | - | - | . | . |
| *Trichosanthes kirilowii* | . | T | **A** | . | . | T | T | . | . | T | G | . | - | - | - | - | - | . | . |
| *Trichosanthes schlechteri* | . | . | **A** | . | . | T | T | . | G | T | . | . | - | - | - | - | - | . | A |
| *Trichosanthes smilacifolia* | . | . | **A** | . | A | T | T | G | . | T | . | . | - | - | - | - | - | . | . |
| *Trichosanthes dafangensis* | . | . | **A** | T | A | T | T | G | . | T | . | . | - | - | - | - | - | . | . |
| *Trichosanthes pedate* | . | T | **A** | . | . | T | T | . | . | T | G | . | - | - | - | - | - | T | . |

(*) The nucleotide numbers arranged vertically represent the nucleotide positions referencing lopang from Riau (*Gymnopetalum cochinchinense DIR118*). A dot (.) indicates that the nucleotide at a specific position is identical to the nucleotide of lopang from Riau (*G. cochinchinense DIR118*). Nucleotides in boxes and bold are critical nucleotides for identification of lopang from Riau (*G. cochinchinense DIR118*).

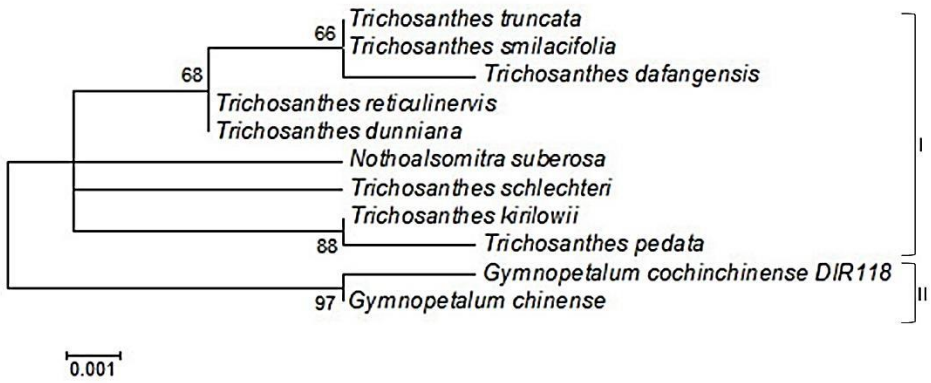


**Figure 5.** Dendogram based on *trnL- trnF intergenic spacer* sequence using the Neighbor Joining method with 1000 times bootstraps.

The dendrogram based on the *trnL-trnF IGS* sequence indicates that the studied lopang (*G. cochinchinense* DIR118) clusters closely with *G. chinense* with a 97% bootstrap value and is distinct from other accessions (Figure 5). These results suggest that lopang from Riau is more closely related to *G. chinense* than to other species in the Cucurbitaceae family.

Comparison of *matK* and *trnL-trnF IGS* sequence similarity (Tables 1 and 3) revealed high similarity between lopang from Riau and *G. chinense*, with 99.73% and 99.76% identity, respectively. According to Andariyusti & Roslim (2021), reliable species identification using BLASTn requires 100% identity and query coverage, and an E-value of zero. No GenBank accession matched the lopang sequences at 100%, suggesting that barcode sequences of *G. chinense* may not yet be available in GenBank. Thus, this study provides the first reported sequences for this species.

Both *matK* and *trnL-trnF IGS* markers effectively distinguish lopang from Riau from other species, owing to sequence variation caused by mutations. These mutations result in critical nucleotide differences that support identification and phylogenetic studies. Thirteen nucleotide variations (1 critical) and no indels were found in *matK*, while *trnL-trnF IGS* showed 19 variations (1 critical) and 5 indels (Tables 2 and 4). The higher variability in the non-coding *trnL-trnF IGS* region, compared to the coding *matK* region, underscores its usefulness in evolutionary and species differentiation studies (Herman et al., 2023). *Gymnopetalum cochinchinense* and *G. chinense* are grouped together with bootstrap values of 89% (*matK*) and 97% (*trnL-trnF IGS*), affirming their genetic relatedness. A bootstrap value above 70% is considered reliable (Afrianti et al., 2023), with >85% indicating strong support (Ho et al., 2021). These results confirm the usefulness of *matK* and *trnL-trnF IGS* markers in *Gymnopetalum* taxonomy, supporting conservation and molecular identification efforts..

## CONCLUSION

The *matK* and *trnL-trnF IGS* sequences obtained from lopang (*G. cochinchinense*) from Riau measured 752 bp and 410 bp, respectively. The *trnL-trnF IGS* region exhibited greater nucleotide variation. Lopang from Riau shows a close genetic relationship to *G. chinense*. This study provides the first DNA barcode data for this plant and highlights the utility of *trnL-trnF IGS* in taxonomic studies within *Gymnopetalum* and Cucurbitaceae. In the future, we also suggest the use of *trnL-trnF IGS* for analysis to solve taxonomic problems in the genus *Gymnopetalum* or family Cucurbitaceae.

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## AUTHOR CONTRIBUTION STATEMENT

DIR & H led the analysis and manuscript writing. DIR & H supervised the project. WL & A contributed to data modeling. FFHA, CWS, and N assisted with data analysis and interpretation. AAFA contributed to the English translation and proofreading.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that no AI tools were used in the generation or analysis of this manuscript. All work was conducted manually by the authors.

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