

# Identification of the Scientific Name of *Curculigo Orchioides* from Kon Ka Kinh National Park in Vietnam, using Morphological and DNA Barcode Methods

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**Abstract:** *Curculigo orchioides* is a perennial plant widely used in traditional medicine for its kidney-tonifying, yang-strengthening, warming, dampness-drying, blood-stagnation-dispersing, paralysis-relieving, and tendon-strengthening effects. In Vietnam, there are 7–8 species of *Curculigo*, some of which are also called *Curculigo orchioides* but do not possess aphrodisiac properties. This study aims to avoid confusion and accurately identify the species of *Curculigo orchioides* collected from Kon Ka Kinh National Park using morphological and DNA barcoding methods.

Morphological observations focused on describing the stem, leaves, flowers, and fruits. The results showed that *Curculigo orchioides* have a short stem covered by overlapping leaf sheaths, with a brown outer layer and a white inner layer. The leaves are sword-shaped with parallel veins, measuring 20–30 cm long and 2–2.5 cm in width. The flowers are yellow, arranged in clusters of 3–5 petals on a short axis at the leaf axils. The capsule fruit is elongated, measuring 1.5–5 cm in length and 0.5–1.1 mm in width, containing 1–4 seeds. The seeds are black, hard, and polyhedral.

DNA barcoding analysis revealed clear, intact DNA bands in electrophoresis images. The sequencing results of the *rbcL* gene segment showed 100% similarity (547/547 nucleotides) to *Curculigo orchioides* Gaertn., as recorded in the genetic database. Based on morphological characteristics and DNA barcoding, the scientific name of the *Curculigo orchioides* species found in Kon Ka Kinh National Park was identified as *Curculigo orchioides* Gaertn., belonging to the family Hypoxidaceae.

**Keywords:** *Curculigo orchioides*, DNA barcode, Scientific Name, Kon Ka Kinh national park.

## 1. INTRODUCTION

*Curculigo orchioides* originates from tropical and subtropical forests of Asia. It has been documented in the flora of several countries for a long time at the species classification level. According to some references, *Curculigo orchioides* are distributed in India, Sri Lanka, China, Japan, Australia, and Southeast Asian countries such as Vietnam, Laos, Thailand, and Malaysia (Asif, 2010).

This plant is also known as "Golden Eye Grass" or "Black Musli." It is a small-sized herbaceous species with rhizomes and thrives in shaded forest environments. It grows well in fertile and moist lowland soils, from sea level up to 2,300 meters in elevation, particularly in rocky crevices and lateritic, humid soils (Ajit, 2012).

The genus *Curculigo* Gaertn. comprises herbaceous species primarily distributed in tropical and subtropical regions of Asia. In Vietnam, there are 7–8 species, among which *Curculigo orchioides* is the smallest. It commonly grows in grassy hills, humid and

cool environments, midland and mountainous provinces such as Lai Châu, Tuyên Quang, Cao Bằng, Hà Giang, and the Central Highlands (Kon Tum, Gia Lai, Đắk Lắk, Lâm Đồng, Đắk Nông), extending to southern provinces like Bà Rịa-Vũng Tàu and An Giang. *Curculigo orchioides* prefers humid, well-lit, and partially shaded environments, often found in relatively fertile soils in valleys, limestone foothills, or near cultivated fields. The plant thrives during the rainy season, with its primary rhizome penetrating deep into the soil, producing flowers and fruits annually. When mature, the fruit dehisces naturally, dispersing seeds around the plant (Do, H. B. *et al.*, 2004). In traditional Chinese and Vietnamese medicine, decoctions of *Curculigo orchioides* rhizomes are used as tonics to restore health and treat body weakness, lower back pain, arthritis, and chronic nephritis. It is also known to increase blood pressure and regulate menstruation. In India, Nepal, and the Philippines, *Curculigo orchioides* is used as a diuretic, aphrodisiac, and treatment for skin diseases, peptic ulcers, hemorrhoids, gonorrhea, leucorrhea, asthma, jaundice, diarrhea, and headaches.

To accurately identify and differentiate *Curculigo orchioides* for breeding research, genetic selection, and

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the development of propagation and cultivation protocols ensuring species authenticity, we conducted a scientific identification study using morphological and molecular marker methods for *Curculigo orchoides* collected from Kon Ka Kinh National Park, Gia Lai Province.

## 2. RESEARCH METHODS

### 2.1. Materials, Location, and Research Timeframe

**Research Materials:** Fresh young leaf samples of *Curculigo orchoides*, labeled as sample DV-SC.050723 were collected from Kon Ka Kinh National Park for DNA extraction and gene sequencing analysis.

**Research Location:** Research Centre for Cultivating and Processing of Medicinal Plants

**Research Timeframe:** January 2023 – December 2023.

### 2.2. Research Methods

#### 2.2.1. Morphological Description Method

Observation, analysis, and recording of morphological characteristics, including stem, leaves, flowers, fruits, and seeds, following the methodology of (Nguyen, N. T., 2007). A camera was used to capture detailed images of plant parts.

- **Stem:** Observed stem color and measured stem diameter using a micrometer (cm).
- **Leaves:** Observed leaf color and shape and measured leaf length and width (cm).
- **Flowers:** Observed flower color and morphology.
- **Fruits and Seeds:** Observed color and shape.

#### 2.2.2. Total DNA Extraction and Purification

Total DNA was extracted from fresh leaves using an improved CTAB extraction and purification method (Doyle & Doyle, 1990).

#### 2.2.3. DNA Amplification by PCR

The rbcL gene fragment, approximately 600 bp in length, was amplified using the primer pair: rbcLa-F: 5'-ATGTCACCACAAACAGAGACTAAAGC-3' (Levin *et al.*, 2003) and rbcLa-R: 5'-GTAAAATCAAGTCCACCRG-3' (Kress & Erickson, 2007)

### PCR Reaction Components

The PCR reaction was performed using the Platinum II Taq Hot-Start DNA Polymerase Kit (Invitrogen, USA). The reaction mixture (50 µL total volume) contained: 10 µL of 5× Platinum™ Buffer, 1 µL of 10 mM dNTP mix, 1 µL of 10 µM rbcL F primer, 1 µL of 10 µM rbcL R primer, 10 µL of Platinum™ GC Enhancer, 2 µL of extracted DNA, 0.4 µL of Platinum™ II Taq Hot-Start DNA Polymerase, Nuclease-free water to a final volume of 50 µL

**PCR Thermal Cycling Conditions,** Initial denaturation: 95°C for 5 minutes, 35 cycles of: 95°C for 30 seconds (denaturation), 60°C for 30 seconds (annealing), 72°C for 30 seconds (extension), Final extension: 72°C for 5 minutes, Storage: 10°C for 20 minutes

#### 2.2.4. DNA Electrophoresis on Agarose Gel

After extraction and purification, DNA was analyzed by electrophoresis on a 2% agarose gel. Following electrophoresis, the gel was stained with Redsafe dye (Bioscience, UK), and the results were recorded.

#### 2.2.5. PCR Product Purification and Sequencing

The PCR products were purified using the Wizard SV Gel and PCR Clean-up System Kit (Promega). After purification, the PCR products were sent to Phu Sa Biochem for sequencing using the Sanger method (Sanger *et al.*, 1977).

#### 2.2.6. Data Analysis and DNA Sequence Comparison

The molecular weight of the DNA fragments was calculated using Gel Analyzer software. The sequencing results were stored in FASTA format and analyzed using the latest version of BioEdit 7.0.5 (Hall, 1999). Species identification was performed using the BLAST algorithm on the NCBI GenBank database.

## 3. RESULTS AND DISCUSSION

### 3.1. Morphological Analysis of *Curculigo Orchoides*

*Curculigo orchoides* is a perennial herbaceous plant belonging to the monocotyledonous group of angiosperms. It withers in winter and resumes growth in the following spring and summer. The plant has a short stem covered by overlapping leaf sheaths. The plant height ranges from 40 to 60 cm, with 3–5 branches, and the stem diameter varies between 1.4 and 2.0 cm.

The leaves are folded, sword-shaped, with green sheaths and narrow, lanceolate leaf blades. The upper surface has sparse hairs, while both surfaces are nearly the same color, with parallel venation. Each stem bears 10–13 leaves, measuring 20–30 cm in length and 2–2.5 cm in width, with petiole lengths ranging from 5 to 10 cm.

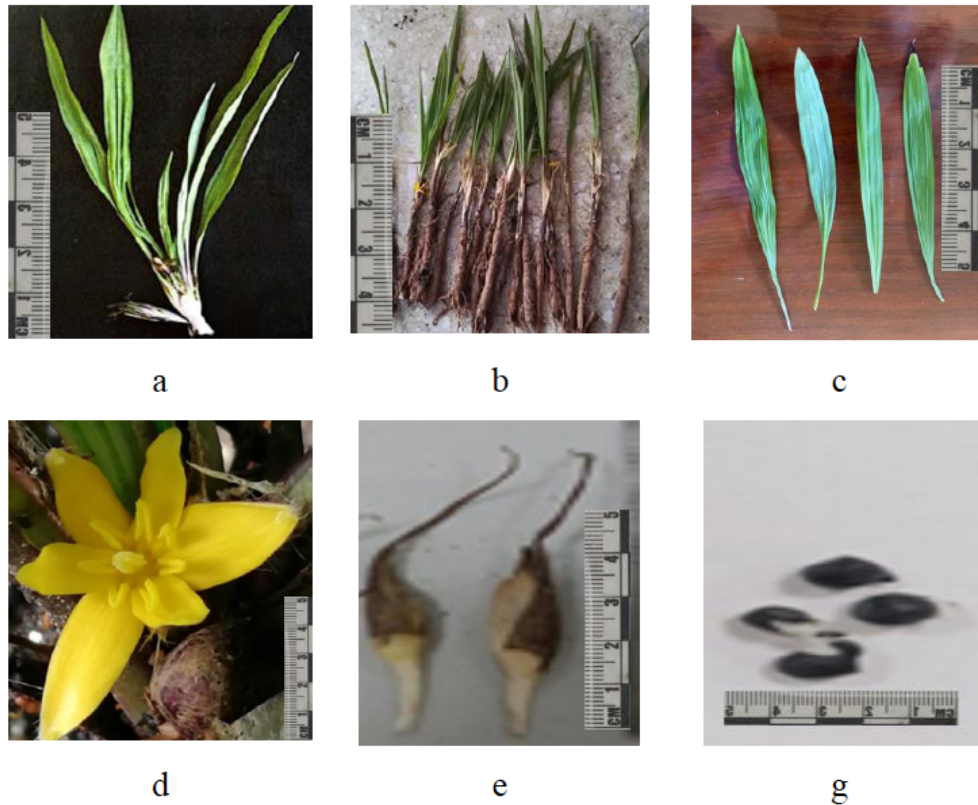
The flowers are yellow and arranged in clusters of 3–5 on a short axis at the leaf axils, enclosed within overlapping ovate bracts. The pedicels are 1–5 cm long, covered with hairs. Each small flower has six tepals: three hairy sepals and three smooth petals. The stamens, numbering six, are arranged in two rows with short filaments. The ovary is fusiform and densely hairy. The flowering period occurs from April to October.

The capsule fruit is elongated and contains 1–4 ivory-colored seeds. The fruit measures 1.5–5.0 cm in length and 0.5–1.1 mm in width. The seeds are polyhedral, black, distinctly ridged, hard, and slightly swollen at one end. The seed size is  $2.1 \times 3.10$  mm, with a weight of 3–6 g per 1,000 seeds.

Upon analyzing the sample labeled DV-SC.050723 and cross-referencing with existing literature and herbarium specimens of *Curculigo orchioides* Gaertn. (specimen numbers NIMM-859, NIMM-5779, NIMM-6661) preserved at the Herbarium of the Center for Medicinal Plant Resources, Institute of Medicinal Materials, the scientific identity of the sample was confirmed as follows:

- Scientific name: *Curculigo orchioides* Gaertn.
- Synonym: *Curculigo orchioides* var. minor Benth
- Vietnamese names: Sâm cau, Ngải cau, Cỏ nóc lan
- Family: Hypoxidaceae

The morphological analysis results, combined with taxonomic keys from various floras and reference specimens preserved in museums (including the Museum of Natural History in Paris and the Herbarium of the National Institute of Medicinal Materials), confirm that the collected genetic sample belongs to *Curculigo orchioides* Gaertn., of the Hypoxidaceae family. These



a: Stem, b: Stem with leaves, c: Leaf, d: Flower, e: Fruit, g: Seed

**Figure 1:** Photograph of the Identified *Curculigo orchioides* Sample.



**Curculigo orchioides chloroplast, complete genome**Sequence ID: [NC\\_053892.1](#) Length: 157472 Number of Matches: 1[See 1 more title\(s\)](#) [See all Identical Proteins \(IPG\)](#)Range 1: 57772 to 58318 [GenBank](#) [Graphics](#)[Next Match](#) [Previous](#)

| Score          | Expect                                                        | Identities    | Gaps      | Strand    |
|----------------|---------------------------------------------------------------|---------------|-----------|-----------|
| 1011 bits(547) | 0.0                                                           | 547/547(100%) | 0/547(0%) | Plus/Plus |
| Query 1        | AAGATTACAAATTGAATTATTATACTCCTGACTACGAAACCAAGATACTGATATCTTGG   | 60            |           |           |
| Sbjct 57772    | AAGATTACAAATTGAATTATTATACTCCTGACTACGAAACCAAGATACTGATATCTTGG   | 57831         |           |           |
| Query 61       | CAGCATTCCGAGTAACCTCTCAACCTGGAGTTCGCCCTGAGGAAGCAGGGGCAGCGGTAG  | 120           |           |           |
| Sbjct 57832    | CAGCATTCCGAGTAACCTCTCAACCTGGAGTTCGCCCTGAGGAAGCAGGGGCAGCGGTAG  | 57891         |           |           |
| Query 121      | CTGCCGAATCTTCTACTGGTACATGGACAACCTGTGTGGACTGATGGACTTACCAGTCTTG | 180           |           |           |
| Sbjct 57892    | CTGCCGAATCTTCTACTGGTACATGGACAACCTGTGTGGACTGATGGACTTACCAGTCTTG | 57951         |           |           |
| Query 181      | ATCGTTACAAAGGACGATGCTACCACATCGAGGCCGTTATTGGGGAGGAAAAATCAATATA | 240           |           |           |
| Sbjct 57952    | ATCGTTACAAAGGACGATGCTACCACATCGAGGCCGTTATTGGGGAGGAAAAATCAATATA | 58011         |           |           |
| Query 241      | TTGCTTATGTAGCTTATCCTTTAGACCTTTTGAAGAAGGTTCTGTTACTAACATGTTTA   | 300           |           |           |
| Sbjct 58012    | TTGCTTATGTAGCTTATCCTTTAGACCTTTTGAAGAAGGTTCTGTTACTAACATGTTTA   | 58071         |           |           |
| Query 301      | CTTCCATTGTGGGTAAATGTATTGGTTTCAAAGCCCTACGAGCTCTACGTCTGGAGGATC  | 360           |           |           |
| Sbjct 58072    | CTTCCATTGTGGGTAAATGTATTGGTTTCAAAGCCCTACGAGCTCTACGTCTGGAGGATC  | 58131         |           |           |
| Query 361      | TGCGAATTCCTCCCTGCTTATCTAAAACTTTCCAAGGCCCGCTCACGGCATCCAAGTTG   | 420           |           |           |
| Sbjct 58132    | TGCGAATTCCTCCCTGCTTATCTAAAACTTTCCAAGGCCCGCTCACGGCATCCAAGTTG   | 58191         |           |           |
| Query 421      | AAAGAGATAAAATTGAACAAGTATGGTCGTCCTGTTGGGATGTACTATTAAACCAAAAT   | 480           |           |           |
| Sbjct 58192    | AAAGAGATAAAATTGAACAAGTATGGTCGTCCTGTTGGGATGTACTATTAAACCAAAAT   | 58251         |           |           |
| Query 481      | TGGGATTATCCGCAAAAAACACGGTAGAGCGGTGTATGAATGTCTACGTGGTGGACTTG   | 540           |           |           |
| Sbjct 58252    | TGGGATTATCCGCAAAAAACACGGTAGAGCGGTGTATGAATGTCTACGTGGTGGACTTG   | 58311         |           |           |
| Query 541      | ATTTTAC 547                                                   |               |           |           |
| Sbjct 58312    | ATTTTAC 58318                                                 |               |           |           |

**Figure 5:** Comparison of the *rbcl* Gene Sequence from the Study Sample with the Published Global Sequence.

The results presented in Figure 5 illustrate the sequence alignment of the *rbcl* gene fragment from the study sample with the globally published sequence. In this comparison, "Query" represents the sequence from the study sample, while "Sbjct" corresponds to the published sequence of *Curculigo orchioides* Gaertn. available in the GenBank database under accession number NC\_053892.1.

The sequencing and alignment results confirm that the genetic sequence of the studied *Curculigo orchioides* sample is identical to that of *Curculigo orchioides* Gaertn. (taxonomy ID: txid681286). This serves as reliable evidence to scientifically validate the species name of Sâm cau cultivated in Kon Ka Kinh National Park, Gia Lai Province, as *Curculigo orchioides* Gaertn., belonging to the Hypoxidaceae family.

### 3.3. Discussion

To support the accurate identification of the scientific name of *Curculigo orchioides*, in addition to analyzing morphological characteristics, DNA barcoding has proven to be an advanced and effective method for species identification worldwide. The *rbcl* DNA barcode has been successfully applied to identify

various plant species, such as *Panax vietnamensis* var. *fuscidiscus* K. Komatsu, S. Zhu & S. Q. Cai (Lai Châu ginseng) and *Panax vietnamensis* Ha & Grushv. (Ngọc Linh ginseng) (Nguyen, T. H. T. *et al.*, 2016). This method has also been effective in distinguishing individual plant species within complex sample mixtures.

Recently, a German research group successfully applied *rbcl* DNA barcoding for classifying the Dipterocarpaceae family in Sumatra, Indonesia (Carneiro *et al.*, 2019). In Vietnam, Do, V. M. *et al.* (2020) also utilized *rbcl* DNA barcoding to classify species of Polyscias.

For *Curculigo orchioides*, this study represents the first application of DNA barcoding for scientific species identification. After extracting, isolating, and sequencing the DNA from Sâm cau leaf samples, the obtained gene sequence was compared with published *rbcl* gene sequences of *Curculigo orchioides* Gaertn. The results showed a 100% sequence match.

Thus, identifying *Curculigo orchioides* using DNA barcoding is a highly reliable and selective method, ensuring precise species classification.



#### 4. CONCLUSION

Using a combination of DNA barcoding and morphological analysis, the *Curculigo orchoides* plant collected from Kon Ka Kinh National Park has been scientifically identified as *Curculigo orchoides* Gaertn., belonging to the Hypoxidaceae family. This result provides a precise determination of the scientific name of the studied specimen using DNA barcoding, with the GenBank accession number NC\_053892.1 and taxonomy ID: txid681286.

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#### CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest related to this study.

If a conflict of interest arises after publication, authors are responsible for notifying the editorial board of appropriate action.

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