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DNA barcoding of “Sibujing” (*Allium chinense* G.Don) accessions from Mindanao, Philippines

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ABSTRACT

Through an integrative taxonomic approach encompassing morphological, phytochemical, and molecular analyses, Mindanao’s famous “Sibujing” was conclusively identified as *Allium chinense* G.Don in 2023. The initial molecular analyses of “Sibujing” were predominantly based on nucleotide sequences from internal transcribed spacer (ITS) region, with sampling limited to few localities. To broaden the scope of the molecular assessment, maturase K (*matK*) region was subsequently utilized to validate the identity of “Sibujing” samples from three major cultivation sites in Mindanao. The *matK* gene was utilized because of its effective species discrimination power, high quality sequence recovery and simple laboratory procedures. The “Sibujing” accessions from (1) Amai Manabilang, Lanao Del Sur (2) Parang, Maguindanao del Norte, and (3) Rangayen, Alamada, Cotabato were molecularly identified as *A. chinense* based on their *matK* partial gene sequences. The result coincides with the previous molecular identification report of this *Allium* species in the Philippines. The findings of this research contribute to the ongoing efforts to resolve the taxonomic ambiguities involving “Sibujing” in the country.

Keywords: *Allium chinense*, ITS, *matK*, partial gene sequences, “Sibujing”, taxonomic ambiguities

Allium chinense G.Don or commonly known as “Sibujing” is an integral component of Maranao’s dishes. It is a species related to leek (*Allium porrum* L.) and is traditionally considered as its wild progenitor (Garcia-Herrera et al. 2014). The classification of *Allium* plants indicated that this particular species is a member of the *Amaryllidaceae* family, known for its perennial herbaceous geophytes (Wheeler et al. 2013). A popular product generated from this harvest is called “palapa,” a seasoned blend or condiment commonly used in Muslim Regions.

For the past 25 years, “Sibujing” has always been regarded as *Allium sativum* L. (Madulid 2001), *Allium ampeloprasum* L. (Añides et al. 2019; Dapanas and Duero 2020), *Allium fistulosum* L. (Casim 2021) and *Allium ascalonicum* L. (Nuñez et al. 2021). In 2023, Vera Cruz et al. reported that the said *Allium* species is *A. chinense*. For further confirmation of this finding, “Sibujing” accessions were obtained from selected major production sites in Mindanao and subjected to deoxyribonucleic acid (DNA) barcoding.



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The DNA barcodes have been developed and utilized to overcome the problems faced by conventional characterization techniques (Saddhe and Kumar 2018). Since it is proven to be unaffected by external or other environmental factors, the markers are considered as a reliable, stable and rapid method for the identification of various organisms (Tnah et al. 2019). To identify “Sibujing” at the molecular level, Vera Cruz et al. (2023) employed internal transcribed spacer gene as their nuclear barcode. Recognizing the value of complementary genetic markers in plant barcoding, the maturase K (*matK*) gene was utilized in the present study to enhance taxonomic resolution. This is to augment the current molecular dataset and explore additional discriminatory power. The *matK* is one of the most rapidly evolving coding sections of the plastid genome (Ford et al. 2009) and has been commonly utilized for identification of plants at the family, genus and even at the species level (Dong et al. 2012). Hence, the Consortium for the Barcode of Life (CBOL) Plant Working Group has recognized its discrimination power as a single barcode region or in combination with other proposed plant barcode sequences (CBOL Working Group et al. 2009).

This research endeavor aimed to validate the genetic identity of “Sibujing” in Mindanao, Philippines using *matK* gene. This gene has vast substitution rates compared to other chloroplast gene sequences and has been proven to be effective in solving phylogenetic problems (Fazekas et al. 2008). The utility of the *matK* gene as a molecular marker for *Allium* taxonomy and genetic diversity has been well-established in various geographical regions. For instance, it has been successfully applied in Iran (Zarei et al. 2020), Kazakhstan (Abugaliev et al. 2017), and Turkey (İpek et al. 2014) to characterize different *Allium* species.

Leaf samples of different “Sibujing” accessions were collected from three production sites in Mindanao: Amai Manabilang, Lanao Del Sur; Parang, Maguindanao; and Rangayen, Alamada (Figure 1). These leaf samples were then submitted to the Philippine Genome Center (PGC) Mindanao for genomic DNA isolation; gene amplification using *matK* gene (Fazekas et al. 2008); agarose gel electrophoresis; gene sequencing; and sequence analyses. Electrophoretograms, generated sequences, and Basic Local Alignment Search Tool (BLAST)n analysis results were sent as data sets by PGC Mindanao via email.

In the advent of modern biotechnology, DNA barcoding techniques have been proven as an efficient procedure for assessing plant phylogenies and presented successful applications in the molecular classification of *Allium* species (Zarei et al. 2020). The DNA barcoding of *matK* nucleotide sequences revealed that “Sibujing” accessions from Amai Manabilang, Lanao Del Sur; Parang, Maguindanao;

and Rangayen, Alamada were 100% homologous to *A. chinense*'s chloroplast genome (Table 1).



Figure 1. “Sibujing” (*Allium chinense* G. Don) samples from (a) Amai Manabilang, Lanao Del Sur (b) Parang, Maguindanao del Norte and (c) Rangayen, Alamada, Cotabato.

This 100% match suggests that the *matK* region, at least for this particular species, is highly conserved. The *matK* gene, while a good barcode due to its variability in some groups, can be highly conserved within a species, especially in cultivated plants (CBOL Working Group et al. 2009). Furthermore, the result also strongly suggests very low genetic variation among the studied samples for this specific gene region.

The primary mode of propagation for “Sibujing” in Mindanao is asexual, utilizing bulbs for vegetative reproduction. Sexual reproduction through seeds is also a common practice. The widespread reliance on bulb propagation leads to the establishment of genetically uniform clones, consequently resulting in minimal intraspecific genetic diversity.

The DNA barcoding results obtained are consistent with the research of Vera Cruz et al. (2023), who initially elucidated the identity of “Sibujing” accessions from Magpet, Cotabato, and Zamboanga del Norte. These findings corroborate the earlier reports, confirming “Sibujing”, a traditional condiment crop of the Maranaos in Mindanao, Philippines, as *A. chinense* G. Don, a species native to China.

Significantly, this study provides the second molecular-based confirmation of “Sibujings” identity in the Philippines, following the initial report of Vera Cruz et al. in 2023.

Table 1. Basic Local Alignment Search Tool (BLAST)n result of *matK* conserved sequences from three “Sibujing” (*Allium chinense* G.Don) samples.

Sample	Basic Local Alignment Search Tool (BLAST)n Best Match (top three hits)	Query cover (%)	E-value	Percent Identity (%)
“Sibujing” from Amai Manabilang, Lanao del Sur	1. <i>Allium chinense</i> chloroplast, complete genome NC 043922.1	100	0.0	100
	2. <i>Allium thunbergia</i> var. <i>deltoides</i> chloroplast, complete genome OQ701006.1	99	0.0	99.87
	3. <i>Allium sacculiferum</i> chloroplast, complete genome NC 070336.1	99	0.0	99.87
“Sibujing” from Parang Maguindanao del Norte	1. <i>Allium chinense</i> chloroplast, complete genome NC 043922.1	100	0.0	100
	2. <i>Allium longistylum</i> chloroplast, complete genome NC 080285.1	99	0.0	99.87
	3. <i>Allium thunbergia</i> var. <i>deltoides</i> chloroplast, complete genome OQ701006.1	99	0.0	99.83
“Sibujing” from Rangayen, Alamada, Cotabato	1. <i>Allium chinense</i> chloroplast, complete genome NC 043922.1	100	0.0	100
	2. <i>Allium longistylum</i> chloroplast, complete genome NC 080285.1	99	0.0	99.92
	3. <i>Allium microdictyon</i> chloroplast, complete genome JF972927.1	99	0.0	99.87

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GENERATIVE AI STATEMENT

The concepts, results, and discussion within this paper are the authors' original work. An AI tool (Gemini 1.5 Pro) was used only for editing and improving the manuscript's readability. It was not involved in the generation of data and interpretation of results.

ETHICAL CONSIDERATIONS

All necessary permits for specimen collection were secured before sampling.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

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