Investigating new barcode markers to distinguish kratom varieties

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INTRODUCTION

Kratom (*Mitragyna speciosa*) is increasingly encountered in forensic casework due to its opioid-like effects and complex legal status. Reliable identification is critical, but traditional methods such as morphology and alkaloid content are limited by variability and lack of DNA data. Unlike animals, no universal plant barcode provides sufficient resolution. Previous studies using *ITS*, *matK*, *rbcL*, and *trnH-psbA* found minimal variation [1]. This study designed kratom-specific primers for four novel chloroplast intergenic regions (*petA-psbJ*, *rpl14-16*, and *trnL-trnF,* and *ndhF-rpl32*). Novel sequences will be submitted to GenBank to expand forensic databases and support robust DNA-based identification tools.

MATERIALS

Table 1. Kratom powder information from product packaging.

ID Number	Manufacturer	Strain	Name	Source Location
1	Kratom Spot	White Indo		Indonesia
2	Kratom Spot	White Borneo		Borneo (Indonesia)
3	Kratom Spot	White Sumatra		Sumatra (Indonesia)
4	Kratom Spot	White Maeng Da		Thailand
5	Kratom Spot	White Bali		Bali (Indonesia)
6	Kratom Spot	Green Thai		Thailand
7	Kratom Spot	Green Indo		Indonesia
8	Kratom Spot	Green Malay		Malaysia
9	Kratom Spot	Green Maeng Da		Thailand
10	Kratom Spot	Red Indo		Indonesia
11	Kratom Spot	Red Thai		Thailand
12	Kratom Spot	Red Sumatra		Southeast Asia
13	Kratom Spot	Red Bali		Indonesia
14	Kratom Spot	Red Maeng Da		Vietnam
15	Kratom Spot	-	Bentuangie	Thailand
16	Remarkable Herbs	Green Indo		Bali (Indonesia)
17	Remarkable Herbs	Green Vietnam		Bali (Indonesia), Thailand, West Kalimantan (Indonesia), Island of Borneo (Indonesia)
18	Remarkable Herbs	Green Thai		Thailand
19	Remarkable Herbs	Red Bali		Indonesia
20	Cali Botanicals	White	White Lightning	Kalimantan (Indonesia)
21	Cali Botanicals		Private Reserve Maeng Da	Bali (Indonesia)
22	Kats Botanicals	White	White Elephant	Island of Borneo (Indonesia)
23	Kats Botanicals	Red	Red Dark Elephant	Southeast Asia
24	Kats Botanicals	Green Bali		Bali (Indonesia)
25	Kats Botanicals	Red	Dark Red Malay	Island of Borneo (Indonesia)
26	Kats Botanicals	Red	Red Chocolate	Southeast Asia
27	Kats Botanicals	Red Bali	Wild Red Bali	Indonesia
28	Kratom Spot	Red, Green and White	Supernova Speciosa Blend	Indonesia and Thailand

RESULTS

Table 2. Summary of sequencing results for two representative samples from the following three barcode regions: *ndhF-rpl32*, *petA-psbJ*, and *rpl14-16.*

	ndhF-rpl32			*petA-psbJ*				*rpl14-16*	
ID Number and Strain	**114,195 Deletion (T)**	**114,246 SNP (C/A)**	**114,550 SNP (C/A)**	**65,276-65,284 Deletion (ATTAGATTA)**	**65,552 SNP (C/A)**	**65,611 SNP (G/T)**	**65,889 SNP (T/G)**	**83,118 Insertion (A)**	**83,234 SNP (G/T)**
3 – White Sumatra	Deletion	A	A	-	A	T	T	A	T
4 – White Maeng Da	Deletion	A	A	Deletion	A	T	G	-	T

petA-psbJ Novel Polymorphisms

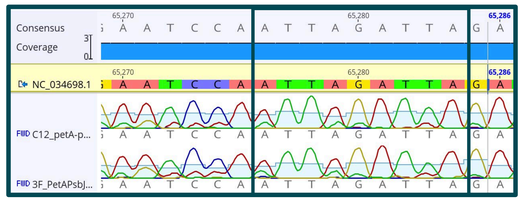

Figure 1. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 3 (*White Sumatra*), which lacks nine-base deletion.

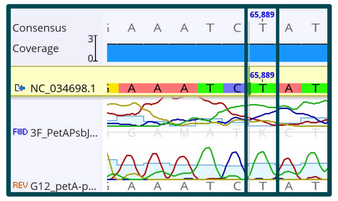

Figure 2. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 3 (*White Sumatra*), exhibiting a thymine at the SNP (G/T) located at the base pair location 65,889.

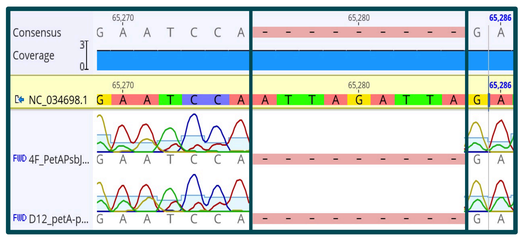

Figure 3. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 4 (*White Maeng Da*), which exhibits nine-base deletion.

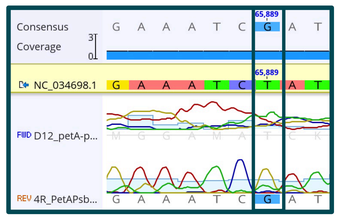

Figure 4. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 4 (*White Maeng Da*), showing a guanine at the SNP (G/T) located at the base pair location 65,889.

ndhF-rpl32 Novel Polymorphisms

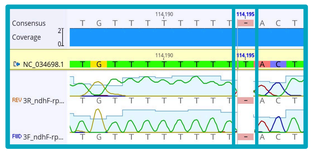

Figure 5. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 3 (*White Sumatra*), which exhibits a deletion at the base pair location 114,195.

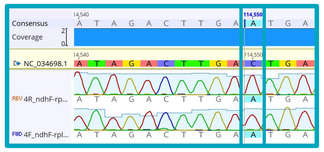

Figure 6. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 4 (*White Maeng Da*), showing an adenine at the SNP (C/A) located at the base pair location 114,550.

rpl14-16 Novel Polymorphisms

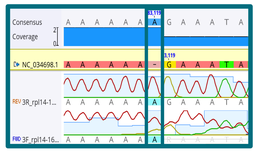

Figure 7. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 3 (*White Sumatra*), exhibiting an insertion of an adenine at base pair location 83,119.

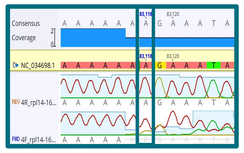

Figure 8. Mapping of whole chloroplast genome reference (NC_034698.1) to sample 4 (*White Maeng Da*), which lacks the insertion of an adenine at base pair location 83,118.

DISCUSSION

- The kratom-specific primers designed for this study reliably amplified the targeted regions and revealed novel polymorphisms, thereby expanding the limited genetic data available for kratom **(Table 2** and **Figs. 3-8)**.
- Although *trnL-trnF* could not resolve kratom varieties, this result is expected given the limited discriminatory power of many plant barcode regions.
- Despite differing stated origins, the products were ultimately obtained from regions that are geographically close and environmentally comparable, which likely contributed to the limited genetic variation detected **(Table 1)**.
- The interpretation of these genetic results is contingent upon the accuracy of the product packaging; therefore, any mislabeling or omission could lead to incorrect assumptions about the varieties present **(Table 1)**.

METHODS

DNA Extraction
Kratom powder was extracted using the DNeasy Plant Mini Kit (QIAGEN).

↓

Polymerase Chain Reaction (PCR)
Type-it Microsatellite PCR Kit (QIAGEN) was used to amplify the following barcode regions: *ndhF-rpl32*, *petA-psbJ*, *rpl14-16*, and *trnL-trnF*.

↓

PCR Product Purification: ExoSAP-IT

↓

Cycle Sequencing: BigDye Terminator v3.1

↓

Sequencing Purification: BigDye XTerminator

↓

Capillary Electrophoresis: ABI 3500 Genetic Analyzer

↓

Sequence Analysis
Geneious Prime (Biomatters) software version 2025.2.2. was used to align and edit *Mitragyna speciosa* sequences to assess intra-species variation.

CONCLUSIONS

- ***petA–psbJ*** showed the highest sequence variation and polymorphism count, but variation did not correspond to strain or geographic origin.
- ***trnL–trnF*** showed no detectable variation, indicating limited utility for intra-species discrimination of kratom.
- ***rpl14–rpl16*** was minimally informative, with only a single adenine insertion distinguishing samples.
- Further evaluation of ***ndhF–rpl32*** is needed to assess its discriminatory value.
- Future studies will include non-powder kratom products (e.g., gels and gummies) to assess kratom DNA detectability.

REFERENCES

[1] Graham K, Cantu C, Houston R. Sequence variation of commercially available kratom products at universal DNA barcode regions. Journal of forensic sciences. 2024;69(4):1421-8. doi: 10.1111/1556-4029. 15547.

ACKNOWLEDGEMENTS

I would like to thank the Department of Forensic Science at Sam Houston State University for laboratory resources and technical assistance. This work was supported in part by institutional funding from the College of Criminal Justice.

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