



BRIEF REPORT

DNA barcoding for the discrimination of *Uncaria gambir* and its closely related species using internal transcribed spacer genes

[version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: *Uncaria gambir* is one *Uncaria* species that exclusively grows in Indonesia. The phytochemical constituents of this species have been widely explored and its extracts are used as traditional medicine. However, the relationship between *Uncaria gambir* and other *Uncaria* species is still unknown. DNA barcoding was used in this study to reveal this relationship.

Methods: Genomic DNA was isolated from four main cultivated variants of *Uncaria gambir* species in Indonesia. ITS primer was used to amplify the specific gene region. Genetic distance analysis was carried out on *Uncaria gambir* and 12 other *Uncaria* species. A phylogenetic tree was created to determine the relationship among *Uncaria* species using the maximum likelihood method.

Results: The ITS primer successfully amplified the ITS region in *Uncaria gambir*. Genetic distance and phylogenetic tree analyses showed that *Uncaria gambir* has a close relationship with *Uncaria scandens*, *Uncaria yunnanensis*, and *Uncaria macrophylla* which is also indicated by Interspecific distance analysis.

Conclusions: Although the DNA barcoding gap is absent in genetic distance analysis, the phylogenetic tree analysis from the ITS region can differentiate *Uncaria gambir* from other *Uncaria* species.

Keywords

Uncaria Gambir, ITS, Phylogenetic analysis, Genetic distance

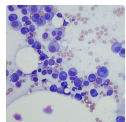
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Approval Status

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version 1 27 Jan 2022	 view	 view

1. Jarrett Phillips , University of Guelph, Guelph, Canada
2. Andrey Yurkov , Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Berlin, Germany

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the **Cell & Molecular Biology** gateway.

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Author roles: **Supri Wardi E:** Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation; **Syukur S:** Conceptualization, Funding Acquisition, Supervision; **Chaidir Z:** Supervision, Writing – Review & Editing; **Jamsari J:** Funding Acquisition, Supervision, Writing – Review & Editing; **Nova B:** Formal Analysis, Resources, Visualization

Competing interests: No competing interests were disclosed.

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Introduction

Gambier, extracted from the twigs and leaves of *Uncaria gambir*, is traditionally used to treat various diseases in Indonesia, such as diarrhea, gastrointestinal diseases, burns, acne, and cancer (Murdja *et al.*, 2018). *Uncaria gambir* is commonly distributed in southeast Asia tropical regions, mostly in Indonesia and Malaysia (Taniguchi *et al.*, 2007). The Sumatera Barat region has become the biggest contributor of gambier with Udang, Cubadak, Riau, and Mancik being the main cultivated variants in Indonesia.

The identification of *Uncaria gambir* is still based on morphological characteristics and phytochemical constituents. These identifications are mainly affected by environmental conditions and conditions of the sample, thus resulting in a biased interpretation (Zhu *et al.*, 2011). To address this problem, DNA barcoding can be a solution to discriminate the species by using short and standardized DNA fragments (Li *et al.*, 2021). The Consortium for the Barcode of Life (CBOL) has approved chloroplast genes sequences, such as *psbA-trnH*, *trnL-trnF*, *ycf1*, and *rpoC1*, as potential DNA barcodes and suggests a combination of *matK* and *rbcL* genes as an alternative barcode for Embryophyta (Hollingsworth *et al.*, 2009). In addition, internal transcribed spacer (ITS) genes (ribosomal region) have been proposed as supplemental barcodes for *matK* and *rbcL* (Hollingsworth *et al.*, 2009).

The ITS gene is located between 18S and 28S genes in the nrDNA repeat unit and covers the ITS1 and ITS2 regions, connected by the 5.8S rRNA gene (Bellemain *et al.*, 2010). Previous studies have used restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) to investigate catechin molecular markers in *Uncaria gambir* (Istino *et al.*, 2013).

Molecular identification using *matK* and *rbcL* genes gives a low amplification success rate and was inaccurate to discriminate among *Uncaria gambir* variants (Wardi *et al.*, 2020). In a recent study, utilization of the ITS-2 gene only gives one species-specific site among *Uncaria gambir* variants and no genetic distance analysis was studied (Wardi *et al.*, 2021b).

In this study, we investigated the utilization of the ITS gene to discriminate *Uncaria gambir* within the other 12 *Uncaria* species. Genetic distance analysis was carried out to provide relationship data between *Uncaria gambir* and the other 12 *Uncaria* species. This study is the first to provide an ITS gene sequence from *Uncaria gambir*.

Methods

DNA extraction, PCR amplification and sequencing

Six fresh leaves of four *Uncaria gambir* variants were collected from Siguntur, West Sumatera Province, Indonesia (1°05'36.8"S 100°28'27.0"E -1.093562, 100.474153). Morphology identification was conducted in the Herbarium of Andalas University. Harvested fresh leaves were frozen immediately in liquid nitrogen and was stored at -80°C for 24 hours prior to DNA isolation.

DNA isolation was performed using the CTAB method (Allen *et al.*, 2006). In total 300 mg of frozen leaf sample was ground and put into a 2 ml Eppendorf tube. 1 ml of 2x CTAB buffer and mercaptoethanol was added and vortexed until homogenized. The solution was incubated at 65°C for 30 minutes and inverted every 10 minutes. 500 µL of phenol:chloroform: isoamylalcohol mixture (25:24:1) was added and vortexed for 1 minute then centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to a new 2 ml Eppendorf tube. 500 µL of chloroform mixture: isoamylalcohol (24:1) was added and vortexed for 10 minutes then centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to a new 1.5 mL Eppendorf tube. Sodium acetate was added as much as 1/10 times the volume of the supernatant, then 1 ml of cold ethanol 99% was added and swirled for 1 minute. The solution was centrifuged at 12,000 rpm for 5 minutes, supernatant was removed and 500 µL of 70% ethanol was added. The solution was centrifuged at 12,000 rpm for 5 minutes, the supernatant was discarded and the DNA was dried in the oven. Finally 100 µL of 1xTE buffer was added to dried DNA and stored at -20°C.

DNA amplification was performed using MyTaq™ HS Red Mix Bioline kit. The final concentrations for a typical 25 µl reaction were as follows: 12.5 µL MyTaq™ HS Red Mix Bioline, 1 µL reverse primer ITS-u4 (RGTTCTTTTCCTC-CGCTTA), 1 µL forward primer ITS-u1 (GGAAGKARA-AGTCGTAACAAGG), 1 µL DNA template, 9.5 µL Nuclease-Free Water to obtain a 25 µL final volume. The thermal cycling conditions (BIO-RAD C1000™ Thermal Cycler) were as follows: (a) Denaturation 96°C for 60 s, (b) Denaturation 96°C for 15 s, (c) Annealing 70°C for 45 s (-1°C decrement each cycle), (d) Extension 72°C for 60 s; (e) repeat step (b-d) for 25 cycles and followed by a final extension at 72 °C for 5 min.

The isolation and amplification results were visualized using electrophoresis with 1.5% agarose gel. The expected amplification product was sequenced by 1st BASE DNA Sequencing Service (Apical Scientific Sdn Bhd) bidirectionally using the specific primer.

Sequence and distance analysis

A total of 132 *Uncaria* ITS sequences were downloaded from National Center for Biotechnology Information (NCBI) and were aligned with *Uncaria gambir* using MEGA X software version 10.2.6 (Tamura *et al.*, 2013). The intraspecific and interspecific distances were computed with Kimura 2-Parameter (K2P) using MEGA X software (Kimura, 1980)(Meier *et al.*, 2006).

Phylogenetic analysis

The sequences were analyzed by the maximum likelihood method for phylogenetic tree construction. The maximum likelihood analyses, including 1000 nonparametric bootstrap replicates, was performed using MEGA X software under the Kimura 2-parameter (K2P)+G model. *Nauclea orientalis* and *Nauclea diderrichi* sequences that are relative to the *Uncaria* genus were downloaded from NCBI and used as an outgroup species.

Results

Sequences and distance analysis

The primer ITS-u1 and ITS-u4 showed 100% amplification efficiency to *Uncaria gambir*. Sequences were deposited in the NCBI GenBank database under the accession numbers MZ927014, MZ927015, MZ927016, and MZ926993 (Table 1). The region's length was obtained from 644 to 646 bp.

The dataset used in this study includes 4 species of *Uncaria gambir* and 132 *Uncaria* sequences of the ITS region downloaded from the NCBI GenBank database (Extended data). This dataset contained 655 conserve sites, 68 variable sites, and 54 parsimony informative sites.

The mean of intraspecific distance (Table 2) and interspecific distance (Table 3) is 0.001% and 0.022%, respectively. The BLAST showed the *Uncaria gambir* Riau, Mancik, Udang, and Cubadak variant had 99.22%, 99.23%, and 99.07% similarity with *U. macrophylla* MF033304, respectively. In

addition, the *Uncaria gambir* Cubadak variant had 99.22% similarity with *U. macrophylla* MF033303.

Phylogenetic analysis

The phylogenetic tree was constructed using 13 *Uncaria* species. *Uncaria gambir* were clustered from other species (Figure 1). The four variants of *Uncaria gambir* had a close relationship with *Uncaria macrophylla* MF033303.

The phylogenetic tree showed *Uncaria gambir* has a close relationship with *Uncaria yunnanensis* but not *Uncaria scandens*. In contrast, the interspecific distance value showed *Uncaria gambir* had a close relationship with *Uncaria yunnanensis* and *Uncaria scandens*.

Discussion

Uncaria is a member of the Rubiaceae family and consists of 34 species (Ridsdale, 1978) that are well known as medicinal plants (Heitzman *et al.*, 2005). The method to identify *Uncaria*

Table 1. Detailed information about ITS sequences from species *Uncaria gambir*.

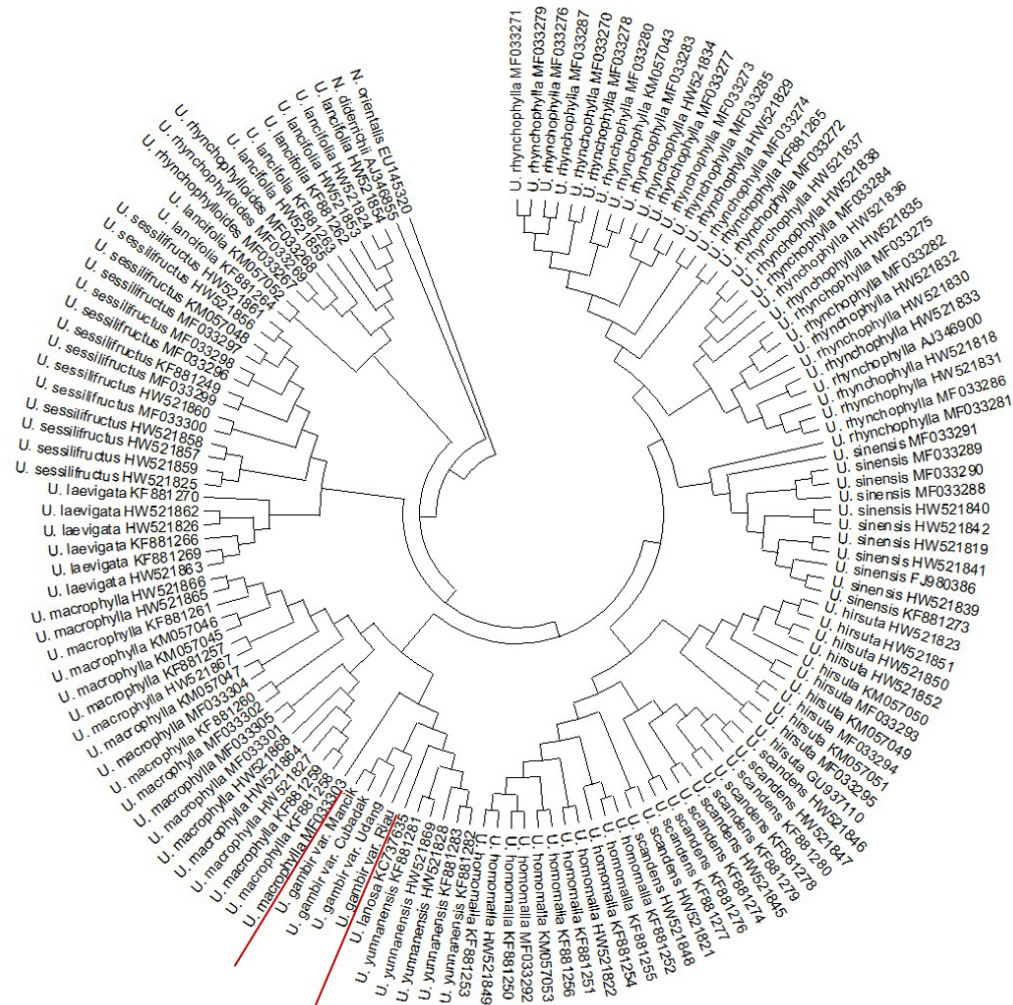
Sample name	Tissue	Length	GC%	Accession number
<i>Uncaria gambir</i> (Cubadak var)	Leaf	645 bp	62.6	MZ926993
<i>Uncaria gambir</i> (Mancik var)	Leaf	644 bp	62.7	MZ927015
<i>Uncaria gambir</i> (Riau var)	Leaf	645 bp	62.8	MZ927016
<i>Uncaria gambir</i> (Udang var)	Leaf	646 bp	62.8	MZ927014

Table 2. Intraspecific distance of 13 *Uncaria* species.

Species	Intraspecific distance
<i>U. rhynchophylla</i>	0.001
<i>U. rhynchophylloides</i>	0.001
<i>U. sinensis</i>	0.001
<i>U. homomalla</i>	0.002
<i>U. hirsuta</i>	0.000
<i>U. sessilifructus</i>	0.001
<i>U. macrophylla</i>	0.001
<i>U. laevigata</i>	0.003
<i>U. lancifolia</i>	0.003
<i>U. scandens</i>	n/c
<i>U. lanosa</i>	0.000
<i>U. yunnanensis</i>	0.000
<i>U. gambir</i>	0.001

Table 3. Interspecific distance of 13 *Uncaria* species.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	<i>U. rhynchophylla</i>													
2	<i>U. rhynchophylloides</i>	0.022												
3	<i>U. sinensis</i>	0.008	0.023											
4	<i>U. homomalla</i>	0.014	0.020	0.015										
5	<i>U. hirsuta</i>	0.015	0.021	0.017	0.010									
6	<i>U. sessilifructus</i>	0.036	0.026	0.037	0.035	0.035								
7	<i>U. macrophylla</i>	0.031	0.021	0.032	0.026	0.027	0.031							
8	<i>U. laevigata</i>	0.026	0.017	0.028	0.024	0.025	0.017	0.024						
9	<i>U. lancifolia</i>	0.019	0.005	0.020	0.017	0.017	0.023	0.017	0.014					
10	<i>U. scandens</i>	0.031	0.021	0.033	0.027	0.027	0.030	0.011	0.024	0.018				
11	<i>U. lanosa</i>	0.013	0.019	0.015	0.007	0.009	0.030	0.024	0.023	0.015	0.025			
12	<i>U. yunnanensis</i>	0.032	0.022	0.033	0.027	0.028	0.031	0.012	0.024	0.018	0.000	0.025		
13	<i>U. gambir</i>	0.031	0.021	0.032	0.026	0.027	0.030	0.008	0.024	0.017	0.003	0.025	0.004	

**Figure 1.** Phylogenetic tree analysis of *Uncaria gambir* to another *Uncaria* species was computed using maximum likelihood method.

gambir is currently limited to morphological identification. This study compares the ITS gene sequence of the four main cultivated variants of *Uncaria gambir* from Indonesia with 132 other *Uncaria* sequences (12 species) downloaded from NCBI.

The interspecific distance analysis showed that *Uncaria gambir* is significantly different from the other 12 *Uncaria* species. *Uncaria scandens* and *Uncaria sinensis* species had the lowest and highest interspecific distance value, respectively. The intraspecific distance analysis showed *Uncaria rhyncho-phylla*, *Uncaria rhynchophylloides*, *Uncaria sessilifructus*, and *Uncaria macrophylla* had a value of 0.001. The intraspecific and interspecific values showed a partial overlap value, thus no gap between the species. Tang *et al.* (2016) showed similar results when comparing *Uncaria rhynchophylla* (Miq.) Jacks with 28 other *Uncaria* species sequences.

It is important in DNA barcoding to have a gap, as it can differentiate unknown from known sequences, as no DNA barcoding gaps may give inaccurate results in differentiating the species (Liu *et al.*, 2014; Rach *et al.*, 2008). The phylogenetic tree was created with the maximum likelihood method. The advantage of this method is that it includes evolutionary models to account for the variation in the sequences compared with maximum parsimony and distance methods (Mount, 2008).

The phylogenetic tree showed all of *Uncaria gambir* species were clustered as monophyletic. All *Uncaria gambir* variants were nested with *Uncaria macrophylla* (MF033303), *Uncaria lanosa*, and *Uncaria yunnanensis*.

Conclusion

In conclusion, the phylogenetic tree ITS sequence can differentiate *Uncaria gambir* from other *Uncaria* species. In

contrast, genetic distance analysis showed different results as the absence of a DNA barcoding gap means it is not reliable to differentiate between species.

Data availability

Underlying data

The ITS sequences from this research are available from NCBI:

GenBank: *Uncaria gambir* isolate Udang, Accession number MZ927014: <https://www.ncbi.nlm.nih.gov/nucleotide/MZ927014>

GenBank: *Uncaria gambir* isolate Mancik, Accession number MZ927015: <https://www.ncbi.nlm.nih.gov/nucleotide/MZ927015>

GenBank: *Uncaria gambir* isolate Riau, Accession number MZ927016: <https://www.ncbi.nlm.nih.gov/nucleotide/MZ927016>

GenBank: *Uncaria gambir* isolate Cubadak, Accession number MZ926993: <https://www.ncbi.nlm.nih.gov/nucleotide/MZ926993>

Extended data

Dryad: GenBank accession numbers of downloaded sequences used in this study <https://doi.org/10.5061/dryad.dfn2z352w> (Wardi *et al.*, 2021a)

This project contains the following extended data:

- Table_E1_GeneBank_Accession_Numbers.docx (This data contains accession numbers from 132 sequence of *Uncaria* species, and two accession numbers from *Nauclea*).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

References

- Allen GC, Flores-Vergara MA, Krasynanski S, *et al.*: **A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide.** *Nat Protoc.* 2006; **1**(5): 2320–2325.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bellemain E, Carlsen T, Brochmann C, *et al.*: **ITS as an environmental DNA barcode for fungi: an *in silico* approach reveals potential PCR biases.** *BMC Microbiol.* 2010; **10**(1): 189.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Heitzman ME, Neto CC, Winiarz E, *et al.*: **Ethnobotany, phytochemistry and pharmacology of *Uncaria* (Rubiaceae).** *Phytochemistry.* 2005; **66**(1): 5–29.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hollingsworth PM, Forrest LL, Spouge JL, *et al.*: **A DNA barcode for land plants.** *Proc Natl Acad Sci U S A.* 2009; **106**(31): 12794–12797.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Istino F, Hamda F, Irfan S, *et al.*: **Characterization of specific random amplified polymorphic (RAPD) DNA fragments related to catechin content for early detection methods in gambier plant (*Uncaria gambir* (Hunter) Roxb.).** *Afr J Biotechnol.* 2013; **12**(15): 1736–1744.
[Publisher Full Text](#)
- Kimura M: **A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences.** *J Mol Evol.* 1980; **16**(2): 111–120.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Li H, Xiao W, Tong T, *et al.*: **The specific DNA barcodes based on chloroplast genes for species identification of *Orchidaceae* plants.** *Sci Rep.* 2021; **11**(1): 1424.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Liu XF, Yang CH, Han HL, *et al.*: **Identifying species of moths (Lepidoptera) from Baihua Mountain, Beijing, China, using DNA barcodes.** *Ecol Evol.* 2014; **4**(12): 2472–2487.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Meier R, Shiyang K, Vaidya G, *et al.*: **DNA Barcoding and Taxonomy in Diptera: A Tale of High Intraspecific Variability and Low Identification Success.** *Syst Biol.* Edited by M. Hedin, 2006; **55**(5): 715–728.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mount DW: **Choosing a Method for Phylogenetic Prediction.** *CSH Protoc.* 2008; **2008**(5): pdb.ip49.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Musdja MY, Rahman HA, Hasan D: **Antioxidant Activity Of Catechins Isolate Of *Uncaria Gambier* Roxb In Male Rats.** *LIFE: International Journal of Health and Life-Sciences.* 2018; **4**(2): 34–46.
[Publisher Full Text](#)
- Rach J, Desalle R, Sarkar IN, *et al.*: **Character-based DNA barcoding allows discrimination of genera, species and populations in *Odonata*.** *Proc Biol Sci.* 2008; **275**(1632): 237–247.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ridsdale CE: **A revision of *Mitragyna* and *Uncaria* (Rubiaceae).** *Blumea:*

Biodiversity, Evolution and Biogeography of Plants. 1978; **24**(1): 43–100.

Reference Source

Tamura K, Stecher G, Peterson D, *et al.*: **MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0**. *Mol Biol Evol*. 2013; **30**(12): 2725–2729.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Tang YL, Wu YS, Huang RS, *et al.*: **Molecular identification of *Uncaria (Gouteng)* through DNA barcoding**. *Chin Med*. 2016; **11**(1): 3.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Taniguchi S, Kuroda K, Doi KI, *et al.*: **[Evaluation of Gambir Quality Based on Quantitative Analysis of Polyphenolic Constituents]**. *Yakugaku Zasshi*. 2007; **127**(8): 1291–1300.

[PubMed Abstract](#) | [Publisher Full Text](#)

Wardi ES, Jamsari J, Irwandi I, *et al.*: **Barkod DNA Pada Tanaman Gambir**

(*Uncaria gambir*(Hunter) Roxb.) Berdasarkan Gen matK Dan rbcL. *Jurnal Ilmiah As-Syifaa*. 2020; **12**(1): 22–28.

Publisher Full Text

Wardi ES, Syukur S, Chaidir Z, *et al.*: **DNA barcoding of *Uncaria Gambir* using Internal Transcribed Spacer (ITS) Marker**. 2021a.

<http://www.doi.org/10.5061/dryad.dfn2z352w>

Wardi ES, Syukur S, Chaidir Z, *et al.*: **Molecular identification of *Uncaria gambir* [Hunter] Roxb. through DNA barcoding**. *IOP Conf Ser: Earth Environ Sci*. 2021b; **741**(1): 012056.

Publisher Full Text

Zhu S, Zhou L, Pang H, *et al.*: **[Molecular identification of one *Uncaria* plant]**. *Zhongguo Zhong Yao Za Zhi*. 2011; **36**(5): 535–7.

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Version 1

Reviewer Report 08 December 2022

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Andrey Yurkov 

Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Berlin, Germany

The article provides an interesting view on molecular recognition of plant species using DNA-barcoding. It has been previously emphasised that species recognitions approaches and the results depend on the used species concepts. Thus, I recommend to introduce in the introduction the presently used species concept (or concepts) of the genus *Uncaria*. This will help readers to better understand the results of this work.

I have two critical points:

1. The results of the phylogenetic analysis should be presented as a distance-based tree or network and not as a cladogram. I also suggest to include statistical support for clades. My feeling is that a phylogenetic network (a distance or parsimony network) might be more suitable for the research question.
2. The conclusions need to be adjusted to reflect the results, i.e. (i) in the absence of a barcoding gap, one cannot distinguish one species from another; (ii) the number of differences (substitutions and gaps) is technically sufficient to identify *Uncaria gambir*; and (iii) if this species is really an independent species (e.g. clade support).

Depending on answers in the second point, I recommend authors to decide on whether they suggest that (i) *Uncaria gambir* is not an independent species or (ii) that ITS is not a good marker for *Uncaria*.

I wish you good luck with your submission.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Fungal taxonomy and systematics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 20 June 2022

<https://doi.org/10.5256/f1000research.77986.r141126>

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Jarrett Phillips 

University of Guelph, Guelph, Canada

Overall, it is my opinion that the present short report is partially scientifically sound. However, further analyses needs to be completed to more easily support overall findings.

Since the success of DNA barcoding relies heavily on the barcode gap, analyses demonstrating the presence or absence of a gap between maximum intraspecific distance and minimum interspecific (i.e., nearest neighbour) distance should be done. This includes dotplots and histograms. There is much in the barcoding literature regarding these visual summaries. The Barcode of Life Data Systems (BOLD) (<http://v4.boldsystems.org>) is a good place to start with this.

Additionally, only mean distance values are reported and the barcode gap is assessed on this basis. This is not correct. The mean has been shown to over exaggerate the presence of a barcode gap. See Meier *et al.* (2008)¹ for details.

I would also encourage you to download relevant *Uncaria* species barcodes from BOLD to supplement the GenBank data. This will help paint a better overall picture within your paper.

The barcode gap is highly sensitive to both the depth and breadth of taxon sampling. Sufficient numbers of specimens need to be collected from across known species' geographic ranges. This should be highlighted in the paper.

There was no mention of BLAST in the manuscript except in the Results. Clearly, a nucleotide BLAST (blastn) was performed, but there was really no mention of details on this aspect. Please add more detail in this respect. It would also be ideal to run the sequences through the BOLD Identification Engine to support findings. BOLD employs global alignment whereas BLAST uses only local alignment to assess overall similarity matches.

Maximum likelihood (ML) with the Kimura-2-Parameter (K2P) model was used for phylogenetic analysis instead of the more common neighbour joining (NJ) method. This was explained because ML allows inclusion of the model of nucleotide substitution. However, no formal comparison of other models was given. Typically one chooses the most parsimonious model on the basis of a model selection criterion such as the Akaike Information Criterion (AIC) or the Bayesian Information Criterion (BIC). I strongly suggest that this is included in the next manuscript version.

Once the above comments are thoroughly addressed, the paper should be in a better place to warrant indexing.

References

1. Meier R, Zhang G, Ali F: The use of mean instead of smallest interspecific distances exaggerates the size of the "barcoding gap" and leads to misidentification. *Syst Biol.* 2008; **57** (5): 809-13
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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, data science, DNA barcoding, machine learning, statistics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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