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ORIGINAL RESEARCH

Molecular identification of Purnajiwa (*Kopsia arborea* Blume. and *Euchresta horsfieldii*) (Lesch.) Benn

Putu Eka Pasmidi Ariati, et al.

Application of DNA Barcoding for authentication of Balinese traditional medicinal plant Purnajiwa (*Kopsia arborea* Blume. and *Euchresta horsfieldii*) (Lesch.) Benn

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Abstract.

Purnajiwa is one of the medicinal plants in Bali. Species information regarding this plant is still unconfirmed. Currently, this plant is considered rare and endangered. Molecular identification

supports authentication at the species level, which will also support conservation efforts. Molecular identification using DNA Barcoding was carried out on purnajiwa from three sampling locations in Bali (Jimbaran, Mambal, and Bedugul) using rbcL primers (rbcLaF and rbcLaR). Data analysis was performed using BLAST with species on GenBank. Pairwise and similarity values were used to measure the proximity of the three samples to the species in the Gen Bank. The phylogenetic tree was constructed with the Maximum Likelihood and Tamura-3-parameter model. The analysis results show a close relationship between the purnajiwa from Jimbaran and Mambal with the species *Kopsia arborea* (KP095079). Meanwhile, the Purnajiwa from Bedugul showed a difference from the other two purnajiwa and was closely related to *Euchresta horsfieldii* (U74225). It can be concluded that the Purnajiwa from Jimbaran and Mambal is a species of *Kopsia arborea* while the Purnajiwa from Bedugul is a species of *Euchresta horsfieldii*.

Keyword : *Euchresta horsfieldii* , DNA Barcoding, *Kopsia arborea,* rbcL

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1. Introduction

Purnajiwa/pranajiwa is one of the medicinal plants empirically used by people in Indonesia, particularly in Bali, to increase stamina and as an aphrodisiac. This plant has been found in the following Bali locations: Jimbaran (lowland), Mambal (medium land), and Bedugul (highland). Morphological identification revealed several differences between the purnajiwa [1]. Some studies mention the pranajiwa as *Kopsia arborea* Blume. or *Euchresta horsfieldii* (Lesch.) Benn. *Kopsia arborea* Blume is a species in the genus Kopsia (family Apocynaceae). *Euchresta horsfieldii* (Lesch.) Benn belongs to the genus Euchresta (family Leguminosae). This plant can be found in

Bhutan, China, India, Indonesia, Laos, Nepal, the Philippines, Thailand, and Vietnam [2]. Researchs on *Kopsia arborea* Blume. and *Euchresta horsfieldii* (Lesch.) Benn are still in its early stages.

Several studies revealed novel and potent phytochemical and biological activities of *Kopsia arborea* Blume. and *Euchresta horsfieldii* (Lesch.) Benn [3–5]. Despite its beneficial phytochemical and pharmacological activities, purnajiwa is considered a rare and endangered plant [1,2]. Genetic studies are an excellent starting point for conservation efforts. Genetic information provides many benefits in aquaculture management and conservation, especially for species with limited numbers and facing extinction. Identification based on complete taxonomy aids in accurately and effectively identifying plant species [6].

DNA Barcoding is a fast and accurate method of species identification [7]. This tool is widely used in ecology, biomonitoring, safety evaluation, species detection, and taxonomic studies, primarily due to its high accuracy and objectivity. DNA Barcoding uses short sequences derived from standardized gene regions[8].

DNA barcoding has also been used to track the provenance of Traditional Chinese Medicine [9,10]. Several DNA barcodes, including internal transcribed spacer 2 (ITS2), psbA-trnH (intergenic spacer region), matK (maturase K), and rbcL (ribulose-1,5-bisphosphate carboxylase/large oxygenase subunit) regions, are used to authenticate medicinal plants [9,11,12]. The most recommended DNA barcodes are generated from chloroplast gene regions, including loci rbcL, matK, and trnH-psbA coding. These gene regions produce the highest universality and discriminatory power, lowest sequencing cost, and highest quality. The chloroplast marker is considered a universal plant barcode. RbcL and matK are known as primary standards in plants [13]. Using these two primers is recommended in the application of plant molecular identification. The rbcL region is constructed from a single rbcL gene encoding eight large subunits of the rubisco

holoenzyme [14]. RbcL has been applied to molecular studies of various plants, especially medicinal plants, and has been successfully used to identify the species level [15,16]. Identification with these loci showed good results up to the genus level, although the species identified have undergone various growth or developmental stages [12].

This research provides novel information about purnajiwa species in Bali. Several studies have been conducted to optimize the amplification conditions in applying purnajiwa barcoding using COI and Ehoscn01a markers [1,16], although there are no published studies on the results of the identification of purnajiwa in Bali using DNA Barcoding. This research was conducted to identify the purnajiwa species from the three locations: Jimbaran, Mambal, and Bedugul, using DNA Barcoding.

2. Material and method

2.1 Sample collection and morphological characterization

The leaf samples of puranjiwa were collected from three locations in Bali, Indonesia : Jimbaran (low land), Mambal (medium land), and Bedugul (highland) in Figure 1.

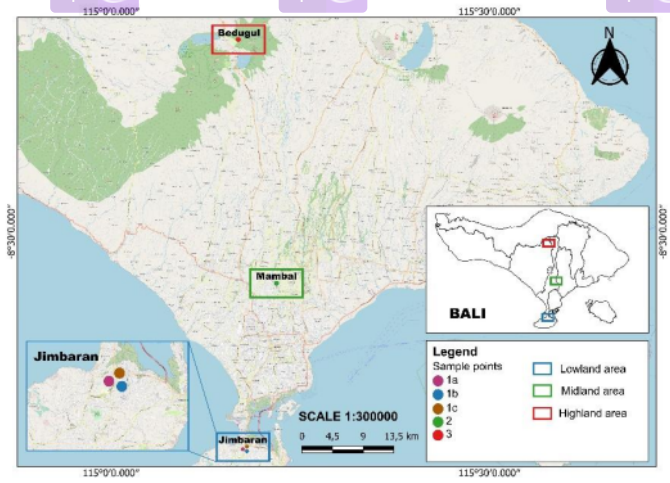

Figure 1. Map of purnajiwa's sampling locations. Purnajiwa leaf samples were taken from three locations in Bali representing lowland, mediumland, and highland areas.

DNA Extraction and PCR Amplification

Total DNA was extracted using the Quick-DNA Plant/Seed Miniprep Kit Kit (Zymo Research, D6020) following the manufacturer's procedure. The DNA of purnajiwa samples collected from Jimbaran (RJ_Jimbaran), Mambal (RM_Mambal), and Bedugul (RB_Bedugul) were amplified using primers designed from the rbcL region referred to[17]. RbcLaF (ATGTCACCCACAAACAGAGACTAAAGC) and RbcLaR (GTAAAATCAAGTCCACCRCG) were used as forward and reverse primer. PCR was conducted using My Taq HS Red Mix BIO-25048 under the following conditions: pre-denaturation (95°C for 3 seconds), 35 cycles of denaturation (95°C for 30 seconds), annealing (53°C for 30 seconds), extension (72°C for 1 minute), and final extension (72°C for 75 seconds). A 1μL PCR product was used for electrophoresis with 1% TBE agarose. The Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) was used to determine the sequence bi-directionally.

Data Analysis

BLAST analysis was used to compare the obtained sequences to the NCBI sequences. ClustalIW was used to select sequences with an e value of 0.0 and a percent identity greater than 90% for the alignment process (MEGA). The maximum likelihood method and the Tamura-3-parameter model were used to build the phylogeny tree. The tree's robustness was assessed using 1000 bootstrap replications.

3. Results

The rbcL primer was able to successfully amplify Purnajiwa from three locations (Figure 2.) The amplification produced DNA sequences with the following base lengths: 588bp (PJ Jimbaran), 588bp (PM Mambal), and 587bp (PB Bedugul). In phylogenetic analysis, the nine Gen Bank accession numbers with the lowest e value and highest percent identity were chosen using BLAST.

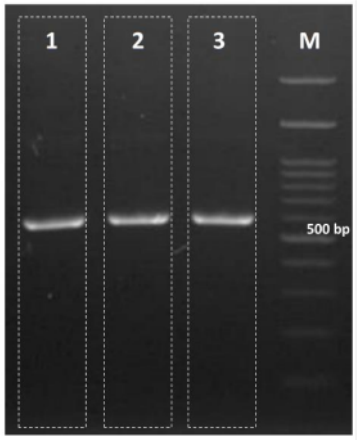

Figure 2. The electrophoresis results of PCR amplification were collected from Jimbaran (PJ_Jimbaran), Mambal (PM_Mambal) and Bedugul (PB_Bedugul).

Table 1. The alignment of the purnajiwa barcoding sequences from the three locations with the best pairwise distance and similarity percentage among several GenBank accession numbers (ID).

Species	Accession No	PJ_Jimbaran		PM_Mambal		PB_Bedugul	
		Pairwise	ID	Pairwise	ID	Pairwise	ID
PJ_Jimbaran	-	-	-	0,002	99.40%	0,010	92.80%
PM_Mambal	-	0,002	99.40%	-	-	0,010	93.00%
PB_Bedugul	-	0,070	92.80%	0,068	93.00%	-	-

Kopsia rosea	MG963245	0,005	99.32%	0,003	99.66%	0,072	93.02%
Kopsia sp.	AB586185	0,005	99.32%	0,003	99.66%	0,072	92.99%
Kopsia fruticosa	X91763	0,005	99.30%	0,003	99.65%	0,073	92.78%
Kopsia arborea	KF496808	0,002	99.65%	0,000	100.00%	0,067	93.25%
Kopsia arborea	KP095079	0,002	99.64%	0,000	100.00%	0,067	93.54%
Rauvolfia verticillata	MN056244	0,024	97.45%	0,022	97.79%	0,080	92.16%
Euchresta horsfieldii	U74225	0,071	92.78%	0,069	93.12%	0,003	99.65%
Euchresta japonica	LC693501	0,072	92.67%	0,070	93.01%	0,005	99.49%
Euchresta japonica	LC690287	0,072	92.67%	0,070	93.01%	0,005	99.49%

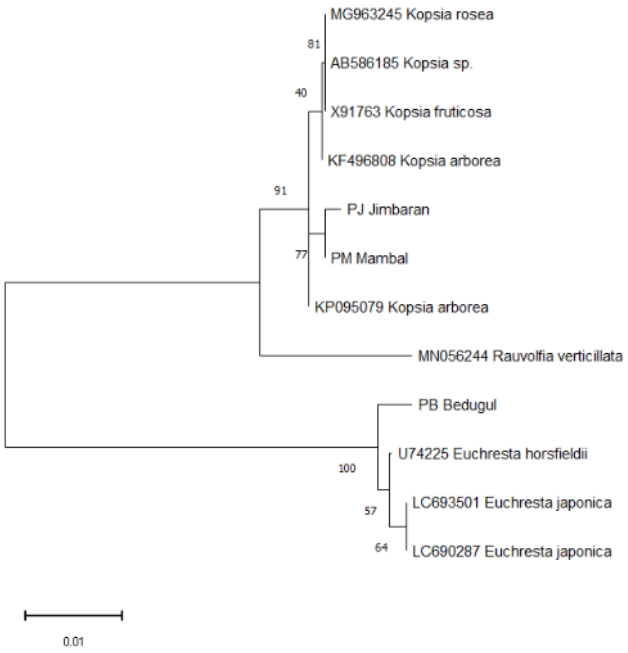

Figure 3. The Purnajiwa rbcL sequence-based phylogenetic tree was built using maximum likelihood and the tamura-3-parameter model.

A pairwise comparison of Purnajiwa from Jimbaran (PJ Jimbaran) and Mambal (PM Mambal) reveals a closeness of 0.02 and a similarity of 99.40%. PJ Jimbaran and PM Mambal have a pairwise value of 0.010 and a similarity of 92.80% and 93% to Bedugul (Table 1). Compared to

several Gen Bank accession numbers, purnajiwa from Jimbaran is closely related to *Kopsia arborea* (KF496808 and KP095079) with a pairwise value of 0.002 and 99.65% and 99.64% similarity, respectively. PM Mambal also produced comparable results, demonstrating closeness to *Kopsia arborea* with a pairwise value of 0.000 and a similarity of 100.00% with both *K. arborea* accession numbers. PB Bedugul is closely related to *Euchresta horsfieldii* (U74255) with a pairwise similarity value of 0.003 and a similarity of 99.65%.

Based on the Maximum likelihood phylogenetic tree and the Tamura-3-parameter model, Purnajiwa from Jimbaran and Mambal belong to the same group and are closely related to *Kopsia arborea* (KP095079). In comparison, purnajiwa from Bedugul were classified with *E. horsfieldii* (U74225) and *E. japonica* (LC693501 and LC690287). PB Bedugul generated a bootstrap value of 100% for *E. horsfieldii* (U74225).

4. Discussion

Molecular tools play an essential role in the development of taxonomical studies. DNA Barcoding offers the most popular, simple, and affordable molecular tool for species identification [18]. This molecular tool is a species identification system that uses standard gene regions as internal species tags. DNA Barcoding is a precise, rapid, automated molecular tool [19]. This method has been utilized successfully in numerous fields, such as species identification, discovering cryptic species, tracking invasive species, community ecology, and conservation [20]. DNA barcoding studies can identify species in a vast array of taxa and investigate their biogeography and phylogeography [21]. As the geographic scale expands, the theory predicts the range of genetic variation. Research conducted on intercontinental spider species demonstrates that DNA barcodes can also be used to identify species geographically. It is possible to state that DNA barcoding is

an efficient method for identifying biogeographical information. Studies confirm the occurrence of this phenomenon in intraspecific species. DNA barcoding is an effective method for identifying species regardless of morphological classification [22].

Properly identifying medicinal plants and evaluating their biological benefits is essential in studying medicinal plants, especially in understanding the evolutionary history of various important plant species. DNA Barcoding has been widely used to identify plant species, including medicinal plants. DNA Barcoding is suitable for demonstrating similarities and differences between families of medicinal plants [23]. Various matK, rbcL, ITS, ITS2, and psbA-trnH gene regions were used to identify medicinal plant species. A molecular barcoding method is reliable for identifying medicinal plants at the genus and species level. This method is consistent and reliable regardless of the sample's age, plant parts, or environmental conditions. The selection of barcoding sequences is vital in applying DNA barcodes for species identification. According to CBOL (Consortium for the Barcode Life), the rbcL and matK regions are essential in identifying medicinal plant species. RbcL and MatK are important regions for similarity and difference studies in various medicinal plant species and have been widely used to study evolution and taxonomy. The chloroplast marker is considered a universal plant barcode. [13,23].

Rubisco is the main regulatory enzyme in the catalysis of CO2 fixation and diphosphate oxygenation reactions in the net determination of photosynthesis. The Rubisco holoenzyme consists of eight small subunits encoded by the nuclear multigene family (rbcS) and eight large subunits encoded by the single gene (rbcL) in the chloroplast genome [24]. The rbcL region is about 500 bp long. The rbcL region is constructed from a single rbcL gene encoding the eight major subunits of the rubisco holoenzyme Ribulose 1,5 bisphosphate carboxylase/oxygenase

(EC4.1.1.39, Rubisco) is an essential biochemical marker, accounting for 50% of the total soluble protein in plant leaves.

RbcL has been applied to several medicinal plants for species identification and conservation efforts. Identifying rubisco rbcL in *Camellia oleifera* to increase tea oil production shows that the rbcL gene is very conservative [24]. Some advantages of using rbcL are its ability as a universal primer standard, high success in DNA amplification, and excellent sequence quality [14]. A comparison of rbcL and matK in Salix taxa showed that rbcL resulted in good quality sequences with high resolution compared to matK. Rare within-taxon substitution can also be detected well, whereas matK primers cannot carry out this detection. In addition, polymorphisms can also be detected well with the use of rbcL. However, matK and rbcL primers could only identify Betula and Slix up to the genus level and not at the species level [14]. The use of rbcL in identifying species and genus of all poisonous medicinal plants in the Chinese Pharmacopoeia (2015) shows that rbcL can be used in identification up to genus and species level by using the identification of blast and distance [15]. The use of rbcL was also able to identify several plants in the Solanaceae, Euphorbiaceae, and Fabaceae families up to the species level [25].

In this study, rbcL primer was able to amplify purnajiwa DNA taken from three locations in Bali. The analysis results show that purnajiwa from Jimbaran and Mambal have a close relationship where the two souls are close to the *Kopsia arborea*. Meanwhile, purnajiwa from Bedugul shows differences from the other two purnajiwa and closeness to *Euchresta horsfieldii*.

The genus Kopsia consists of 24 species distributed in several countries, especially Southeast Asia, India, China, northern Australia, and Vanuatu. Kopsia species typically contain potent and diversely bioactive indole alkaloid compounds [3,11]. The species *Euchresta horsfieldii* (Lesch.) Benn belongs to the genus Euchresta (family Leguminosae). *E. horsfieldii* is a perennial plant

found in several countries, including Bhutan, China, India, Indonesia, Laos, Nepal, the Philippines, Thailand, and Vietnam. The habitat of *E. horsfieldii* in Indonesia is rainforests between 1300 and 2400 meters above sea level. This plant is found in some Indonesian regions, including Sumatra, Java, and Bali [2]. *Euchresta horsfieldii* contains numerous bioactive compounds, including isoflavonoids, and has been used historically to treat hyperlipidemia. Several studies have evaluated the pharmacological activity of *E. horsfieldii* as an antitumor, antioxidant, aphrodisiac, and lipid-lowering agent [2,5].

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Missing "," Review the rules for using punctuation marks.

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Coord. Conjunction Review the rules for combining sentences.

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S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.

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Confused You have used either an imprecise word or an incorrect word.

Article Error You may need to remove this article.

Prep. You may be using the wrong preposition.

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Run-on This sentence may be a run-on sentence.

P/V You have used the passive voice in this sentence. You may want to revise it using the active voice.