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"LUBEG" (Philippine Cherry), *Syzygium lineatum* (Roxb.) (DC.) Merr & Perry: Its Taxonomic and Molecular Identification

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Abstract

Objectives: This study aimed to taxonomically classify "Lubeg", Philippine Cherry and molecularly identify this indigenous species thru DNA barcoding. **Methods:** Fresh samples of "Lubeg" leaves, unripe fruits and flowers were collected and submitted to Bureau of Plant Industry, Manila for authentication. The Key to Species of *Syzygium* Gaertn was used in the taxonomic classification of "Lubeg". Molecular identification of the species used the plastid DNA (ptDNA) with two genes *rbcL*and *matK,* together with ITS 2. Leaf extracts were submitted to Macrogen, South Korea for the complete barcoding of the plant. The procedures were: gDNA extraction, PCR amplification, purification, sequencing and BLAST BI report. **Results:** "Lubeg", Philippine Cherry belongs to family Myrtacaea under species *Syzygium*(Gaertn). It was identified as *Syzygium lineatum* (Roxb.) (DC.) Merr & Perry.*'Syzygium'* was derived from the Greek term *'syzygos,'* meaning the paired leaves are opposite in arrangement and *'lineatum'* means stripes, netted veins on the leaves. The amplification was only successful using the *rbcL* gene and furthermore, based on the sequences, the plant sample belongs to the genus *Syzygium*. It was noted that this was the first sequence of *Syzygium lineatum* under the *rbcL* gene. Using the phylogenetic tree, the "Lubeg" sequence is closest to *Syzygium malaccense*, and the out-group is *Eucalyptus globulus.* **Improvements:** The process of DNA barcoding with the same DNA markers be carried out with the same plant species to further verify the result of this study.

Keywords: DNA Barcoding, "Lubeg", Molecular Identification, Taxonomic Classification

1. Introduction

The species of *Syzygium lineatum* is a native to Borneo, Cambodia, China (Guangxi), Philippines, Java, Laos, Malaysia, Myanmar, Small Suda Islands, Singapore, Sumatra, Thailand and Vietnam where it lives in humid forest[1].

Statistically, in the Plant List, there are 1,374 scientific plant names of species rank for the genus *Syzygium.* Of these 1,123 are accepted names and one of these is *Syzygium lineatum.*

"Lubeg", "Malubeg" and "Alebadu" are the common names of Philippine Cherry, *Syzygium lineatum*(Roxb.) Merr & Perry. It inhabits some areas of Cagayan, Apayao and Isabela provinces of Region 02. In Cagayan, it is noted that "Lubeg" trees thrive mostly and abundantly in the municipality of Lal-lo.

Its classification had been established but further verification should be done to the "Lubeg" species that thrive in the municipality of Lal-lo, Cagayan. Phytochemical screening results of "Lubeg" leaves and fruits are also varied[2–5]. It contains flavonoids, tannins, saponins, steroids, coumarins and quinones[7].

Syzygium sp. has been reported to its abundance of bioactive phytochemicals to have anticancer potentials[6],

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Development of food products from "Lubeg" *Syzygium lineatum* such as wine, jam and jelly had been developed into technology[3]. These articles on the medicinal potentials of "Lubeg" and food production from "Lubeg" are the evidences on the relevance of this species in the scientific community.

Molecular identification of plant species was considered since DNA barcoding serves as a means to identify the species and the purity and identity of botanical products such as commercial herbal medicines and dietary supplements[8].Hence, this study was induced to come up with the DNA barcode for its molecular identification and the proper taxonomic classification of "Lubeg" especially the ones that thrive in Cagayan Valley.

2. Objectives

This study aimed to determine the taxonomic classification and molecular identification through DNA barcoding of "Lubeg", (Philippine Cherry) *Syzygium lineatum.*

3. Methodology

3.1 Taxonomic Classification

Fresh samples of "Lubeg" leaves, unripe fruits and flowers were collected in Lal-lo, Cagayan. These samples were brought to Bureau of Plant Industry, Manila for authentication. A letter of request was done addressed to the OIC director of the Bureau of Plant Industry, Manila together with photos of the "Lubeg" flowers, fruits and leaves were submitted for proper authentication of the species.

3.2 Molecular Identification (DNA Barcoding)

The Consortium for the Barcode of Life (CBOL) Plant Working group has forwarded the use of the plastid DNA (ptDNA) genes *rbcl* and *matK* standard DNA barcode markers primarily because of the availability of published primers of these genes together with the high level of taxonomic resolution when these primers are used. In this study, these two genes, together with the ITS2 gene were targeted for the barcoding of *Syzygium lineatum*, locally known as "Lubeg".

"Lubeg" leaves were collected in Lal-lo, Cagayan, transported and submitted in fresh sample at College of Science Laboratory, University of the Philippines- Baguio, Baguio City where it was extracted.

"Lubeg" leaves were subjected to genomic DNA extraction using the methods of Liu et al (2000). However, repeated efforts resulted to negative results. Norgen-Biotek Plant DNA Isolation Kit was also employed, which was also unsuccessful. The last option was to send the leaf samples to Macrogen, South Korea for the complete barcoding of the plant. The procedures were: gDNA extraction, PCR amplification, purification, sequencing and last was the BLAST BI report. The DNA Barcoding Report was emailed back to the researcher.

4. Results and Discussion

4.1 Taxonomic Classification

Kingdom Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magniliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Specific epithet: *lineatum*

Species *Syzygiuml ineatum* (Roxb.) Merr Perry ; *Syzygium lineatum* (DC.) Merr & Perry

Keys to Species of Syzygium **Gaertn**[9].

Flowers terminal or in axils of leaves or fallen leaves, rarely on old wood, in cymes or panicles; calyx-tube often turbinate, campanulate or obconical, whether or not stipitate at base, produced beyond the ovary' calyx-lobes 4-5, rarely more, well-developed or minute; petals 4-5, rarely more, patent or coherent into small hood, mostly early caduceus; disk inside insertion of innermost stamens whether or not provided with an inwardly protruding, naked edge (disk margin); stamens numerous, in 1-numerous rows; filaments filiform; anthers dorsified; ovary 2-4 celled, many -ovuled; berry mostly 1-seeded; cotyledons thick-fleshy, free, enclosing the radicle. Leaves opposite, sometimes 3-verticillate or sub-opposite, pinnately nerved. Trees or shrubs, always glabrous.

1a. Flower-buds thinly clavate; calyx-tube at least 3 1?2 times as long as broad; ovary-cells with 2 long rows of ovules; stamens much shorter than calyx-tube. . . . . .

1b. Flower buds mostly comparatively thicker; ovules not biseriate. 2

2a. Calyx-tube 2-2 ½ mm long, turbinate, base not stalk-like; teeth 4, wide apart, very small; petals white, minute, rather soon caduceus; stamens 1-seriate, 1 ½ mm; style 1 ½ mm; berry depressed-globose, black, 6-10 mm diam.; inflorescences mostly fascicled at apices of acuminate, coriaceous, not or very sparsely dark-dotted beneath, 7-12 cm by 2 ½ -4 ½ cm; lateral nerves 6-8 on either side midrib, curved, thin; intra-marginal nerve -1-2 mm (*Eugenia magnoliaefolia* (Bl.) K. & V. . . . . .S. magnoliaefolia (Bl.) DC.

2b.Calyx tube at least 4 mm long, or if not so the flowers panicledin axils of fallen leaves 3

3a. Calyx-tube cylindricorobconical, much prolonged beyond ovary, mostly longitudinally plicate, covered by a white or yellowish waxy layer, less than 4 mm broad apex, shortly stipitate towards base, stipe distinctly shorter than the rest of calyx (incl. pedicel) 4-6 mm long;calyx-lobes 5-6 in all or most flowers, obvious, erect, sub-equal, ½ - 1 mm long; petals minute, often coherent to small calyptra, falling off when the flowers expand; stamens 4-8 mm; style 5-8 mm; berry sub-globose, white, 1 cm long at most; flowers in racemes, heads or small panicles. Leaves all or most of them shorter than 10 cm; petiole shorter than ½ cm. . . . . .

3b.Calyx otherwise; lobes mostly 4, very rarely 5 or minute and irregularly shaped; tube smooth, straited or finely ribbed. 4

4a. Style 1 ½ - 2 mm long; stigma 2-lobed; flowers on defoliate twig-parts, small; cotyledon with lobed inner surfaces. . . . . .

4b. Style longer, or if not so the flowers axillary or terminal, often combination of both. 5

5a. Mature flowers subtended by 2 conspicuous bracteoles; inflorescences corymbiform, repeatedly fasciculately branched; calyx-tube obconical, 5-7 mm long; teeth 4, wide apart, very short, acute, petals coherent to small calyptra; inner margin of disk distinctly prominent; stamens 5; syle 3-4 mm; berry ellipsoid, 1 cm long; cotyledons joining each other with lobed inner surfaces; radicle longish. Leaves obovate-oblong, base long- tapering, apex shortly acuminate, coriaceous, dotted beneath, 7-14 cm by 3-5 cm; lateral nerves close-set, thin; petiole 1 ½ - 2 cm. Tree. (*Eugenia fastigiata* (Bl.) K. & V., *E. confertiflora* K. & V., non A. Gray) . . . . . .S. fastigiatum (Bl.) Merr& Perry

5b. Bracteoles absent at base of flower, or minute and soon caducous. 6

6a. Style 1 ½ mm long at most; calyx-tube gradually (though distinctly) stipitate towards base, 4 mm long, campanulate-turbinate; teeth 4, small, obtuse; petals 1 ½ - 2 mm long; stamens in 1 row, up to 1 ½ mm; ovary 2-3 celled; fruit unknown; inflorescences axillary and terminal, shorter than 5 cm. Brachletsterete or sub-angular; leaves elliptic-oblong, long-acuminate, acute, mostly distinctly dark green-dotted beneath, 6 ½ - 13 cm by 2 ½ - 5 cm lateral nerves close-set, thin; intra-marginal nerve 1 mm from margin; petiole ½ - 1 cm. Tree with copper-red, thin, flaky bark . 10.00-35.00; II,X;W.E.; forest; local (*Eugenia microcyma* K. & V. . . . . S. microcymum (K. & V.)Amsh.

6b. Style at least 2 mm long. 7

7a. Calyx-lobes 1 mm long at most, usually shorter, sub-membranous, sometimes obscure; tube 4-7 mm long; staminiferous disk insconspicuous . . . . . .

7b. Calyx-lobes well-developed, at least 1 mm long. 8

8a. Calyx-tube 5-7 mm long; lobes soon caduceus; two outer ones up to 2 mm long, half as long as two inner ones, strongly imbricate; staminiferous disk obscure; berry crowned by truncate calyx-limb. . . . . .

8b. Calyx-lobes mostly not semi-persistent; staminiferous disk well-developed, obvious in blown flowers; berry usually crowned by erect (rarely incurved or reflexed) calyx-lobes. 9

9a.Flowers on defoliate twig-parts in many-flowered, 5-13 cm long panicles with widely patent triangular, 1 ½ mm long; petals 4-6 mm long, early caduceus; disk orbicular, thick filaments 4-8 mm; style 7-8 mm; berry whether or not crowned by calyx-limb, dark purple, 2 ½ - 3 ½ cm diam. Leaves sub-sessile, cordate at base, sub-amplexicaul, oblong, oblong lanceolate or sub-obovate, acuminate, pellucidly dotted or not, 11-25 cm by 4-10 1?2 cm, youngones purple; lateral nerves on either side of

midrib 12-14. Tree 8.00-20.00; V – IX, W.C.E 200-1800; secondary forest; also often cultivated in gardens as a fruit tree (*Eugenia polycephala*Miq.) . . . . . S. polycephalum (Miq.) Merr & Perry

9b. Inflorescences terminal or axillary, or bearing few large flowers on defoliate twig parts. . . 10

10a. Lateral nerves strongly approximated, thin; intra-marginal nerve 1 mm from margin. Inflorescence terminal, corymbose. 11

10b. Larger lateral nerves all or for the greater part more than ½ cm (often more than 1 cm) distant from each other . . . . .

11a. Leaves obovate-oblong, base very acute, apex shortly obtusely acuminate, coriaceous shining, pellucid-dotted, 6-13 ½ cm by 2 ½ -5 ½ cm, petiole 1 ½ -2 ½ cm. Corymbs 3-22 flowered, usually few-flowered; calyx-tube subterete, sub-quadrangular, yellowish green with a red flush, 1 - 1 ½ cm high, slightly produced beyond the ovary; lobes ovate-triangular , 2 mm long; petals coherent, more or less tinged with red; filaments 3-7 mm; style 3mm; stigma 2-lobed; berry ellipsoid-obovoid, dark red, 2- 2 ½ cm long; cotyledons oppressed against each other with their lobed inner surfaces; radicle half as long as the cotyledons. Tree 5.00-10.00; IV-IX; from the Moluccas; in Java locally cultivated (*Eugeniacaryophyllus* (Spreng.) Bullock & Harris) . . . . . .S. aromaticum (L.) Merr & Perry

11b. Leaves elliptic or elliptic-oblong, base acute, apex acuminate. Inflorescence many-flowered, dense; calyx-tube long, obconical; petals free, white; berry crowned by calyx-limb. 12

12a. Corymbs 8-12 cm across; calyx-tube 6-8 mm; lobes 2-3 mm long; petals 5 mm across filaments and style ½ - 1 ½ cm long; disk inside insertion of stamens with naked inner margin; berry sub-globose, 1 ½ cm diam. Leaves thickly coriaceous, conspicuously striate-veined on both surfaces, especially above, 7-10 cm by 3-4 ½ cm; petiole 5-7 mm. Tree 15.00 XII, I; on Galunggung (W); 1300-1400; forest (*Clavimyrtus firma* BL. non *Syzygium firmum* THW., *Eugenia firma* DC., - *Eugenia ampliflora* K. & V. . . . . .S. ampliflorum (K. & V.)Amsh.

12b. Corymbs 2 ½ - 8 cm across; calyx-tube 5-6 mm; lobes 1 ½ - 2 mm; petals 3- 4 ½ mm long; stamens and style ½ - 1 ½ cm; disk nearly entirely beset with stamens; berry separated from calyx-limb by a constriction, ellipsoid, black 1 ½ cm long; cotyledons superposed, semi-globose; radicle very short. Leaves thin-coriaceous, conspicuously straite-veined, especially on lower surface, 5-12 cm by 2-5 cm; petiole 6-12 mm. Shrub or tree, occasionally with stilt roots. 1.00 - 21.00, rarely higher; VI - I; W.-half; 50-1600; primary and secondary forest (*Eugenia lineata*(DC.) Duthie, - *E. teysmanni* (Miq.) K. . . . . .S. lineatum **(DC.) Merr. & Perry**

4.2 Morphology

"Lubeg" *Syzygium lineatum* (Roxb.) Merr Perry belongs to the family Myrtaceae. This tree is medium with a height of 5 to 7 meters, perennial with flaky, corky, grayish brown bark. Figure 1 shows that it has green leaves, smooth and leathery, simple unifoliate, opposite in arrangement and lanceolate shape. The flower of "Lubeg" is bisexual, white in color, in clusters or inflorescence and with axillary

a.

b.

c.

Figure 1. *Syzygium lineatum* (Roxb.) Merr & Perry. **(a)** Fruits – ripe and unripe. **(b)** Flowers. **(c)** Leafy branch.

spike. "Lubeg" are cherry-like fruits, oblong, white and gradually turns to red to purple when ripe.

4.3 Distribution

Southeast China, Myanmar, Vietnam, Thailand, Malaysia, Singapore, Brunei, Indonesia and the Philippines. In the Philippines, *Syzygium*is distributed in a diverse habitat types. In the Philippines, there are 180 sp. of Syzygium distributed in the country. *Syzygium lineatum* , "Lubeg" is one of the native tree species identified at Minalungao Park, Nueva Ecija[10]. "Lubeg" trees also thrive in the North- Western, Cagayan where they grow abundantly specially in the town of Lallo. "Lubeg" trees were found planted and grown vigorously, naturally and endemically in the in Region 2 and in the municipality of Lal-lo. Most of the collection of samples for propagation[11] and its phytochemical study[6] were collected in the said municipality. *Syzygium lineatum*, "Malibago" which is an indigenous edible plant were collected and identified in six towns of Southern, Isabela[12]. It was noted that this species was also a lesser known species inhabiting the province of Apayao[3].

4.4 Habitat and Ecology

Secondary forest, Cultivated

4.5 Phenology

Flowering specimens were collected in July to August.

4.6 ETYMOLOGY

Genus epithet 'Syzygium' derived from Greek term 'syzygos' (joined), alluding to the opposite paired leaves. Species epithet 'lineatum' means "with lines or stripes", a reference to the dense network of veins on the leaves. (https://florafaunaweb.nparks.gov.sg).

The name of the genus is the combination of the Greek words *syn*= together and *zygon* = yoke, which is attributed to joined petals in some species. 'Lineatum', a, um, indicated by the many secondary veins of the leaf[12].

4.7 Vernacular Name

"Lubeg" or "Malubeg" (Iokano), "Alebadu" (Ibanag)

4.8 Molecular Identification

The Consortium for the Barcode of Life (CBOL) Plant Working group has been forwarding the use of the plastid DNA (ptDNA) genes *rbcL* and *matK* as standard DNA barcode markers primarily because of the availability of published primers for these genes together with the high level of taxonomic resolution when these primers are used[13]. In this research, these two genes, together with the ITS 2 gene were targeted for the barcoding of *Syzygium lineatum*, locally known as "Lubeg". (Table 1)

The yield of 1.08 ng/µL is very low. This signifies the difficulty of extracting DNA from the sample. They were only successful in amplifying the rbcl gene and thus this was the only gene that was sequenced. The amplification of the matK and the ITS 2 gene sequences were unsuccessful.

Basic Local Alignment Search tool of both sequences resulted to the identification of the plant sample as belonging to the genus *Syzygium*. Unfortunately, the GenBank database does not have any sequence of *Syzygium lineatum.* If ever, this is the first sequence of the *rbcL*gene for *Syzygium lineatum.*

Figure 2, is a phylogenetic tree generated by the Molecular Evolutionary Genetics Analysis software using the maximum likelihood algorithm[11,14,15]. It shows that the "Lubeg" sequence is closest to *Syzygium malaccense.* The out-group is *Eucalyptus globulus.*

An infrageneric classification of *Syzygium* (Myrtaceae),[16] revealed six subgenera of *Syzygium* supported by nuclear and plastid DNA sequence data.

Table 1. Summary of the sequences of the primers used in barcoding the plant sample

	Forward	Sequence	Reverse	Sequence
1	rbclaF	ATGTCACCACAAACAGAGACTAAAGC	rbclaR	GTAAAATCAAAGTCCACCCRCG
2	MatK 472_F	CCCRTYCATCTGGAAATCTTGGTTC	MatK 1248_R	GCTRTRATAATGAGAAAGATTTCTGC
3	matK-xf	TAATTTACGATCAATTCATTC	matK-MALP	ACAAGAAAGTCGAAGTAT
4	ITS5a	CCTTATCATTTAGAGGAAGGAG	ITS4	TCCTCCGCTTATTGATATGC
5	ITS1	TCCGTAGGTGAACCTGCGG	ITS4	TCCTCCGCTTATTGATATGC
6	ITS_S2F	ATGCGATACTTGGTGTGAAT	ITS_S2R	GACGCTTCTCCAGACTACAAT

Fasta file of the sequence of the rbcL gene using the forward primer: >180103-R02_A01_Plant_p1_rbcL_F.ab1 605

AAGGAAGGGTAAAACGACCTTA ACTGACTTATTATACCCCC TGACAATGA
AACCAACGATACTGATATATTGGCAGCATTCCGAGTAACTCCTCAACCTG
GAGTTCCTCCTGAGGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACT
GGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGCCTTGATCGTTA
TAAAGGAAGATGCTACCACATCGAGCCTGTTGCTGGAGAAGAAAATCAAT
ATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTT
ACTAATATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCT
GCGCGCTCTACGTCTGGAGGATCTGCGAATCCCTACTTCCTATACGAAAA
CTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAATTGAAC
AAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTT
ATCCGCTAAGAACTACGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGAC
TGGATTTTACATACTCATGGAATGGATCTTATCCTTCCCCCTTCTGCTGA
CCTTT

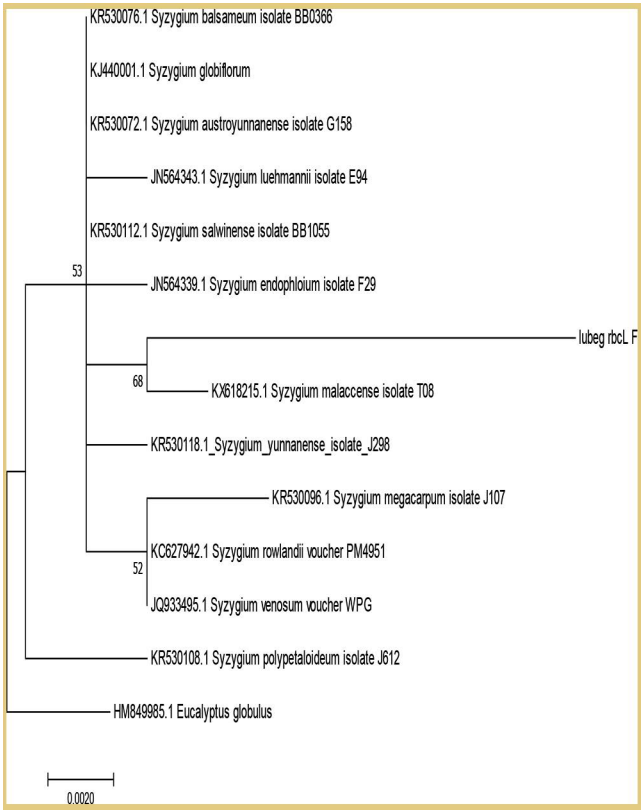

Figure 2. Molecular phylogenetic analysis by maximum likelihood method[15].

Based on Figure 2, the sample is closest to S. malaccense. The relationship established in the phylogram is not convincing because of the low bootstrap value (the number at the base of the branch which is 68). This means that only 68% sure that this is true.

5. Conclusion and Recommendation

"Lubeg," scientifically named as *Syzygium lineatum* (Roxb.) (DC.) Merr & Perry belongs to Division Magniliophyta, Class Magnoliopsida, Order Mystales and Family Myrtacaea. It is closed to *Syzygium malaccense* an out-group Eucalyptus globulus but the likelihood to be was not convincing thus it is recommended that the process of DNA barcoding be repeated with the same DNA markers, *rbcl*and *matK* using plastid and mitochondrial DNA to verify the result of this study.

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