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ORIGINAL ARTICLE

DNA Barcoding of the Ilongot-Eǵongot Ethnobotanicals of Bayanihan, Maria Aurora, Aurora, Philippines

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ABSTRACT
The Philippines is an immensely diverse country with numerous plant species with pharmacological potential. Among these are the ethnobotanicals used by ethnic communities as part of their primary healthcare. These plants are mostly wild and endemic as they are largely found in the highlands and other areas that are not yet reached by industrialization. One of these ethnic communities is the Ilongot Eǵongots that inhabit the vast domains of Sierra Madre. The richness of their domains include expansive areas with ethnobotanicals that are utilized in their traditional way of medication. However, these ethnobotanicals are generally unstudied and unexplored. Identification of these ethnobotanicals is the first and essential step in their study and will further support researches conducted in the future. Molecular methods such as DNA barcoding is a reliable and manageable method of identification. It uses a molecular marker such as rbcL which is a standard barcode for plants. This study reports mlecular identification of the six ethnobotanicals with pharmacological values using the rbcL: Tinospora pandacaqui, Andrographis paniculata, Artemisia argyi, Mikania macrantha, Tabernaemontana crassa, and Senna alata.
Keywords*: Ethnobotanicals, DNA, rbcL, Ilongot Eǵongot*

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INTRODUCTION

The Philippines is an immensely diverse country with numerous plant species [1]. However, this diversity and high endemism is threatened. The IUCN (2015) reported that the Philippines is one of the hotspots in the world that is prone to the extinction of biodiversity. Biodiversity of plants are mostly found in highlands and other areas that are not generally reached by industrialization [2] and are particularly unstudied. These include plants that are utilized by ethnic communities for their primary healthcare known as ethnobotanicals.

Ethnobotany deals with plants and its practical uses based on the traditional knowledge of the people or a local community [3,4].The uses of plants, especially its medicinal properties, are being applied by the people therefore linking ethnobotany to the field of medicine [5]. One of the ethnic groups in the Philippines that utilizes ethnobotanicals in their traditional medicine are the Ilongots Eǵongots that inhabit the rich domains of Bayanihan, Maria Aurora, Aurora, Philippines and adjacent areas. Their domains are vast areas of ethnobotanicals which are unstudied and unexplored. However, identities of these ethnobotanicals are not completely recorded in the scientific community [6]. Moreover, the first and essential step in scientific researches is the identification of species. Additionally, identification is a pressing need due to its vulnerability to extinction because of rapid decrease of biodiversity [7].

Traditionally, the identification is through morphological characteristics [8]. Although manageable, morphological identification has limitations especially when an organism undergoes development. To resolve the this problem, DNA barcoding has been employed [9]. DNA barcoding uses a short sequence from a standardized region of a genome [10]. Plants have standard barcodes that includes the rbcL gene which is characterized with slower evolution rate appropriate for identification [11]. Furthermore, *rbcL* marker allows a wide and good baseline for comparison with other barcoded land plants since 17,000 sequences of land plants are deposited in Genbank as of 2005.

MATERIALS AND METHODS

Collection of Samples

Survey of the plants with pharmacological potential was conducted in the ethnic community through the permission, support and assistance of the provincial and local chieftains as well as the members of the ethnic community. Collection was done at the Ilongot-Eǵongotancestral domain located at Bayanihan, Maria Aurora, Aurora Philippines with the assistance of a field guide for the proper identification of the plants. Shoots of the plants were collected.

DNA Extraction

DNA extraction was done using CTAB method. Samples were powderized using liquid nitrogen and sterilized mortar and pestle. Powderized samples were place inmicrotubes. Equal amounts of 400ul of 2x CTAB buffer were added to the ependorf,vortex for 30 seconds and were incubated for 65 degrees Celsius for 30 minutes to 1hour. Afterwards it was cooled down and 400ul chloroform were added, vortex and centrifuge at 13,000 rpm for 5 minutes. The supernatant of upper part were transferred toa new microtube. Four hundred microliter of ice-cold isopropanol was added in eachmicrotubes, homogenized it for five minutes and were allowed to stand at room temperature for five minutes. The samples were centrifuge for 10 minutes at 13,000 rpm. After the centrifugation, the supernatant part of each samples were discarded and the pellets were remained in the microtubes. Air drying of the pellets were done for three to four hours. Isopropanol was dried and evaporated. Fifty microliter of TE buffer were added, provided that the buffer to be added was pre-heated in a water bath at 37^0C. The pellets were dissolved into the buffer, then were allowed to stand and
incubated for 30 minutes at room temperature.

Polymerase Chain Reaction

PCR Amplification rbcL gene marker was modified following the protocols of Kress *et al.* [12]. The PCR condition that was used by Kress *et al.,* [12] was followed as the starting point in optimization: initial denaturation at 95°C, 2 minutes, followed by 40 cycles of 30seconds denaturation at 95°C, 30 seconds annealing ranges at 59°C to 57°C, and 30 seconds extension at 72°C, final extension was done for 2 minutes at 72°C. The holding temperature was 4°C.Two microliter of PCR products were mixed with 2ul of loading dye containing gel red and were run in 1.5% Agarose gel electrophoresis for 70 minutes under 150 volts. The amplicons were sent to 1st Base Corporation for sequencing. Forward and reverse sequences were assembled in Global Alignment Tool of Basic Local Alignment Search Tool (BLAST) to estimate the quality of generated sequences. The assembled sequences were used to identify the species Basic Local Alignment Search Tool (BLAST).

RESULTS AND DISCUSSION

Six ethnobotanicals with local names Makabuhay, Serpentina, Herba buena, Ola ola, Pandakaki and Bensola. were identified successfully using the gene marker *rbcL*. Table 1 shows the results of BLAST analysis which includes the accession number, accession name, total score, percentage query, and percentage identity.

Makabuhay matched the identity of *Tinospora crispa* with Genbank Accession Number of KY365710.1, 99% maximum identity, and 92% query. Serpentina matched the identity of *Andrographis paniculata* Genbank Accession Number of JQ922118.1, 99% maximum identity, and 97% query. Herba Buena matched the identity of *Artemisia argyi* with Genbank Accession Number of KM386991.1, 99% maximum identity, and 98% query. Ola-ola matched the identity of *Mikania micrantha* with Genbank Accession Number of MF135326.1, 99% maximum identity, and 95% query. Pandakaki matched the identity of *Tabernaemontana pandacaqui* with Genbank Accession Number of KM895696.1, 99% maximum identity, and 92% query. Bensola matched the identity of *Senna alata* with Genbank Accession Number of GQ436678.1, 99% maximum identity, and 94% query.

Table1. Identities of the collected ethnobotanicals using BLAST with Genbank Accession numbers

Local Name	Best Genbank Match		Total Score	Query %	Identity %
	Accession Number	Accession Name			
Makabuhay	KY365710.1	*Tinospora crispa* voucher Chen ZD s.n. (PE) ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	977	92%	99 %
Serpentina	JQ922118.1	*Andrographis paniculata* voucher MICET P00101 ribulose 1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1026	97%	99 %
Herba buena	KM386991.1	*Artemisia argyi* chloroplast, complete genome	1033	98%	99 %
Ola ola	MF135326.1	*Mikania micrantha* voucher Li ZY, Jin XH, Xu SZ 13352 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1014	95%	99 %
Pandakaki	KM895696.1	*Tabernaemontana pandacaqui* voucher 1076420303 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene partial cds; chloroplast	957	92%	99%
Bensola	GQ436678.1	*Senna alata* voucher PS1362MT02 ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	998	94%	99%

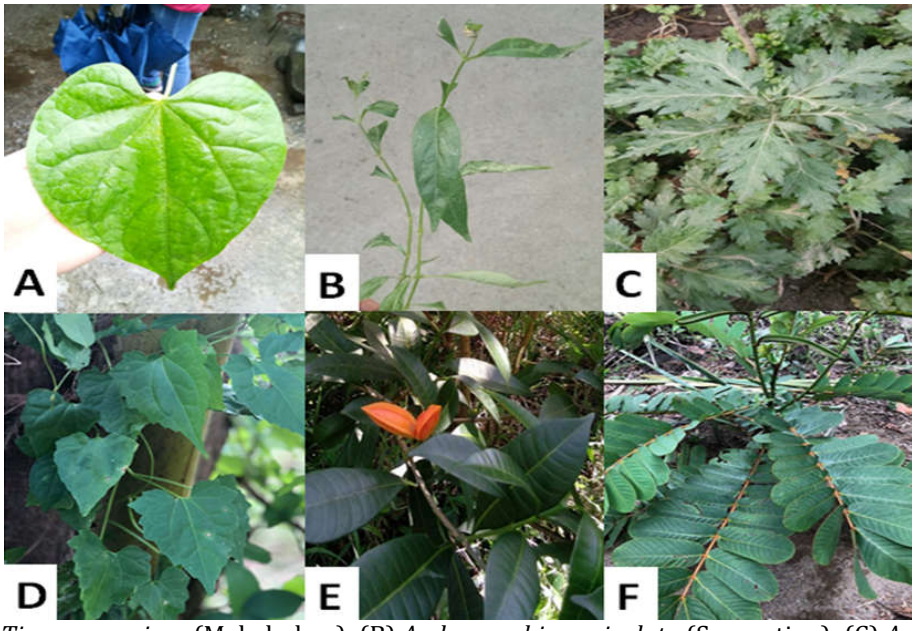

Figure1. (A) *Tinospora crispa* (Makabuhay); (B) *Andrographis paniculata* (Serpentina); (C) *Artemisia argyi* (Herba buena)i; (D) *Mikania micrantha* (Ola ola; (E) *Tabernaemontana pandacaqui* (Pandakaki); and (F) *Senna alata* (Bensola)

Tinospora crispa (L.) Hook.f. & Thomson (Figure 2.A), (Ilongot local name: Makabuhay) is aherbaceous vine that belongs to the family Menispermaceae (13). The old stems are fleshy, with prominent blunt tubercles. The leaves are large and heart shaped. Petioles are glabrous. It contains two or three small and yellow or greenish yellow color flowers which are fascicled (14). It extensively grows in tropical and subtropical regions of Southeast Asia including the Philippines (15,14). In the Ilongot Eǵongot community, it is used for toothache (16). In Bangladesh traditional medicine, it is used to treat intestinal disorders (17). Various medicinal attributes include therapeutic uses for the treatment of fever, jaundice, hyperglycemia, hypertension, wounds, intestinal worms, skin infections, tooth and stomach ache, cough, asthma and pleurisy (18). It is used as an anti-diabetes in Indian traditional medicine (19).

Andrographis paniculata (Burm. f.) Wall. ex Nees (Figure 2.B) (Ilongot local name: Serpentina) is an annual, branched, herbaceous plant that belongs to the family Acanthaceae (20). It grows in moist shady places with stem acutely quadrangular, and much branched. Leaves are simple, opposite, lanceolate, and glabrous. The flowers possess botanical features of upper lip oblong with white and yellowish top; and lower lip broadly with white and violet markings. Seeds are very small, and subquadrate (21). It grows abundantly in southeastern Asia: India (and Sri Lanka), Pakistan and Indonesia but cultivated extensively in China and Thailand, the East and West Indies, and Mauritius (21). In the Ilongot Eǵongot community, it is used for the treatment of diabetes (16). It is popular in Scandinavia as a cold and influenza remedy and used in traditional medicine in India. Pharmacological studies suggest its anti-inflammatory (22,23), anti-pyretic (24,25), antiviral (26), and immunostimulatory (27) properties.

Artemisia argyi H. Lév & Vaniot (Figure 2.C), (Ilongot local name: Herba Buena), is a perennial herb that belongs to the family Lamiaceae (28). It is strongly aromatic, 80-100 cm tall, with stoloniferous rhizomes. Stems are striate, incanous arachnoid-pubescent or tomentose [29]. It is native to China [30,31] and Japan and mainly found in Asia, Europe, and North America. The greatest distribution is found in Asia including the Philippines (32,33). In the Ilongot Eǵongot community, it is used for body pain and abortifacient (16). It has anti-fungal properties [30], anti-tumor, and immunomodulatory activities [34]. It is used as traditional herbal medicine for treating microbial infections, inflammatory diseases, diarrhea, hepatitis, malaria, cancer, circulator disorders [35,36], and blunt-liver-complaint [37].

Mikania micrantha Kunth. (Figure 2.D), (Ilongot local name: Ola ola), is a perennial plant that belongs to the family Asteraceae (38). It is a pest in plantation crops and commercial forests (39). It is a scrambling vine of the family Asteraceae that can reproduce both sexually and vegetative reproduction. The vine can produce a large number of seeds that are small and light and are dispersed by the wind and human invasion (40,41). It is native to central tropical and South America. It is also found in Mauritus to West Africa and across Asia [24]. It is introduced in Southern China from the Pacific islands and Southeast Asia [42,38].The Ilongot Eǵongot community uses this plantas abortifacient [16]. It is used by the Zeme Tribe on North Cachar Hills District in treating diarrhea of pigs, hens, and dogs(43). Pharmacological activities include activity in respiratory tract, anti-inflammatory, anti-allergic, analgesic, antioxidant and even in central nervous system [44].

Tabernaemontana pandacaqui Poir. (Figure 2.E) (Ilongot local name: Pandakaki) is a shrub or a small tree that belongs to the family Apocynaceae [45]. Leaves are opposite and often a pair unequal in size. The head of mature corolla bud is rounded or obtuse, tube glabrous inside. Sepals are either ciliated or not. Fruit is a paired follicles having obliquely ellipsoid to elongated shape. Seeds are covered in a fleshy aril, obliquely ellipsoid [46]. It is found in India [47,48], Australia [49] and also in the Philippines [50 ,51]. In the Ilongot Eǵongot community, it is used for the treatment of urinary tract infection and as a laxative [16]. Scientific studies proved the antipyretic, antinociceptive, and anti-pyretic properties [45] as well as high antimalarial activity [52] of this plant. In Iloilo, Philippines, it is used to treat cuts, wounds, and dermatological diseases [50] while in Surigao Del Sur, it is used to treat antibacterial infections [51].

Senna alata L. (Roxb.) (Figure 2.F) (Ilongot local name: Bensola) is an ornamental annual or biannual shrub [53] that belongs to Fabaceae family. The leaves are yellowish-green and broad (54). It is native in forest areas of Africa and introduced in many tropical countries including the Philippines (55,54) and locally used in Nigeria [53]. In the Ilongot Eǵongot community it is used to treat fungal infections and wounds [16]. In other places the stem bark is used to also treat fungal infections such as ringworm. It is also a common ingredient in soaps, shampoos and lotions because of its antifungal properties [56]. The leaves are reported to be useful in treating convulsion, veneral diseases (syphilis and gonorrhoea), heart failure, abdominal pains, oedema, stomach problems, fever, asthma, snake bite and is also used as a purgative (53). It is traditionally used for poisonous bites [15]. The ointment is used to treat against bovine dermatophilosis (57). In Cameroon, the leaves and stem bark are used to treat hepatitis, skin diseases, jaundice, gastroenteritis, intestinal helminthiasis, eczema, tryphoenteritis and ringworm (58). It is also used in other ethnic tribes in the Philippines, such as the Ivatan Tribe of Batan, for ascariasis,

chicken pox, head lice, herpes, ringworm, and scabies (55) and the Kalanguya Tribe of Tinoc, Ifugao to treat scabies and expel round worms in the stomach (59).

CONCLUSION

Six etnobotanicals with pharmacological potential used by the Ilongot Eǵongot of Bayanihan, Maria Aurora, Aurora were identified using the molecular marker *rbcL*: *Tinospora crispa* (Makabuhay), *Andrographis paniculata* (Serpentina), *Artemisia argyi* (Herba Buena), *Mikania micrantha* (Ola ola), *Tabernaemontana pandacaqui* (Pandakaki), and *Senna alata* (Bensola).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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