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Identification of plant species in multi flower honey by using Ribulose-Bisphosphate carboxylase gene (RBC L) coding region as barcode marker, Mizoram, Northeast India: An Indo: Burma hotspot region

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Abstract
Honey is mostly used for human nutrition due to its therapeutic properties. Honey is produced from living parts, nectar of plants and also from plant sucking insect's excreation. Honey is sweet in taste, produced by honey bees. In the current study, we investigated ribulose-Bisphosphate carboxylase gene (RBC *L*) coding region ability to identify the plant species/botanical composition/pollen origins in multi flower honey. Honey also shows diverse plant compositions in diverse geographical locations. We collected 19 honey samples in Aizawl (AZL; 23.72° & 92.71°) and Champhai (23.45° & 93.32°), Mizoram, India, were examined by using the *rbcL* coding regions as barcode markers. A DNA barcoding reference database was retrieved from Gen Bank, NCBI and used for identification of the plant species/pollen origin/taxonomic/botanical composition in multiflower honey in Mizoram, Northeast India (NEI). Our results showed that total 11 plant species were recognized in two honey sample districts (AZL and Champhai). All 11 plant species were emerged from a mix of common plants belonging to Betulaceae, Euphorbiaceae, Juglandaceae, Asteraceae, Lamiaceae, Berberidaceae, Lacistemataceae, and Paracryphiaceae taxa respectively. In two different honey sampling districts, at least 1 endemic plant was found which in turn giving a clear cut geographical origin and botanical composition/pollen origin of these honeys products. Further, the rbcL barcode was found to be effective in identifying the true botanical origin of honey samples with 99 to 100 percent confidence with E- value is about 0.0 mostly.

Keywords: Botanical composition/pollen origin, Gen Bank, geographical location, multi flower honey, Ribulose-Bisphosphate carboxylase gene (RBC L)

Introduction
Honey is produced from living parts, nectar of plants and also from plant sucking insect's excreation and is sweet in taste, produced by honey bees. Carbohydrates, organic acids, proteins, amino acids, minerals, poly phenols, vitamins and aroma compounds are present in honey [1]. Honey contains medicinal values (anti-microbial, anti-viral, anti-parasitary, anti-inflammatory, anti-oxidant, anti-mutagenic and anti-tumoral effects) which in turn depends on plant composition [2-6]. Therefore the knowledge on honey plant composition is extremely significant for human health [7]. The yield of crops is mostly dependent on pollinator insects especially honeybees [8]. During the procedure of foraging for nectar and pollen, honeybees with efficiency accumulates pollen in honey as an embedded matrix thereby creating pollen embedded honey as a possible biomarker to ascertain the plant source [9]. Honey are often classified as unifloral, when arising predominantly from a single botanical origin (represents more than 45% of the total pollen content) and multifocal, once when it's sourced from a mixture of flower from different plant species. Unifloral honey is extremely valued in food and pharmaceutical industry and is mostly deemed as premium product. Therefore, unifloral honey is at risk of intentional mislabeling or adulteration (with common honey, beet sugar syrup, rice syrup and corn sugar syrup etc.) to fetch premium value. Strayer and his associates [10], termed the food products that are adulterated for money advantage as Economically-Motivated Adulteration (EMA). Traditionally, the true source of honey has been deduced by its taste, flavour, aroma and colour [11, 12].

Whereas, true absolute botanical origin depends on 4 innate characteristics such as physical (refractive index, density, viscosity, water content, water activity, pH, electrical conductivity sugars and ash content) [13], physicochemical (Nuclear Magnetic Resonance (NMR) spectroscopy [9]; Liquid Chromatography coupled with mass spectroscopy (LC/MS), High Pressure Liquid chromatography (HPLC) [14], sensory (phenolic content, antioxidant activity and colour) [12], and biochemical properties (carbohydrate, proteins, minerals and volatile compounds) were also reported as suitable biomarker for identification of true honey source and geographical origins of honey [15-21]. However, these methods are laborious, time-consuming, difficult to understand the results, requires extensive botanic knowledge and unable to identify individual species and botanical origins [22].

Recently, molecular techniques have applied to overcoming these limitations [23-26] to analyze and characterize the composition of honey and pollen from environmental sediments [27, 28] by using 'barcode' markers [29] (18S rDNA, rbcL and trnL) and probes specially designed to recognize and establish a universal and reliable molecular identification system [30, 31] in attaining utmost universality and highest inequity power in local plant species in honey [32-35] by contrasting sequences of the same *ITS* region with a reference database [30, 36]. Barcode markers *rbcL* and *matK* (CBOL-Consortium for the Barcode of Life; http://www.barcoding.si.edu/plant_working_group) as a core barcodes to analyse and identify very close plant species [37, 38]. Hence, the aim of the present study was to evaluate the efficacy of ribulose-Bisphosphate carboxylase gene (*rbcL*) coding region as a DNA barcoding marker for identifying plant species/ botanical composition/pollen origin in multiflower honey.

2. Materials and Methods

2.1 Study area and honey sampling

The study areas are the localities of 2 districts [Aizawl (23° 43' 59.88''N, 92° 43' 0.12''E) and Champhai (23° 28' 28''N, 93° 19' 32''E)], Mizoram, Northeast India: An Indo-Burma hot spot region, were selected to examine the plant species/ botanical composition/pollen origin of multiflower honey by using *rbcL* coding region. Aizawl is located north of the Tropic of Cancer in the northern part of Mizoram. It is situated on a ridge 1,132 meters (3715 ft) above sea level, with the Tlawng river valley to its west and the Tuirial river valley to its east [32]. Aizawl has a mild, sub-tropical climate due to its location and elevation. Under the Köppen climate classification, Aizawl features a humid subtropical climate (Cwa) but very rainy. In the summer the temperature ranges from 20–30 °C (68–86 °F), and in the winter 11–21 °C (52–70 °F). Champhai is another district, occupies an area of 3185.83 km².Champhai district has a moderate climate. In winter the temperature varies from 10 °C to 20 °C and in summer, the temperature varies between 15 °C and 30 °C. Aizawl and Champhai districts are one of the eight districts of Mizoram state in India [39]. The districts are bounded on the north by Churachandpur district of Manipur state, on the west by Serchhip district, and on the south and east by Myanmar. The sampling sites of two districts, Aizawl (23° 43' 59.88''N, 92° 43' 0.12''E) sampling sites namely Aizawl (23.73° Lat. & 92.74° Long.), Durtlang (23.79° Lat. & 92.72° Long.), Hlimen (23.77° Lat. & 92.66° Long.), Melthum (23.69° Lat. & 92.72° Long.), Sairang (23.80° Lat. & 92.65° Long.), Sakawrtuichhun (23.76° Lat. & 92.67° Long.), Sihphir (23.81° Lat. & 92.73° Long.) and Tanhril (23.74° Lat. & 92.67° Long.). Champhai ((23° 28' 28''N, 93° 19' 32''E) sampling sites namely Chawngtlai (23.44° Lat. & 93.19° Long.), Hmunhmeitha (23.4° Lat. & 93.2° Long.), Khawzawl (23.37° Lat. & 93.12° Long.), Mualkawi (23.41° Lat. & 93.33° Long.), N. Khawbung (23.54° Lat. & 93.31° Long.), Ruantlang (23.44° Lat. & 93.34° Long.), Tlangsa (23.46° Lat. & 93.34° Long.) and Zote (23.49° Lat. & 93.35° Long.) were selected as study areas to identify the plant species/botanical composition/pollen origin of multiflower honey collected by honeybees (Table 1 and Fig. 1). The locations and field studies did not compromise the health of endangered or protected plant species. All the samples were stored at - 20° C as aliquots for DNA extraction.

2.2 DNA extraction and Purification

The collected honey samples were stored at -20° C for DNA extraction. After dilution, each honey samples were subjected to DNA extraction and purification (100µL of sample) was carried out using plant D Neasy Isolation and Purification kit (Qiagen, Milan, Italy). DNA purification of each sample was carried out by using fluorometrically, Nano Drop™ 1000 Spectrophotometer (Thermo Scientific, USA) by measuring the absorbance (Abs) at 260 nm and by comparison of ethidium bromide-stained band intensities with λ DNA standard on an agarose gel. DNA extracts were used as template for DNA barcoding analyses when they showed a minimum concentration of10 ng/µL.

2.3 Reference database, DNA barcoding analysis and Taxonomic assignments

In this study, plant sequence database used as a dedicated DNA barcoding reference database, retrieved from Gen Bank, NCBI for two districts (Aizawl and Champhai) of Mizoram, India. A total of 8 taxa were newly characterized by *rbcL* DNA barcode sequences in the reference database from fresh samples collected in the study areas during 2016 – 2018 [33-35, 40]. Our group sequences, 20 species were generated by during previous DNA barcoding studies. Records from Gen Bank were chosen and selected after a careful evaluation of each specimen, voucher codes and accession characteristics to avoid misidentification in the next bioinformatics analyses (i.e., availability of voucher details and sequence overlapping with those generated in this study). All samples were vouchered, following the protocol specified by the Global Registry of Biodiversity Repositories (http://sci-hub.io/http://grbio.org/), and the data standards for BARCODE Records [41]. DNA barcoding analysis was conducted using a portion of the plastidial *rbcL* gene. According to Fay *et al.* 1998; Galimberti *et al.* 2014, PCR amplification, sequencing and primers combinations (1F:5'ATGTCACCACAAACAGAAAC3': 724R:5'TCGCATGTACCTGCA GTAGC-3') of the plastidial *rbcL* gene were recovered [35, 42]. PCRs were performed starting from 10 ng of DNA by using pu Re Taq Ready-To-Go PCR beads (Amersham Bioscience, Freiburg, Germany) in a 25 µL reaction according to the manufacturer's instructions. PCR cycles consist following steps: Initial denaturation at 95° C for 7 min., following 35 cycles denaturation at 95° C for 40 sec. annealing at 48.5° C for 35 sec. extension at 69.8° C for 1 min. and final extension at 72° C for 5 min. Agarose gel (1.2%) was utilized for investigation of amplified PCR products. The sequencing PCR were set by using Big Dye Terminator V3.1 Cycle Sequencing kit (Thermo Fischer Scientific, USA). The samples were

sequenced on ABI 3730XL DNA Analyzer (outsourced from Scigenom Labs Pvt. Ltd., Kerala, India). Raw traces and subsequent alignments of forward and reverse sequences were manually edited with Bioedit v. 7.2.5 [43] and forward and reverse runs were aligned and assembled using Clustal W v. 2.1 [44] and analyzed with MEGA 5.1 [45]. Processed nucleotide sequence information was submitted to Gen Bank (NCBI) [46] and can be retrieved using their accession numbers. Nucleotide similarity searches were performed by BLAST tool at NCBI database [47, 48]. Similarity identity was used to assign the identity to each query sequence and blast result (with high identity percentage, high query coverage, and E-value < cutoff) of the query sequence was used to associate with the authenticity of the source of honey samples.

Table 1: Sampling sites of two districts Aizawl and Champhai with their latitudes and Longitudes

Sampling sites	Latitude	Longitude	Number of samples collected
Aizawl (23° 43' 59.88''N, 92° 43' 0.12''E)			
Aizawl	23.73°	92.74°	5
Durtlang	23.79°	92.72°	7
Hlimen	23.77°	92.66°	5
Melthum	23.69°	92.72°	7
Sairang	23.80°	92.65°	5
Sakawrtuichhun	23.76°	92.67°	3
Sihphir	23.81°	92.73°	12
Tanhril	23.74°	92.67°	9
Total samples collected			53
Champhai (23◦ 28' 28''N, 93◦ 19' 32''E)			
Chawngtlai	23.44°	93.19°	11
Hmunhmeitha	23.4°	93.2°	5
Khawzawl	23.37°	93.12°	17
Mualkawi	23.41°	93.33°	4
N.Khawbung	23.54°	93.31°	7
Ruantlang	23.44°	93.34°	8
Tlangsam	23.46°	93.34°	2
Zote	23.49°	93.35°	5
Total samples collected			59

Table 2: Molecular identification of plant species detected in honey from Aizawl (AZL). The species matches in reference database (RD) and accession number obtained from NCBI Gen Bank are reported for the *rbcL* barcode region

Sample ID/Voucher Number	Accession No.	Identified Plants	Species match in Reference Database (RD)
2C	KX966374	*Alnus incana*	*Alnus incana*
4C	KX966375	*Alnus glutinosa*	*Alnus cremastogyne*
			Alnus nepalensis
			Alnus glutinosa
			Alnus incana
5C	KX966376	*Alnus glutinosa*	*Alnus cremastogyne*
			Alnus nepalensis
			Alnus glutinosa
			Alnus incana
2E	KX966377	*Macaranga aleuritoides*	*Macaranga aleuritoides*
2S	KX966378	*Alnus nepalensis*	*Alnus cremastogyne*
			Alnus nepalensis
			Alnus glutinosa
			Alnus incana
NKB	KX966379	*Juglans cinerea*	*Juglans cinerea*
16E	KX966380	*Macaranga denticulate*	*Macaranga denticulata*
15D	KX966381	*Mikania scandens*	*Mikania micrantha*
			Mikania scandens
			Mikania micrantha
			Mikania cordata
7E	KX966382	*Prostanthera calycina*	*Prostanthera calycina*
14E	KX966383	*Berberidopsis corallina*	*Berberidopsis corallina*

Table 3: Molecular identification of plant species detected in honey from Champhai. The species matches in reference database (RD) and accession number obtained from the NCBI Gen Bank are reported for the *rbcL* barcode region

Sample ID/Voucher Number	Accession No.	Identified Plants	Species match in Reference Database (RD)
2E1	KX966384	*Macaranga aleuritoides*	*Macaranga aleuritoides*
2S1	KX966385	*Alnus glutinosa*	*Alnus cremastogyne*
			Alnus nepalensis
			Alnus glutinosa
			Alnus incana
NKB1	KX966386	*Juglans cinerea*	*Juglans cinerea*

16E1	KX966387	*Macaranga denticulate*	*Macaranga denticulate*
15D1	KX966388	*Mikania scandens*	*Mikania micrantha*
			Mikania scandens
			Mikania micrantha
			Mikania cordata
9E	KX966389	*Berberidopsis corallina*	*Berberidopsis corallina*
5E	KX966390	*Berberidopsis corallina*	*Berberidopsis corallina*
4E	KX966391	*Lacistema aggregatum*	*Tapiscia sinensis Lacistema aggregatum*
4D	KX966392	*Paracryphia alticola*	*Paracryphia alticola*

Table 4: Statistical simulation of BLAST Sequence homology *Alnus nepalensis*, *Alnus glutinosa*, *Macaranga aleuritoides*, *Juglans cinerea*, *Macaranga denticulate*, *Mikania scandens*, *Prostanthera calycina*, *Berberidopsis coralline*, *Lacistema aggregatum*, and *Paracryphia alticola* with *rbcL* primers respectively

Identified species	Family	Genome region	Taxonomic level	BLAST similarity	Sequence cover	E-Value (BLAST)
Alnus nepalensis	Betulaceae	*rbcL*	species	100%	100%	0.0
Alnus glutinosa	Betulaceae	*rbcL*	species	100%	100%	0.0
Alnus glutinosa	Betulaceae	*rbcL*	species	100%	100%	0.0
Macaranga aleuritoides	Euphorbiaceae	*rbcL*	species	100%	100%	0.0
Alnus nepalensis	Betulaceae	*rbcL*	species	100%	100%	0.0
Juglans cinerea	Juglandaceae	*rbcL*	species	100%	100%	0.0
Macaranga denticulate	Euphorbiaceae	*rbcL*	species	100%	100%	3e-115
Mikania scandens	Asteraceae	*rbcL*	species	100%	100%	0.0
Prostanthera calycina	Lamiaceae	*rbcL*	species	100%	100%	1e-174
Berberidopsis corallina	Berberidopsidaceae	*rbcL*	species	100%	100%	0.0
Macaranga aleuritoides	Euphorbiaceae	*rbcL*	species	100%	100%	0.0
Alnus glutinosa	Betulaceae	*rbcL*	species	100%	100%	0.0
Juglans cinerea	Juglandaceae	*rbcL*	species	100%	100%	0.0
Macaranga denticulate	Euphorbiaceous	*rbcL*	species	100%	100%	3e-115
Mikania scandens	Asteraceae	*rbcL*	species	100%	100%	0.0
Berberidopsis corallina	Berberidopsidaceae	*rbcL*	species	100%	100%	0.0
Berberidopsis corallina	Berberidopsidaceae	*rbcL*	species	100%	100%	0.0
Lacistema aggregatum	Lacistemataceae	*rbcL*	species	100%	100%	9e-110
Paracryphia alticola	Paracryphiaceae	*rbcL*	species	100%	100%	0.0

Table 5: Dot distribution of plant species in the two districts (Aizawl, Champhai) along with their latitude and longitudes. Plant typology (tree, flower, and shrub) and sampling sites are indicated.

Species	Typology	Site of sampling	
		Aizawl (23.72° & 92.71°)	Champhai (23.45° & 93.32°)
Alnus incana	Tree	●	-
Alnus glutinosa	Tree	●	-
Alnus glutinosa	Tree	●	-
Macaranga aleuritoides	Tree	●	-
Alnus nepalensis	Tree	●	-
Juglans cinerea	Tree	●	-
Macaranga denticulate	Tree	●	-
Mikania scandens	Plant	●	-
Prostanthera calycina	Shrub	●	-
Berberidopsis corallina	Plant	●	-
Macaranga aleuritoides	Tree	-	●
Alnus glutinosa	Tree	-	●
Juglans cinerea	Tree	-	●
Macaranga denticulate	Tree	-	●
Mikania scandens	Plant	-	●
Berberidopsis corallina	Plant	-	●
Berberidopsis corallina	Plant	-	●
Lacistema aggregatum	Plant	-	●
Paracryphia alticola	Tree	-	●

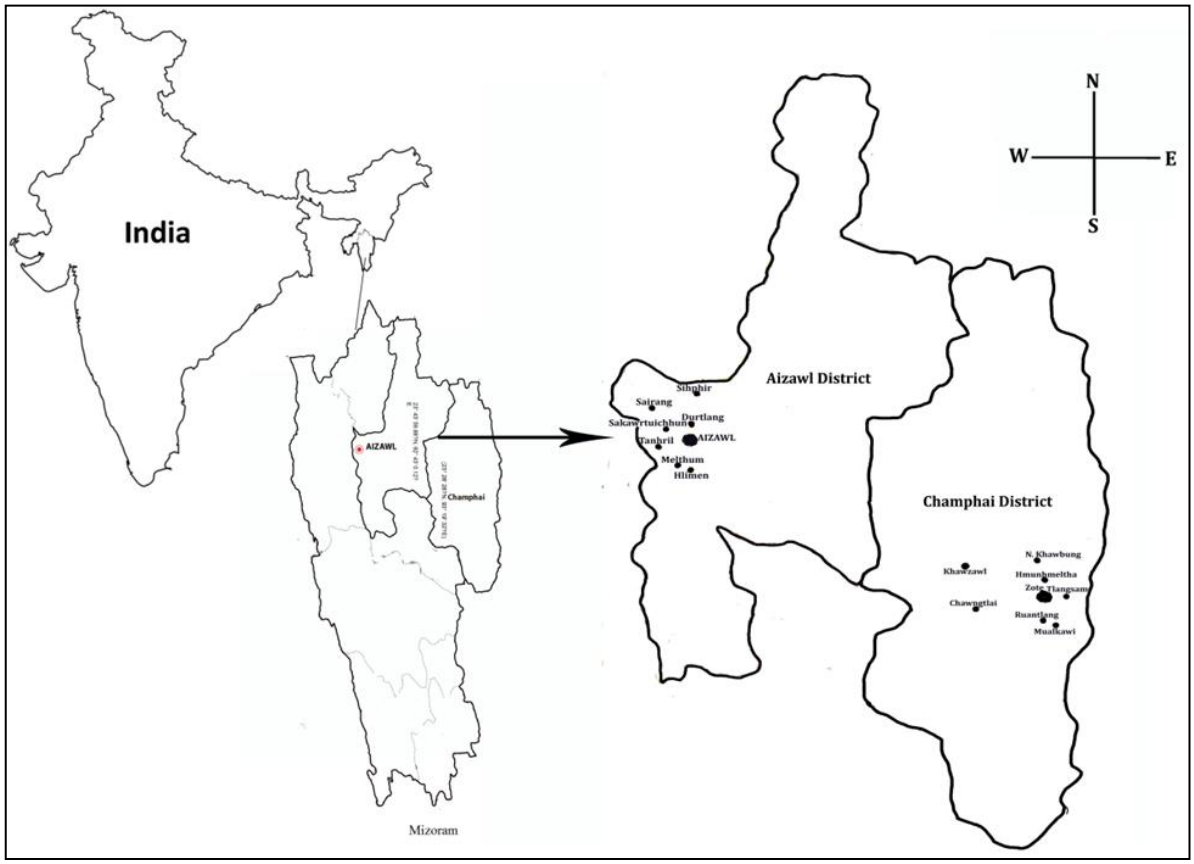

Fig 1: Distribution map of honey production sites within Aizawl (AZL) and Champhai, Mizoram, India: An Indo-Burma hot spot region. The full names and geographic coordinates for the Aizawl (AZL) & Champhai and the collection sites are provided with black colour dot.

3. Results and Discussion

3.1 DNA isolation, quality and quantity

The DNA extracted from the Twenty honey samples and the plant reference samples were of high quality (ratios of absorbance, A260/280 and A260/230~ 1.80 and >1.90, respectively) and an acceptable concentration yield was (10 ng/µL for each sample). Amplification of DNA was successful (i.e., non-specific bands were absent) with standard primer pairs and thermal conditions was obtained for *rbcL*. No short inverted repeat regions were found. Maximum length of aligned sequences was 627 bp for *rbcL*. Accession numbers of DNA barcode sequences for each species are provided in (Table 2 and Table 3). Very few literatures were available regarding the effective high pollen DNA yielding protocol with respect to pollen embedded in honey [21, 33, 40, 49-52].

3.2 DNA Barcoding

DNA barcoding data could be characterized complex food matrices, small quantities of pollen and origin of honey at the molecular level for a relatively low price. Sampling can be done at any life stage (a typical problem when only the morphological recognition is used) of collecting plants in cooperation with local laypersons or natural history museums because of sampling is usually limiting factor for DNA barcoding analysis. BOLD plant database (http://www.boldsystems org/) and local floras are currently very poor in characterization (pollen identification and honey origin) of rare and endemic plant taxa [34] due to lack of molecular characterization, which in turn demands the availability of local dedicated databases [53].The efficiency of *rbcL* coding region as a DNA barcoding marker, to characterizing the botanical composition/pollen origin of multiflower honey and also facilitated taxonomic assignment at the species level [34, 53]. Very high-quality DNA was acquired and *rbcL* marker was very easily amplified and sequenced from all samples, although *rbcL* had limited discrimination power (most of the MOTUs determine up to the genus level only), especially of congeneric taxa [53, 54]. The present study suggested that the use of universal primer *rbcL* for DNA barcoding investigations was adequate to typify or identification of botanical composition or pollen origin of honey samples among study sites (Table 2 and Table 3) and also successful for amplification, identification and discrimination of plant species mentioned in this research work.

3.3 Bioinformatics analysis

BLAST (Basic Local Alignment Tool) was used to detect DNA Sequence homology of conserved region (*rbcL*). All identified plants were matched with reference database (such as *Alnus incana, Alnus cremastogyne, Alnus nepalensis, Alnus glutinosa, Macaranga aleuritoides, Juglans cinerea, Macaranga denticulata, Mikania micrantha, Mikania scandens, Mikania micrantha, Mikania cordata, Prostanthera calycina, Berberidopsis coralline, Tapiscia sinensis, Lacistema aggregatum, and Paracryphia alticola* respectively (Table 2 and Table 3). The homology of all identified Plant species with *rbcL* was *Alnus nepalensis* (100%), *Alnus glutinosa* (100%), *Macaranga aleuritoides* (100%), *Juglans cinerea* (100%), *Macaranga denticulate* (100%), *Mikania scandens* (100%), *Prostanthera calycina* (100%), *Berberidopsis corallina* (100%), *Lacistema aggregatum* (100%), and *Paracryphia alticola (100%)* respectively with E-value is 0.0 mostly (Table 4). However, *rbcL* is a coding region with mononucleotide repeats which in turn causes

species misidentification [53] as a result of sequencing error. The latest techniques such as appropriate polymerases and ideal PCR conditions [44], recover reference barcodes due to high-quality sequences. The present condition is not revelationing regarding identification of at the species level [34, 53]. The *rbcL* is appropriate, for representation the nature of honey as well as Characterization of pollen grains collected by Honeybees [40, 55] with well-known flora from limited sampling sites since the sampling area is not in public databases, which in turn essential for a wider variety of taxa. However, this marker was selected for this study as per CBOL (Consortium for the Barcode of Life) guidelines. DNA barcoding and micro-morphological analysis is good in morphological and molecular identification with local reference database, to differentiate pollens from different honey samples. Moreover, DNA barcoding is a faster, standardized, suitable, universal and routine DNA technique to analyze complex food and environmental matrices without any botanical knowledge of different species [55, 56]. It would also be probable to process, determine and analyze a vast number of pollen, pollen products and vast collections of samples with next-generation sequencing [57]. In several cases, the BLAST analysis performed with *rbcL* was not able to identify a species with more than 89% similarity. For example, the *rbcL* did not distinguish *Alnus nepalensis* from *Anogeissus acuminate*. The data obtained from using *rbcL* permitted us to identify 11 plant species from the sampling sites (Aizawl (AZL) and Champhai) (Table 2 and Table 3), with a minimum of 6 tree species, 3 plant species and 1 shrub from the both districts (Aizawl (AZL) and Champhai) respectively (Table 5). The analyzed data displayed prevalence of pollen types from a variety of floral sources widely distributed in Aizawl and Champhai, including: *Alnus nepalensis, Berberidopsis coralline, Macaranga denticulate* in the Aizawl (AZL) honey samples (Table 2); *Juglans cineria, Maracaranga species* in the Champhai honey samples (Table 3) were detected. Most of the plant species identified by DNA barcoding were entomophilous; however, some anemophilous and both (entomophilous and anemophilous) species such as *Alnus nepalensis* and *Juglans cineria* were detected. The current study results showed that geographical origin and botanical composition/pollen origin of some common plants and at least one endemic plant connected the honey samples to sixteen sampling sites of two districts (Aizwal (AZL) and champhai) in Mizoram and also contributed new data to Gen Bank for several plant species. These species, should display the maximum flowering in August and September (*Mikania scandens)* and some were also found in May and June, respectively (*Macaranga denticulate*) which inturn explained the period of pollen or honey sampling. Honeybees collected pollen from the same species *Alnus glutinosa, Alnus nepalensis, Macaranga aleuritoides, Macaranga denticulate, Mikania scandens, Berberidopsis corallina* and *Juglans cineria* from the both the districts (Aizawl and Champhai). These observations reinforce and characterize the environmental community structure and type of pollinators including honeybees, of the Aizawl and Champhai [58-60].

In the current study, results showed that honeybee pollen collection activity is subjected to rapid and continuous changes related to different local plant phenology. Plant phenology also influences pollen diversity, which depends on local biodiversity and environmental alterations, collected by honeybees. Pollen from closely related species varied in its nutritional proprieties (slightly) and biochemical composition (largely; protein, carbohydrate, lipid, vitamin, and mineral composition and nutraceutical properties) were observed among families and orders for humans [36, 61-65]. After a short period of observations, species composition of pollen collected from beehives distinguishably changed as a result of changes in local flora and fauna. So, assessment of local plant phenology and molecular characterization is vital to collect the ‘‘curative’’ pollen as well as pollen composition. The current study based on a few honey samples, support DNA barcoding act as a potential tool in distinguishing fraudulent or mistaken labelling of honey, confirming geographical provenance of pollen-based products and for honey traceability at different stages of production and distribution [36]. We identified pollen of several plants of the Aizawl and Champhai. The occurrence of these plants can be considered a clear signature of botanical and geographical provenance. The limitations of DNA barcoding strategy are inability, lack of a general consensus because of various identifications, selections and quantifications amongst different plant groups [30]. Additional molecular tools, qRT PCR (SCAR) with DNA barcoding markers, could be used to obtain and detect relative abundances for plant species as well as possible contaminants in honey samples [55]. The universality of DNA barcoding strategy is a second limitation, which can be determined with well-populated reference database for the local flora surrounding the honeybees, as was the case in this study.

4. Conclusion

Various health related advantages of honey to the people, is based on the quality of honey is available in the agro-markets which in turn depends on the plants in the foraging range of the honeybees. Nowadays, mislabeled honey is finding in the market which affects the consumer’s health. Hence, it is important to assess the quality of honey in a short time span and low cost. In this current study, we demonstrate that DNA barcoding analysis with the *rbcL* marker is a suitable method for the identification of plant species/pollen origin/botanical composition in multiflower honey which in turn addresses desired honeybee products. DNA barcoding is an alternative source can be used to determine the quality of honey. However, DNA barcoding with next generation sequencing, could be identified more species without cloning. Further, honey beekeepers and stakeholders should take seriously to this opportunity.

5. Acknowledgements

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