DNA Barcoding Medicinal Plant Species from Indonesia

Ria Cahyaningsih 1,2,* , Lindsey Jane Compton 1, Sri Rahayu 2, Joana Magos Brehm 1 and Nigel Maxted 1

1 School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK; l.j.compton@bham.ac.uk (L.J.C.); joanabrehm@gmail.com (J.M.B.); n.maxted@bham.ac.uk (N.M.)
2 Research Center for Plant Conservation, Botanical Gardens and Forestry, National Research and Innovation Agency, Bogor 16122, Indonesia; srir005@brin.go.id
* Correspondence: ria.cahyaningsih@brin.go.id

Abstract: Over the past decade, plant DNA barcoding has emerged as a scientific breakthrough and is often used to help with species identification or as a taxonomical tool. DNA barcoding is very important in medicinal plant use, not only for identification purposes but also for the authentication of medicinal products. Here, a total of 61 Indonesian medicinal plant species from 30 families and a pair of ITS2, matK, rbcL, and trnL primers were used for a DNA barcoding study consisting of molecular and sequence analyses. This study aimed to analyze how the four identified DNA barcoding regions (ITS2, matK, rbcL, and trnL) aid identification and conservation and to investigate their effectiveness for DNA barcoding for the studied species. This study resulted in 212 DNA barcoding sequences and identified new ones for the studied medicinal plant species. Though there is no ideal or perfect region for DNA barcoding of the target species, we recommend matK as the main region for Indonesian medicinal plant identification, with ITS2 and rbcL as alternative or complementary regions. These findings will be useful for forensic studies that support the conservation of medicinal plants and their national and global use.

Keywords: DNA barcoding; medicinal plants; conservation; forensic; Indonesia

1. Introduction

Plant identification has formerly been formed using morphological characteristics that could be observed visually. Currently, DNA is also used to help species identification and to build bioinventories [1]. DNA barcoding was introduced by Hebert and colleagues in 2003 and involves the identification of species through universal, short, and standardized DNA regions [2]. DNA material for the barcoding can be obtained from living plants, herbarium specimens [3], and market products [4,5].

In plants, plastid DNA (rbcL, matK, trnL, and trnH-psbA regions) and nuclear DNA (ITS and ITS2 regions) are often used in DNA barcoding [6–8]. The rbcL and matK regions are recommended by the Consortium for the Barcode of Life (CBOL) as a standard two-locus barcode for global plant databases because of their species discrimination ability [8].

The process entails registering the DNA of identified species into a barcoding library and matching the DNA of unidentified species against the genetic data available in the library [6,9]. The library or the database can be accessed online for species identification and taxonomic clarification [10], namely through the NCBI GenBank (https://www.ncbi.nlm.nih.gov; accessed on 1 February 2022) [10] and the Barcode of Life Data (BOLD) (http://www.boldsystems.org; accessed on 1 February 2022) [11].

DNA barcoding has become an important taxonomic tool because of its accuracy, repeatability, and rapidity. It can also be used to identify species under legislative protection, or under threat of extinction, and to check the authenticity of biological products [6,9]. It is particularly powerful as identification is not influenced by the morphological diversity of species, growth phases, and environmental factors [12–15]. In the forensic field, even an inexperienced user is able to assign a taxonomic name to an unidentified plant specimen.
with relative ease [16,17]. It is an effective conservation tool as it is able to prevent substitution of important commercial species, protect species from theft [6,18], and help to define species richness in underexplored areas [6].

DNA barcoding is valuable in terms of medicinal plant (MP) species identification compared to traditional morphological identification for conservation and use, as it is able to identify species and ensure a genuine product rather than a substitute [6,18]. Identifying the plant correctly protects consumer rights [19], even with respect to small and damaged plant parts used in botanical forensics [10,20–22]. Several studies conducted on DNA barcoding of medicinal plants have indicated the effectiveness of ITS2 and matK. For example, these regions are able to distinguish *Rauvolfia serpentina* (L.) Benth. Ex Kurz, of which root extracts act as an antihypertensive drug from other species in the genus [5,23] and are able to authenticate *Eurycoma longifolia* Jack, of which all plant extracts (particularly roots) are a useful drug for cough, anticancer, and aphrodisiac activities [24]. MatK is also known to give the best identification for Philippine ethnomedicinal Apocynaceae [25]. However, DNA barcoding from only one specific sequence region has been applied for most medicinal plants. For example, the ITS2 region has been used as a DNA barcode for authenticating many medicinal plants, their relatives, and broader species [14,26], although it was found that this region could not authenticate all Chinese medicinal *Bupleurum* L. (Apiaceae) species [27]. For Indian medicinal plants (Ayurveda), the *rbcL* region has been used for DNA barcoding [19], while for medicinal plants of the Philippines, *rbcL*, matK, and *trnL*-F regions have been used based on their efficiency [28].

Indonesia is famous for its plant diversity and richness, particularly in medicinal plants and their uses [29–31]. Different forms of medicinal plants are used, regardless of being fresh or dried, for curing illness and disease [32]. Thus, the primary purpose of undergoing the barcoding process, apart from enriching the DNA barcoding database, is determining the identity of medicinal plants. DNA barcoding is an advanced technology for plant diversity inventories, and its high cost makes it both an issue and challenge for biodiversity conservation in Indonesia [33]. Nevertheless, DNA barcodes are useful for conservation and even for commercial purposes, and they will be widely used in the future as DNA sequencing technologies become simpler and cheaper [6]. This study aimed to assess how four different DNA barcoding regions (ITS2, matK, rbcL, and trnL) can aid 61 species identifications and conservation efforts, and investigate their effectiveness for DNA barcoding of Indonesian medicinal plants. The finding will allow for broader and more comprehensive use in the future with respect to medicinal plant conservation both nationally and globally.

2. Results and Discussions

2.1. Understanding the Use of DNA Barcoding for Indonesian Medicinal Plants

Of the 61 sampled Indonesian medicinal plants, 55 species are native to Indonesia (of which 29 are endemics), and six are introduced [34]. Some of the medicinal plants may need to be prioritized in terms of conservation, namely those assessed as threatened (VU, EN, CR) or near threatened (NT) according to the IUCN Red List [35], the 19 species listed in the CITES Appendices I, II, or III (UNEP-WCMC database) [36], and the 12 rare medicinal plants [37]. Two species were assessed as VU, namely *Aquilaria hirta* Ridl. [38] and *Etlingera solaris* (Blume) R.M.Sm. [39] and are considered to be facing a high extinction risk in the wild in the near future [40]. The 19 species listed in CITES II may become extinct if their trade is not controlled because they are collected from the wild and there is no sufficient data with respect to artificial propagation for commercial purposes [36]. Of the 61 sequence target species, 13 sequences were not found in BOLD, although their DNA sequence data was available in NCBI; a further 10 species did not have DNA sequences stored in either NCBI or BOLD. Detailed information for each of the 61 species is presented in Table 1.
Table 1. The Indonesian medicinal plants (n = 61) used in this study with related information from literature study.

| No. | Species                          | Author | Family.   | N/I | Important Sp. | Sp. No. per Genus | BOLD (NCBI) Database |
|-----|----------------------------------|--------|-----------|-----|---------------|-------------------|----------------------|
| 1   | Justicia gendarussa              | Burm.f.| Acanthaceae| N   | No            | 921               | yes                  |
| 2   | Staurogyne elongata              | (Nees) Kuntze | Acanthaceae| N   | No            | 148               | yes                  |
| 3   | Pangium edule                    | Reinv. | Achariaceae| N   | Yes (P)       | 1                 | yes                  |
| 4   | Spondias malayana                | Kosterm.| Anacardiaceae| N   | No            | 19                | no (yes)             |
| 5   | Toxicodendron succedaneum        | (L.) Kuntze | Anacardiaceae| I   | No            | 27                | yes                  |
| 6   | Anastrocladus tectorius          | (Lour.) Merr. | Anastrocladaceae| N   | No            | 21                | yes                  |
| 7   | Anaxagorea javanica              | Blume  | Annonaceae | N   | Yes (P)       | 25                | no (yes)             |
| 8   | Dasysmatschalan                  | (Blume)| Annonaceae | N   | No            | 27                | yes                  |
| 9   | Alstonia macrophylla             | Wall. Ex. G.Don | Apocynaceae| N   | No            | 44                | yes                  |
| 10  | Alstonia scholaris               | (L.) R. Br. | Apocynaceae| N   | Yes (P)       | 106               | yes                  |
| 11  | Alidia reinwardtii               | Blume  | Apocynaceae| N   | Yes (P)       | 521               | no (yes)             |
| 12  | Hoya diversifolia                | Blume  | Apocynaceae| N   | No            | 74                | yes                  |
| 13  | Rauvolfia seropentina            | Schott | Araceae   | N   | No            | 22                | yes (yes)            |
| 14  | Trevesia burckii                 | R.Br.  | Araliaceae | N   | No            | 8                 | yes (yes)            |
| 15  | Cibotium barometz                | (L.) J.Sm. | Cibotiacae| N   | Yes (II)      | 10                | yes                  |
| 16  | Decalobanthus mammosus           | A.R.Simoes & Staples | Convolvulaceae| I   | No            | 13                | no (yes)             |
| 17  | Erycibe malaccensis              | C.B. Clarke | Convolvulaceae| N   | No            | 70                | no (no)              |
| 18  | Rheedodendron macgregoriae       | F. Muell.| Ericaceae | N   | Yes (E)       | 1057              | no (no)              |
| 19  | Acalypha grandis                 | Benth. | Euphorbiaceae| N   | No            | 428               | no (no)              |
| 20  | Euphorbia tirucalii              | L.      | Euphorbiaceae| I   | Yes (II)      | 1976              | yes                  |
| 21  | Millettia sericafolia            | (Vent.) Benth. | Fabaceae| N   | No            | 187               | yes                  |
| 22  | Parkia timoria                   | (DC.) Merr. | Fabaceae| N   | No            | 40                | yes                  |
| 23  | Panthera fulva                   | (Korth.) Benth. | Fabaceae| N   | Yes (E)      | 90                | no (no)              |
| 24  | Orthosiphon aristatus            | (Blume) Miq. | Lamiaecae| N   | No            | 44                | yes                  |
| 25  | Premna serratifolia              | L.      | Lamiaecae | N   | No            | 131               | yes                  |
| 26  | Vitez glabrata                   | Gaertn. | Lamiaecae| N   | No            | 203               | yes                  |
| 27  | Cinnamomum rhynchophyllum        | Miq.    | Lauraceae | N   | No            | 241               | no (yes)             |
| 28  | Ficus deltoidea                  | Jack    | Moraceae  | N   | Yes (P)       | 874               | yes                  |
| 29  | Myristica succedanea             | Blume  | Myristicaceae| N   | Yes (E)       | 175               | no (no)              |
| 30  | Neophanes ampullaria             | Jack    | Nepentheaceae| N   | Yes (P, II)  | 165               | yes                  |
| 31  | Nepenthes gracilis               | Korth.  | Nepentheaceae| N   | Yes (P, II)  | yes               | yes                  |
| 32  | Nepenthes mirabilis              | (Lour.) Druce | Nepentheaceae| N   | Yes (P, II)  | yes               | yes                  |
| 33  | Neophanes reinwardtiana          | Miq.    | Nepentheaceae| N   | Yes (P, E, II)| yes               | yes                  |
| 34  | Acriopsis liliifolia var. liliifolia | (J.Koenig) | Orchidacae| N   | Yes (P, II)  | 10                | no (yes)             |
| 35  | Cymbidium aloifolium             | Ormerod | Orchidacae| N   | Yes (P, II)  | 74                | yes                  |
| 36  | Cymbidium ensifolium             | (L.) Sw. | Orchidacae| I   | Yes (II)     | yes               | yes                  |
| 37  | Dendrobium crumenatum            | Sw.     | Orchidacae| N   | Yes (P, II)  | 1547              | yes                  |
| 38  | Dendrobium purpureum             | Roxb.   | Orchidacae| N   | Yes (P, E, II)| no (no)           | yes                  |
Table 1. Cont.

| No. | Species                          | Author                                      | Family. | N/I | Important Sp. | Sp. No. per Genus | BOLD (NCBI) Database |
|-----|----------------------------------|---------------------------------------------|---------|-----|---------------|-------------------|----------------------|
| 40  | *Dendrobium salaccense*          | (Blume) Lindl.                             | Orchidaceae | N   | Yes (P, II)   |                   | yes                  |
| 41  | *Grammatophyllum speciosum*      | Blume                                      | Orchidaceae | N   | Yes (P, II)   | 13                | yes                  |
| 42  | *Nervilia concolor*              | (Blume) Schltr.                            | Orchidaceae | N   | Yes (P, II)   | 77                | yes                  |
| 43  | *Nervilia plicata*               | (Andrews) Schltr.                          | Orchidaceae | N   | Yes (P, II)   |                   | yes                  |
| 44  | *Oberonia lycopodioides*         | Ormerod, (Lindl.) Schuit., (Y.P. Ng & H.A. Pedersen) | Orchidaceae | N   | Yes (P, II)   | 305               | no (no)              |
| 45  | *Strongylaria pannea*            | Y.P. Ng & H.A. Pedersen                    | Orchidaceae | N   | Yes (P, II)   | 4                 | no (yes)             |
| 46  | *Gelearia filiformis*            | (Blume) Boerl., (Kurz) Callim. & Buerki    | Pandaceae | N   | Yes (E)      | 5                 | yes                  |
| 47  | *Benstonea affinis*              |                                            | Pandanaeae | N   | No            | 61                | yes                  |
| 48  | *Phyllanthus oxyphyllus*         | Miq.                                       | Phyllanthaceae | N   | No            | 1016              | yes                  |
| 49  | *Arsidia complanata*             | Wall.                                      | Primulaceae | N   | No            | 719               | no (no)              |
| 50  | *Arsidia crenata*                | Sims                                       | Primulaceae | I   | No            |                   | yes                  |
| 51  | *Ventilago madraspatana*         | Boerl.                                     | Rhamnaceae | N   | No            | 41                | no (yes)             |
| 52  | *Psychotria montana*             | Blume                                      | Rubiaceae | N   | No            | 1531              | no (yes)             |
| 53  | *Lunasia amara*                  | Blanco                                     | Rutaceae | N   | Yes (P)      | 1                 | yes                  |
| 54  | *Melicope lunu-ankenda*          | (Gaertn.) T.G. Hartley                   | Rutaceae | N   | No            | 241               | no (yes)             |
| 55  | *Kadsura scandens*               | (Blume) Blume                              | Schisandraceae | N   | Yes (P)      | 17                | yes                  |
| 56  | *Smilax calophylla*              | Wall. ex A.D.C.                            | Smilaceae | N   | No            | 262               | yes                  |
| 57  | *Smilax zeylanica*               | L.                                         | Smilaceae | N   | Yes (P)      |                   | yes                  |
| 58  | *Aquilaria hirta*                | Ridl.                                      | Thymelaceae | N   | Yes (P, VU)  | 21                | no (yes)             |
| 59  | *Amomum hochreutinieri*          | Valeton                                    | Zingiberaceae | N   | Yes (E)      | 102               | no (no)              |
| 60  | *Etlingera solaris*              | (Blume) R.M. Sm.                          | Zingiberaceae | N   | Yes (E, VU)  | 143               | no (no)              |
| 61  | *Meistera aculeata*              | (Roxb.) Skornick. & M.F. Newman            | Zingiberaceae | N   | No            | 41                | no (yes)             |

Note: Scientific names (1st and 2nd columns were collected from POWO (2022); Species: R for rare medicinal plant (MP), E for endemic to Indonesia, VU for Vulnerable (IUCN Red List), P for Priority, and II for CITES Appendix II; N = Native, I = Introduced.

The contribution of the DNA barcoding information from each species to DNA banks and to the correct identification of medicinal plants with high conservation status was classified using categories A–M, where category A denotes the contribution of new data to DNA banks and DNA barcoding information that can strongly assist MP conservation; at the opposite end of the spectrum, letter M denotes the least substantial contribution, where DNA barcoding needs to be clarified further before using it directly for identification. Figure 1 indicates how the four DNA barcodes are useful for the conservation and use of Indonesian medicinal plants with respect to the availability of their data in the DNA bank. The number of medicinal plant species per criteria are provided in Table A1. Sequences grouped in categories A-D can be of direct use to conservation efforts due to the correct identification of related medicinal plants. The A-B categories can be used in botanic forensics (in cases of medicinal plant adulteration and illegal trading) for medicinal plant identification [10,21–24], as the plants are listed as species that need to be prioritized in terms of conservation. There are 19 families of Indonesian medicinal plants consisting of 31 species, that could be identified accurately to the family level by DNA barcoding. Two major families of Indonesian medicinal plants that were successfully sequenced and correctly identified were Orchidaceae (13 sequences) and Apocynaceae (10 sequences). It is highlighted that correct identification was defined after the validation step, which
is cross-checked to morphological identification result by taxonomists (indicated in the species identity card).

Figure 1. Summary of DNA barcoding use for medicinal plant (MP) conservation in Indonesia. Letters represent the DNA barcoding contribution of a species to the DNA bank data and its importance in conservation in the following order; A = new DNA barcoding and can strongly assist MP conservation; B = can strongly assist MP conservation; C = new DNA barcoding and can assist MP conservation; D = can assist MP conservation; E = new DNA bank data and new DNA barcoding and may strongly assist MP conservation; F = new DNA barcoding and may strongly assist MP conservation; G = may strongly assist MP conservation; H = new DNA bank data and new DNA barcoding and may assist MP conservation; I = new DNA barcoding and may assist MP conservation; J = may assist MP conservation; K = new DNA bank data and new DNA barcoding but sequences need to be clarified further; L = new DNA barcoding, but sequences need to be clarified further; M = sequences need to be clarified further.

2.2. Understanding The Effectiveness of Each DNA Barcoding Region Used for Indonesian Medicinal Plants Identification

A total of 61 studied species were analyzed for DNA barcoding of four regions (ITS2, matK, rbcL, and trnL). There were some failures in DNA amplification and sequencing assembly, with the results of each step presented in Table 2.

Table 2. Success or failure in each DNA barcoding step.

| Observed Parameter                      | ITS2 (%) | matK (%) | rbcL (%) | trnL (%) |
|-----------------------------------------|----------|----------|----------|----------|
| No PCR amplicon obtained                | 1.64     | 27.87    | 1.64     | 16.39    |
| Mixed sequences—no use                 | 8.20     | 0        | 1.64     | 3.28     |
| Sequence provided                      | 90.16    | 72.13    | 96.72    | 80.33    |
| Assembled consensus sequence           | 88.52    | 65.57    | 96.72    | 73.77    |
| Unidirectional sequence                | 1.64     | 6.56     | 0        | 6.56     |

* 4 matK regions with the second primer excluded.
The sequence quality is based on the easily done assembly of both the forward and reverse regions into a single consensus sequence (Table 2). When both forward and reverse sequences were available and were of good quality, obtaining the assembled consensus sequence was straightforward. If one direction of the sequence was mixed, then no assembly could occur and only the unidirectional sequence could be used. The matK region, which is known to have the lowest amplification success among the regions used for DNA barcoding [3,41], could only be amplified in 72% samples, compared with successful amplification in 83–98% samples for the other regions (Table 2). This is consistent with previous work indicating matK has a lower PCR success rate than rbcL for DNA amplification of Indonesian plants [42]. The PCR amplification failure likely occurred due to a high level of sequence variation within the matK regions complementary to the primers [43].

There were only 212 sequences of ITS2, matK, rbcL, and trnL obtained from 61 Indonesian medicinal plants instead of the expected 244 sequences resulting from the sequencing (Table A2). Each species was annotated with its key information, such as whether it is native, how the species became important to be conserved, and all generated sequences from ITS2, matK, rbcL, and trnL regions with identification results from BLAST, whether correct, ambiguous, correct at genus or family level, or incorrect.

2.3. Description of ITS2, matK, rbcL, and trnL Regions of Indonesian Medicinal Plants

The descriptive statistics of the sequence regions ITS2, matK, rbcL, and trnL are portrayed in Figure 2. The minimum and maximum lengths (bp) of ITS2, matK, rbcL, and trnL regions varied between 233–984, 384–1142, 382–1122, and 416–962, respectively, for all studied species; the average lengths of each region were 591.2, 676.9, 636.1, and 735.8, respectively. The range of GC Content (%) for ITS2, matK, rbcL, and trnL regions varied between 30.94–66.83, 27.86–65.43, 27.72–63.64, and 29.26–67.74, respectively, for all Indonesian medicinal plant species, whilst the average GC contents were 48.34, 41.64, 43.52, and 39.10, respectively.

Figure 2. Box plots of the sequence length (upper) and GC content (lower) of ITS2, matK, rbcL, and trnL of Indonesian medicinal plants.
The relationships between MP species identification accuracy and sequence length (bp), GC content (%), species number per genus, and percentage of identity are presented in Figure 3. With respect to sequence length, the longer the ITS2 and \textit{rbcL} sequence regions, the lower the identification accuracy, while the other regions indicated no such relationship. With respect to GC content (%), all regions except ITS2 tended to be less accurate for identification when the GC content increased. In terms of species number per genus, \textit{matK}, \textit{rbcL}, and \textit{trnL} regions all tended to have no correlation with the species number per genus, but the ITS2 sequence region was more accurate in identification when the species number per genus was higher. However, this result will depend on the available DNA information in the data bank. All regions indicated a positive relationship of percentage identity (through a BLASTN search) with identification accuracy.

Figure 3. Scatterplot of identification accuracy vs. sequence length (bp), GC Content (%), species number per genus, and percentage of identity. Scale 0–3 represents the identification accuracy (0 = incorrect, 1 = correct at the family level, 2 = correct at the genus level, 3 = correct at the species level).

Among the sequence regions produced for Indonesian medicinal plants, ITS2 generally had the lowest minimum length, smallest average sequence, and highest GC content (Figures 1 and 2) and hence gives the highest efficiency of identification, with only a short DNA sequence needed for correct identification. After ITS2, \textit{matK} follows second with respect to having the smallest average sequence length. A short DNA sequence may make the process of DNA barcoding technically easier and more economical from extraction to sequencing, as Kress and colleagues suggested [44]. Meanwhile, in terms of GC content (%), only ITS2 had higher identification accuracy when the GC content increased. In some plant DNA sequences, GC content has a positive correlation with exon sites, i.e., the coding regions [45]. This might mean that the longer the exons, the higher the GC content; thus, DNA regions with high GC content are expected to have more accurate identification.
2.4. Identification of Indonesian Medicinal Plants Using Sequences of Their ITS2, matK, rbcL, and trnL Regions

Identification of the sequence regions resulting from the BLAST method that have been matched with samples morphologically identified are presented in Table 3. The highest correct identification in the set of medicinal plant species was reached by the \textit{matK} region, followed by ITS2 and \textit{rbcL}, although the percentage values among them were not significantly different (i.e., 31.15\% compared to 29.51\%). In contrast, \textit{trnL} had the lowest correct identification, approximately 15\% lower than that of \textit{matK}. The highest incorrect identification was reached by the ITS2 region, followed by the \textit{rbcL} region. Overall, the most accurate of the four regions was \textit{matK} because it has the highest identification rate at the species level, lowest at the family level, and resulted in no incorrect identifications [3,41,42].

Table 3. Identification success rates of each region through the BLAST method after validating with the species name from morphology identification.

| Identification Measure                  | ITS2 (%) | \textit{matK} * (%) | \textit{rbcL} (%) | \textit{trnL} (%) |
|----------------------------------------|----------|---------------------|------------------|-----------------|
| Correct identification at species level| 29.51    | 31.15               | 29.51            | 16.39           |
| Correct identification at genus level  | 32.79    | 47.54               | 52.46            | 55.74           |
| Correct identification at family level | 6.56     | 0                   | 9.84             | 8.20            |
| Incorrect identification               | 22.95    | 0                   | 4.92             | 0               |

* 4 \textit{matK} regions with the second primer excluded.

Some ambiguous (correct at the genus and family level) and incorrect identification of Indonesian medicinal plants occurred. This might have happened because the world plant data has more than 1.2 million species names [34], while the DNA barcoding data for plants contains only 234,692 barcodes and only 5942 plants are recorded from Indonesia (http://www.boldsystems.org; accessed on 6 February 2020). As such, the available DNA bank to be cross-checked with the samples is far from complete.

The correct identification of unique species by singular regions and by combinations of regions can be visualized in the Venn diagrams (Figure 4). ITS2 was the most accurate region with unique correct identification, followed by \textit{rbcL}, \textit{matK}, and \textit{trnL}. A combination of three regions gave the same number of unique correct identifications, and a combination of all gave the highest correct identification. With respect to unique correct identification at the genus level, \textit{rbcL} gave the most accurate identification, followed by ITS2, \textit{trnL}, and finally \textit{matK}. A combination of \textit{matK}, \textit{rbcL}, and \textit{trnL} gave the best unique accurate identification compared to the other three combinations, and the combination of all gave the largest number of unique species among all possibilities. The highest unique correct species at the family level were obtained by using \textit{rbcL}, then ITS2, and finally \textit{trnL}.

Figure 4. Venn diagrams for correct identification of species at different taxonomic levels. From left to right: at the species level, at the genus level, and at the family level.
As presented in Table 4, the overall averages of the barcoding regions describing the genetic distance between the two compared species were very similar to one another, i.e., above 1.1% and below 1.2%, except for ITS2, which indicated an average of 1.29%. The lower the taxon unit relation, the lower the percentage, while the higher the taxon unit relation, the higher the percentage. Only the minimum distance of the matK region could describe species in the same genera. Nevertheless, the maximum distance of each region describes the highest level of the different species in a family. In principle, the genetic distance of interspecific related species (within the genus level and above) should be greater than that of the intraspecific species (within species level). It can be stated that more genetic distance lies between two different species with a different family than two different species with the same family. Species within the same genus have the least genetic distance.

Table 4. K2P pairwise genetic distances (%) of each region at different species levels.

| Region | Observation | Value (%) | Related Species |
|--------|-------------|-----------|-----------------|
| ITS2   | Overall average | 1.29503 | Nepenthes reinwardtiana and Nervilia concolor *** |
|        | Minimum distance | 0.00440 | Nepenthes mirabilis and N. ampullaria * |
|        | Maximum distance | 2.70903 | Erycibe malaccensis and Acalypha grandis *** |
|        | Overall average | 1.12567 | Nepenthes reinwardtiana and Parkia timoriana *** |
| matK   | Minimum distance | 0.00615 | Nepenthes mirabilis and N. ampullaria * |
|        | Maximum distance | 2.62368 | Nepenthes reinwardtiana and Parkia timoriana *** |
|        | Overall average | 1.19148 | Nepenthes mirabilis and N. ampullaria * |
| rbcL   | Minimum distance | 0.00350 | Anomum hochreutineri and Etlingera solaris ** |
|        | Maximum distance | 2.62587 | Phyllanthus oxyphyllus and Galearia filiformis *** |
|        | Overall average | 1.11310 | Phyllanthus oxyphyllus and Galearia filiformis *** |
| trnL   | Minimum distance | 0.02887 | Alstonia scholaris and Rauvolfia serpentina ** |
|        | Maximum distance | 2.59858 | Millettia sericea and Cymbidium aloifolium *** |

Notes: *: MP species in the same genera; **: MP species in the same family; ***: MP species in the different family.

The percentage of the sequences identified for each of the regions (ITS2, matK, rbcL, and trnL) was directly proportional to the accuracy of the identification. The higher the percentage, the more accurate the identification. MatK could correctly lead to identification of species with the highest percentages, followed by rbcL and ITS2 (Table 2). Only the matK region could differentiate species at the same genus level and species in different families compared to other regions. In contrast, ITS2 could not differentiate all species distances appropriately (Table 4).

In addition, it should be considered that using BLAST in a DNA barcoding study with any regions/primers is a basic step in identifying species [25–28,42]. BLAST analysis is the approach to the most similar species, and it depends on the species information stored in DNA bank. Therefore, the validation step to confirm the most accurate or most possible species is still required. When the used samples were clear species [42] like in this study, morphological identification by the experts was used, but when the samples were unable to be identified morphologically due to damage or derivative form or/and lack of taxonomic expert, generating a phylogenetic tree amongst medicinal plant groups such as a neighbor-joining (NJ) tree [23,25,26,42], maximum parsimony (MP), and maximum likelihood (ML) [42], and even analyzing chemical compound products [24] might be needed.

Considering Hollingsworth and colleagues’ findings with respect to DNA barcoding, it could serve two purposes. The first would be to provide information into the species-level taxon unit, and the second would be to help identify an unknown specimen to a known species. Thus, all the regions tested are valuable, depending on the purpose [43]. We emphasize that having a phylogenetic tree in the barcoding study would be beneficial, particularly when experts assume the medicinal plants are unidentified or a cryptic species. Thus, identification, authentication, and even conservation plan and action can be properly defined and applied.
3. Materials and Methods

3.1. Plant Samples and Literature Survey

This study used 61 different species of medicinal plants belonging to 30 families and 50 genera (Table 1). Plant samples were collected from a living collection with written permission from botanic gardens, including Bogor Botanic Gardens and Cibodas Botanic Gardens in Indonesia, and Hortus Botanicus Leiden in the Netherlands. All species had been taxonomically identified using morphological features as viewed on their identity card. Their scientific names were cross-checked online using POWO (2022) [34]. A leaf sample was collected from each species, except for Alstonia scholaris (L.) R. Br. and Spondias malayana Kosterm, from which bark samples were taken. This was due to A. scholaris and S. malayana Kosterm being high trees with unreachable leaves. Each sample (approximately 25 g) was collected and stored in a teabag with silica gel [46–48].

A literature study was conducted to collect all scientific information with respect to each of the sampled plant species, which can help the conservation status of every species. Information about available DNA data—i.e., whether the species already had DNA barcoding or genetic information that could be accessed from DNA banks—was identified using BOLD [11] and NCBI [10]. Data on species origin, including whether the species are native or introduced to Indonesia, and, if native, whether they are endemic, were collected from POWO (http://www.plantsoftheworldonline.org; accessed on 1 February 2022) [34]. Threatened species status was collected from the IUCN Red List of Threatened Species (https://www.iucnredlist.org; accessed on 6 February 2022), with species classified as Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in The Wild (EW), or Extinct (EX) [35]. Global legislation regulating trade, i.e., based on whether the species is included in CITES Appendices I, II, or III, was collected from the UNEP-WCMC Checklist of CITES species (https://checklist.cites.org; accessed on 1 February 2022) [36]. The information on rare medicinal plants, was compiled from the Indonesian Biodiversity Strategy and Action Plan (IBSAP) National Document [37]. Endemic plants or plants mentioned in the IUCN Red List, CITES Appendices I, II, or III, endemic, and priority lists were considered to be important species that need to be prioritized for conservation [49].

3.2. Molecular Analysis

Molecular analysis was performed at the University of Guelph laboratory, Canada. The barcoding method involves genomic DNA extraction, DNA amplification, and DNA sequencing, and taxonomic identification against available DNA banks. For DNA extraction, genomic DNA was extracted from plant samples using the Maxwell® RSC Purefood GMO and Authentication Kit and the Maxwell® RSC Instrument (Promega). For DNA amplification, primers targeting the ITS2, matK, rbcl, and trnL genes of plants were used to amplify the DNA (Table 5). Each PCR reaction mix (25 µL) contained 1x HotStarTaq master mix (Qiagen), 0.4 µM of each (forward and reverse) primers, 0.15 µg of BSA and 2 µL of template DNA. PCR thermal cycling was conducted by using a GeneAmpTM PCR System 9700 (Applied Biosystems, Waltham, MA, USA). The PCR cycling conditions were as follows: 95 °C for 10 min for DNA denaturation, 45 cycles of 95 °C for 15 sec for DNA annealing with the primer, followed by 55 °C for 30 sec and 72 °C for 1 min for DNA extension, and finally 72 °C for 7 min.

PCR products were visualized on 2% agarose gels to check whether DNA amplification was successful. PCR products were then purified using a NucleoFast® 96 PCR clean-up kit (Macherey-Nagel). The purified PCR fragments were sequenced bidirectionally, using the same primers as for the PCR, with the help of an ABI 3730 Genetic Analyzer (Applied Biosystems). The retrieved sequences were analyzed using ABI Prism™ Sequencing Analysis software (Applied Biosystems) to obtain a consensus sequence (Q > 20) for each sample.
Table 5. Primers used for amplification of DNA regions of ITS2, matK, rbcL, and trnL.

| Gene Region | Name | Sequence | Reference |
|-------------|------|----------|-----------|
| rbcL        | rbcLa-F | ATGTCACCACAAACAGAGACTAAAGC | [50] |
|             | rbcLa-R | GCTAAATCAATCCACCCRGCG | |
| matK        | matK472F | CCCRTYCACTGGAAATCTTGGTGTC | [41] |
|             | matK1248R | GCCRTRATAATGAGAAAGATTTCTGC | |
| matK a      | matKxF | TAATTTACGATCAATTCATTC | [23] |
|             | matK5R | GTTCAGACACAGAAAGTACG | |
| ITS2        | ITS2F | ATCGGATACCTTGGTGTAAT | [51] |
|             | ITS3R | GACGCTTCTCCAGACTACAT | |
| trnL        | trnL-F | ATTTGAACTGGTGACGAG | [7] |
|             | trnL-c | CGAACATCGGTAGACGCTACG | |

Note: matK a is an alternative to matK that is used when the PCR reaction fails to have an amplificon. F denotes the forward primer sequence and R is the reverse primer sequence.

3.3. Sequence Analyses and Data Interpretation

For each sample, the consensus sequence was compared with the nucleotide sequences in the BOLD species ID engine and the NCBI GenBank using BLASTN (https://blast.ncbi.nlm.nih.gov; accessed on 7 January 2022) [52] with the program selection as "Highly Similar Sequences (Megablast)" [53] for taxonomic identification. When no result was obtained from Megablast due to the sequence being too short, the sequence was queried with the program selection as, “Somewhat similar sequences (nBlast) for an alternative”.

PCR amplification, sequencing, and identification success rates were calculated as percentages. Only one best-matched species was selected from the BLASTN identification that is approached from the most similar sequence species recorded in DNA bank. Where there was more than a single match, the best-matched species was selected as the one with the lowest E value and the highest coverage; otherwise, any species was the closest-related species to the query (species). The results were then validated with studied medicinal species’ ID from botanical gardens where they have been morphologically identified by taxonomic expert.

The BLAST identification results were the initial step to identify species with DNA barcoding [25–28,42]. It was considered to be the correct species if the highest percentage of identification referred to the right species, i.e., when the species name from sequence identification matched the morphologically identified species. Otherwise, when the sequence was identified as a different species within a genus or a different species within a family, the result was considered to be an ambiguous species or genus. Ambiguous identifications were counted as correct identification, as per the study by Amandita et al. [42]. Sequences with an identification percentage of 99% or more were included in the novel sequence data for specific DNA barcoding for a species. Novel sequence data will be deposited in the GenBank database to assist in future identification.

Descriptive, statistical, and scatter plot analyses were used to gain understanding of the ITS2, matK, rbcL, and trnL regions and the relationship between factors in the BLAST analysis, with the identification being completed using the MINITAB Statistical Software.

In addition, Venn diagrams generated by Bioinformatics and Evolutionary Genomics (http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.htmpl; accessed on 2 January 2022) were used to depict how many species were correctly identified by singular regions and by multiple combinations of regions, whether or now there was a correct identification within species, genus, or family level. Information about the species number per genus was obtained from POWO [34].

Sequence alignments were performed using the Muscle program. The nucleotide composition of all sequences obtained from the ITS2, matK, rbcL, and trnL regions were computed, and their genetic distances were calculated with Kimura 2 parameters (K2P) [54]. The K2P pairwise genetic distance is the percentage of nucleotide sequence divergence that
was used by Hebert and colleagues [2]. All analyses were performed with the Molecular Evolutionary Genetics Analysis (MEGA X) software [55].

All the medicinal plant species information collected was analyzed and interpreted according to the use of the data in DNA barcoding with respect to conservation. Any correct identification can be used for DNA barcoding for related species and can be subsequently helpful for medicinal plant conservation, although the DNA barcoding can only be used for identification at species level and cannot estimate variation within species [56]. Any ambiguous identification can be used as an approach to species identification and thus may also be valuable for medicinal plant conservation.

Any new sequence or new DNA barcoding that is not available in NCBI or BOLD constitutes novel data. Species included in at least one of the following categories: IUCN Red List [40], CITES Appendixes I, II, or III [36], rare medicinal plants species [37], or Native and Endemic species [34] would require DNA barcoding more urgently than the non-listed species. Therefore the species were categorized in priority order A-M as follows: new DNA barcoding and can strongly assist medicinal plant (MP) conservation (A), can strongly assist MP conservation (B), new DNA barcoding and can assist MP conservation (C), can assist MP conservation (D), new to DNA bank data and new DNA barcoding and may strongly assist MP conservation (E), new DNA barcoding and may strongly assist MP conservation (F), may strongly assist MP conservation (G), new to DNA bank data and new DNA barcoding and may assist MP conservation (H), new DNA barcoding and may assist MP conservation (I), may assist MP conservation (J), new to DNA bank data and new DNA barcoding but sequences need to be clarified further (K), new DNA barcoding but sequences need to be clarified further (L) and sequences need to be clarified further (M).

4. Conclusions

Based on the results of this study, we conclude that no single region is perfectly ideal for DNA barcoding. Nonetheless, according to the observed criteria, we recommend \textit{matK} as the core DNA barcoding region for Indonesian medicinal plant identification. In addition, due to its unique correct species identification, we recommended the ITS2 and \textit{rbcL} regions as alternative or complementary regions to the core barcoding DNA using \textit{matK}. DNA barcoding for 33 Indonesian medicinal plant species was provided; of these 33 species, 21 species were newly DNA barcoded; of these 21 species, three contributed novel DNA barcoding data to DNA bank. In the future, this guide and associated data will facilitate a means to identify Indonesian medicinal plants, particularly those that need to be conserved strongly, to assure a valid species rather than a substitute in herbal medicines and to prevent illegal trade.

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\textbf{Institutional Review Board Statement:} This study did not require ethical approval.

\textbf{Informed Consent Statement:} Not applicable.

\textbf{Data Availability Statement:} All data resulting from this study has been stored and could be accessed at \url{http://www.boldsystems.org} under Project-MPIN DNA BARCODING STUDY OF MEDICINAL PLANTS OF INDONESIA FOR ASSISTING THEIR CONSERVATION AND USE.

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of samples are from BBG, except *Amomum hochreutineri* Valeton, *Etlingera solaris* (Blume) R.M.Sm., *Psychotria montana* Blume, *Rhododendron maccropogoniae* F.Muell., *Smilax calophylla* Wall. ex A.DC. and *Staurogyne elongate* (Nees) Kuntze are from CBG, and *Aglaonema commutatum* Wall., *Cymbidium ensifolium* (L.) Sw. and *Hoya diversifolia* Blume are from HBL.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Appendix A**

Table A1. DNA barcoding regions used for medicinal plant (MP) conservation in Indonesia.

| DNA Barcoding Use for MP Conservation in Indonesia | ITS2 | matK | rbCL | trnL |
|--------------------------------------------------|------|------|------|------|
| **A. new DNA barcoding and can strongly assist MP conservation** |      |      |      |      |
| *Anaxagorea javanica* | 1    | 1    | 2    | 1    |
| *Aquilaria hirta* | 1    |      |      |      |
| *Strongyleria pannea* | 1    | 1    | 1    |      |
| **B. can strongly assist MP conservation** | 11   | 12   | 8    | 6    |
| *Alstonia scholaris* | 1    | 1    | 1    | 1    |
| *Algeia reinwardtii* | 1    | 1    | 1    |      |
| *Cymbidium aloifolium* | 1    | 1    | 1    |      |
| *Dendrobium crumenatum* | 1    | 1    |      |      |
| *Dendrobium salacense* | 1    |      | 1    | 1    |
| *Euphorbia tirucalli* |      |      |      | 1    |
| *Ficus deltoidea* |      |      |      |      |
| *Galenia filiformis* |      |      | 1    | 1    |
| *Kadsura scandens* |      |      |      | 1    |
| *Lunasia amara* |      | 1    | 1    | 1    |
| *Nepenthes gracilis* |      |      |      | 1    |
| *Nepenthes reinwardtiana* |      |      | 1    | 1    |
| *Nervilia plicata* |      | 1    | 1    | 1    |
| *Pangium edule* |      |      | 1    | 1    |
| *Parkia timoriana* |      |      |      |      |
| *Rauvolfia serpentina* |      | 1    | 1    | 1    |
| **C. new DNA barcoding and can assist MP conservation** |      |      |      | 1    |
| *Aglaonema commutatum* |      |      |      |      |
| *Meistera aculeata* |      |      |      |      |
| **D. can assist MP conservation** | 5    | 6    | 7    | 3    |
| *Alstonia macrophylla* |      | 1    | 1    |      |
| *Ancistrocladus tectorius* |      | 1    | 1    | 1    |
| *Ardisia crenata* |      |      | 1    | 1    |
| *Dasysmacalum dasysmacalum* |      |      |      | 1    |
| *Justicia gendarussa* |      | 1    | 1    | 1    |
| *Orthosiphon aristatus* |      |      |      | 1    |
| *Phyllanthus oxycaphylus* |      | 1    |      |      |
| *Prema serratifolia* |      |      |      | 1    |
| *Toxicodendron sucedaneum* |      | 1    | 1    | 1    |
| **E. new to DNA bank data and new DNA barcoding and may strongly assist MP conservation** | 6    | 4    | 6    | 7    |
| *Anomum hochreutineri* |      | 1    | 1    |      |
| *Dendrobium purpureum* |      | 1    | 1    | 1    |
| *Etlingera solaris* |      | 1    | 1    | 1    |
| *Myristica sucedanea* |      |      | 1    | 1    |
| *Obregonia lycopodioides* |      | 1    | 1    | 1    |
| *Planera fulva* |      |      | 1    |      |
| *Rhododendron maccropogoniae* |      | 1    | 1    | 1    |
| **F. new DNA barcoding and may strongly assist MP conservation** | 2    | 3    | 2    | 2    |
| *Acriopsis liliifolia var. liliifolia* |      | 1    | 1    | 1    |
| *Anaxagorea javanica* |      |      | 1    |      |
| *Aquilaria hirta* |      |      | 1    |      |
Table A1. Cont.

| DNA Barcoding Use for MP Conservation in Indonesia | ITS2 | matK | rbcl | trnL |
|---------------------------------------------------|------|------|------|------|
| **G. may strongly assist MP conservation**        | 3    | 8    | 12   | 12   |
| Alyxia reinwardtii                                 |      |      |      |      |
| Cibotium barometz                                   |      |      |      |      |
| Cymbidium aloifolium                                |      |      |      |      |
| Cymbidium ensifolium                                | 1    | 1    |      |      |
| Dendrobium crumenatum                               | 1    |      |      |      |
| Dendrobium salacense                                | 1    |      |      |      |
| Euphorbia tirucalli                                 | 1    |      |      |      |
| Ficus deltoidea                                    | 1    | 1    | 1    |      |
| Grammatophyllum speciosum                           | 1    | 1    |      |      |
| Kadsura scandens                                    | 1    | 1    |      |      |
| Lunasia amara                                       | 1    |      |      |      |
| Nepenthes ampullaria                                | 1    | 1    | 1    |      |
| Nepenthes gracilis                                  | 1    |      |      |      |
| Nepenthes mirabilis                                 | 1    | 1    | 1    | 1    |
| Nepenthes reinwardtiana                             | 1    | 1    |      |      |
| Nervilia concolor                                   | 1    |      |      |      |
| Pangium edule                                       | 1    |      |      |      |
| Parkia timoriana                                    | 1    |      |      |      |
| Smilax zeqlanica                                    | 1    | 1    |      |      |
| **H. new to DNA bank data and new DNA barcoding and may assist MP conservation** | 2    | 2    | 3    | 3    |
| Acalypha grandis                                    | 1    | 1    |      |      |
| Ardisia complanata                                  | 1    | 1    | 1    |      |
| Erycibe malaccensis                                 | 1    | 1    | 1    |      |
| **I. new DNA barcoding and may assist MP conservation** | 4    | 6    | 7    | 6    |
| Aglaonema commutatum                                 | 1    | 1    |      |      |
| Cinnamomum rhynchophyllum                           | 1    | 1    | 1    |      |
| Decalobanthus mammosus                              | 1    |      |      |      |
| Hoya diversifolia                                   | 1    | 1    | 1    |      |
| Meistera aculeata                                   |      |      |      |      |
| Melicope lunu-ankenda                               | 1    | 1    | 1    |      |
| Psychotria montana                                   | 1    | 1    | 1    |      |
| Spondias malagana                                   | 1    |      |      |      |
| Ventilago madraspatana                              |      |      |      |      |
| **J. may assist MP conservation**                   | 7    | 6    | 8    | 9    |
| Alstonia macrophylla                                | 1    | 1    |      |      |
| Ancistroclados tectorius                             |      |      |      |      |
| Ardisia crenata                                     | 1    | 1    |      |      |
| Benstonea affinis                                   | 1    | 1    |      |      |
| Dasymaschalon dasymaschalum                         | 1    |      |      |      |
| Meliletta sericea                                    | 1    | 1    | 1    |      |
| Orthosiphon aristatus                               |      |      |      |      |
| Phyllanthus oxyphyllus                              |      |      |      |      |
| Premna serratifolia                                  |      |      |      |      |
| Smilax calophylla                                   |      |      |      |      |
| Staurogyne elongata                                 | 1    | 1    | 1    | 1    |
| Trevesia burckii                                    | 1    | 1    | 1    | 1    |
| Vitex glabrata                                       | 1    |      |      |      |
| **K. new to DNA bank data and new DNA barcoding, but sequences need to clarify further (K)** | 2    | 1    |      |      |
| Acalypha grandis                                    |      |      |      |      |
| Myristica succedane                                 |      |      |      |      |
| Phanera fulva                                       |      |      |      |      |
| **L. new DNA barcoding, but sequences need to clarify further** | 2    |      |      |      |
| Aglaonema commutatum                                 |      |      |      |      |
| Ventilago madraspatana                              |      |      |      |      |
Table A1. Cont.

| DNA Barcoding Use for MP Conservation in Indonesia | ITS2 | matK | rbcL | trnL |
|--------------------------------------------------|------|------|------|------|
| M. new DNA barcoding and may strongly assist MP conservation | 10   | 2    |      |      |
| Benstonea affinis                                   | 1    |      |      |      |
| Cibotium barometz                                   | 1    |      |      |      |
| Dasymaschalon dasymaschalum                        | 1    |      |      |      |
| Galeria filiformis                                 | 1    |      |      |      |
| Grammatophyllum speciosum                          | 1    |      |      |      |
| Nervilia concolor                                  | 1    |      |      |      |
| Nervilia plicata                                   | 1    |      |      |      |
| Pangium edule                                      | 1    |      |      |      |
| Parkia timoriana                                   | 1    |      |      |      |
| Smilax calophylla                                  | 1    |      |      |      |
| Smilax zeylanica                                   | 1    |      |      |      |

Table A2. Summary of DNA barcoding result per species.

| No. | Species [38] | Author | Fam. | Region | Max Score | Total Score | Query Cover | E Value | Per. Ident | Best Matched Species | Sum. | Notes |
|-----|--------------|--------|------|--------|-----------|-------------|-------------|---------|------------|----------------------|------|-------|
| 1   | Justicia gendarussa | Burm.f. | Acanth. | ITS2   | 562       | 562         | 0.73        | 5.00E-156 | 0.9968     | Justicia gendarussa   |      | c     |
|     |              |        |      | matK   | 1330      | 1330        | 0.96        | 0        | 0.9986     | Justicia gendarussa   |      | c     |
|     |              |        |      | rbcL   | 1055      | 1055        | 0.97        | 0        | 1          | Justicia gendarussa   |      | c     |
|     |              |        |      | trnL   | 1487      | 1487        | 0.92        | 0        | 0.9975     | Justicia gendarussa   |      | c     |
| 2   | Staurogyne elongata | (Nees) | Kuntze | Acanth. | ITS2   | 597         | 0.89        | 1.00E-166 | 0.9526     | Ophiophriziphilum microcephalum |      | a ** |
|     |              |        |      | matK   | 1273      | 1273        | 0.97        | 0        | 0.9821     | Staurogyne concinnum | a *  |
|     |              |        |      | rbcL   | 939       | 939         | 0.91        | 0        | 0.9923     | Staurogyne concinnum | a *  |
|     |              |        |      | trnL   | 1013      | 1427        | 0.99        | 0        | 0.9732     | Staurogyne trinitissi   | a *  |
| 3   | Pangium edule | Reinw. | Achari. | ITS2   | 163       | 163         | 0.15        | 1.00E-35  | 0.9286     | Celastraceae sp. | i    |
|     |              |        |      | matK   | 1387      | 1387        | 1           | 0        | 0.9974     | Pangium edule | c    |
|     |              |        |      | rbcL   | 972       | 972         | 0.91        | 0        | 0.982      | Pangium edule | c    |
|     |              |        |      | trnL   | 1158      | 1741        | 0.98        | 0        | 0.982      | Ryparosa kurrangii | a *  |
| 4   | Spondias malayana | Kosterm. | Anacardi. | ITS2   | 636       | 636         | 1           | 3.00E-178 | 0.9332     | Spondias tuberosa | a *  |
| 5   | Toxicodendron succedaneum | (L.) | Kuntze | Anacardi. | ITS2   | 660       | 660         | 0.75        | 0         | 1         | Toxicodendron succedaneum | c    |
|     |              |        |      | matK   | 1452      | 1452        | 0.99        | 0        | 1         | Toxicodendron succedaneum | c    |
|     |              |        |      | rbcL   | 1038      | 1038        | 0.97        | 0        | 1         | Toxicodendron succedaneum | c    |
|     |              |        |      | trnL   | 1598      | 1598        | 1           | 0        | 1         | Toxicodendron succedaneum | c    |
| 6   | Ancistrocladius tectorius | (Lour.) | Merr. | Ancistroclad. | ITS2   | 774       | 774         | 1           | 0         | 0.9953   | Ancistrocladius benemensis | c    |
|     |              |        |      | matK   | 1387      | 1387        | 1           | 0        | 0.9987   | Ancistrocladius heptaneus | a *  |
|     |              |        |      | rbcL   | 1053      | 1053        | 1           | 0        | 1         | Ancistrocladius tectorius | c    |
|     |              |        |      | trnL   | 1663      | 1663        | 1           | 0        | 0.9903   | Ancistrocladius tectorius | c    |
| 7   | Anaxagorea javanica | Blume | Annon. | ITS2   | 1502      | 1502        | 0.97        | 0        | 0.9928   | Anaxagorea luzonensis | a *  |
|     |              |        |      | matK   | 1502      | 1502        | 0.97        | 0        | 0.9928   | Anaxagorea luzonensis | a *  |
|     |              |        |      | rbcL   | 1013      | 1013        | 0.94        | 0        | 1         | Anaxagorea javanica | c    |
| 8   | Dasymaschalon dasymaschalum | (Blume) | I.M.Turner | ITS2   | 237       | 237         | 0.38        | 3.00E-58  | 0.9474   | Acer palmatum | i    |
|     |              |        |      | matK   | 1382      | 1382        | 1           | 0        | 0.9947   | Dasymaschalon clusiflorum | c    |
|     |              |        |      | rbcL   | 1020      | 1020        | 0.97        | 0        | 1         | Demo dasymaschalum | c    |
|     |              |        |      | trnL   | 1565      | 1565        | 0.95        | 0        | 0.9965   | Dasymaschalon megalamutham | a *  |
| No. | Species [38]        | Author                | Fam.            | Region | Max Score | Total Score | Query Cover | E Value | Per. Ident | Best Matched Species        | Sum. | Notes |
|-----|---------------------|-----------------------|-----------------|--------|-----------|-------------|-------------|---------|------------|-----------------------------|------|-------|
| 9   | Alstonia macrophylla | Wall. Ex. G.Don       | Apocyn.         | ITS2   | 763       | 763         | 0.98        | 0       | 0.9976     | Alstonia scholaris           | a *  |       |
|     |                     |                       |                 | matK   | 1386      | 1386        | 1           | 0       | 0.9987     | Alstonia scholaris           | c    | 13/14 is a * with the same coverage |
|     |                     |                       |                 | rbcL   | 857       | 857         | 1           | 0       | 0.9876     | Alstonia scholaris           | c    |       |
|     |                     |                       |                 | trnL   | 1557      | 1557        | 1           | 0       | 0.9908     | Alstonia scholaris           | a *  |       |
|     |                     |                       |                 | ITS2   | 457       | 457         | 0.62        | 3.00E-124| 0.9772     | Alstonia scholaris           | c    |       |
| 10  | Alstonia scholaris   | (L.) R. Br.           | Apocyn.         | matK   | 1380      | 1380        | 1           | 0       | 0.9887     | Alstonia scholaris           | c    | 1/9 a is a * with same coverage |
|     |                     |                       |                 | rbcL   | 1051      | 1051        | 1           | 0       | 0.9983     | Alstonia scholaris           | c    |       |
|     |                     |                       |                 | trnL   | 1589      | 1589        | 1           | 0       | 0.9977     | Alstonia scholaris           | c    |       |
|     |                     |                       |                 | ITS2   | 614       | 614         | 0.8         | 1.00E-171| 0.9912     | Alyxia reinwardtii           | c    | 1/2 is a * |
|     |                     |                       |                 | matK   | 1317      | 1317        | 0.95        | 0       | 0.9972     | Alyxia reinwardtii           | c    | 1/2 is a * with higher coverage |
| 11  | Alyxia reinwardtii   | Blume                 | Apocyn.         | rbcL   | 1020      | 1020        | 0.96        | 0       | 1          | Alyxia reinwardtii           | c    |       |
|     |                     |                       |                 | trnL   | 1524      | 1524        | 0.98        | 0       | 0.9929     | Alyxia grandis              | a *  |       |
|     |                     |                       |                 | ITS2   | 507       | 507         | 0.63        | 3.00E-139| 1          | Hoya glabra                 | a *  |       |
|     |                     |                       |                 | matK   | 1347      | 1347        | 1           | 0       | 1          | Hoya vitellinoides           | a *  |       |
|     |                     |                       |                 | rbcL   | 1051      | 1051        | 0.99        | 0       | 1          | Hoya petiolaris              | a *  |       |
|     |                     |                       |                 | trnL   | 1539      | 1539        | 0.98        | 0       | 0.9988     | Hoya sp. serpentina          | c    |       |
|     |                     |                       |                 | ITS2   | 617       | 617         | 0.73        | 1.00E-172| 1          | Rauvolfia serpentina         | c    |       |
| 12  | Hoya diversifolia    | Blume                 | Apocyn.         | matK   | 1380      | 1380        | 0.99        | 0       | 1          | Rauvolfia serpentina         | c    |       |
|     |                     |                       |                 | rbcL   | 1057      | 1057        | 0.99        | 0       | 1          | Rauvolfia serpentina         | c    |       |
|     |                     |                       |                 | trnL   | 1395      | 1395        | 0.89        | 0       | 0.9873     | Rauvolfia serpentina         | c    |       |
| 13  | Rauvolfia serpentina | (L.) Benth. ex Kurz.  | Apocyn.         | ITS2   | 501       | 501         | 0.59        | 2.00E-137| 0.9964     | Thunbergia coccinea          | i    |       |
|     |                     |                       |                 | matK   | 1384      | 1384        | 1           | 0       | 0.9974     | Aglaonema coccineum          | a *  |       |
|     |                     |                       |                 | rbcL   | 1022      | 1022        | 0.97        | 0       | 1          | Aglaonema commutatum         | c    |       |
|     |                     |                       |                 | trnL   | 1650      | 1650        | 1           | 0       | 0.9989     | Aglaonema crispum            | a *  |       |
|     |                     |                       |                 | ITS2   | 745       | 745         | 0.95        | 1.00E-183| 0.9988     | Trevesia palmata             | a *  |       |
|     |                     |                       |                 | matK   | 1393      | 1393        | 1           | 0       | 1          | Brassiaopsis gracilis       | a *  |       |
|     |                     |                       |                 | rbcL   | 1048      | 1048        | 0.98        | 0       | 0.9982     | Trevesia palmata             | a *  |       |
|     |                     |                       |                 | trnL   | 1668      | 1668        | 0.99        | 0       | 0.9989     | Brassiaopsis ciliata        | a *  |       |
| 14  | Aglaonema commutatum | Schott                | Ar.             | ITS2   | 501       | 501         | 0.59        | 2.00E-137| 0.9964     | Cucumis sativus              | i    |       |
|     |                     |                       |                 | matK   | 1384      | 1384        | 1           | 0       | 0.9974     | Cibotium barometz            | i    |       |
|     |                     |                       |                 | rbcL   | 1022      | 1022        | 0.97        | 0       | 1          | Cibotium barometz            | i    |       |
|     |                     |                       |                 | trnL   | 1650      | 1650        | 1           | 0       | 0.9989     | Cibotium barometz            | a ** |       |
| 15  | Trevesia buckii      | R.Br.                 | Arali.          | ITS2   | 348       | 348         | 0.75        | 3.00E-91 | 0.9896     | Merremia peltata             | a *  |       |
|     |                     |                       |                 | rbcL   | 965       | 965         | 0.94        | 0       | 0.9872     | Merremia peltata             | a *  |       |
| 16  | Cibotium barometz    | (L.) J.Sm.            | Cibot.          | trnL   | 1629      | 1629        | 0.96        | 0       | 0.9955     | Acera tataricum              | a *  |       |
|     |                     |                       |                 | ITS2   | 272       | 272         | 0.35        | 1.00E-68 | 0.9808     | Acera tataricum              | i    |       |
|     |                     |                       |                 | rbcL   | 1062      | 1062        | 0.99        | 0       | 1          | Acera tataricum              | i    |       |
|     |                     |                       |                 | trnL   | 1729      | 1729        | 1           | 0       | 0.9886     | Acera tataricum              | a *  |       |

**Table A2. Cont.**
| No. | Species [38]                      | Author          | Fam.   | Region | Max Score | Total Score | Query Cover | E Value | Per. Ident | Best Matched Species | Sum. | Notes          |
|-----|----------------------------------|-----------------|--------|--------|-----------|-------------|-------------|---------|------------|----------------------|------|-----------------|
| 21  | *Euphorbia tirucalli*           | L. Euphorbi.    |        |        | 1046      | 1046        | 0.98        | 0       | 0          | *Euphorbia raulii*     | a *  |
|     |                                 |                 |        |        | 712       | 712         | 0.94        | 0       | 0          | *Millettia pulchra*     | a *  |
|     |                                 |                 |        |        | 1332      | 1332        | 0.97        | 0       | 0          | *Dioseteria piretata*   | a *  |
|     |                                 |                 |        |        | 1042      | 1042        | 0.97        | 0       | 0          | *Millettia piretata*    | a *  |
|     |                                 |                 |        |        | 1543      | 1543        | 1           | 0       | 0          | *Parkia timoriana*      | c    |
| 22  | *Millettia sericea*             | (Vent.) Fab.    |        |        | 1376      | 1376        | 0.98        | 0       | 0          | *Parkia biglandulosa*    | a *  |
|     |                                 |                 |        |        | 1000      | 1000        | 0.95        | 0       | 0.9927     | *Magnoliodaphya sp.*     | i    |
|     |                                 |                 |        |        | 1814      | 1814        | 0.99        | 0       | 0.99       | *Parkia biglandulosa*    | a *  |
|     |                                 |                 |        |        | 475       | 475         | 0.68        | 7.00E-130| 0.9477     | *Rudinia sp.*           | a *  |
|     |                                 |                 |        |        | 1016      | 1016        | 0.96        | 0       | 0.9982     | *Embryophyllum environmental* | a ** |
|     |                                 |                 |        |        | 1404      | 1404        | 0.78        | 0       | 0.9974     | *Phanaea vahlii*         | a ** |
|     |                                 |                 |        |        | 562       | 562         | 0.69        | 5.00E-156| 1          | *Orthosiphon aristatus*  | c    |
|     |                                 |                 |        |        | 1042      | 1042        | 0.98        | 0       | 1          | *Clerodendranthus spicatus* | a ** |
| 23  | *Parkia timoriana*              | (DC.) Merr.     | Fab.   |        | 1376      | 1376        | 0.98        | 0       | 0.996      | *Premna microphylla*     | a *  |
| 24  | *Phanera fulva*                 | (Korth.) Benth. | Fab.   |        | 1040      | 1040        | 0.97        | 0       | 1          | *Premna serratifolia*     | c    |
| 25  | *Orthosiphon aristatus*         | (Blume) Miq.   | Lami.  |        | 651       | 651         | 0.91        | 0       | 0.9558     | *Vitex carvalhoi*        | a *  |
|     |                                 |                 |        |        | 1587      | 1587        | 1           | 0       | 0.9968     | *Vitex glabrata*         | a *  |
|     |                                 |                 |        |        | 1050      | 1050        | 1           | 0       | 0.9982     | *Vitex doniana*          | a *  |
|     |                                 |                 |        |        | 1411      | 1411        | 0.94        | 0       | 0.9923     | *Vitex triflora*         | a *  |
|     |                                 |                 |        |        | 1375      | 1375        | 0.99        | 0       | 0.9987     | *Cinnamomum camphora*     | a *  |
|     |                                 |                 |        |        | 1055      | 1055        | 1           | 0       | 0          | *Cinnamomum dubium*      | a *  |
|     |                                 |                 |        |        | 1587      | 1587        | 1           | 0       | 0          | *Cinnamomum pittosporoides* | a *  |
|     |                                 |                 |        |        | 616       | 616         | 0.78        | 4.00E-172| 1          | *Ficus deltoidea*        | c    |
|     |                                 |                 |        |        | 1051      | 1051        | 0.98        | 0       | 0.9983     | *Ficus ochroleuca*       | a *  |
|     |                                 |                 |        |        | 1664      | 1664        | 0.99        | 0       | 0.9967     | *Ficus benjamina*        | a *  |
|     |                                 |                 |        |        | 185       | 185         | 0.17        | 2.00E-42 | 0.9251     | *Riododendron milloides*  | i    |
|     |                                 |                 |        |        | 1476      | 1476        | 0.92        | 0       | 0.9988     | *Myristica fragrans*     | a *  |
|     |                                 |                 |        |        | 1057      | 1057        | 1           | 0       | 0          | *Horsfieldia amygadalina* | a *  |
|     |                                 |                 |        |        | 1371      | 1371        | 0.83        | 0       | 0.9987     | *Myristica iners*        | a *  |
|     |                                 |                 |        |        | 1375      | 1375        | 0.99        | 0       | 0.9973     | *Nepenthes mapuluenis*    | a *  |
|     |                                 |                 |        |        | 1042      | 1042        | 1           | 0       | 1          | *Nepenthes mirabilis*     | a *  |
|     |                                 |                 |        |        | 1648      | 1648        | 1           | 0       | 0.9956     | *Nepenthes mirabilis*     | a *  |
|     |                                 |                 |        |        | 1371      | 1371        | 1           | 0       | 0.9973     | *Nepenthes gracilis*      | c    |
|     |                                 |                 |        |        | 1046      | 1046        | 1           | 0       | 1          | *Nepenthes mirabilis*     | a *  |
|     |                                 |                 |        |        | 961       | 961         | 0.57        | 0       | 0.9962     | *Nepenthes amplenularia*   | a *  |
| 31  | *Nepenthes ampullaris*           | Jack Nepenth.   |        |        | 857       | 857         | 1           | 0       | 0.9979     | *Nepenthes reinwardiana*   | a *  |
| 32  | *Nepenthes gracilis*             | Korth. Nepenth. |        |        | 1371      | 1371        | 1           | 0       | 0.9973     | *Nepenthes mapuluenis*    | a *  |
|     |                                 |                 |        |        | 1038      | 1038        | 1           | 0       | 0.9965     | *Nepenthes graciliflora*   | a *  |
|     |                                 |                 |        |        | 959       | 959         | 0.57        | 0       | 0.9943     | *Nepenthes sanguinea*      | a *  |
|     |                                 |                 |        |        | 861       | 861         | 1           | 0       | 0.9979     | *Nepenthes reinwardiana*   | c    |
|     |                                 |                 |        |        | 1376      | 1376        | 1           | 0       | 0.9965     | *Nepenthes reinwardiana*   | c    |
|     |                                 |                 |        |        | 1042      | 1042        | 0.98        | 0       | 0.9965     | *Nepenthes reinwardiana*   | c    |
|     |                                 |                 |        |        | 948       | 948         | 0.57        | 0       | 0.9924     | *Nepenthes reinwardiana*   | a *  |

**Table A2. Cont.**
| No. | Species [38]                                      | Author                  | Fam.                 | Region  | Max Score | Total Score | Query Cover | E Value   | Per. Ident | Best Matched Species                                      | Sum. Notes |
|-----|--------------------------------------------------|-------------------------|----------------------|---------|-----------|-------------|-------------|-----------|------------|----------------------------------------------------------|------------|
| 35  | Acriopsis longibracteatum var. lilifolia         | (J.Koenig) Ormerod      | Orchid.              | ITS2    | 394       | 394         | 0.94        | 2.00E-105 | 0.8428     | Cymbidium ensifolium                                      | a **       |
|     |                                                  |                         |                      | matK    | 1408      | 1408        | 1           | 0         | 0.9987     | Aciopsis sp.                                               | a *        |
|     |                                                  |                         |                      | rbcL    | 911       | 911         | 1           | 0         | 0.9824     | Cymbidium ensifolium                                      | a *        |
|     |                                                  |                         |                      | trnL    | 824       | 1591        | 0.91        | 0         | 0.9265     | Cymbidium ensifolium                                      | a **       |
| 36  | Cymbidium aloifolium                            | (L.) Sw.                | Orchid.              | ITS2    | 468       | 468         | 0.61        | 1.00E-127 | 0.9884     | Cymbidium aloifolium                                      | c          |
|     |                                                  |                         |                      | matK    | 1386      | 1386        | 1           | 0         | 0.9987     | Cymbidium aloifolium                                      | c          |
|     |                                                  |                         |                      | rbcL    | 1048      | 1048        | 0.98        | 0         | 0.9982     | Cymbidium aloifolium                                      | c          |
|     |                                                  |                         |                      | trnL    | 989       | 989         | 0.79        | 0         | 0.953      | Cymbidium aloifolium                                      | a *        |
| 37  | Cymbidium ensifolium                            | (L.) Sw.                | Orchid.              | ITS2    | 387       | 387         | 0.66        | 4.00E-103 | 0.9072     | Cymbidium ensifolium                                      | a *        |
|     |                                                  |                         |                      | matK    | 1293      | 1293        | 0.99        | 0         | 0.9889     | Cymbidium ensifolium                                      | a *        |
| 38  | Dendrobium crateratum                          | Sw.                    | Orchid.              | ITS2    | 577       | 577         | 0.7         | 2.00E-160 | 0.9968     | Dendrobium crateratum                                    | c          |
|     |                                                  |                         |                      | matK    | 1400      | 1400        | 0.99        | 0         | 0.9961     | Dendrobium crateratum                                    | c          |
|     |                                                  |                         |                      | rbcL    | 1038      | 1038        | 0.97        | 0         | 0.9982     | Dendrobium crateratum                                    | a *        |
| 39  | Dendrobium purpureum                            | Roxb.                  | Orchid.              | ITS2    | 481       | 537         | 0.86        | 2.00E-131 | 0.9005     | Dendrobium purpureum                                     | a *        |
|     |                                                  |                         |                      | matK    | 1360      | 1360        | 1           | 0         | 0.9947     | Dendrobium purpureum                                     | a *        |
|     |                                                  |                         |                      | rbcL    | 1042      | 1042        | 0.98        | 0         | 0.9965     | Dendrobium purpureum                                     | a *        |
|     |                                                  |                         |                      | trnL    | 562       | 998         | 0.98        | 8.00E-156 | 0.9814     | Dendrobium purpureum                                     | a *        |
| 40  | Dendrobium salacense                            | (Blume) Lindl.         | Orchid.              | ITS2    | 627       | 627         | 0.79        | 2.00E-175 | 0.9914     | Dendrobium salacense                                     | a *        |
|     |                                                  |                         |                      | matK    | 1382      | 1382        | 0.99        | 0         | 0.9987     | Dendrobium salacense                                     | a *        |
|     |                                                  |                         |                      | rbcL    | 1031      | 1031        | 1           | 0         | 1          | Dendrobium salacense                                     | c          |
|     |                                                  |                         |                      | trnL    | 1328      | 1328        | 0.81        | 0         | 0.9959     | Dendrobium salacense                                     | c          |
| 41  | Grammatophyllum speciosum                       | Blume                   | Orchid.              | ITS2    | 809       | 38152       | 1           | 0         | 1          | Grammatophyllum papuanum                                  | i          |
|     |                                                  |                         |                      | matK    | 1378      | 1378        | 0.99        | 0         | 0.996      | Grammatophyllum papuanum                                  | a *        |
|     |                                                  |                         |                      | rbcL    | 1037      | 1037        | 0.97        | 0         | 0.9947     | Grammatophyllum papuanum                                  | a **       |
|     |                                                  |                         |                      | trnL    | 568       | 1103        | 0.93        | 2.00E-157 | 0.9905     | Grammatophyllum papuanum                                  | i          |
| 42  | Nervilia concolor                               | (Blume) Schltr.         | Orchid.              | ITS2    | 828       | 828         | 1           | 0         | 1          | Liparis siccitans                                         | i          |
|     |                                                  |                         |                      | rbcL    | 1062      | 1062        | 0.99        | 0         | 1          | Nervilia siccitans                                         | i          |
|     |                                                  |                         |                      | trnL    | 1585      | 1585        | 1           | 0         | 0.9834     | Nervilia siccitans                                         | i          |
| 43  | Nervilia plicata                                | (Andrews) Schltr.       | Orchid.              | ITS2    | 721       | 721         | 0.88        | 0         | 0.9741     | Nervilia plicata                                          | i          |
|     |                                                  |                         |                      | matK    | 1413      | 1413        | 0.97        | 0         | 0.9987     | Nervilia plicata                                          | c          |
|     |                                                  |                         |                      | rbcL    | 1005      | 1005        | 0.94        | 0         | 1          | Nervilia plicata                                          | c          |
|     |                                                  |                         |                      | trnL    | 1663      | 1663        | 0.99        | 0         | 0.9967     | Nervilia plicata                                          | c          |
| 44  | Oberonia caulescens                             | (J.Koenig) Ormerod      | Orchid.              | ITS2    | 398       | 398         | 0.88        | 1.00E-106 | 0.8765     | Oberonia caulescens                                       | a *        |
|     |                                                  |                         |                      | matK    | 1205      | 1205        | 0.93        | 0         | 0.9732     | Oberonia caulescens                                       | a *        |
|     |                                                  |                         |                      | rbcL    | 922       | 922         | 1           | 0         | 0.9921     | Oberonia caulescens                                       | a **       |
|     |                                                  |                         |                      | trnL    | 592       | 1078        | 0.91        | 2.00E-164 | 0.8734     | Oberonia caulescens                                       | a **       |
| 45  | Stronganthera pauperea                           | (Lindl.) Schuit., Y.F.Ng & H.A.Federsen | Orchid.              | ITS2    | 431       | 431         | 0.59        | 2.00E-116 | 0.959      | Liparis lobellii                                           | c          |
|     |                                                  |                         |                      | matK    | 1375      | 1375        | 1           | 0         | 0.996      | Mycaranthes pannae                                        | c          |
|     |                                                  |                         |                      | rbcL    | 1055      | 1055        | 1           | 0         | 0.9965     | Mycaranthes pannae                                        | c          |
|     |                                                  |                         |                      | rbcL    | 433       | 433         | 0.99        | 4.00E-117 | 0.8552     | Mycaranthes pannae                                        | c          |
| 46  | Galatia filiformis                              | (Blume) Boerl.          | Pand.                | ITS2    | 1393      | 1393        | 1           | 0         | 1          | Populus nigra                                              | i          |
|     |                                                  |                         |                      | matK    | 1393      | 1393        | 1           | 0         | 1          | Galatia filiformis                                         | c          |
|     |                                                  |                         |                      | rbcL    | 1042      | 1042        | 0.98        | 0         | 1          | Galatia filiformis                                         | c          |
|     |                                                  |                         |                      | trnL    | 1744      | 1744        | 1           | 0         | 0.9969     | Galatia filiformis                                         | c          |
| No. | Species [38] | Author | Fam. | Region | Max Score | Total Score | Query Cover | E Value | Per. Ident | Best Matched Species | Sum. | Notes |
|-----|--------------|--------|------|--------|-----------|------------|-------------|---------|------------|----------------------|------|-------|
| 47  | Benstonea affinis (Kurz) Calm. & Buerki | Pandan. | ITS2 | 124 | 124 | 0.24 | 6.00E-24 | 0.8611 | | Magnolia henryi | i | |
|     |              |        | matK | 1397 | 1397 | 0.91 | 0 | 0.9935 | Pandanus orbatus | a* | |
|     |              |        | rbcL | 1057 | 1057 | 1 | 0 | 1 | Pandanus adindebregts | a* | |
|     |              |        | trnL | 1705 | 1705 | 1 | 0 | 0.9989 | Pandanus baptistii | a* | |
| 48  | Phyllanthus oxyphyllus Miq. | Phyllanth. | ITS2 | 621 | 621 | 0.74 | 9.00E-174 | 0.9971 | | Phyllanthus oxyphyllus | c | 1/2 is a * with higher coverage |
|     |              |        | matK | 1375 | 1375 | 1 | 0 | 0.9973 | Phyllanthus oxyphyllus | c | |
|     |              |        | rbcL | 1059 | 1059 | 1 | 0 | 1 | Phyllanthus emblica | a* | |
|     |              |        | trnL | 989  | 989  | 0.58 | 0 | 0.9945 | Phyllanthus emblica | a* | |
| 49  | Ardisia complanata Wall. | Primul. | ITS2 | 667 | 667 | 0.78 | 0 | 0.9973 | Ardisia dasypodismatica | a* | |
|     |              |        | matK | 1574 | 1574 | 1 | 0 | 0.9931 | Ardisia manillata | a* | |
|     |              |        | rbcL | 1031 | 1031 | 0.99 | 0 | 0.9965 | Ardisia crenata | a* | |
|     |              |        | trnL | 1483 | 1483 | 1 | 0 | 0.9951 | Ardisia dasypodismatica | a* | |
| 50  | Ardisia crenata Sims | Primul. | ITS2 | 617 | 617 | 0.74 | 1.00E-172 | 0.997 | | Ardisia crenata | c | |
|     |              |        | matK | 1404 | 1404 | 0.88 | 0 | 0.9887 | Ardisia cornu-dentata | c | 1/2 is a * |
|     |              |        | rbcL | 1048 | 1048 | 1 | 0 | 1 | Ardisia cornu-dentata | c | |
| 51  | Ventilago madraspatana Boerl. | Rhamn. | ITS2 | 1476 | 1476 | 0.99 | 0 | 0.9988 | Hibiscus panderformis | i | |
|     |              |        | matK | 1347 | 1347 | 1 | 0 | 0.9444 | Ventilago leucarpia | a* | |
|     |              |        | rbcL | 1022 | 1022 | 0.96 | 0 | 0.9947 | Ventilago leucarpia | a* | |
|     |              |        | trnL | 1574 | 1574 | 1 | 0 | 0.9722 | Ventilago kurzii | a* | |
| 52  | Psychotria montana Blume | Rubi. | ITS2 | 1376 | 1376 | 0.99 | 0 | 0.996 | Psychotria asatica | a* | |
|     |              |        | matK | 1504 | 1504 | 0.96 | 0 | 0.9826 | Psychotria adenophylla | a* | |
|     |              |        | rbcL | 1029 | 1029 | 0.96 | 0 | 1 | Psychotria adeniophylla | a* | |
|     |              |        | trnL | 1654 | 1654 | 0.96 | 0 | 0.9826 | Psychotria asatica | a* | |
| 53  | Lunasia amara Blanco | Rut. | ITS2 | 579 | 579 | 0.74 | 6.00E-161 | 0.9971 | | Lunasia amara | c | |
|     |              |        | matK | 1243 | 1243 | 0.88 | 0 | 0.9854 | Flindersia braylegana | c | |
|     |              |        | rbcL | 1026 | 1026 | 0.97 | 0 | 0.9947 | Lunasia amara | c | |
|     |              |        | trnL | 1668 | 1668 | 0.95 | 0 | 0.9946 | Lunasia amara | c | |
| 54  | Melicope luni-ankenda (Gaertn.) T.G. Hartley | Rut. | ITS2 | 787 | 787 | 1 | 0 | 0.9823 | Melicope pleiophylla | a* | |
|     |              |        | matK | 1408 | 1408 | 1 | 0 | 0.9987 | Melicope pleiophylla | a* | |
|     |              |        | rbcL | 1031 | 1031 | 0.98 | 0 | 0.9965 | Melicope pleiophylla | a* | |
|     |              |        | trnL | 1168 | 1168 | 1 | 0 | 0.9953 | Melicope grisea | a* | |
|     |              |        | rbcL | 1050 | 1050 | 0.99 | 0 | 0.986 | Melicope grisea | a* | |
|     |              |        | trnL | 1635 | 1635 | 0.99 | 0 | 0.986 | Melicope grisea | a* | |
| 55  | Kadsura scandens (Blume) Schisandr. | Rubi. | ITS2 | 558 | 558 | 0.69 | 7.00E-155 | 0.9967 | | Kadsura philippinensis | c | |
|     |              |        | matK | 1376 | 1376 | 1 | 0 | 0.9947 | Kadsura philippinensis | c | |
|     |              |        | rbcL | 1050 | 1050 | 0.99 | 0 | 0.986 | Kadsura philippinensis | c | |
|     |              |        | trnL | 1635 | 1635 | 0.99 | 0 | 0.986 | Kadsura philippinensis | c | |
| 56  | Smilax calophylla Wall. ex A.DC. | Smilac. | ITS2 | 821 | 821 | 1 | 0 | 0.9933 | Smilax micrantha | a* | |
|     |              |        | rbcL | 1048 | 1048 | 0.98 | 0 | 0.9982 | Smilax micrantha | a* | |
| 57  | Smilax ceplinica L. | Smilac. | ITS2 | 274 | 274 | 0.35 | 3.00E-69 | 0.9809 | | Acer tataricum | i | |
|     |              |        | matK | 1371 | 1371 | 1 | 0 | 1 | Smilax micrantha | a* | |
|     |              |        | rbcL | 1044 | 1044 | 0.98 | 0 | 1 | Smilax micrantha | a* | |
|     |              |        | rbcL | 702  | 702  | 0.82 | 0 | 0.9948 | Smilax ocreata | a* | |
|     |              |        | trnL | 987  | 987  | 0.67 | 0 | 0.9945 | Smilax ocreata | a* | |

**Table A2. Cont.**
Table A2. Cont.

| No. | Species [38]                     | Author          | Fam.          | Region | Max Score | Total Score | Query Cover | E Value | Per. Ident | Best Matched Species | Sum. | Notes |
|-----|----------------------------------|-----------------|---------------|--------|-----------|-------------|-------------|---------|------------|----------------------|------|-------|
| 59  | Amomum hochreutineri             | Valeton         | Zingiber.     | ITS2   | 616       | 616         | 0.79        | 4.00E-172| 0.9884     | Sunanamos hastilabrum | a ** |       |
|     |                                  |                 |              | rbcL   | 1044      | 1044        | 0.98        | 0       | 1          | Amomum villosum var.  | a *  |       |
|     |                                  |                 |              |        |           |             |             |         |            | xanthioides          |      |       |
|     |                                  |                 |              |        |           |             |             |         |            | Amomum fulciceps      | a *  |       |
|     |                                  |                 |              |        |           |             |             |         |            | Horstedtia conica    | a ** |       |
|     |                                  |                 |              |        |           |             |             |         |            | Alpinia arundelliana  | a ** |       |
|     |                                  |                 |              | trnL   | 1568      | 1568        | 0.98        | 0       | 0.9311    | Elingera yunnanensis  | a ** |       |
| 60  | Elingera solaris (Blume) R.M.Sm.  |                 | Zingiber.     | ITS2   | 656       | 656         | 0.89        | 0       | 0.9764    | Amomum aculeatum      | c    |       |
|     |                                  |                 |              | rbcL   | 1053      | 1053        | 0.99        | 0       | 1          | Amomum daliacyti      | a *  |       |
|     |                                  |                 |              | trnL   | 1622      | 1622        | 0.99        | 0       | 0.9551    |                    |      |       |
| 61  | Meistera aculeata (Roxb.) Skornick. & M.F. Newman | | Zingiber. | ITS2 | 592  | 592 | 0.72 | 7.00E-165 | 1 | Amomum aculeatum |
|     |                                  |                 |              | rbcL   | 1020      | 1020        | 0.96        | 0       | 1          |                    | c    |       |

Note: Result summary: c = correct, a*: ambiguous or correct in genus level, a **: ambiguous or correct in family level, i = incorrect.

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