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Electrochemical fingerprint collection and taxonomic investigation of *Michelia spp.*

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Michelia belongs to Magnoliaceae and has important timber, medicinal, oil and ornamental values. The wide morphological variation of this genus and the hybridization among some species make it difficult to classify. There are controversies about the systematic relationships and status of species within the genera. In this communication, we have made the first taxonomic survey of species of the genus *Michelia* using electrochemical fingerprinting. The molecules corresponding to the oxidation peaks in the electrochemical fingerprints were explored. The electrochemical fingerprints collected under different conditions were used to construct density pattern. Texture features and local binary pattern algorithms are used to the identification of different species. The results of the phylogenetic tree generated based on electrochemical fingerprinting differed significantly from those of traditional morphological taxonomy. However, these results are in good agreement with the phylogenetic trees generated by chloroplast sequencing.

Keywords: Electrochemical fingerprint; *Michelia*; Chloroplast Genome; Pattern recognition; Texture feature

1. INTRODUCTION

The genus *Michelia* Linn. belongs to the Magnoliaceae family. It is the second largest genus in the Magnoliaceae family and is the more evolved taxon in the family. The genus *Michelia* is mainly distributed in the tropical and subtropical regions of Asia. In China, it is mainly distributed in the southwestern to southeastern regions, occupying an important position in the evergreen broadleaf forests. The genus has a wide range of morphological variation and some hybridization among species, thus making it difficult to classify. The intrageneric groupings and systematic relationships among species are controversial.

Linnaeus established the genus *M. champaca* with axillary flowers as the type species [1]. Since then, scholars from all over the world have been debating the taxonomic status of the genus *Michelia* and the division of the taxa under the genus. After extensive palynological studies, it was found that the morphological characters of the genus are relatively variable and there are many intermediate transition types [2,3]. In 1866, Baillon proposed the concept of *Magnolia* Linn. in a broad sense and combined all the previously published genera of the Magnolia family such as *Manglietia*, *Yulania*, and *Michelia* [4]. However, Dandy [5]and most scholars since then have argued that the genus *Michelia* should be separated from the genus *Magnolia*. Table 1 shows the classical classification system under the genus *Michelia*. As can be seen from the table, the genus *Michelia* is highly divergent in the definition of the genus category, the division of subgenus rank, and the establishment of species.

Table 1. Classification system of *Michelia*.

Dandy (1974) [5]	Law (1984)	Liang & Nooteboom (1996) [6]	Law (1996)
Sect. *Michelia*	Subg. *Michelia*	Sect. *Michelia*	Subg. *Michelia*
	Sect. *Michelia*		Sect. *Michelia*
Sect. *Micheliopsis*	Sect. *Micheliopsis*	Sect. *Micheliopsis*	Sect. *Micheliopsis*
	Subg. *Metamichelia*		Subg. *Metamichelia*
Sect. *Dichlamys*	Sect. *Dichlamys*	Sect. *Dichlamys*	Sect. *Dichlamys*
	Sect. *Aniochlamys*	Sect. *Aniochlamys*	
Sect. *Aniochlamys*	Sect. *Calathochlamys*	Sect. *Paramichelia*	Sect. *Aniochlamys*
	Sect. *Dolichochlamys*	Sect. *Tsoongiodendron*	

Phytochemical studies also have an important role in plant taxonomy. A study by Zhong et al. [7] found that the main chemical composition of the essential oil of *M. maudiae* was not consistent with that of *M. chapensis* and *M. foveolata*. Ma et al. [8] showed that the relationships between the volatile chemical constituents of *M. wilsonii* and *M. sphaeran* were relatively similar. Xiong et al. [9] represented eucalyptol and notoginseng as eucalyptane sesquiterpenes. Gimane type sesquiterpenes represented by wide mullein endolipids and chamomile endolipids. Sesquiterpene endolipids and guaiacane type sesquiterpenes represented by smilax endolipids were mainly distributed in *Michelia spp*. However, taxonomic investigations based on phytochemistry can only trace a limited number of chemical components. On the contrary, electrochemical techniques allow the quantitative and qualitative analysis of molecules with electrochemical activity in plant tissues. Electrochemical fingerprinting-based plant taxonomy is a new technique that has received attention within the last few years. A series of reports have proven it to be an effective and inexpensive technique for plant taxonomic surveys [10–15].

In this work, we collected electrochemical fingerprints of a total of 15 species of *Michelia spp*. Different fingerprint profiles were used for species identification through different conditions of tissue extraction and electrochemical condition. In addition, the taxonomic results based on electrochemical fingerprinting were used for comparison with other taxonomic techniques.

2. EXPERIMENTAL

2.1. Reagents and instruments

All reagents used in this work were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD. All reagents were analytical grade and used without the further purification. Table 1 shows the detail of the species (13 species from *Michelia spp*. and 2 species from *Parakmeria spp.*) used in this work.

All electrochemical experiments were conducted using a CHI760E electrochemical workstation. A glassy carbon electrode, a Ag/AgCl (3M KCl) and a Pt wire were used as working electrode, reference electrode and counter electrode, respectively.

Table 2. Information of the species used in this work.

No.	Botanical name	Abbreviation
1	*Michelia figo*	MFO
2	*Michelia floribunda*	MFBA
3	*Michelia ‘Zhongshanhanxiao’*	MZO
4	*Michelia foveolata*	MFA
5	*Michelia foveolata* var. cinerascens	MFC
6	*Michelia chapensis*	MCS
7	*Michelia compressa*	MCA
8	*Michelia maudiae*	MME
9	*Michelia crassipes*	MCS
10	*Michelia cavaleriei* var. platypetala	MCP
11	*Manglietia fordiana*	MFDA
12	*Manglietia patungensis*	MPS
13	*Magnolia grandiflora*	MGA
14	*Parakmeria nitida*	PN
15	*Parakmeria lotungensis*	PL

2.2. Preparation leaf extract for electrochemical fingerprinting

2 g of shredded leaves were cut off from the leaves. The shredded leaves were added to a centrifuge tube along with 3 steel beads. 1.5 mL of water or ethanol was added to the centrifuge tube.

The centrifuge tube was placed in a cell grinder for extraction. The extracted mixture was filtered and the extract was added to 10 mL of electrolyte (phosphate buffer solution, PBS or acetate buffer solution, ABS) and mixed thoroughly.

2.3. Electrochemical fingerprinting

The three-electrode system was inserted to the above-mentioned mixture. Differential pulse voltammetry (DPV) was used to record the electrochemical fingerprinting of plants. The window of scanning potential was from 0 V to 1.3 V.

3. RESULTS AND DISCUSSION

Figure 1 and 2 show the electrochemical fingerprints of 13 species from *Michelia spp*. and 2 species from *Parakmeria spp.* recorded under ABS after extraction by water. As can be seen from the figure, electrochemically active molecules were involved in the electrochemical oxidation in the extracts of each species. Different oxidation peaks represent different molecules involved in the reaction. The type and amount of these molecules are mainly genetically determined. *Michelia spp.* exhibited a variety of biological activities, mainly including terpenoids, lignans, alkaloids, etc. The terpenoids in *Michelia spp.* are monoterpenoids, sesquiterpenoids and their oxygenated derivatives [16–18]. Monoterpenes and sesquiterpenes are mainly found in the volatile oils of the above-ground parts of plants, among which, monoterpenes are mostly found in the low-boiling components of volatile oils, and sesquiterpenes are mostly found in the high-boiling components of volatile oils [19–21]. The acyclic monoterpenes in *Michelia spp.* have been reported to be mainly myrcene, ocimene type acyclic monoterpenes and their oxygen-containing derivatives [22]. Menthane, eucalyptus oleoresin-like monocyclic monoterpenes are present in most *Michelia spp.* The bicyclic monoterpenes are mainly thujane, carane, pinane, fenchane and camphene terpenes and their oxygen-containing derivatives. Sesquiterpenoids are FPPs composed of three molecules of isoprene, which are derived from GPP or NPP and one molecule of IPP by enzymatic condensation [23,24]. Its skeleton type is one of the most abundant terpenoids and is often present in plants as alcohols, ketones, and lactones [25]. The acyclic sesquiterpenes in the *Michelia spp.* are mainly farnesane terpenes and their derivatives. The skeletons of monocyclic monoterpenes are mainly bisabolane, germacrane, humulane and elemane. The skeletons of bicyclic sesquiterpenes are mainly cadinane, eudesmane, eremophilane, acroane, santalane, guaiane and carryophyllane [26]. The skeletons of tricyclic sesquiterpenes are mainly copane, aromadendrane, cubebane, cuparane and cedrane [27].

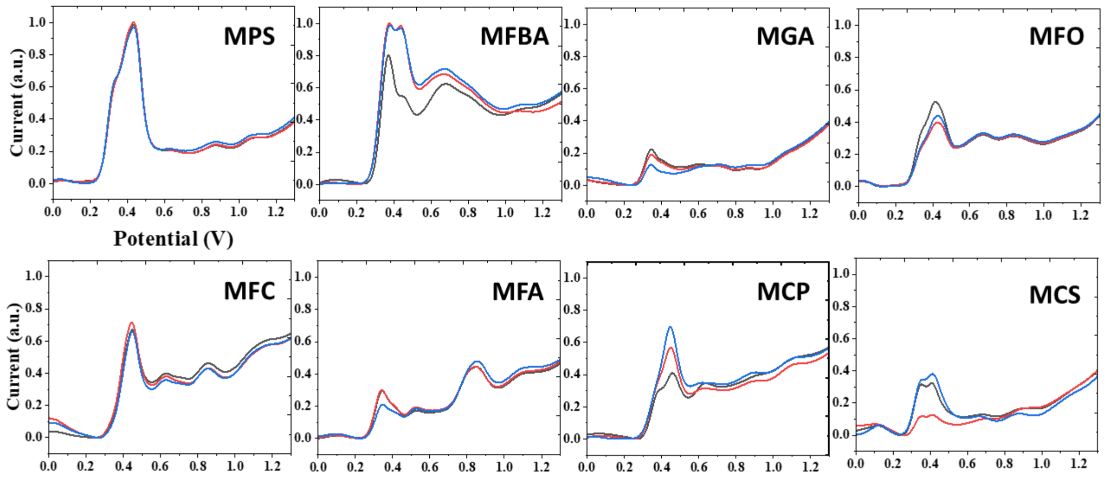

Figure 1. Electrochemical fingerprints of MPS, MFBA, MGA, MFO, MFC, MFA, MCP and MCS recorded using ABS after the extraction using water as solvent.

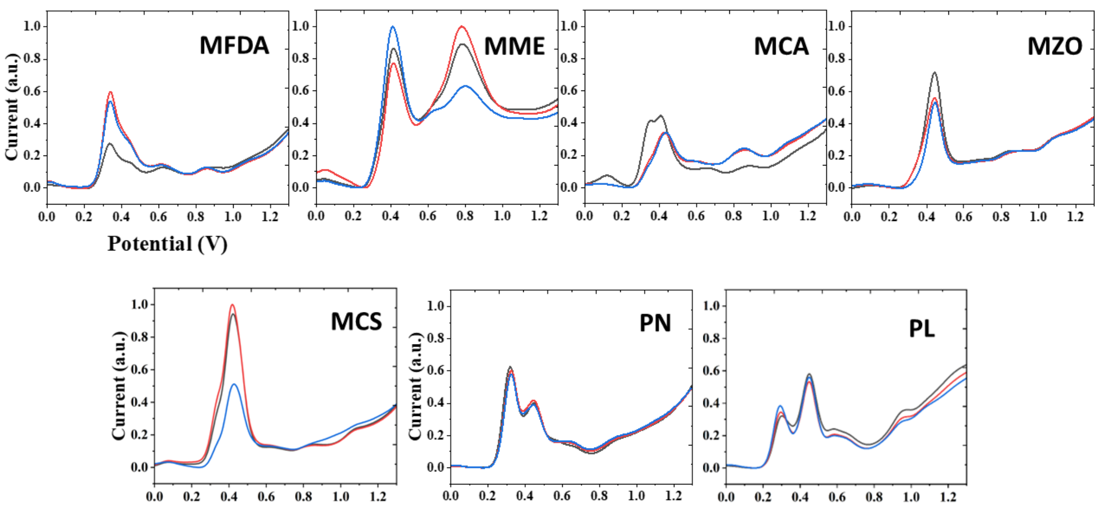

Figure 2. Electrochemical fingerprints of MFDA, MME, MCA, MZO, MCS, PN and PL recorded using ABS after the extraction using water as solvent.

Figure 3 and 4 shows the electrochemical fingerprints of 13 species from *Michelia spp*. and 2 species from *Parakmeria spp.* recorded under PBS after extraction by ethanol. Lignans, which are formed by the polymerization of dimeric phenylpropanoids, are another common macromolecule in *Michelia spp.* and belong to the phenolic group [28]. Lignan compounds possess a wide range of biological activities in anti-cancer, anti-viral and hepatoprotective activities [29,30]. According to previous literature, the lignans involved in electrochemical reactions may contain piperitylmagnolol, piperitylhonokiol, dipiperitylmagnolol, bornylmagnolol, magnolignan, magnaldehyde, magnoloside, magnolol, honokiol, randainal, randaiol and houpulin [31,32].

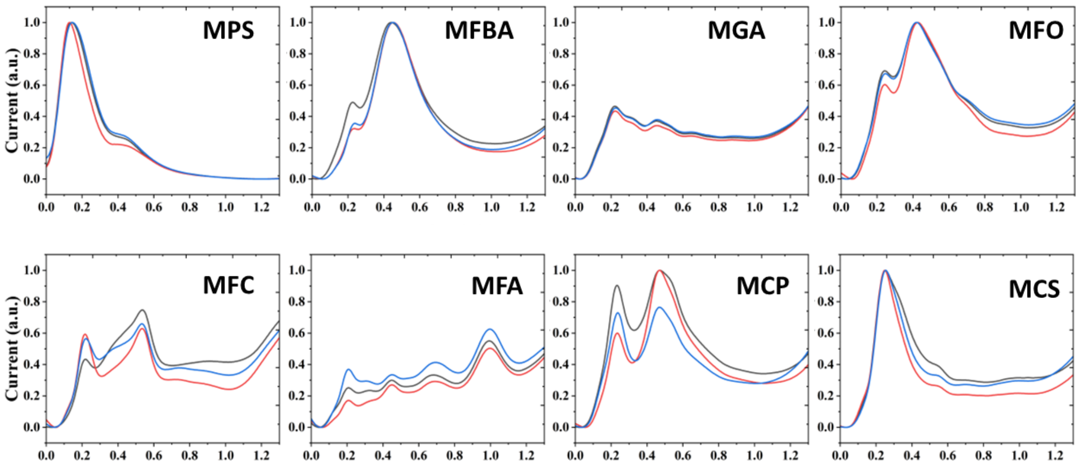

Figure 3. Electrochemical fingerprints of MPS, MFBA, MGA, MFO, MFC, MFA, MCP and MCS recorded using PBS after the extraction using ethanol as solvent.

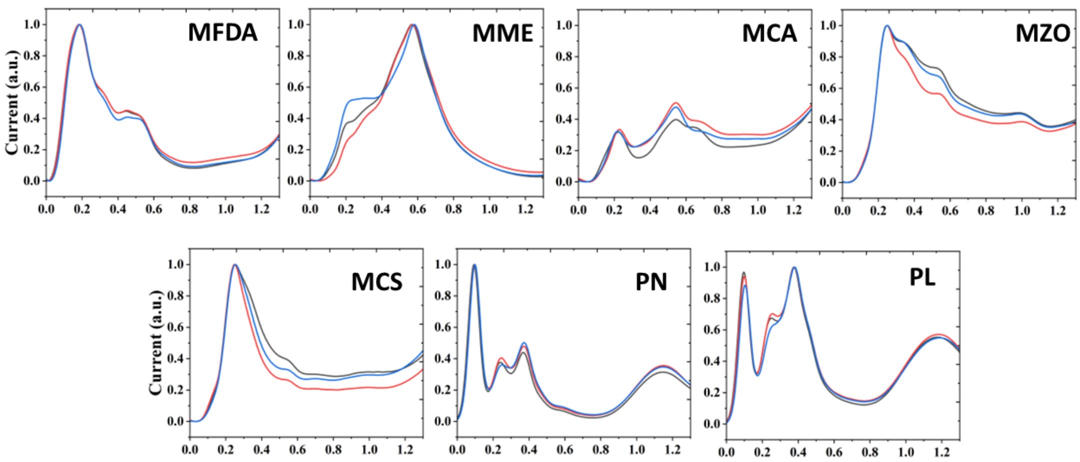

Figure 4. Electrochemical fingerprints of MFDA, MME, MCA, MZO, MCS, PN and PL recorded using PBS after the extraction using ethanol as solvent.

The combination of the two sets of electrochemical fingerprints allowed the construction of density patterns. Figures 5 and 6 show the density patterns of 13 species from *Michelia spp.* and 2 species from *Parakmeria spp.* We believe that such patterns can be identified using texture features. A texture feature is defined as a visual feature to express homogeneous regions in an image that reflects the properties of periodically varying or progressively varying surface pixel arrangement distribution in the image [33]. Texture features are numerical representations of the grayscale distribution within individual pixels and their neighborhoods. Statistical texture features are also included, among which grayscale co-occurrence matrix and local binary pattern are more effective and widely used [34]. However, the above-mentioned feature extraction methods also have their disadvantages, such as the grayscale co-occurrence matrix, which is more discriminative but brings a very large amount of computation and is very time-consuming [35]. We believe that local binary patterns (LBP) texture features can achieve the recognition of plants [36]. Compared with other algorithms, LBP has the advantages of low computational complexity, gray scale and rotation invariance in the computation of texture features.

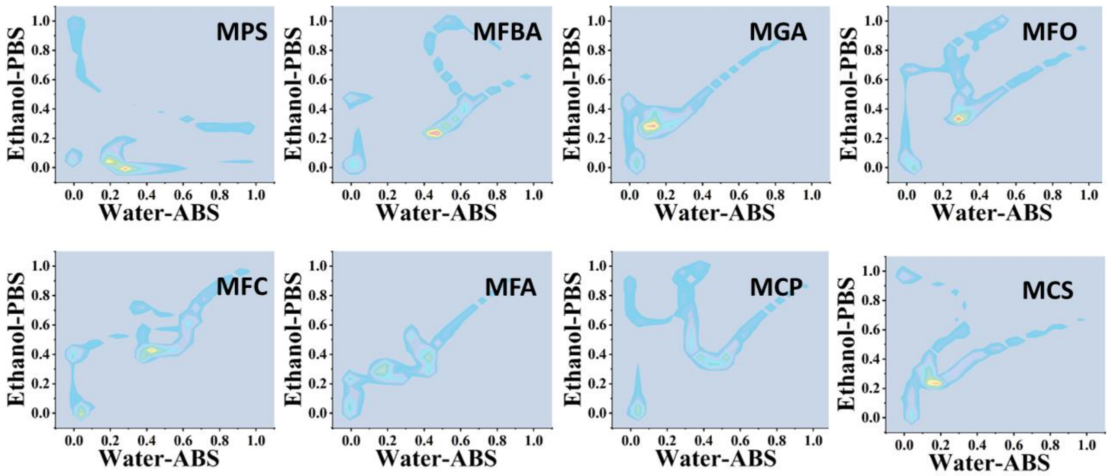

Figure 5. Density pattern of MPS, MFBA, MGA, MFO, MFC, MFA, MCP and MCS deduced from electrochemical fingerprint.

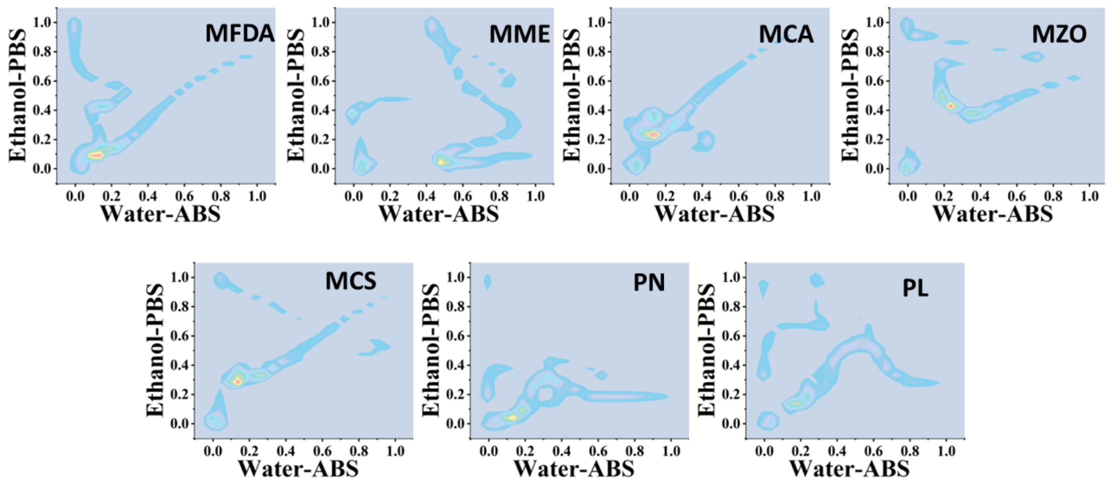

Figure 6. Density pattern of MFDA, MME, MCA, MZO, MCS, PN and PL deduced from electrochemical fingerprint.

Since the signals generated by electrochemical fingerprinting and the types and amounts of electrochemically active molecules in the extracts show a positive correlation, we can discuss the taxonomy of *Michelia spp.* using electrochemical fingerprinting. Figure 7 shows the dendrogram of 13 species from *Michelia spp.* and 2 species from *Parakmeria spp.* It can be seen from the figure that PN and PL are not clustered with the other species. They were used as outgroup in this work to verify whether electrochemical fingerprinting could be used to investigate the intermediate relationships of *Michelia spp*. The results showed that they were significantly distant from all the other *Michelia spp.*

and represented that the electrochemical fingerprinting technique has potential in the taxonomic study of plants.

The dendrogram of *Michelia spp.* has been divided to two clades. The first clade contains *M. fordiana*, *M. patungensis*, *M. grandiflora* and *M. maudia*. The second clade contains *M. foveolata* var. *cinerascens*, *M. cavaleriei* var. *platypetala*, *M. 'Zhongshanhanxiao'*, *M. floribunda*, *M. compressa*, *M. foveolate*, *M. crassipes* and *M. figo*. The clades in this study differed significantly from previous morphology-based analysis. In contrast, our results are more similar to the dendrogram generated by the chloroplast gene spacer association sequence (*psb*A-*trn*H, *psb*B-H, *trn*L-F, *trn*C-ycf6, *trn*D-E) [37–40]. Morphological characteristics are determined by both the external environment and the genetic base, while cpDNA is more about the similarity of the genetic base, which can better reflect the phylogenetic characteristics of *Michelia spp*.

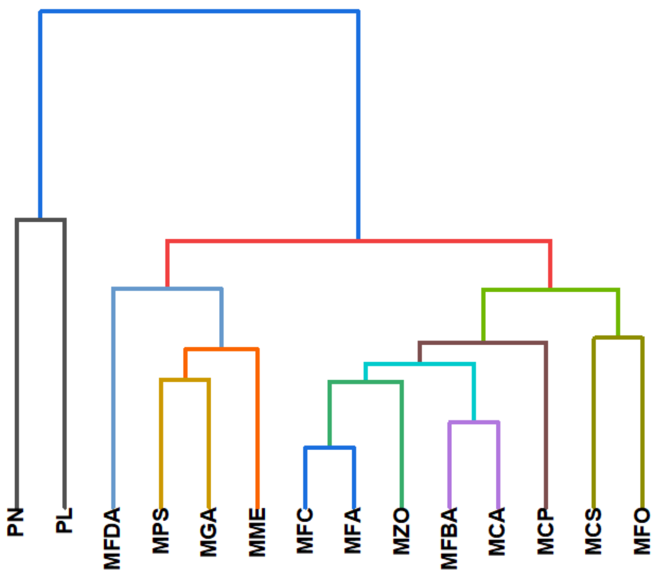

Figure 7. Dendrogram of MPS, MFBA, MGA, MFO, MFC, MFA, MCP, MCS, MFDA, MME, MCA, MZO, MCS, PN and PL based on the voltammetric fingerprints.

4. CONCLUSION

In this work, the electrochemical fingerprints of 13 species from *Michelia spp.* and 2 species from *Parakmeria spp.* have been recorded. Water and ethanol have been used as solvent, while the ABS and PBS have been used as electrolyte. Two sets of electrochemical fingerprints of each species were recorded for species recognition and phylogenetic investigation. The results showed that 2 species from *Parakmeria spp.* are not clustered with the other species. The dendrogram of *Michelia spp.* has been divided to two clades. The results of the phylogenetic tree generated based on electrochemical fingerprinting differed significantly from those of traditional morphological taxonomy. However, these results are in good agreement with the phylogenetic trees generated by chloroplast sequencing.

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