Analytical Chemistry

Recording the Electrochemical Profile of *Pueraria* Leaves for Polyphyly Analysis

Mingjun Zhang,[a] Bo Pan,*[b] Yangyang Wang,[a] Xinpeng Du,[a] Li Fu,*[a] Yuhong Zheng,[c] Fei Chen,[a] Weihong Wu,[a] Qinwei Zhou,[a] Su Ding,[a] and Shichao Zhao[a]

Electrochemical profiles of *Pueraria bouffordii*, *P. montana* var. *lobata*, *P. montana* var. *montana*, *P. montana* var. *thomsonii*, *Haymondia wallichii*, *Teyleria stricta* and *Toxicopueraria yunnanensis* were recorded from leaf tissue after different solvent extractions. The voltammetric data recorded after different solvent extractions can be derived as patterns for species identification. The electrochemical behavior of plant tissue contains its electrochemical active compounds profile. As the distribution of chemical compounds in plants is controlled by genes, these profiles can reflect differences at the genetic level between species. The dendrogram deduced from the electrochemical profile has been used for polyphyly analysis. The result suggests the Teyleria stricta showed very distant relationships with other species. *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii* showed a close relationship because they were varietas. Interestingly, *H. wallichii* showed a close infrageneric relationship within these species, which disagrees with other morphological studies. In addition, the result also provides insight into phylogenetic status of the regionally *Toxicopueraria yunnanensis*.

Introduction

Pueraria is a genus of 20 species of plants native to Asia. *Pueraria* species have been widely used as food, medicine, papermaking and weaving since ancient times. The molecular phylogenetic study indicated the widespread polyphyly within the genus *Pueraria*.[1] Our previous work proposed a modified taxonomic profile of *Pueraria*. More specifically, we reinstated the genus *Neustanthus*, established two new genera: *Haymondia* and *Toxicopueraria*.[2] In addition, a species of *Pueraria* has been moved to the *Teyleria*. These circumscriptions were deduced from the morphological characteristics of samples. Morphological diversity is the classical evidence for the plant phylogenetic study. However, modern molecular techniques often produce different results from traditional taxonomy. Furthermore, different molecular techniques can result in different phylogenetic results. Therefore, the verification of taxonomic results using an alternative technique can help to determine the phylogenetic position of species.[3]

Chemotaxonomy is a method of subsidiary classification. It is based on the differences of certain chemical components in different plant species to determine their genetic distance. Since the chemical composition of plants is controlled by genes, the differences in chemical composition could reflect the differences at the gene level.[4–7] However, the traditional chemotaxonomy method has several drawbacks. For example, it only tags a few compounds, so it can only provide a small amount of genetic information. Furthermore, plant composition analysis requires the use of complex instruments and sample preprocessing. In 2015, the Doménech-Carbó group began exploring the plant species analysis by recording the electrochemical profile of plant tissue.[8] The voltammogram of the plant tissue provides the information of electro-active compounds, such as polyphenols, aldehydes and alkaloids.[9] Our previous works demonstrated the possible phylogenetic analysis based on the electrochemical-based taxonomy using *Lycoris* and *Chimonanthus* as examples.[10–13]

In this work, disposable screen-printed electrodes (SPEs) were used for leaf tissue modification and subsequent electrochemical profile recording. *Pueraria bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta* and *Toxicopueraria yunnanensis* were submitted for analysis. The profiles of each species recorded after five solvents extractions with two buffer solutions were used for pattern generation. Then, the electrochemical information of seven species was analyzed and compared with the polyphyly result deduced from the morphological analysis.

Results and Discussion

The schematic diagram of the electrochemical profile recording has been illustrated in Figure 1. Plant tissue was firstly ground

[a] *M. Zhang, Y. Wang, X. Du, L. Fu, Prof. F. Chen, Prof. W. Wu, Q. Zhou, Dr. S. Ding, S. Zhao*
College of Materials and Environmental Engineering, Hangzhou Dianzi University, Hangzhou, 310018, P.R. China
E-mail: fuli@hdu.edu.cn
[b] *B. Pan*
Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, P.R. China
E-mail: pb@xtbg.org.cn
[c] *Prof. Y. Zheng*
Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing Botanical Garden, Mem. Sun Yat-Sen, Nanjing 210014, P.R. China
Supporting information for this article is available on the WWW under https://doi.org/10.1002/slct.202001100

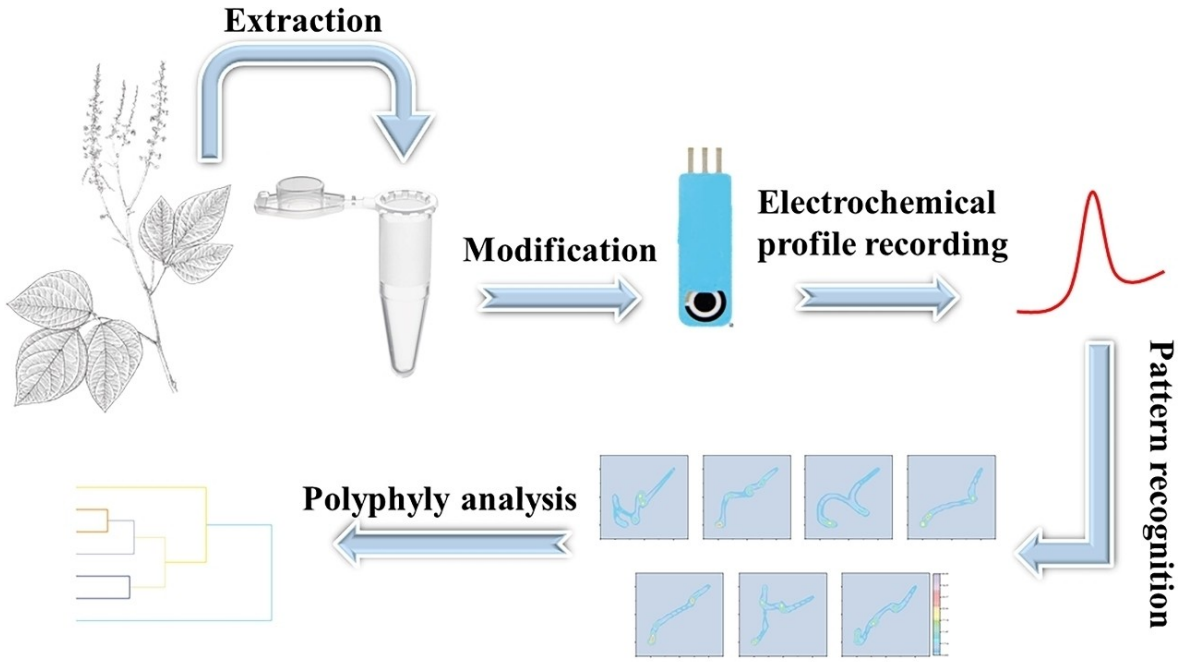

Figure 1. Scheme of recording the electrochemical profile of *Pueraria* for polyphyly analysis.

before sonication to achieve fast extraction. Five different solvents include water, methanol, ethanol, glycol and DMF were used for each species for ensuring the comprehensive representation of electrochemical active compounds during the voltammetric scan. Then, the voltammograms were used for generating the 2D density pattern, which could be used for plant species identification. In addition, the voltammograms were used for polyphyly analysis.

Figure 2 shows the DPV curves of the *P. bouffordii* recorded under 0.1 M PBS using water, methanol, ethanol, glycol and DMF as extraction solvent (DPV curves of *P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta* and *Toxicopueraria yunnanensis* were shown in Figure S3-8). Figure 3 shows the DPV curves of the *P. bouffordii* recorded under 0.1 M ABS using water, methanol, ethanol, glycol and DMF as extraction solvent (DPV curves of *P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta* and *Toxicopueraria yunnanensis* were shown in Figure S9-14). It is pertinent to note that the plant tissue all exhibited several peaks during the voltammetric scan after the extraction with different solvent, indicating some electro-active compounds were oxidized. Because of the complexity of the chemical components in plant tissues, it is difficult to identify specific components by voltammetry alone. However, previous studies have confirmed the electrochemical activity of polyphenols,[14,15] flavonoids[14,16] and alkaloids[17,18] in plant tissues, which can be oxidized at low potentials. The aim of this work is not to identify a single compound in plants, but to analyze the total profile of all electrochemically active com-

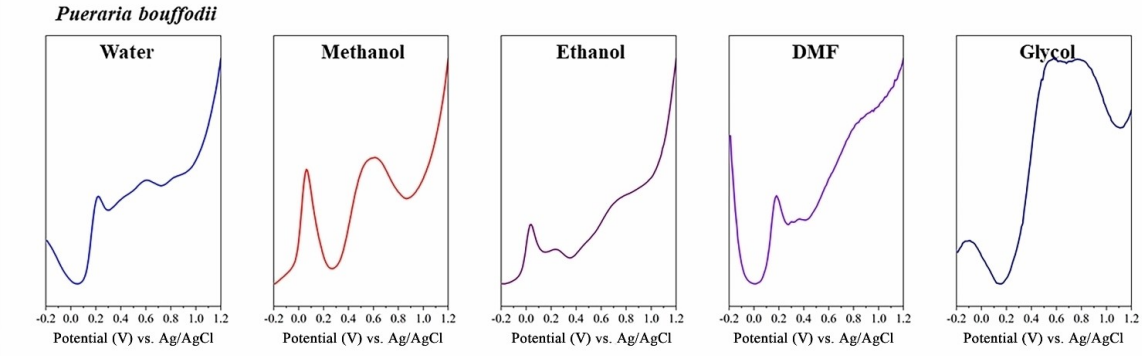

Figure 2. DPV curves of *P. bouffordii* recorded using water, methanol, ethanol, DMF and glycol as extraction solvent in 0.1 M PBS.

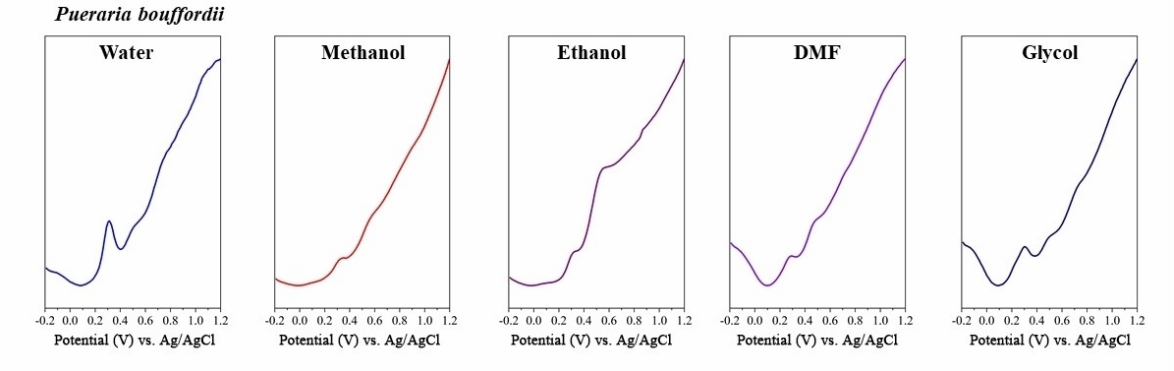

Figure 3. DPV curves of *P. bouffordii* recorded using water, methanol, ethanol, DMF and glycol as extraction solvent in 0.1 M ABS.

pounds in plants. The recorded voltammograms show the types and proportions of electrochemically active compounds in the electrode modifier. Furthermore, these compounds are controlled by genes, so differences in voltammetry reflect differences in plant genes.[19,20] As shown in Figure 2, the *P. bouffordii* showed profile differences after extraction with different solvents. This phenomenon can be explained by the extraction of different electrochemical active substances with different solvents. Therefore, combining the electrochemical behavior of plant tissues after extraction with different solvents can reveal a more comprehensive profile of the electrochemical active compound.

Figure 4 shows the parallel coordinate plot of the normalized current of seven species recorded after five solvents extraction. The parallel coordinate plot is a common method to analyze and display multivariate data. As shown in the plot, the signal of each species exhibited different tendencies, suggesting different species show discernible differences after extraction with different solvents due to the different chemical compositions. Therefore, the electrochemical profile of the plant tissue showed the potential of using the voltammetric data for species discrimination. As shown in the Figure S2, although the target plants have different flower colors and sizes in the florescence, it is difficult to identify them only by morphological features in the non-flowering season. Distinguishing *Pueraria* has potential commercial value because some of these species are used for food and medicine production.[21,22] For example, *P. montana* var. *lobata* and *P. montana* var. *thomsonii* showed very similar morphological features but the *P. montana* var. *lobata* is a medicinal plant while the *P. montana* var. *thomsonii* is a food plant.[23] The electrochemical analysis is a portable technique can be used for on-field rapid sample test. Therefore, the development of a fast recognition method for *Pueraria* is valuable for wild resource investigation.

2D density plot is an effective method for data visualization. In this work, we tried to make a 2D density plot for pattern recognition by using the current data of plants in the voltammetry after same solvent extraction with different electrolytes for the first time. Figure 5 shows the 2D density

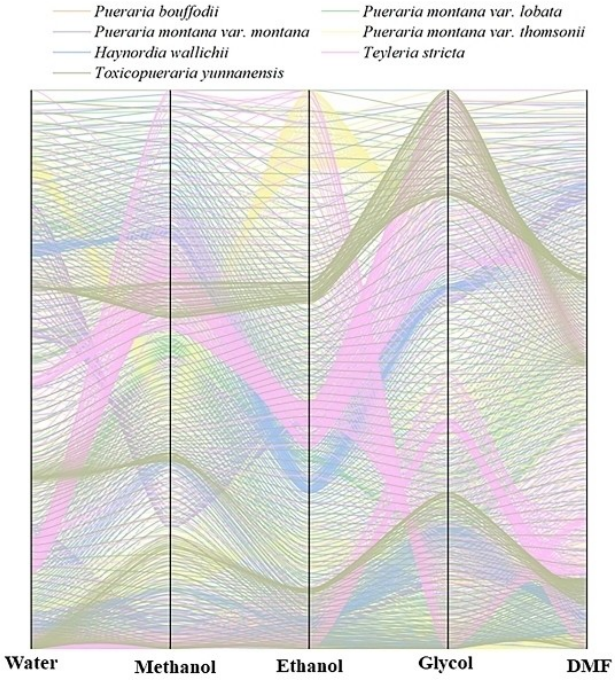

Figure 4. Parallel coordinate plot of normalized currents of *P. bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta and Toxicopueraria yunnanensis* recorded after water, methanol, ethanol, DMF and glycol extractions in 0.1 M PBS.

plots of *P. bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta and Toxicopueraria yunnanensis* generated using the current data recorded under PBS and ABS after the water extraction. As shown in the figure, each species displayed a different pattern. Plant with unknown species can be identified by matching patterns or even by locating high-density areas with the database. It is pertinent to note that the 2D density plot of some species has some similarities, such as *P. montana* var. *lobata, Haymondia wallichii* and *P. montana* var. *montana*, probably due to the three species have similar electro-active compounds extracted in water. In order to accurately distinguish them, voltammograms extracted from methanol can be

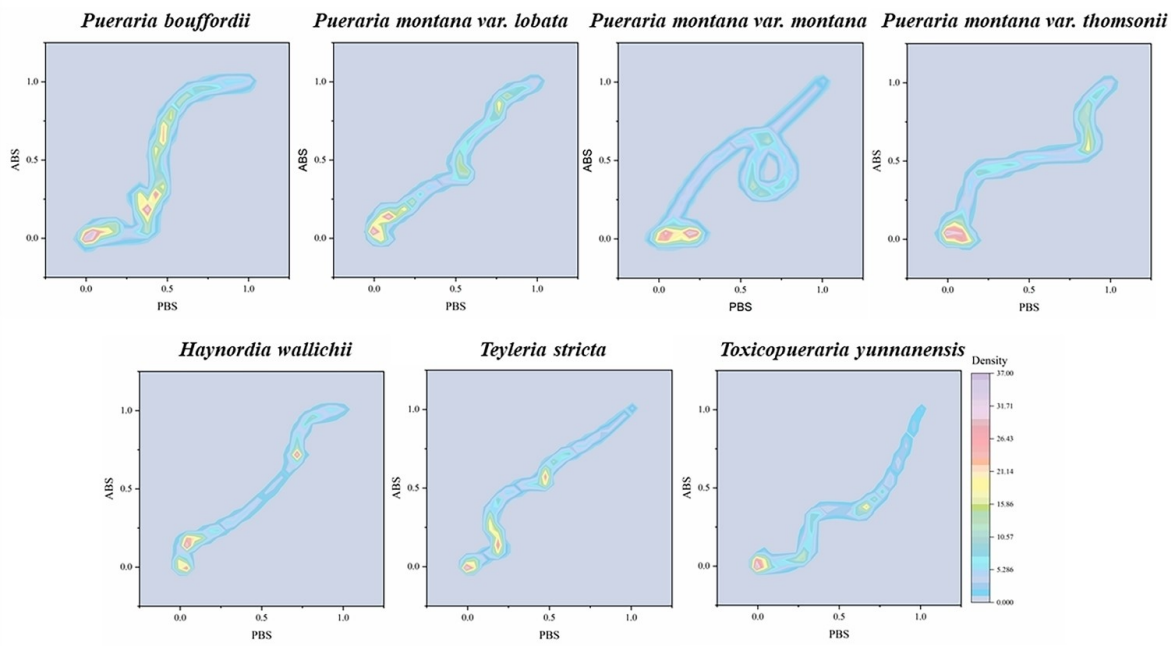

Figure 5. 2D density patterns of *P. bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta and Toxicopueraria yunnanensis* using normalized current recorded after water extractions in 0.1 M PBS and ABS.

used to create extra 2D density plots. As shown in Figure S15, the 2D density plots of *P. montana* var. *thomsonii, Haymondia wallichii* and *Toxicopueraria yunnanensis* showed a very large difference.

Based on the above results, the infrageneric identification can be easily achieved using the electrochemical behavior of plant tissues. The DPV curves of the plant tissue using only one solvent with different electrolyte have less information about the profile of electro-active compounds. Therefore, we only use the species recorded under 0.1 M PBS with five solvents extractions for following infrageneric analysis. Principal component analysis (PCA) is often used for dimensionality reduction of high dimensional variables. Our previous studies suggested that PCA analysis of electrochemical voltammetric data does not have a high interpretative capability.[10–13] However, as shown in Figure 6, the three factors extracted within the voltammetric data can reach nearly 90% interpretative capability, suggesting there were significant differences in electrochemical profiles among the plant species studied in this work. This further confirms the obvious differences in electrochemical active compounds among plant species, reflecting that there may also be significant differences at the gene level. According to the location of each species, *P. montana* var. *lobata,* and *Haymondia wallichii* were closely related, while *P. montana* var. *thomsonii* and *Toxicopueraria yunnanensis* were in a group. In addition, *Teyleria stricta* can be considered as an outlier among the species.

Although ecology has a great influence on the type and distribution of chemicals in plant tissues, genes are still the most important factor. Since the electrochemical behavior of plant tissues is positively correlated with the distribution and amount of electrochemical active compounds, we attempted to use voltammetric data for dendrogram analysis. Figure 7 shows the dendrogram of *P. bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta* and *Toxicopueraria yunnanensis* deduced from the voltammetric data recorded in five solvents.

The dendrogram was divided into three clades. Among them, *Teyleria stricta* showed very distant relationships with other species, which is in good agreement with the PCA analysis. *Teyleria stricta* a former name of *Pueraria stricta* reported by Lackey[24] because it contains canavanine, a free amino acid not usually found in *Pueraria.* Later on, Van der Maesen[25] disagreed with Lackey's suggestion and restored the *P. stricta.* Lee and Hymowitz[26] reported a molecular phylogenetic study based on the chloroplast DNA rps16 intron sequences. Their findings indicate the *P. stricta* is closely related with the *T. koordersii* rather than the *Pueraria.* Our previous mophological, nuclear and chloroplast data also suggested the *P. stricta* should nested within *Teyleria.*[1,2] In this work, the electrochemical profile also showed the distinct difference of the electrochemical active compounds profile of *Teyleria stricta* among other species.

As expected, *P. montana* var. *lobata, P. montana* var. *montana* and *P. montana* var. *thomsonii* showed a close relationship becuase they were varietas of *P. montana.* Interestingly, *H. wallichii* showed a close infrageneric relationship within these species. The phylogenetic position of the

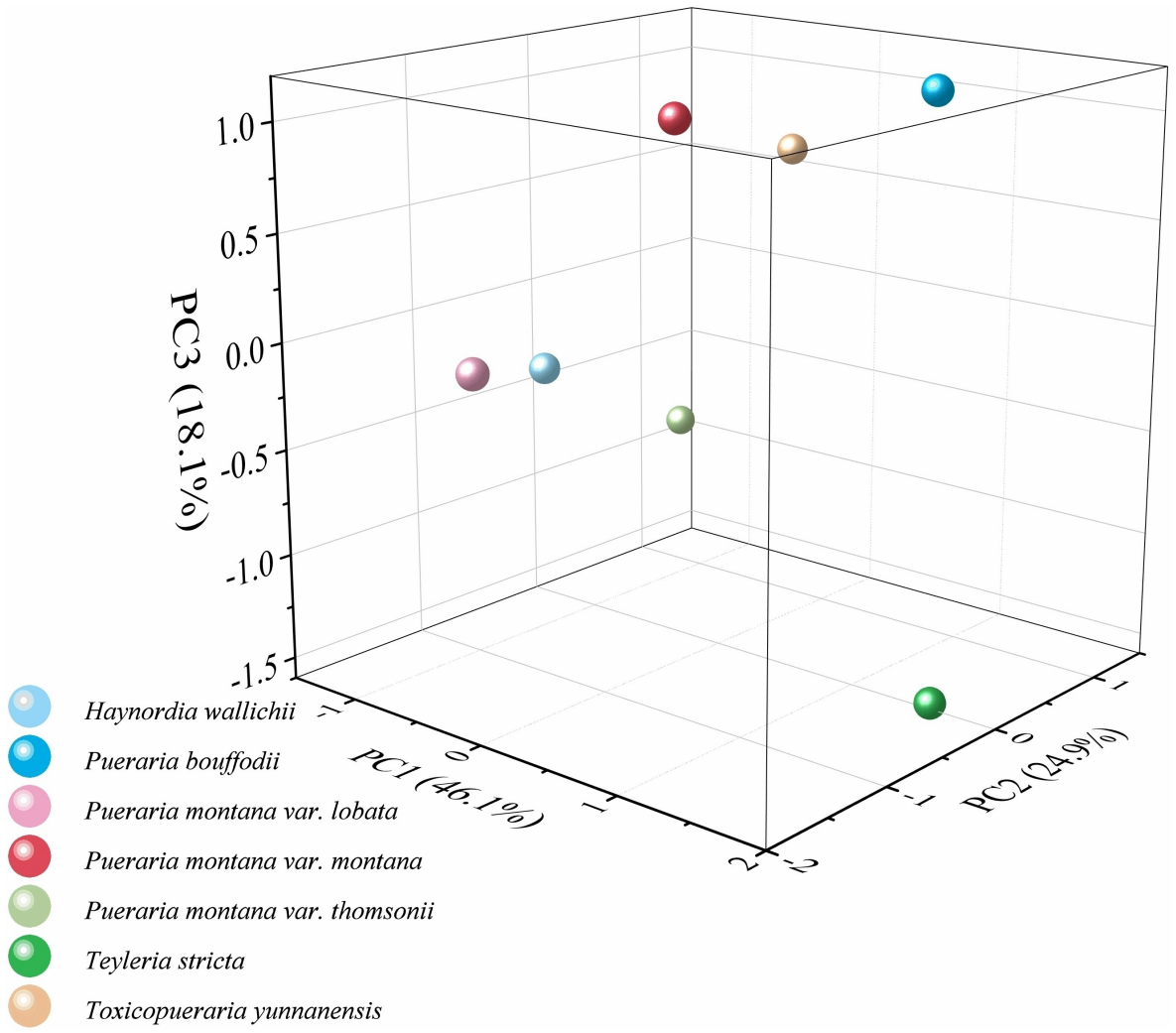

Figure 6. 3D PCA analysis of *P. bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta and Toxicopueraria yunnanensis* using normalized current recorded after five solvents extractions.

Haymondia wallichii is a controversial issue throughout the polyphyly analysis of *Pueraria. Pueraria wallichii* was originally described with *P. tuberosa* because they resemble each other.[27] Both Lackey and Van der Maesen stood equivocal opinion for *P. wallichii* after careful examination of its morphological features.[24,25] The result deduced from chloroplast DNA rps16 intron sequences also suggested the ambiguous position of *P. wallichii.* However, none of these works suggested the exclusion of *P. wallichii* from *Pueraria.* The previous study of *Pueraria* with other phaseoloid genera described *Haymondia* as a distinct phylogenetic lineage.[28] Based on the electrochemical profile recorded for *Haymondia wallichii*, it is necessary to further study its molecular phylogenetics.

The etymology of the *Toxicopueraria* is derived from the latin toxicus ("poisoned") because the aboriginals in Yunnan (China) was use of ground stems and roots as an insecticide and fish poison. The phylogenetic study of the *Toxicopueraria yunnanensis* has not been carried out based on either chemical or molecular data. Our results suggested the *Toxicopueraria yunnanensis* could have a very close relationship with other *Pueraria* even it contains poisonous toxin.

Conclusions

In conclusion, the electrochemical profiles of *P. bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta* and *Toxicopueraria yunnanensis* were recorded using plant leaf tissue after five solvents extractions. Based on the recorded voltammetric data, these species can be effectively identified using pattern recognition based on the 2D density plot. In addition, the polyphyly analysis was deduced from the recorded voltammetric data. We found the *Teyleria stricta* had a distant relationship with other species, which is in a good agreement with the morphological study. However, the *Haymondia wallichii* showed an unexpected close phylogenetic position with other *Pueraria.* The result also provides insight into the regional species of *Toxicopueraria yunnanensis.*

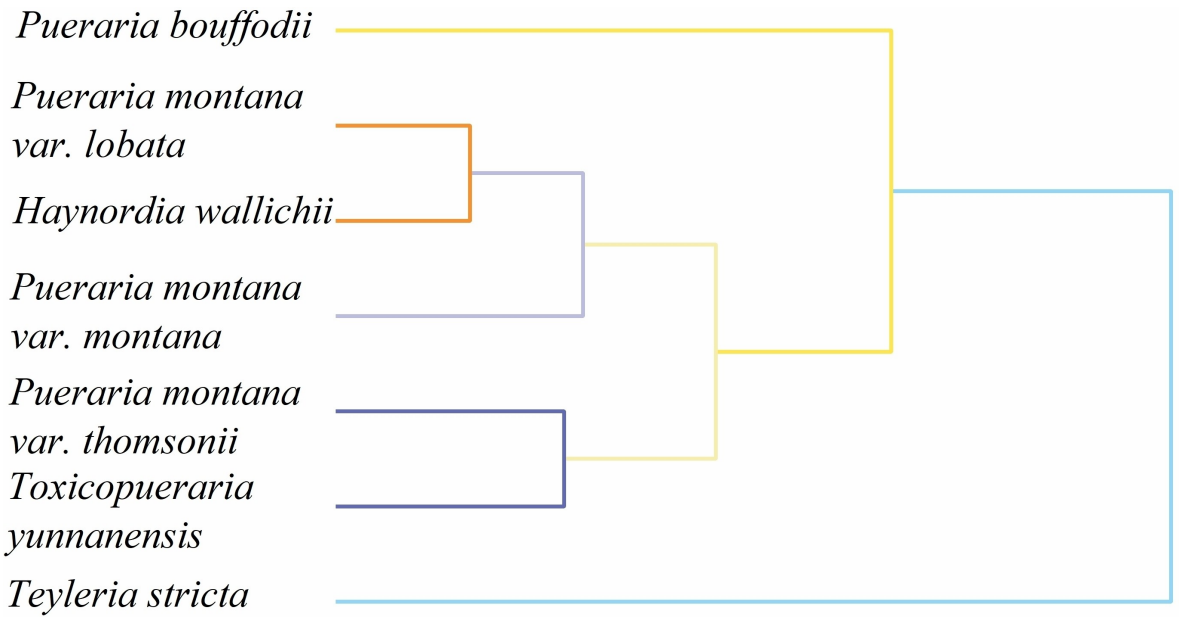

Figure 7. Dendrogram of *P. bouffordii, P. montana* var. *lobata, P. montana* var. *montana, P. montana* var. *thomsonii, Haymondia wallichii, Teyleria stricta and Toxicopueraria yunnanensis* based on the voltammetric behavior recorded in five solvents.

Acknowledgements

This work has been financially supported by Zhejiang Province Natural Science Foundation of China (LQ18E010001); China Postdoctoral Science Foundation (2018 M640523).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Electrochemistry · *Pueraria* · Polyphyly analysis · Phytochemistry · Leaf extract

[1] A. N. Egan, M. Vatanparast, W. Cagle, *Mol. Phylogenet. Evol.* **2016**, *104*, 44–59.
[2] A. N. Egan, B. Pan, *Phytotaxa* **2015**, *218*, 201–226.
[3] A. N. Egan, J. Schlueter, D. M. Spooner, *Am. J. Bot.* **2012**, *99*, 175–185.
[4] M. Xu, S. Heidmarsson, E. S. Olafsdottir, R. Buonfiglio, T. Kogej, S. Omarsdottir, *Phytomedicine* **2016**, *23*, 441–459.
[5] I. Baek, M. Kim, I. Lee, S.-I. Na, M. Goodfellow, J. Chun, *Front. Microbiol.* **2018**, *9*, 834.
[6] R. Singh, *J. Med. Plants* **2016**, *4*, 90–93.
[7] M. Wink, F. Botschen, C. Gosmann, H. Schäfer, P. G. Waterman, *Annual Plant Reviews Online* **2018**, 364–433.
[8] A. Doménech-Carbó, I. Domínguez, P. Hernández-Muñoz, R. Gavara, *Food Chem.* **2015**, *172*, 318–325.
[9] G. Cebrián-Torrejón, A. Doménech-Carbó, B. Figadère, E. Poupon, A. Fournet, *Phytochem. Anal.* **2017**, *28*, 171–175.
[10] L. Fu, M. Wu, Y. Zheng, P. Zhang, C. Ye, H. Zhang, K. Wang, W. Su, F. Chen, J. Yu, *Sens. Actuators B* **2019**, *298*, 126836.
[11] L. Fu, Y. Zheng, P. Zhang, H. Zhang, W. Zhuang, H. Zhang, A. Wang, W. Su, J. Yu, C.-T. Lin, *Biosens. Bioelectron.* **2018**, *120*, 102–107.
[12] L. Fu, Y. Zheng, P. Zhang, H. Zhang, M. Wu, H. Zhang, A. Wang, W. Su, F. Chen, J. Yu, *Bioelectrochemistry* **2019**, *129*, 199–205 .
[13] Y. Lu, Y. Xu, H. Shi, P. Z. H. Zhang, L. Fu, *Int. J. Electrochem. Sci.* **2020**, *15*, 758–764.
[14] C. Capannesi, I. Palchetti, M. Mascini, A. Parenti, *Food Chem.* **2000**, *71*, 553–562.
[15] R. Gusmão, V. López-Puente, I. Pastoriza-Santos, J. Pérez-Juste, M. F. Proença, F. Bento, D. Geraldo, M. C. Paiva, E. González-Romero, *RSC Adv.* **2015**, *5*, 5024–5031.
[16] S. Ahmed, S. Tabassum, F. Shakeel, A. Y. Khan, *J. Electrochem. Soc.* **2012**, *159*, F103–F109.
[17] J. Skopalová, J. Vacek, B. Papoušková, D. Jirovský, V. Maier, V. Ranc, *Bioelectrochemistry* **2012**, *87*, 15–20.
[18] D. Stanković, E. Mehmeti, L. Svorc, K. Kalcher, *Microchem. J.* **2015**, *118*, 95–100.
[19] A. Doménech-Carbó, A. M. Ibars, J. Prieto-Mossi, E. Estrelles, F. Scholz, G. Cebrián-Torrejón, M. Martini, *New J. Chem.* **2015**, *39*, 7421–7428.
[20] I. Domínguez, A. Doménech-Carbó, *Sens. Actuators B* **2015**, *210*, 491–499.
[21] T. Kamiya, A. Takano, Y. Matsuzuka, N. Kusaba, M. Ikeguchi, K. Takagaki, K. Kondo, *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1511–1517.
[22] A. K. Maji, S. Pandit, P. Banerji, D. Banerjee, *Nat. Prod. Res.* **2014**, *28*, 2111–2127.
[23] E. Buhlmann, C. Horváth, J. Houriet, E. Kiehlmann, J. Radtke, L. Marcourt, J.-L. Wolfender, C. Wolfrum, S. Schröder, *Phytomedicine* **2019**, *64*, 153075.
[24] J. Lackey, *Bot. J. Linn. Soc.* **1977**, *74*, 163–178.
[25] L. Van der Maesen, *Revision of the Genus Pueraria DC with Some Notes on Teyleria Backer (Leguminosae)*, Taylor & Francis, **1985**.
[26] J. Lee, T. Hymowitz, *Am. J. Bot.* **2001**, *88*, 2064–2073.
[27] A. P. de Candolle, *Mémoires Sur La Famille Des Légumineuses*, A.Belin, **1825**, p. 72.
[28] A. N. Egan, C. F. Puttock, *Thai Forest Bulletin (Botany)* **2016**, *44*, 26–31.

Submitted: March 18, 2020
Accepted: April 21, 2020