Int. J. Electrochem. Sci., 16 **(2021)** Article Number: 211041, doi: 10.20964/2021.10.56

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Identification of Species in *Lycoris spp.* from stigmatic exudate using electrochemical fingerprints

Weiting Ye[1,#], Yuhong Zheng[2,#], Pengcong Zhang[3], Boyuan Fan[1], Yongfang Li[4], Li Fu[1,]*

[1] Key Laboratory of Novel Materials for Sensor of Zhejiang Province, College of Materials and Environmental Engineering, Hangzhou Dianzi University, Hangzhou, 310018, PR. China
[2] Institute of Botany, Jiangsu Province & Chinese Academy of Sciences (Nanjing Botanical Garden Mem. Sun Yat-Sen), Nanjing 210014, PR. China
[3] Hangzhou Botanical Garden, Hangzhou, 310013, PR. China
[4] Xiangshan County Agricultural Technology Extension Centre, Xiangshan, 315700, PR. China
[*]E-mail: fuli@hdu.edu.cn
# These authors contributed equally.

Received: 8 July 2021 / *Accepted:* 16 August 2021 / *Published:* 10 September 2021

The use of electrochemical fingerprints for plant identification is an emerging application in biosensors. In this work, stigmatic exudate was collected from plants and the electrochemical fingerprints were taken. *Lycoris anhuiensis, L. longituba, L. straminea, L. guangxiensis, L. haywardii, L. sprengeri, L. aurea, L. chejuensis, L. squamigera, L. qinlingensis, L. albiflora, L. radiata, L. incarnata* and *L. chinensis* were collected for this purpose. Different electrochemical fingerprints were obtained in accordance with the differences of electrochemically active substances in the stigmatic exudate. These electrochemical fingerprint profiles can be adopted to construct different patterns of recognition strategies and further applied in the identification of species. In addition, since the electrochemical fingerprints of stigmatic exudate contain the overall information of electrochemically active substances, they can be adopted to study the relationship among different species. A phylogenetic tree was successfully constructed based on the data of electrochemical fingerprints. The deduced infrageneric relationship among species is more persuasive than that in classical chemotaxonomic studies. The results can rival those obtained from modern molecular taxonomic methods and provide insights for future genetic studies.

Keywords: Electroanalysis; Stigmatic exudate; Plant identification; Fingerprints; Biometrics

1. INTRODUCTION

A stigma is usually located at the tip of the pistil and is slightly expanded or extended into different shapes to facilitate the admission of more pollen to the stigma [1,2]. The surface of the plant

stigma varies in shape, some of which appear concave and convex, while others have elevated epidermal cells in the form of papillae or outgrow into trichomes. The successful completion of sexual reproduction in flowering plants relies on a series of interactions between pollen and the tissues of the pistil. Only compatible pollen can be recognized by the stigma, while incompatible pollen cannot be recognized, which is a screening process that is the first interaction between pollen and pistil recognition [3–5]. The completion of this recognition process is the most important biological function of the stigma. The pollen and stigma of plants exhibit co-evolution [6].

Mature wet stigmas secrete liquid exudate on their surfaces. The main components of the exudate are water, proteins, lipids, polysaccharides and phenols, which confer multiple biological functions to the stigma exudate [7–11]. Firstly, the sticky exudate promotes pollen adhesion and avoids the loss of water in the stigma. Secondly, the proteins, lipids and polysaccharides of the exudate may be involved in the mutual recognition between the stigma and pollen. Thirdly, certain kinds of proteins and polyphenols may play a protective role in resisting the fungal and bacterial attack. The specific recognition between stigma and pollen allows us to hold the opinion that the study of stigma exudate may be a biometric strategy. The composition of the stigma exudate of plants varies, thus it is possible that different plants can be identified by the composition of the stigma exudate. A biosensor to differentiate stigma exudate has the potential to differentiate among species.

Species identification with electrochemical fingerprints of plant tissues is an identification method that has emerged in recent years [12–18]. The principle of this technique is the variability of electrochemically active components in the tissues of different plants, which reflects, to some extent, the genetic differences among species. However, the recording of electrochemical fingerprints with different plant tissues has different accuracies, the reason for which is that the composition in some plant organs varies considerably with the growth environment. For example, the reproducibility of electrochemical fingerprints in plant bulbs is poor due to the interference of starch [19]. Despite the good reproducibility of the electrochemical fingerprints of plant flowers, these fingerprints are not well used to reflect genetic differences among species, which is resulted from the fact that the electrochemical signal of pigments in plant flowers is too strong to mask other electrochemically active substances. Hence the use of stigma exudate as a study object for species identification has not been explored. In this study, we have tried for the first time to use the stigma secretion of *Lycoris* spp. for the identification of species. *Lycoris* is a genus that mainly distributes in Asia, containing less than twenty species [20–22]. The reproductive isolation among them is not strict, thus natural hybridization is very common, which makes their interspecific relationships very complex [23–25]. In this study, stigmatic exudate was collected from 14 *Lycoris* spp. and the possibility of the identification of species was explored. In addition, their interspecific relationships were also studied on the basis of the statistical differences in electrochemical fingerprints.

2. EXPERIMENTAL METHODS

Stigmatic exudate of *Lycoris anhuiensis, L. longituba, L. straminea, L. guangxiensis, L. haywardii, L. sprengeri, L. aurea, L. chejuensis, L. squamigera, L. qinlingensis, L. albiflora, L. radiata, L. incarnata* and *L. chinensis* were supplied by Nanjing Botanic Garden and Hangzhou Botanic Garden.

All the stigmatic exudate was collected during the flowering season of each species in 2020. In the process of collecting, the surface of the stigma was wiped with a cotton swab to absorb stigmatic exudate. All samples were kept frozen before analysis. All electrochemical fingerprint recordings were conducted with a CHI760 electrochemical workstation (data acquisition was conducted with CHI760 software). A commercial glassy carbon electrode (GCE, 3 mm in diameter), an Ag/AgCl electrode and a Pt electrode were adopted as the working electrode, reference electrode and counter electrode, respectively. Differential pulse voltammetry (DPV) was adopted for electrochemical fingerprint recording

Before recording the electrochemical fingerprints, the swabs with adsorbed exudate were extracted with ethanol. Specifically, the swabs containing the exudate was inserted into 2 mL of ethanol and extracted for 2 min under sonication. Afterwards, 5 μL of the extracted solution was coated on the GCE surface and dried naturally. Phosphate buffered solution (PBS, pH=7) and acetate buffered solution (ABS, pH=4.5) were used to collect electrochemical fingerprints of the stigmatic exudate. The electrochemical fingerprints had been recorded 3 times for each sample.

Before biometrics recognition, all data were first treated with a normalization process, where the ratios between the current and the maximum peak current were obtained at different potentials. These normalized data were also applied in PCA analysis and phylogenetic tree generation.

3. RESULTS AND DISCUSSION

The leaves of *Lycoris* spp. are very similar, however, at the time of flowering, various species have different color petals. Figure 1 shows the digital photos of each species when the stigma exudate was collected. From the photos, it can be seen that the petal color and petal morphology of different species can vary, while some species still have great similarity to each other, such as *L. anhuiensis/L. guangxiensis, L. squamigera/L. incarnata, and L. aurea/L. chinensis*.

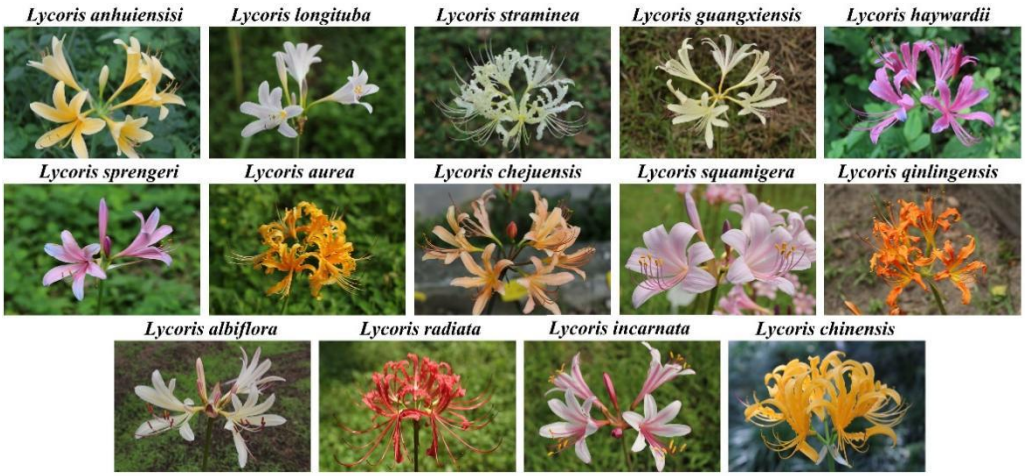

Figure 1. Digital photo of *Lycoris anhuiensis, L. longituba, L. straminea, L. guangxiensis, L. haywardii, L. sprengeri, L. aurea, L. chejuensis, L. squamigera, L. qinlingensis, L. albiflora, L. radiata, L. incarnata* and *L. chinensisa*.

The electrochemical fingerprints of the stigmatic exudate under PBS are shown in Figure 2. A series of oxidation peaks can be seen between -0.1 and 1.5 V for any species, representing the involvement of substances in the electrochemical oxidation reaction in the stigmatic exudate. As mentioned in the introduction, the stigmatic exudate contains proteins, lipids, polysaccharides and phenols, among which phenolics can be electrochemically oxidized at a lower potential [26–28]. Figure 2 presents that the phenolics vary in species for the reason that they show oxidation at different potentials. The differences in overpotentials are often due to the differences in the structure of the molecules involved in the reaction [29]. Meanwhile, it can be noted that there are some species with two distinct electrochemical oxidation peaks, such as *L. longtiuba*, *L. haywardii*, *L. aurea*, *L. squamigera* and *L. chinensis*. However, some other species have three electrochemical oxidation peaks, such as *L. anhuiensisi*, *L. guangxiensis*, *L. sprengeri* and *L. radiata*. Despite that there appears to be some variability in the DPV of these species, some of them are still difficult to be identified directly, such as *L. straminea/L. chejuensis*, and *L. haywardii/L. qinlingensis*. Therefore, it is highly inaccurate to directly use the DPV recorded in one condition for the identification of species. As shown in the Figure 2, DPV curves can vary somewhat between different samples in the same species (black and red lines). These differences are resulted from the differences in the accumulation of substances caused by various growth microenvironments, such as soil nutrients and sunlight. Nevertheless, the electrochemical fingerprints of stigmatic exudate were found to be less reproducible than those of other plant organs we investigated previously, such as leaves and pollen [15–17].

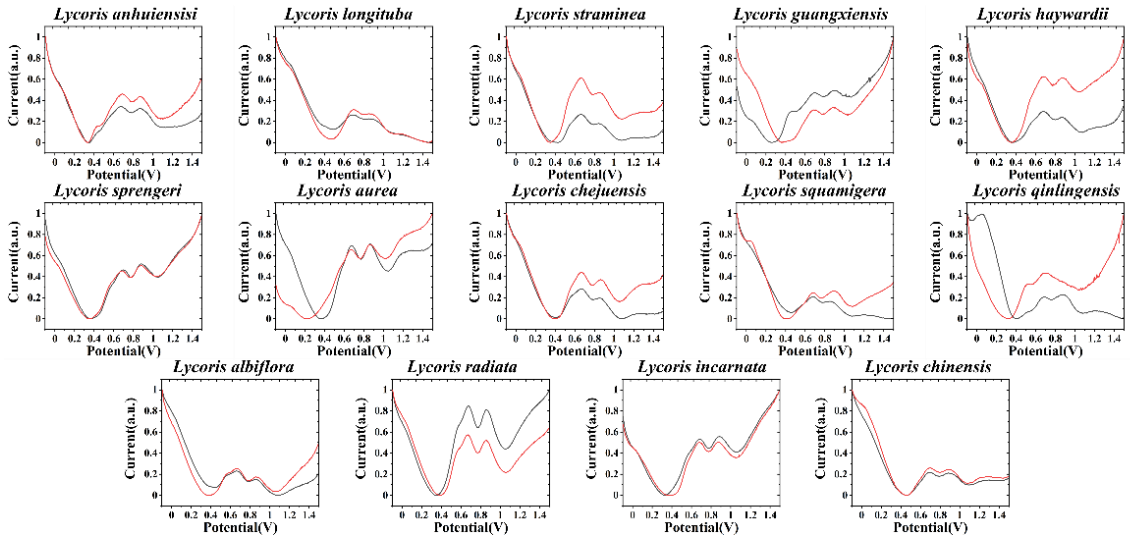

Figure 2. Electrochemical fingerprint of *L. anhuiensis*, *L. longituba*, *L. straminea*, *L. guangxiensis*, *L. haywardii*, *L. sprengeri*, *L. aurea*, *L. chejuensis*, *L. squamigera*, *L. qinlingensis*, *L. albiflora*, *L. radiata*, *L. incarnata* and *L. chinensisa* recorded in PBS (pH 7.0).

Figure 3 shows the electrochemical fingerprint records of the stigmatic exudate of all species under ABS. Very similar to the case of PBS, all species also have a series of oxidation peaks under ABS. However, the substances involved in the electrochemical oxidation are different and the behaviors of the

electrochemical oxidation are also different due to the different pH environments [30]. It is difficult to directly identify these species by only looking at their electrochemical fingerprints in the ABS environment. Some of them are very similar, such as *L. anhuiensis/L. sprengeri* and *L. aurea/L.chejuensis*. However, if the electrochemical fingerprints of species in both PBS and ABS are combined, the accuracy of identification can be increased.

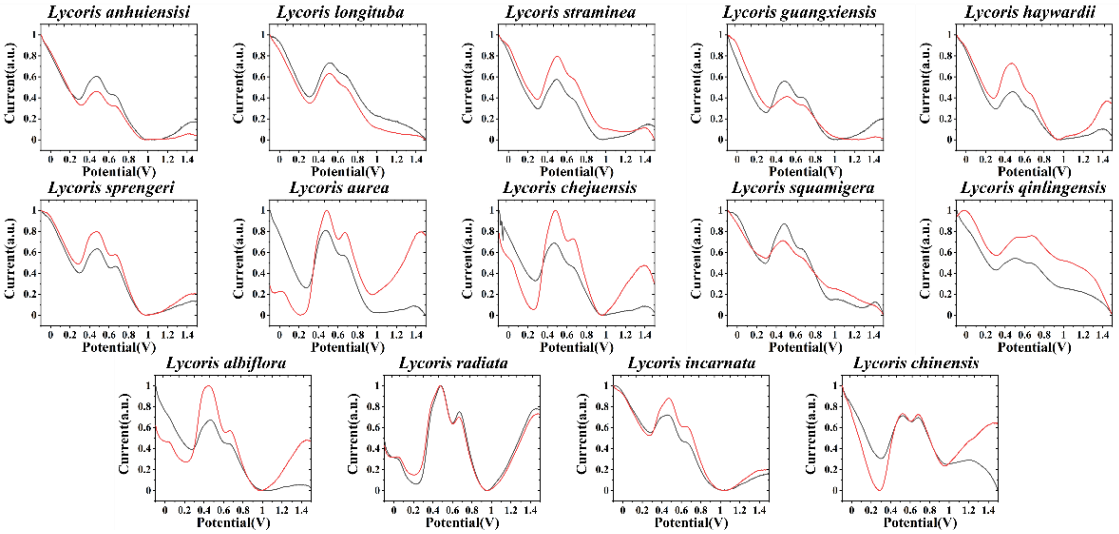

Figure 3. Electrochemical fingerprint of *L. anhuiensis*, *L. longituba*, *L. straminea*, *L. guangxiensis*, *L. haywardii*, *L. sprengeri*, *L. aurea*, *L. chejuensis*, *L. squamigera*, *L. qinlingensis*, *L. albiflora*, *L. radiata*, *L. incarnata* and *L. chinensis* recorded in ABS (pH 4.5).

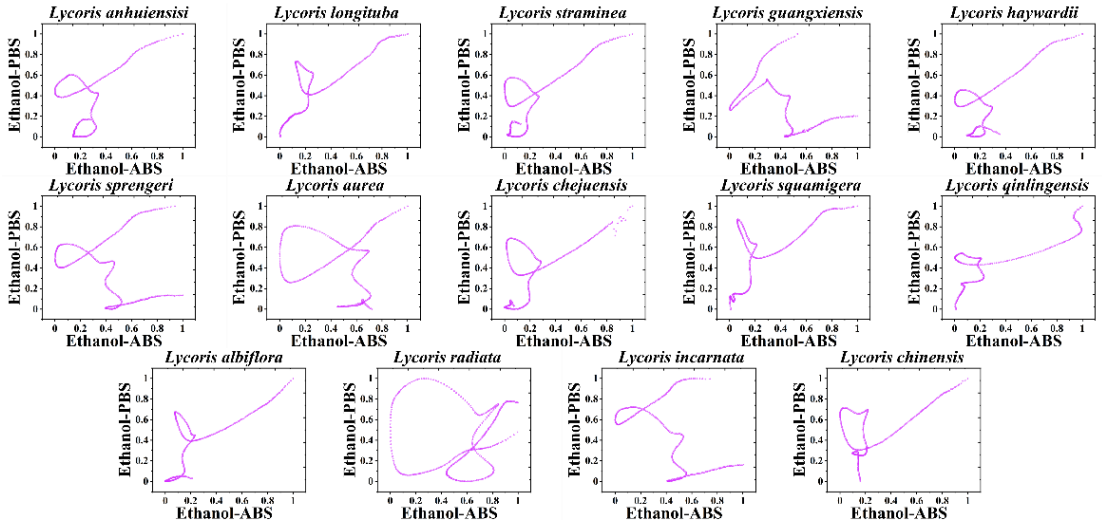

Figure 4. Scatter patterns of *L. anhuiensis*, *L. longituba*, *L. straminea*, *L. guangxiensis*, *L. haywardii*, *L. sprengeri*, *L. aurea*, *L. chejuensis*, *L. squamigera*, *L. qinlingensis*, *L. albiflora*, *L. radiata*, *L. incarnata* and *L. chinensis.*

As shown in Figure 4, a scatter pattern plot can be formed by combining the normalized current values in PBS and ABS. The originally similar electrochemical fingerprint profiles can be easily identified in the scatter plot. For example, the DPV curves of *L. anhuiensisi* and *L. sprengeri* have great similarity under ABS, but significant differences could be detected in the scatter plot. The DPV curves of *L. haywardii* and *L. qinlingensis* were highly similar under PBS, but also showed significant differences in the scatter plot.

Although the scatter plot can represent the difference among species more intuitively than DPV, it is not easy to count the scatter points in the scatter plot directly. Therefore, a two-dimensional density map can be used to strengthen the weights of some data points. In a two-dimensional density map, data points that are clustered closer together will appear in a darker color. Thus the identification of species can be achieved based on the position of these key regions [31]. The advantage of this pattern recognition is that the amount of data for image recognition can be reduced.

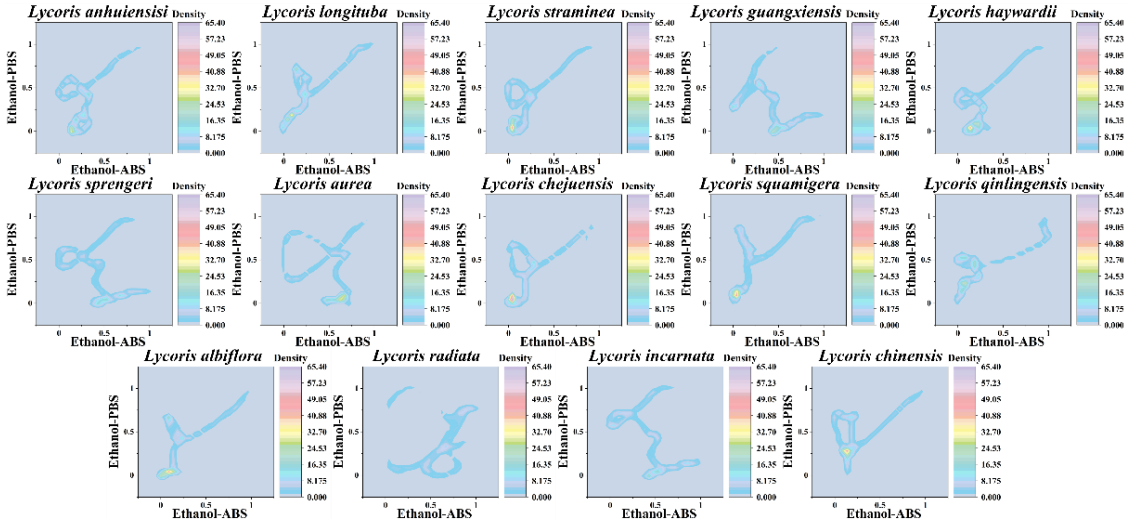

Figure 5. 2D density map of *L. anhuiensis*, *L. longituba*, *L. straminea*, *L. guangxiensis*, *L. haywardii*, *L. sprengeri*, *L. aurea*, *L. chejuensis*, *L. squamigera*, *L. qinlingensis*, *L. albiflora*, *L. radiata*, *L. incarnata* and *L. chinensis.*

In addition to using the 2D density map to determine the focus areas, it is also possible to divide all image areas. According to the number of data points within each region, a corresponding heat map can be formed. Figure 6 reveals that the heat map can be used to explicitly calculate the similarity among different species. Despite that these pattern recognition approaches are all based on the data from electrochemical fingerprinting, they apply different recognition strategies. It can be seen from all these pattern recognition approaches that electrochemical fingerprinting techniques have a very strong ability to identify plant species, the reason for which is that the electrochemical fingerprints contain information about the response of electrochemically active substances in the stigmatic exudate. Although these substances cannot be identified individually in electrochemical fingerprinting, they contribute to the overall collection of information, just like the omics that is based on other macromolecules [32].

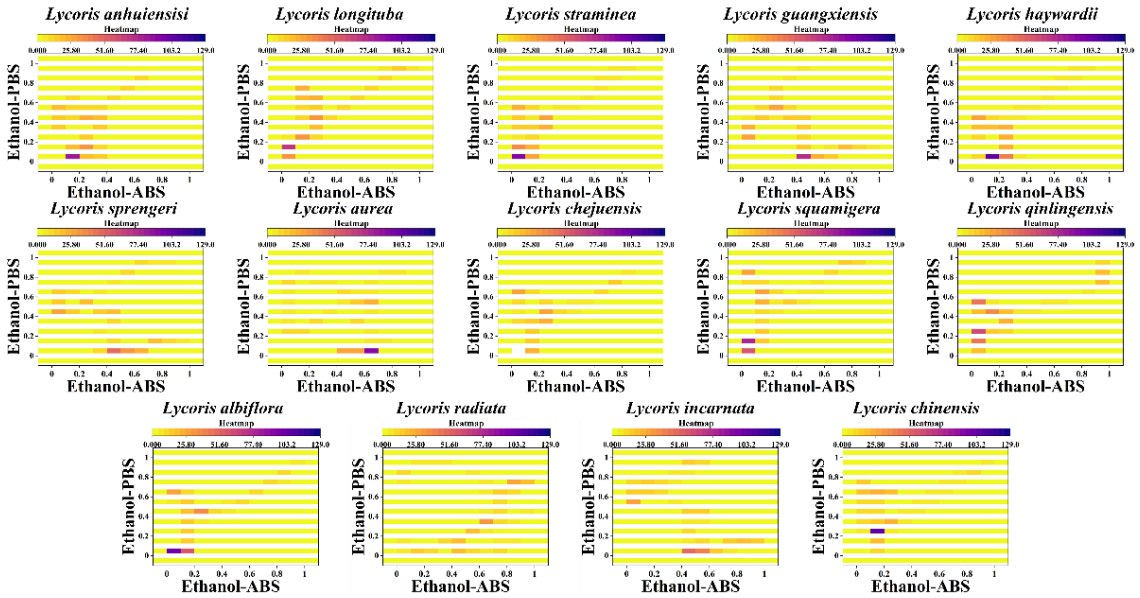

Figure 6. Heatmap of *L. anhuiensis*, *L. longituba*, *L. straminea*, *L. guangxiensis*, *L. haywardii*, *L. sprengeri*, *L. aurea*, *L. chejuensis*, *L. squamigera*, *L. qinlingensis*, *L. albiflora*, *L. radiata*, *L. incarnata* and *L. chinensis.*

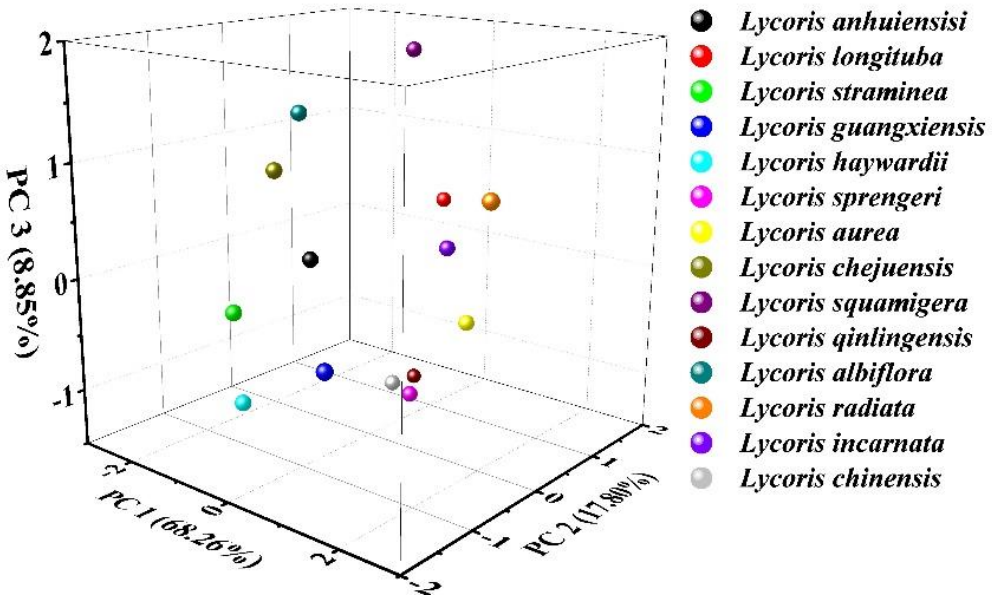

Figure 7. PCA analysis of *L. anhuiensis*, *L. longituba*, *L. straminea*, *L. guangxiensis*, *L. haywardii*, *L. sprengeri*, *L. aurea*, *L. chejuensis*, *L. squamigera*, *L. qinlingensis*, *L. albiflora*, *L. radiata*, *L. incarnata* and *L. chinensis.*

The differences in electrochemical fingerprints can be applied not only in identifying different species, but also in exploring the relationship among different species. We firstly studied the electrochemical fingerprints of different species by principal component analysis (PCA). As shown in

Figure 7, after extracting three factors, PCA could reach more than 93% interpretation. Therefore, the electrochemical fingerprints contain representative information points that can be adopted to represent different data sets, which further indicates that electrochemical fingerprinting with stigmatic exudate can be applied in the identification of species.

Previous studies were carried out on the interspecific relationships of *Lycoris* via HPLC data [33]. However, there was a large discrepancy between the HPLC results and those derived from molecular phylogenetics, the reason for which may be the weak representativeness of HPLC, due to the tracking and identification of only a few alkaloids in the plant. However, the information in the electrochemical fingerprint map is a collection of all electrochemically active substances and is therefore more representative. Figure 8 shows the phylogenetic tree constructed based on electrochemical fingerprinting. The entire phylogenetic tree is divided into three main clades. The first clade includes *L. anhuiensis, L. straminea, L. haywardii, L. chejuensis* and *L. albiflora*. The second clade includes *L. longituba, L. squamigera, L. qinlingensis* and *L. chinensis*. The third clade includes *L. guangxiensis, L. sprengeri, L. incarnata* and *L. aurea*. Figure 8 shows that although *L. aurea* and *L. chinensis* have similar morphologies, a distant relationship exists, which is in consistent with the results from RAPD study [34]. Moreover, the *L. aurea* and *L. sprengeri* show a close relationship, which is in good agreement with the results from inter-simple sequence repeat (ISSR) markers analysis [35]. *L. chejuensis* and *L. qinlingensis* are two new species that have not been involved in our previous studies. This work located the phylogenetic position of these two species for the first time and further refines our phylogenetic study of *Lycoris* spp.

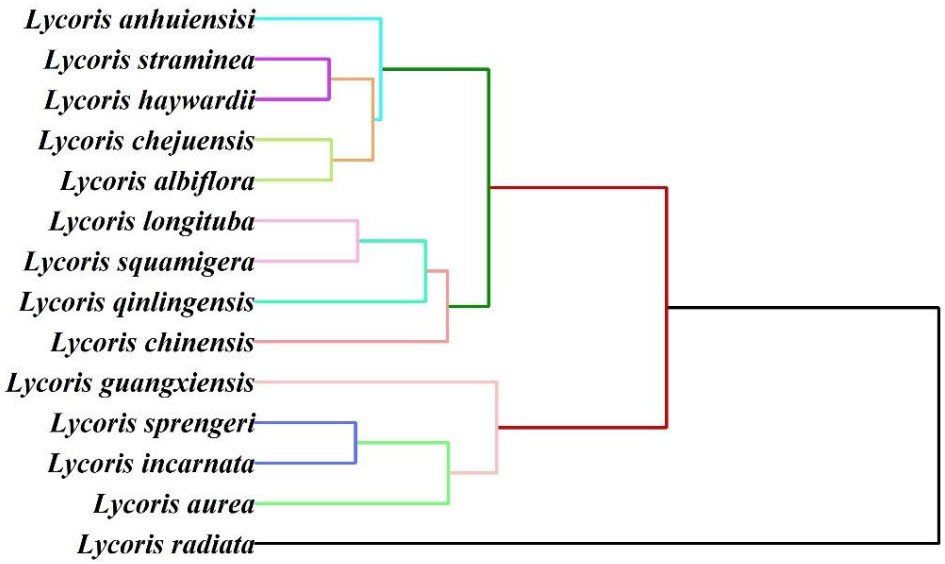

Figure 8. Dendrogram of *L. anhuiensis, L. longituba, L. straminea, L. guangxiensis, L. haywardii, L. sprengeri, L. aurea, L. chejuensis, L. squamigera, L. qinlingensis, L. albiflora, L. radiata, L. incarnata* and *L. chinensis* based on normalized data.

4. CONCLUSION

In conclusion, the electrochemical fingerprints of the *L. anhuiensis, L. longituba, L. straminea, L. guangxiensis, L. haywardii, L. sprengeri, L. aurea, L. chejuensis, L. squamigera, L. qinlingensis, L. albiflora, L. radiata, L. incarnata* and *L. chinensis* were recorded with stigmatic exudate under PBS and ABS. The electro-active compounds in different species vary for the reason that they show oxidation at different potentials. It is highly inaccurate to directly use the DPV recorded in one condition for the identification of species. However, the pattern constructed with these electrochemical fingerprints can be adopted more effectively for different identification strategies. Furthermore, the electrochemical fingerprint signal of stigmatic exudate can also be adopted as a signal ensemble to study the relationship among different species. The entire phylogenetic tree is divided into three main clades, among which the first clade includes *L. anhuiensis, L. straminea, L. haywardii, L. chejuensis* and *L. albiflora*, the second clade includes *L. longituba, L. squamigera, L. qinlingensis* and *L. chinensis*, and the third clade includes *L. guangxiensis, L. sprengeri, L. incarnata* and *L. aurea*.

ACKNOWLEDGMENTS
This research was supported by the National Natural Science Foundation of China (22004026).

References

1. E. Dhooghe, D. Reheul, T. Versluys, M.-C. Van Labeke, *Euphytica*, 214 (2018) 1–13.
2. K. Katano, T. Oi, N. Suzuki, *Frontiers in Plant Science*, 11 (2020) 989.
3. H. Benjlil, K. Elkassemi, M.A. Hamza, T. Mateille, J.N. Furze, K. Cherifi, Z. Ferji, *Applied Soil Ecology*, 147 (2020) 103362.
4. P. Saumitou-Laprade, P. Vernet, X. Vekemans, V. Castric, G. Barcaccia, B. Khadari, L. Baldoni, *Evolutionary Applications*, 10 (2017) 860–866.
5. Z. Zulkarnain, E. Eliyanti, E.I. Swari, *Ornamental Horticulture*, 25 (2019) 158–167.
6. S. Rivest, J.R. Forrest, *New Phytologist*, 225 (2020) 1053–1064.
7. Z. Zulkarnain, E. Eliyanti, E.I. Swari, *Ornamental Horticulture*, 25 (2019) 158–167.
8. M. Raina, V. Kaul, *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 89 (2019) 867–875.
9. R. Shakya, *Vegetos*, 33 (2020) 376–384.
10. I.C. da Silva-Batista, F.G.C.M. da Costa, T.S. de Assunção, C. Koschnitzke, R.C. Vieira, C.P. Bove, *Flora*, 278 (2021) 151799.
11. C.M. Alves, A.K. Noyszewski, A.G. Smith, *Plant Reproduction*, 32 (2019) 341–352.
12. J. Zhou, Y. Zheng, J. Zhang, H. Karimi-Maleh, Y. Xu, Q. Zhou, L. Fu, W. Wu, *Analytical Letters*, 53 (2020) 2517–2528.
13. L. Fu, W. Su, F. Chen, S. Zhao, H. Zhang, H. Karimi-Maleh, A. Yu, J. Yu, C.-T. Lin, *Bioelectrochemistry* (2021) 107829.
14. L. Fu, Y. Zheng, P. Zhang, H. Zhang, M. Wu, H. Zhang, A. Wang, W. Su, F. Chen, J. Yu, W. Cai, C.-T. Lin, *Bioelectrochemistry*, 129 (2019) 199–205.
15. Y. Xu, Y. Lu, P. Zhang, Y. Wang, Y. Zheng, L. Fu, H. Zhang, C.-T. Lin, A. Yu, *Bioelectrochemistry*, 133 (2020) 107455.
16. L. Fu, Y. Zheng, P. Zhang, H. Zhang, W. Zhuang, H. Zhang, A. Wang, W. Su, J. Yu, C.-T. Lin, *Biosensors and Bioelectronics*, 120 (2018) 102–107.
17. L. Fu, Y. Zheng, P. Zhang, H. Zhang, Y. Xu, J. Zhou, H. Zhang, H. Karimi-Maleh, G. Lai, S. Zhao, W. Su, J. Yu, C.-T. Lin, *Biosensors and Bioelectronics*, 159 (2020) 112212.

18. M. Zhang, B. Pan, Y. Wang, X. Du, L. Fu, Y. Zheng, F. Chen, W. Wu, Q. Zhou, S. Ding, *ChemistrySelect*, 5 (2020) 5035–5040.
19. L. Fu, M. Wu, Y. Zheng, P. Zhang, C. Ye, H. Zhang, K. Wang, W. Su, F. Chen, J. Yu, A. Yu, W. Cai, C.-T. Lin, *Sensors and Actuators B: Chemical*, 298 (2019) 126836.
20. C.H. Park, H.J. Yeo, Y.E. Park, S.-A. Baek, J.K. Kim, S.U. Park, *Biology*, 8 (2019) 63.
21. H.J. Yeo, Y.J. Kim, B.V. Nguyen, Y.E. Park, C.H. Park, H.H. Kim, J.K. Kim, S.U. Park, *Horticulturae*, 7 (2021) 5.
22. P. Salachna, R. Piechocki, *World News of Natural Sciences*, 26 (2019) 72–79.
23. T. Shi, Y. Yue, M. Shi, M. Chen, X. Yang, L. Wang, *Plants*, 8 (2019) 422.
24. Y. Yue, J. Liu, T. Shi, M. Chen, Y. Li, J. Du, H. Jiang, X. Yang, H. Hu, L. Wang, *Plants*, 8 (2019) 53.
25. P. Wang, S. Yu, X. Han, J. Xu, Q. He, S. Xu, R. Wang, *PloS One*, 15 (2020) e0230177.
26. M.M. Lounasvuori, D. Kelly, J.S. Foord, *Carbon*, 129 (2018) 252–257.
27. J.R. Oliveira-Neto, S.G. Rezende, C. de Fátima Reis, S.R. Benjamin, M.L. Rocha, E. de Souza Gil, *Food Chemistry*, 190 (2016) 506–512.
28. D. Vega, L. Agüí, A. González-Cortés, P. Yáñez-Sedeño, J. Pingarrón, *Talanta*, 71 (2007) 1031–1038.
29. H. Sharifian, D. Kirk, *Journal of the Electrochemical Society*, 133 (1986) 921.
30. J. Iniesta, P. Michaud, M. Panizza, G. Cerisola, A. Aldaz, C. Comninellis, *Electrochimica Acta*, 46 (2001) 3573–3578.
31. A. Rolland-Lagan, M. Amin, M. Pakulska, *The Plant Journal*, 57 (2009) 195–205.
32. Z. Feng, B. Lin, Y. Xu, L. Zhu, J. Liu, J. Gao, *Molecular Biology Reports*, 40 (2013) 1861–1866.
33. S. Shi, Y. Sun, L. Wei, X. Lei, K.M. Cameron, C. Fu, *Botanical Journal of the Linnean Society*, 176 (2014) 115–126.
34. L. Zhang, Y.-M. Cai, Q. Zhuge, L.-H. Lou, H.-Y. Zou, M.-R. Huang, M.-X. Wang, *Acta Genetica Sinica*, 29 (2002) 915–921.
35. S. Shi, Y. Qiu, L. Wu, C. Fu, *Scientia Horticulturae*, 110 (2006) 285–291.