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Discrimination of *Pinus yunnanensis*, *P. kesiya* and *P. densata* by FT-NIR

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ABSTRACT

Pinus yunnanensis Franch., P. kesiya var. Langbianensis and P. densata Masters have taken on an obvious geographical overlapped region and were difficult to be distinguished. In this study, we discriminated these three species by Fourier transform near infrared (FT-NIR) spectroscopy. The discriminant analysis model incorrectly assessed the P. kesiya to P. yunnanensis species in cross areas, and optimized one was able to estimate the unknown samples exactly while the overlapped P. kesiyain dividuals have been abandoned, which highly matched the geographical distribution of P. yunnanensis, P. densata and P. kesiya. The close relationship between P. yunnanensis and P. kesiya probably resulted from the gene flow among species in cross areas.

Key words: *P. yunnanensis*; closely related species; FT-NIR; discriminant analysis

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INTRODUCTION

P. yunnanensis Franch. is a principal species of wooding and afforestation in Yunnan, which composed up to 70 percent of the forest and varied from 700m to 3200m in altitude[1].*P. yunnanensis* originated and occupied the middle part of Yunnan Plateau[2, 3]. Moreover, it neighbored *P. densata* in Hengduan Mountainous of northwest of Yunnan and joined *P. kesiya* var. Langbianensis in the southwestern Yunnan. There existed difficulty in identifying these three species for introgression and hybridization occurred in cross areas and the some kind of cenospecies were formed[4]. Therefore, different opinions of relationships among them were argued[5]. The point that the closer correlation between *P. yunnanensis* and *P. kesiya* than that between *P. yunnanensis* and *P. densata* was provided by both systematics and cytology researches[6-8].Armitage and Burley[9] recognized *P. yunnanensis* and *P. kesiya* var. Langbianensis as the varieties of *P. kesiya* while Wang[10] hold differing views. Ecological researches suggested that they were geographical substitute species with southeast oriented, and *P. kesiya* expanded towards northeast and bordered *P. yunnanensis* in Ailao Mountain11]. *P. yunnanensis*, *P. densata* and *P. kesiya* belong to the same genus, with the similarity in morphology and gene flow at complex terrain, which decreased their distinction[4]。

Bark, which is defined as all the tissues external to and surrounding the vascular cambium, comprises about 9-15% of a typical log by volume or 13-21% on a dry weight basis[12,13]. Bark differs from wood in terms of chemical compositions. Generally, bark consists of polysaccharides (cellulose, hemicelluloses), pectic substances, phenolic polymers including lignin and high molecular weight tannins, andcross-linked polyesters such as suberin and cutin. The holocellulose in bark generally contains a higher proportion of mannose and the lignin in some conifer barks can be more highly cross linked than wood lignin. In addition, some low molecular weight components such as low molecular weight phenolics, fatty acids and resins can also be found in bark[14].The chemical composition of bark varies with tree species (hardwood or softwood), tree parts (root, stem or branch), tree stress (normal wood, tension wood or compression wood), tree parts (root, stem or branch), geographic location, climate and soil conditions[15].Thus, new methods are demanded for distinguishing species through chemical components in barks

of a species in varies habitats or diverse species at same environment.

Fourier transform near infrared (FT-NIR) spectroscopy is rapidly developed during the last thirty years[16], with the advantage of efficiency, rapid and easily detection, and lower cost as well. NIR has already been widely used in scientific research, including crops[17-24],traditional Chinese medicine[25-28], tea[29], fruit[30-32], forestry[33] et al. However, few works has been done in discrimination of *P. yunnanensis* and its related species.

In this study, FT-NIR of *P. yunnanensis*, *P. kesiya* and *P. densata* were detected and then being distinguished using the discriminant analysis method. Improving our understanding will provide new insights into the relationship of these three species.

EXPERIMENTAL SECTION

SAMPLES COLLECTION

A total of 185 individuals (130 for *P. yunnanensis*, 30 for *P. kesiya* and 25 for *P. densata*) from Yunnan, Sichuan and Tibet provinces were obtained during 2010-2012 (Table 1).The rectangular (portrait: landscape = 3:1) barks at one meter above ground each plant were naturally dried and then smashed to 0.25mm. All samples were kept at -4℃.

Table 1 185 samples of *Pinu s*genus from Yunnan, Sichuan and Tibet provinces

ID	Species	Locations	Altitude (m)	Long.(E)	Lat.(N)
1-5	*P. yunnanensis*	Baoshan, Eshan, Yuxi, Yunnan	1897.999-1900.374	102.305772-102.306638	24.229955-24.230105
6-10	*P. yunnanensis*	Gasa, Xinping, Yuxi, Yunnan	2009.047-2036.670	101.539555-101.539725	23.975998-23.976283
11-15	*P. yunnanensis*	Shigu, Lijiang, Yunnan	1998.541-2036.533	99.945060-99.945637	26.929408-26.929433
16-20	*P. yunnanensis*	Taizhong, Jingdong, Pu'er, Yunnan	2601.003-2603.005	101.085160-101.085180	24.530821-24.530822
21-25	*P. yunnanensis*	Tianchi, Yunlong, Lijiang, Yunnan	2655.000-2720.000	99.270282-99.270267	25.860870-25.864192
26-30	*P. yunnanensis*	Huashan, Zhanyi, Qujing, Yunnan	2057.950-2059.951	103.956777-103.956976	25.786104-25.786305
31-35	*P. yunnanensis*	Dongadi, Nanjian, Dali, Yunnan	1624.567-1716.890	100.582998-100.592978	24.767995-24.786995
36-40	*P. yunnanensis*	Tianshengqiao,Nanjian, Dali, Yunnan	1765.187-1767.983	100.543387-100.549396	24.808520-24.808715
41-45	*P. yunnanensis*	Dajiantang, Yongping, Dali, Yunnan	1738.308-1750.000	99.527318-99.529405	25.481587-25.482887
46-50	*P. yunnanensis*	Qushi, Tengchong, Baoshan, Yunnan	1572.440-1596.420	98.573293-98.574050	25.223950-25.224760
51-55	*P. kesiya*	Simao, Pu'Er, Yunnan	1378.040-1443.129	100.974450-100.974708	22.716907-22.717525
56-60	*P. kesiya*	Xiaoheijiang, Jinggu, Pu'er, Yunnan	933.314-935.005	100.964500-100.969658	23.187003-23.187201
61-65	*P. densata*	Dongjiu, Linzhi, Tibet	2464.264-2510.779	94.850957-94.802030	29.948778-29.962737
66-70	*P. yunnanensis*	Datian, panzhihua, Sichuan	1744.570-1747.918	101.787163-101.787193	26.252105-26.252210
71-75	*P. yunnanensis*	Xiaguan, Dali, Yunnan	2188.890-2278.330	100.233538-100.265343	25.502935-25.556702
76-80	*P. yunnanensis*	Guishan, Shilin, Kunming, Yunnan	2022.154-2039.661	103.499353-103.501383	24.678573-24.679518
81-85	*P. yunnanensis*	Tuodian, Shuangbai, Chuxiong, Yunnan	1993.000-2018.400	101.581587-101.582627	24.756695-24.758563
86-90	*P. yunnanensis*	Zhulin, Guangnan, Wenshan, Yunnan	1450.480-1455.420	104.593985-104.593997	23.965587-23.965692
91-95	*P. yunnanensis*	Toutang, Wenshan, Yunnan	1516.400-1518.990	104.240445-104.240473	23.419363-23.419402
96-100	*P. yunnanensis*	Yaoguan, Shidian, Baoshan, Yunnan	1802.710-1852.920	99.246190-99.246888	24.618433-24.618782
101-105	*P. yunnanensis*	Zhesang, Funing, Wenshan, Yunnan	1488.000-1493.000	106.024500-106.024500	23.839003-23.8392215
106-110	*P. yunnanensis*	Waicang, Anding, Jingdong,, Pu'er, Yunnan	1531.462-1535.452	100.634000-100.634325	24.697000-24.697236
111-115	*P. yunnanensis*	Baishiyan, Anding, Jingdong, Pu'er, Yunnan	1785.390-1787.813	100.721401-100.721632	24.708600-24.708613
116-120	*P. densata*	Milin airport, milin, Linzhi, Tibet	2948.692-2980.474	94.267108-94.275360	29.246997-29.248407
121-125	*P. densata*	Gedinggou, Linzhi, Tibet	3083.798-3096.123	94.166230-94.166292	29.747805-29.747878
126-130	*P. yunnanensis*	Hutiaoxia, Shangri-La, Diqing, Yunnan	2660.397-2687.312	99.956525-99.957000	27.355485-27.356270
131-135	*P. densata*	Xiaozhongdian, Shangri-La, Diqing, Yunnan	3236.120-3266.116	99.846920-99.847248	27.426223-27.426247
136-140	*P. densata*	Napahai, Shangri-La, Diqing, Yunnan	3495.064-3536.580	99.621032-99.621482	27.927030-27.927267
141-145	*P. kesiya*	Gaoligongshan, Longling, Baoshan, Yunnan	1296.070-1330.41	98.873482-98.873640	24.788785-24.789028
146-150	*P. yunnanensis*	Zhaojiadian, Dayao, Chuxiong, Yunnan	1853.571-1880.874	101.471538-101.471723	25.803338-25.803383
151-155	*P. yunnanensis var. pygmaea*	Xinjian, Dayao, Chuxiong, Yunnan	2035.000-2038.000	101.120460-101.120482	25.492320-25.492420
156-160	*P. yunnanensis var. pygmaea*	Xiaobaihu, Luliang,Qujing, Yunnan	1909.423-1909.446	103.356100-103.356235	25.022462-25.022473
161-165	*P. yunnanensis*	Zhaoyang, Zhaotong, Yunnan	2001.926-2006.618	103.523845-103.523892	27.278280-27.278292
166-170	*P. yunnanensis*	Mengmeng, Shuangjiang,Lincang, Yunnan	1730.267-1741.362	99.928217-99.929100	23.495852-23.496725
171-175	*P. kesiya*	Gucheng, Zhenyuan, Pu'er, Yunnan	1061.000-1081.990	101.137820-101.138158	23.742728-23.743167
176-180	*P. kesiya*	Qima, Mojiang, Pu'er, Yunnan	1553.47-1557.89	101.677550-101.677572	23.555468-23.555503
181-185	*P. kesiya*	Zhongliangzi, Mojiang, Pu'er, Yunnan	1634.373-1715.475	101.593267-101.593183	23.367438-23.367805

DETECTING WITH NT-NIR

Data was collected by Fourier transform infrared spectrometer (Nicolet Antaris^{TM}, USA) after pre-heating for 30min. The RESULT^{TM} package and TQ Analyst 8.6.12 (Thermo Nicolet, USA) software were used to carry out the spectrum data of all samples. Data range for scanning was as follows: 10000～4000cm^{-1}; Resolution:8 cm^{-1}; Number of scan: 72 times.

DATA ANALYSIS

The principal component analysis- mahalanobis distance (PCA-MD) method was used for modeling the discrimination of spectrum of *P. yunnanensis*, *P. densata* and *P. kesiya*.

RESULTS AND DISCUSSION

PRE-PROCESSING OF SPECTRUM

Generally, a series of pre-processing, like enhancing signal, speckle reduction et al, were required in building the model of infrared correction method, for the data obtained was always accompanied changing, overlaid information. Derivative was the most common means in decreasing the effects that caused of spectral line shifts. The NIR spectra was pre-processed with second derivative to decrease the systemic error and then Norris derivative filter(5, 5) was used in filtering of spectra.

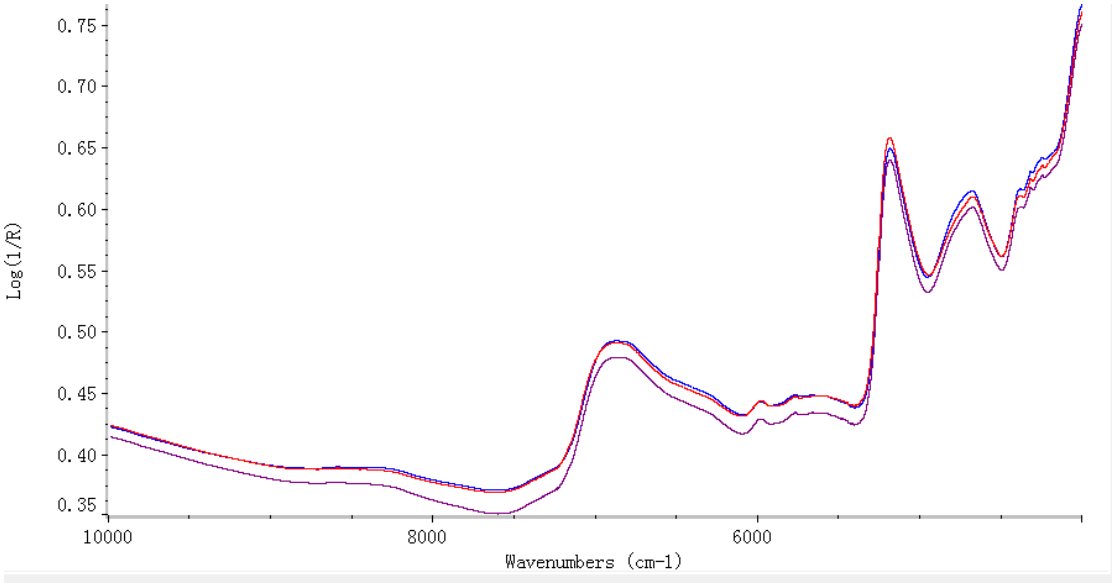

Fig.1 The spectrum of NIR scanning in randomized samples

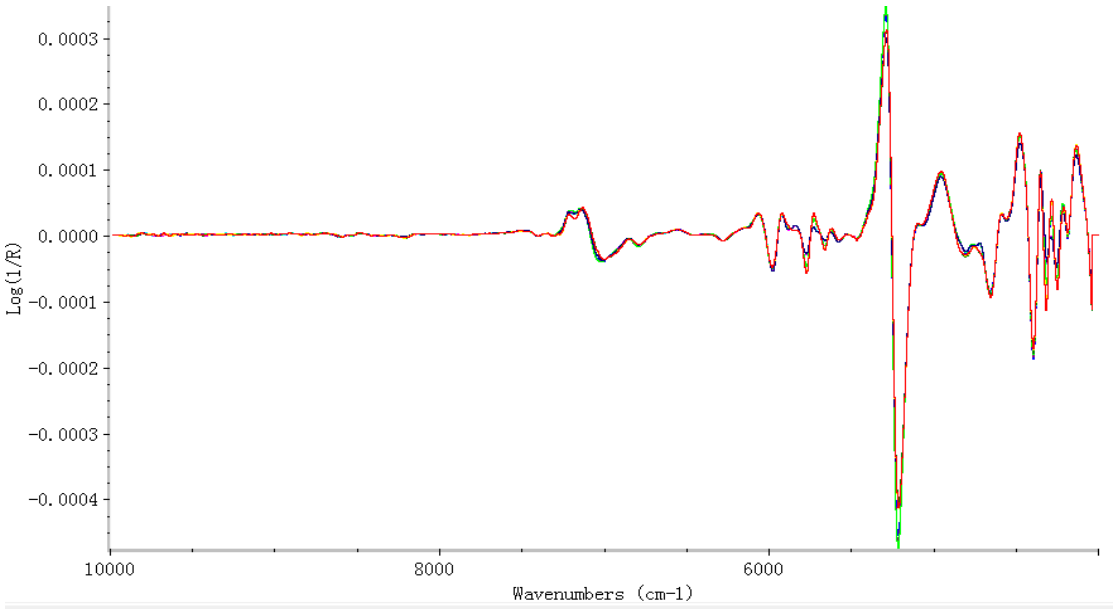

Fig.2 The spectrum corrected with second derivative

THE CRITERIA DEFINITIONS

Discriminant analysis was carried out with PCA-Mahalanobis according to the products spectrum of each step. Mahalanobis distance usually acts as the measurement between the barycentre of groups in clustering, with the correlations among samples being considered. Samples would coincide with each other when the same spectrum have been described, or separate at entirely distinctive wave.

PCA-Mahalanobis：

$$A_{m\times n} = \begin{bmatrix} X_{11} & X_{12} & \cdots & X_{in} \\ X_{21} & X_{22} & \cdots & X_{2n} \\ \vdots & \vdots & \cdots & \vdots \\ X_{m1} & X_{m2} & \cdots & X_{mn} \end{bmatrix}$$

Then mahalanobis distance were calculated as follows:

$$D_i^2 = (\mathbf{t}_i - \bar{\mathbf{t}})^{\mathrm{T}} \mathbf{M}^{-1} (\mathbf{t}_i - \bar{\mathbf{t}})$$

$$\bar{\mathbf{t}} = \frac{1}{m} \sum_{i=1} \mathbf{t_i}$$

Wherem denotes the number of values, D_i^2 denotes the distance square, t_i is a multivariate vector, M denotes the covariance matrix and $\bar{t}$ denotes equals overall sample mean.

PCA-MAHALANOBIS MODEL

Referring to the discrimination analysis model, FT-NIR spectrum optimized suggested that samples of *P. densata* assembled to a single group and the rest individuals gathered into *P. yunnanensis* and *P. kesiya*, respectively (Fig.3). However, eight *P. kesiya* samples were incorrectly evaluated to be *P. yunnanensis*, which blended one another at the cross areas.

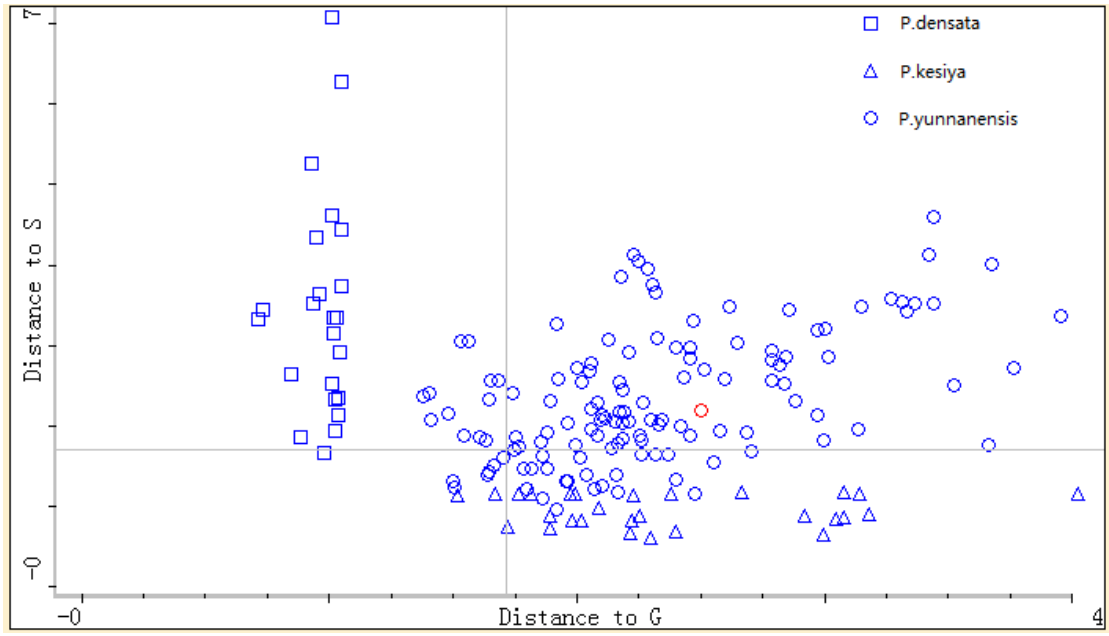

Fig.3 Individuals of *P. yunnanensis*, *P. densata* and *P. kesiya* being classified based on the PCA- Mahalanobis distance

Characteristics of samples would perform a high similarity while the variation of locus being at the lower level on the basis of the Mahalanobis distance, which measures the variation of clusters, and completely coincided with each other at zero of Mahalanobis distance.

Table 2 Mahalanobis distance within and among *P. yunnanensis*, *P. densata* and *P. kesiya* clusters

	P. yunnanensis	*P. kesiya*	*P. densata*
P. yunnanensis	0.971	2.370	2.320
P. kesiya	1.245	0.910	2.349
P. densata	2.245	3.592	0.975

The mahalanobis distance of clusters were analyzed and the mean distance within and between each pair of clusters were listed in table 2. It can be concluded that the mean distance within *P. yunnanensis* was 0.971 and that between

P. densata, *P. kesiya* were 2.320, 2.370. *P. kesiya* gave less mean distancewhen compared to that of *P. yunnanensis*, which is 0.910 within clusters and 1.245, 2.349 distanced from*P. yunnanensis*, *P. densata*. Mean distance for *P. densata* within clusters was 0.975 and 2.245, 3.592 between *P. yunnanensis*, *P. kesiya*, respectively. At total, each species, which took on less mean distance within species than that among species, assembled to a separate cluster. A fraction of *P. kesiya*individuals overlapped to*P. yunnanensis*, with the distance of 1.245.This result match the result that eight *P. kesiya* had been incorrectly discriminated to be *P. yunnanensis*.

Table 3 showed the sample plots where *P. kesiya* and *P. yunnanensis* got overlapped. That the two species went through the long-term convergent evolution in same habitat led to the their close relationship for they had been similar to each other in morphology and chemical components[11]. Thus, we eliminated eight *P. kesiya* samples overlapped with *P. yunnanensis* and then optimized the discrimination analysis model.

P. yunnanensis, *P. kesiya* and *P. densata* were assembled to their respective clusters when the eight overlapped samples of *P. kesiya* had been abandoned. Perfectly match was obtained between discriminant analysis modeling chart and their geographical distribution correspondingly. *P. yunnanensis* occupied the middle part and neighbored to *P. densata* at the Hengduan Mountainous of northwest of Yunnan, and joined the *P. kesiya* in southwestern Yunnan, and was distance from *P. densata*, additionally.

Table 3 Eight *P. kesiya* individuals were incorrectly assessed to be *P. yunnanensis*

ID	Species	Locations	Province
52	*P. kesiya*	Simao, Pu'er	Yunnan
53	*P. kesiya*	Simao, Pu'er,	Yunnan
181	*P. kesiya*	Zhongliangzi, Mojiang, Pu'er	Yunnan
182	*P. kesiya*	Zhongliangzi, Mojiang, Pu'er	Yunnan
183	*P. kesiya*	Zhongliangzi, Mojiang, Pu'er	Yunnan
184	*P. kesiya*	Zhongliangzi, Mojiang, Pu'er	Yunnan
141	*P. kesiya*	Gaoligongshan, Longling, Baoshan	Yunnan
145	*P. kesiya*	Gaoligongshan, Longling, Baoshan	Yunnan

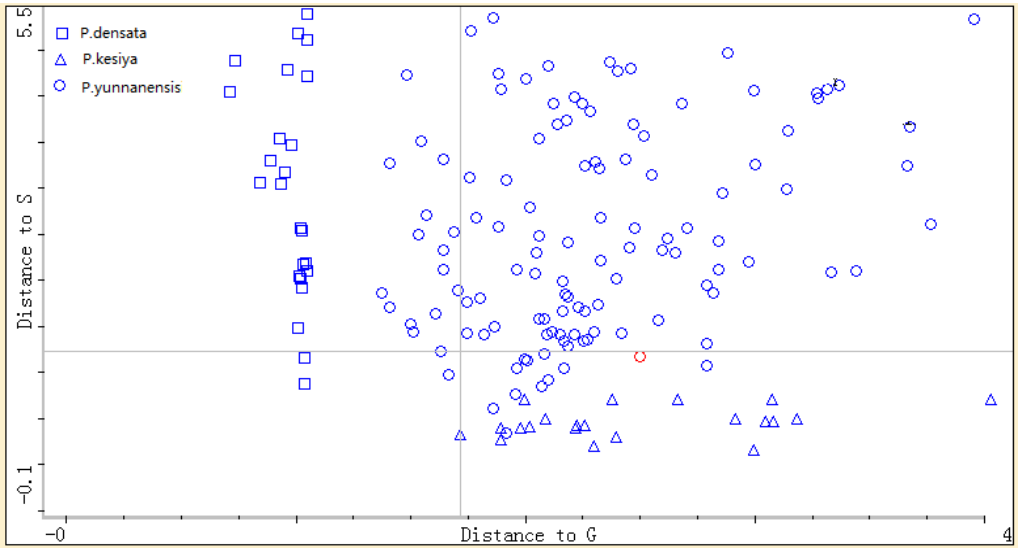

Fig. 4 Clusters of three species being classified without eight individuals of *P. kesiya* in cross area

Table 4 Mahalanobis distance within and among *P. yunnanensis*, *P. densata* and *P. kesiya* clusters without eight individuals of *P. kesiya* in corss area

	P. yunnanensis	*P. kesiya*	*P. densata*
P. yunnanensis	0.971	3.293	2.321
P. kesiya	1.353	0.962	2.493
P. densata	2.245	3.493	0.975

The mean Mahalanobis distance within and between each pair of clusters were calculated, without eight samples in table 3. The mean distance within *P. yunnanensis* was same to that in table 4 (0.971) and were 2.321, 3.293 between *P. densata*, *P. kesiya*. *P. kesiya* was 0.962 within clusters and the distances were 1.353, 2.493 from *P. yunnanensis*, *P. densata*. Mean distance for *P. densata* within clusters was 0.975 and 2.245, 3.493 between *P. yunnanensis*, *P. kesiya*, respectively. These three species varied more among species than that within species. Distance between *P. yunnanensis* and *P. kesiya* was relatively lower (1.353) educed from the optimized discriminant analysis model

(eight overlapped *P. kesiya* were abandoned), and proper assessments were obtained.

CERTIFYING OF OPTIMIZED PCA- MAHALANOBIS ANALYSIS MODEL

The NIR of 20 individuals (14 individuals for *P. yunnanensis*, 3 of both for *P. densata* and *P. kesiya* , as table 5 shows) were distinguished by optimized discriminant analysis model. Only one *P. kesiya* was incorrectly regard as *P. yunnanensis*, and the rest were identified exactly (table 6).

It could be concluded from table 6 that the optimized discriminant analysis model without eight overlapped *P. kesiya* individuals could correctly distinguish the unknown samples. However, a *P. kesiya* sample marked XL039-C which from Number 80 Simao of Pu'er, Yunnan was determined to *P. yunnanensis*. The similar habitats and long-term convergent evolution of these two species was a possible reason[11].

Table 5 Twenty individuals for certifying the optimized PCA-discriminant analysis model

ID	Species	Location	Altitude (m)	Long.(E)	Lat.(N)
XL027-C	*P. yunnanensis*	Wuliang, Nanjian, Dali, Yunnan	1716.800	100.536697	24.824105
XL063-C	*P. yunnanensis*	Anding, Jingdong, Pu'er, Yunnan	1531.473	100.634000	24.697020
XL072-C	*P. yunnanensis*	Toutang, Wenshan, Yunnan	1518.990	104.240473	23.419363
XL077-C	*P. yunnanensis*	Yaoguan, Shidian, Baoshan, Yunnan	1819.240	99.246190	24.618708
XL088-C	*P. yunnanensis*	Zhaojiadian, Dayao, Chuxiong, Yunnan	1861.324	101.471618	25.803353
XL102-C	*P. yunnanensis var. pygmaea*	Xiaobaihu, Luliang, Yunnan	1909.446	103.356100	25.022462
XL105-C	*P. yunnanensis*	Mengmeng, Shuangjiang, Lincang, Yunnan	1741.362	99.928680	23.496555
XL120-C	*P. yunnanensis*	Datian, Panzhihua, Panzhihua, Sichuan	1747.918	101.787177	26.252117
XL145-3	*P. yunnanensis*	Xiaguan, Dali, Yunnan	2278.330	100.265343	25.502935
XL150-C	*P. yunnanensis*	Qushi, Tengchong, Baoshan, Yunnan	1580.64	98.574050	25.2243302
XL170-C	*P. yunnanensis*	Gasa, Xinping, Yuxi, Yunnan	2012.040	101.539657	23.976092
XL175-C	*P. yunnanensis*	Shigu, Lijiang, Yunnan	2036.533	99.945280	26.929440
XL179-C	*P. yunnanensis*	Hutiaoxia, Shangri-La, Diqing, Yunnan	2647.300	99.956525	27.355485
XL125-C	*P. yunnanensis*	Taizhong, Jingdong, Pu'er, Yunnan	2600.529	101.08517	24.53082
XL161-C	*P. kesiya*	Gaoligongshan, Longling, Baoshan, Yunnan	1297.563	98.873482	24.788785
XL039-C	*P. kesiya*	80th, Simao, Pu'Er, Yunnan	1378.040	100.974252	22.717372
XL061-C	*P. kesiya*	Zhongshan, Jinggu, Pu'er, Yunnan	1678.854	100.546660	23.508732
XL187-C	*P. densata*	Xiaozhongdian, Shangri-La, Diqing, Yunnan	3251.900	99.847058	27.426243
XL130-C	*P. densata*	Milin, Linzhi, Tibet	2970.863	94.267417	29.247008
XL135-C	*P. densata*	Linzhi, Tibet	3096.060	94.166292	29.747878

Table 6 Twenty samples assessed byoptimized discriminant analysis model

ID	Ci	Cd	D	P/F	ID	Ci	Cd	D	P/F
XL027-C	*P. yunnanensis*	*P. yunnanensis*	1.09	Pass	XL170-C	*P. yunnanensis*	*P. yunnanensis*	0.81	Pass
XL063-C	*P. yunnanensis*	*P. yunnanensis*	0.83	Pass	XL175-C	*P. yunnanensis*	*P. yunnanensis*	1.02	Pass
XL072-C	*P. yunnanensis*	*P. yunnanensis*	1.21	Pass	XL179-C	*P. yunnanensis*	*P. yunnanensis*	1.30	Pass
XL077-C	*P. yunnanensis*	*P. yunnanensis*	0.53	Pass	XL125-C	*P. yunnanensis*	*P. yunnanensis*	1.00	Pass
XL088-C	*P. yunnanensis*	*P. yunnanensis*	1.10	Pass	XL161-C	*P. kesiya*	*P. kesiya*	1.17	Pass
XL102-C	*P. yunnanensis*	*P. yunnanensis*	1.00	Pass	XL039-C	*P. kesiya*	*P. yunnanensis*	0.91	Pass
XL105-C	*P. yunnanensis*	*P. yunnanensis*	0.90	Pass	XL061-C	*P. kesiya*	*P. kesiya*	0.92	Pass
XL120-C	*P. yunnanensis*	*P. yunnanensis*	0.98	Pass	XL187-C	*P. densata*	*P. densata*	0.94	Pass
XL145-3	*P. yunnanensis*	*P. yunnanensis*	0.79	Pass	XL130-C	*P. densata*	*P. densata*	1.05	Pass
XL150-C	*P. yunnanensis*	*P. yunnanensis*	1.37	Pass	XL135-C	*P. densata*	*P. densata*	1.14	Pass

Ci: Clusters being identified. Cd: Clusters being assessed by optimized discriminant analysis model. D: Distance. P/F: Pass or fail.

CONCLUSION

The FT-NIR analysis illustrated that the mixed distribution of different species appeared in both discriminant analysis modeling and samples plot. The optimized model without the eight overlapped individuals eliminated, could correctly identify the unknown samples, which matched the geographical distribution of *P. yunnanensis*, *P. kesiya*and *P. densata*.

P. yunnanensis inhabited the middle part in geography and neighbored to *P. densata* at Hengduan Mountainous of northwest of Yunnan, and joined *P. kesiya* in southwestern Yunnan as well. There existed gene flow at the cross areas[4], which led to the difficulty in sample discrimination. In this study, accurate identification of *P. yunnanensis* and *P. densata* has been due to their niche divergence, while eight *P. kesiya* individuals in cross areas led to the incorrect discrimination. In addition, the optimized discriminant analysis model without individuals in cross areas abandoned, could exactly assess the unknown samples.

Genetic studies have suggested that *P. densata* is a homoploid hybridization between *P. tabuliformis* and *P. yunnanensis*[34-40]. Isolation has taken on in *P. densata*[41-44] and adaptive evolution promoted in the specialized habitats, with high altitude, low temperature and moisture[36,36, 45-46].The superior physiological properties, rapid

growing rate, resistances against cold, drought and UV-radiation in extreme environments were improved.[45,47]. Mao and Wang's findings provide evidence of a distinct niche shift in *P. densata* and support the hypothesis that local adaptation and geographic isolation help maintain and reinforce between-species differences and reproductive isolation in the species complex[48]. Consequently, *P. densata* has taken the advantages than its two parental species in their respective natural habitats and occupied the ecological niches of the extreme environments[49-52].

Neither geographical nor reproductive isolation had existed in *P. yunnanensis* and *P. kesiya*. Furthermore, short distance between these two species mostly referring to their gene flow at cross areas and convergent adaptation in the similar habitats.

Discriminant analysis results indicated FT-NIR was a valid resource in distinguishing *p. yunnanensis* and its closely related species. However, the models estimated cannot tell the origin of them. As may be expected, a further study of molecular technology and genetic analysis are requested to understand the genetic relationships or classification of *Pinus* genus.

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REFERENCES

[1] Flora of China Editorial Committee. *Flora of China*, **1978**, 7: 255,
[2] Jin ZZ, Peng J. *Pinus yunnanensis*. pp.6-332, **2004**.
[3] XuYL, Cai NH, Kang XY, Li GQ, He CZ, Duan AA. *Journal of Plant Genetic Resources*, **2011**, 12(6):982-985.
[4] Yu H, Ge S, Huang RF, Jiang HQ. *Acta Botanicasinica*, **2000**, 42(1):107-110.
[5] XuYL, Cai NH, Kang XY, Li GQ, He CZ, Duan AA. *Journal of Northwest Forestry University*, **2012**, 27(1):98-102.
[6] Gu ZJ, Li MX. *Acta Botanica Yunnanica*, **1982**, 4(2):185-190.
[7] Li LC, Qian J. *Aeta Botanica Yunnanica*, **1993**, 15(1):47-56.
[8] Saylor LC. *Silvae Genet*, **1964**, 13(6):165-170.
[9] Armitage FB, Burley J. *Pinuskesiya, department of forestry*, **1981**.
[10] Wang CM, Wang J, Jiang HQ. *Journal of southwest forestry college*, **2003**, 23(4):4-6.
[11] Wu ZL. *Journal of ShanxiNormalUniversity: Natural Science Edition*, **1993,** (2):45-49.
[12]USUD. Barks and its possible use.Department of Agriculture.Forest Service Forest Products Laboratory. Madison, Wisconsin, **1971**.
[13] Diamantopoulou M. *Computers and Electronics in Agriculture*, **2005**, (48):235-44.
[14)Hon DNS, Shiraishi N. *Chemistry of bark*, **2001**, 243-74.
[15] Feng SH, Cheng S, Yuan ZS, Leitch M, Xu CB. *Renew a bleand Sustainable Energy Reviews*, **2013** (26):560-578.
[16] Lu WZ. *Modem Near Infrared Spectroscopy Analytival Technology*, **2007**.
[17] Zhu DZ, Wang K, Zhou GH, Hou RF, Wang C. *Spectroscopy and Spectral Analysis*, **2010**, 30(12):3217-3221.
[18] Zhou ZQ, Chen B, Yan H. *Journal of the Chinese Cereals and Oils Association*, **2011**, 26(9):115-117.
[19] Wu WJ, Wang HW, Chen SJ, Guo TT, Wang SJ, Su Q, Sun M, An D. *Spectroscopy and Spectral Analysis*, **2010**, 30(5):1248-1251.
[20] Xia LY, Shen SG, Liu ZH, Sun HW. *Spectroscopy and Spectral Analysis*, **2013**, 33(1):102-105.
[21] Zhou ZL, Zhang Y, He Y, Li XL, Shao YN. *Transactions of the CSAE*, **2009**, 25(8):131-135.
[22] Liang L, Liu ZX, Yang MH, Zhang YX, Wang CH. *J. Infrared Millim. Waves*, **2009**, 28(5):353-356,391.
[23] Feng WS, Wu SH, Gu YY, Zhang Y, Gao HT, Wang WD, Zhang LS, Zhang XP, Ma F, Zhang CJ. *Agricultural Science & Technology*, **2012**, 13(12):2615-2619.
[24] Huang YY, Zhu LW, Li JH, Wang JH, Sun BQ, Sun Q. *Spectroscopy and Spectral Analysis*, **2011**, 31(3):661-664.
[25] Lin B, Lin JM, Shao MH, Sun JY, Qin LP. *Journal of Pharmaceutical Practic*, **2012**, 30(3):194-196.
[26] Huang YW, Wang JH, Li XY, Jacqueline JS, Lei L, Han DH. *Spectroscopy and Spectral Analysis*, **2010**, 30(11):2954-2957.
[27] Luo WQ, Yang HQ, Li Y, Xu N. *Infrared (Monthly)* , **2013**, 34(1):38-40.
[28] Wang GL, Shi Y, Wei YH, Wang HC, Xu XJ, Lin RC. *Chinese Traditional and Herbal Drugs*, **2006**, 37(10):1569-1571.
[29] Zhou J, Cheng H, Ye Y, Wang LY, He W, Liu X, Lu WY. *Acta Optica Sinica*, **2009**, 29(4):1117-1120.
[30] Cao F, Wu i, He Y, Bao YD. *ACTA OPTICA SINICA*, **2009**, 29(2):537-540.
[31] Li XL, Hu XY, He Y. J. *Waves*, **2006**, 25(6):417-420.
[32] Pang YP, Liu K, Yan JY, Li PS. *Modern Food Science and Technolog*, **2013**, 29(5):1160-1162**.**

[33] Liu YM, Chai YF, Qi YP, Fan GR, Wu YT. *Chinese Traditional Patent Medicine*, **2004**, 26(12):1049-1051.
[34] Wang XR, Szmidt AE. *Theoretical and Applied Genetics*, **1990**, 80: 641-647.
[35] Wang XR, Szmidt AE, Lewandowski A, Wang ZR. *Theoretical and Applied Genetics*, **1990**, 80: 635-640.
[36] Wang XR, Szmidt AE. *Evolution*, **1994**, 48: 1020-1031.
[37] Wang XR, Szmidt AE. *Scandinavian Journal of Forest Research*, **2001**, 16: 199-220.
[38] Wang XR, Szmidt AE, Savolainen O. *Genetics*, **2001**, 159: 337-346.
[39] Song BH, Wang XQ, Wang XR, DingKY, Hong DY. *Molecular Ecology*, **2003**, 12: 2995-3001.
[40] Song BH, Wang XQ, Wang XR, Sun LJ, Hong DY, Peng PH. *Molecular Ecology*, **2002**, 11: 1057-1063.
[41] Butlin R. *Trends in Ecologyand Evolution*, **1987**, 2: 8-13.
[42] Turelli M, Barton NH, Coyne JA. *TrendsinEcology and Evolution*, **2001**, 16: 330- 343.
[43] Widmer A, Lexer C, Cozzolino S. *Heredity*, **2008**, 102: 31-38.
[44] Liu YL, Mao JF, Wang XR, Li Y. *Plant Diversity and Resources*, **2011**, 33(3):269-274.
[45] Ma F, Zhao C, Milne R. *New Phytologist*, **2010**, 185: 204-216.
[46] Ma XF, Szmidt AE, Wan g XR. *Molecular Biology and Evolution*, **2006**, 23: 807- 816.
[47] Wang CM, Wang J, Jiang HQ. *Journal of West China Forestry Science*, **2009**, 38(1):23-27.
[48] Mao JF, Wang XR. *American Naturalist*, **2011**, 177: 424–439.
[49] Rieseberg LH, Carney SE. *NewPhytologist*, **1998**, 140: 599-624.
[50] Buerkle CA, Morris RJ, Asmussen MA. *Heredity*, **2000**, 84: 44l-451.
[51] Rieseberg LH, Raymond O, Rosenthal DM. *Science*, **2003**, 301: 1211-12l6.
[52] Seehausen O. *Trends in Ecology and Evolution*, **2004**, 19: 198-207.