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Authentication of the family Polygonaceae in Chinese pharmacopoeia by DNA barcoding technique

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ABSTRACT

Ethnopharmacological relevance: Medicinal plants belonging to the family Polygonaceae in Chinese pharmacopoeia possess important medicinal efficacy in traditional Chinese medicines.
Aim of the study: DNA barcodes are first used to discriminate the Polygonaceae in Chinese pharmacopoeia and their adulterants.
Materials and methods: DNA samples, extracted from thirty-eight specimens belonging to eighteen species in Polygonaceae, were used as templates. Eight candidate barcodes were amplified by polymerase chain reaction. Sequence analysis was accomplished by CodonCode Aligner V 2.06 and DNAman V 6. Species identification was performed using MEGA V 4.0.
Results: The amplification efficiency of six candidate DNA barcodes (*rbcL*, *trnH-psbA*, *ndhJ*, *rpoB*, *rpoC1*, *accD*) was 100%, while the efficiency of *YCF5* and nrITS was 56% and 44%, respectively. The interspecific divergence was highest for the *trnH-psbA* (20.05%), followed by the nrITS (14.01%) across all species pairs, while intraspecific variation both within populations and between populations was absent (0.0%). The *trnH-psbA* can not only distinguish ten species of Polygonaceae in Chinese pharmacopoeia, but also recognize eight other species of Polygonaceae including their adulterants.
Conclusion: Our findings show that DNA barcoding is an efficient tool for identification of Polygonaceae in Chinese pharmacopoeia and their adulterants.

1. Introduction

Traditionally, biological species are authenticated according to their morphological features. Even until now, morphological keys still are main basis of taxonomy (Heinrich, 2007). However, traditional taxonomy study usually requires the expertise of an experienced professional taxonomist. In the case that the diagnostic morphological trait of a specimen is lacking, it becomes difficult even for the specialists to recognize a species correctly. Authentication using DNA barcodes overcomes these problems (Chen et al., 2007). DNA barcode is a short fragment of the genome and species identification using DNA barcoding has been completed in several studies (Gregory, 2005; Lahaye et al., 2008) when morphological characteristics are absent. DNA barcoding technique is a useful tool for taxonomists (Schindel and Miller, 2005) and is likely to usher the taxonomic research into a new era (Miller, 2007). In animals, a mitochondrial gene called cytochrome *c* oxidase subunit 1 (*CO1*) as a DNA barcode varies enough to distinguish most animal lineages (Hebert et al., 2003a,b, 2004; Marshall, 2005; Hajibabaei et al., 2006). In plants, several candidate DNA barcodes have attracted the attention of many researchers (Kress et al., 2005; Pennisi, 2007; Sass et al., 2007; Newmaster et al., 2007; Taberlet et al., 2007; Kress and Erickson, 2007; Lahaye et al., 2008). Here, we propose seven candidate barcodes (*trnH-psbA*, *rbcL*, *rpoB*, *rpoC1*, *ndhJ*, *accD*, *YCF5*) in the plastid genome and one (rDNA ITS) in the nuclear genome to classify the Polygonaceae in Chinese pharmacopoeia.

There are five genera and ten species of the family Polygonaceae in Chinese pharmacopoeia (Chinese Pharmacopoeia Commission, 2005). These species possess important medicinal properties and healthcare efficacies. For example, anthraquinones from rhubarb, *Rheum palmatum*, is able to inhibit the growth of cancer (Huang et al., 2007) and the DNA replication of Hepatitis B virus (HBV) (Li et al., 2007), the rhizomes of *Fagopyrum dibotrys* (Wang et al., 2005)

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Table 1
Plant samples of the family Polygonaceae used in the present study.

Species	Collected place	Date of collection	Statement	Voucher number	Remark
Rheum officinale Baill.	Mianyang, Sichuan, China	October 2007	Cultivated	PS2902MT11	Population 1
	Mianyang, Sichuan, China	October 2007	Cultivated	PS2902MT12	Population 1
	Mianyang, Sichuan, China	October 2007	Cultivated	PS2902MT05	Population 1
	Mianyang, Sichuan, China	October 2007	Cultivated	PS2902MT02	Population 1
	Mianyang, Sichuan, China	October 2007	Cultivated	PS2902MT06	Population 1
	Wanyuan, Sichuan, China	October 2007	Cultivated	PS2902MT09	Population 2
	Wanyuan, Sichuan, China	October 2007	Cultivated	PS2902MT10	Population 2
	Wanyuan, Sichuan, China	October 2007	Cultivated	PS2902MT04	Population 2
	Mao, Sichuan, China	July 2007	Cultivated	PS2902MT01	Population 3
	Mao, Sichuan, China	July 2007	Cultivated	PS2902MT07	Population 3
	Songpan, Sichuan, China	July 2007	Cultivated	PS2902MT03	Population 4
	Youyang, Chongqing, China	July 2007	Cultivated	PS2902MT08	Population 5
Rheum tanguticum Maxim. ex Balf.	Ruoergai, Sichuan, China	July 2007	Wild	PS2904MT01	Population 1
	Ruoergai, Sichuan, China	July 2007	Wild	PS2904MT02	Population 1
	Ruoergai, Sichuan, China	July 2007	Wild	PS2904MT03	Population 1
	Ruoergai, Sichuan, China	July 2007	Wild	PS2904MT04	Population 1
	Ruoergai, Sichuan, China	July 2007	Wild	PS2904MT05	Population 1
	Banma, Qinghai, China	August 2007	Cultivated	PS2904MT07	Population 2
	Banma, Qinghai, China	August 2007	Cultivated	PS2904MT08	Population 2
	Banma, Qinghai, China	August 2007	Cultivated	PS2904MT09	Population 2
	Maqu, Ganshu, China	August 2007	Cultivated	PS2904MT06	Population 3
Rheum palmatum L.	Mao, Sichuan, China	July 2007	Cultivated	PS2903MT01	Population 1
	Mao, Sichuan, China	July 2007	Cultivated	PS2903MT02	Population 1
Fagopyrum dibotrys (D. Don) Hara	Nanning, Guangxi, China	November 2007	Cultivated	PS0797MT01	
Fallopia aubertii (L. Henry) Holub	Beijing, China	September 2007	Cultivated	PS0799MT02	NO[a]
Polygonum bistorta L.	Beijing, China	September2007	Cultivated	PS0801MT01	
Polygonum aviculare L.	Beijing, China	September 2007	Cultivated	PS0785MT02	
Persicaria orientalis (L.) Spach (Syn. "*Polygonum orientale* L.")	Beijing, China	September 2007	Cultivated	PS0790MT01	
Fallopia multiflora (Thunb.) Haraldson (Syn. "*Polygonum multiflorum* Thunb.")	Beijing, China	September 2007	Cultivated	PS0789MT01	
Fallopia japonica (Houtt.) Ronse Decr. (Syn. "*Reynoutria japonica* Houtt.")	Beijing, China	September 2007	Cultivated	PS0792MT02	
Rheum rhaponticum L.	Beijing, China	July 2007	Cultivated	PS0804MT02	NO
Rumex acetosa L.	Beijing, China	July 2007	Cultivated	PS2905MT01	NO
Oxyria digyna Hill	Chengdu, Sichuan, China	December 2007	Cultivated	PS0803MT02	NO
Polygonum plebeium R. Br.	Putian, Fujian, China	December 2007	Wild	PS0806MT01	NO
Polygonum chinense L. (Syn. "*Persicaria chinensis* (L.) H. Gross")	Putian, Fujian, China	December 2007	Wild	PS0794MT02	NO
Rumex japonicus Houtt.	Putian, Fujian, China	December 2007	Wild	PS0807MT01	NO
Persicaria lapathifolia (L.) Gray (Syn. "*Polygonum lapathifolium* L.")	Putian, Fujian, China	December 2007	Wild	PS1683MT01	NO
Persicaria tinctoria (Ait.) H. Gross (Syn. "*Polygonum tinctorium* Ait.")	Beijing, China	January 2008	Bought	PS2901MT01	

[a] NO: the species are not in Chinese pharmacopoeia.

and the roots, leaves and stems of *Polygonum cuspidatum* (Huang et al., 2008) exhibited high antioxidant activity, rhubarb offers therapeutic potential for cardiovascular diseases (Wang et al., 2008). The rhubarb extract from *Rheum officinale* significantly attenuated radiation induced lung toxicity and improved pulmonary function (Yu et al., 2008). *Polygonum multiflorum* water extract exerts a preventive effect against cognitive deficits in Alzheimer's disease (Um et al., 2006). However, it is arduous to identify the species of *Rheum*, *Fallopia*, *Persicaria* and *Polygonum* of the family Polygonaceae because of morphological similarity and frequent variation. On TCM markets and medicinal plant production areas, roots and rhizomes of *Rheum rhaponticum* of section Rhapontica are sometimes confused with official rhubarb of section Palmata (Yang et al., 2004a), *Fallopia aubertii* is an adulterant of *Fallopia multiflora*. This situation has threatened the safety of using these medicines. Furthermore, emodin is a naturally occurring anthraquinone present in the roots of rhubarb, it is reported that it was considered a potential carcinogen and macroscopic lesions associated with concentration of emodin and exposed to weeks were present in the gallbladder and kidney of mice (National Toxicology Program, 2001). Consequently, rapid and correct authentication of rhubarb and Polygonaceae is very important to ensure drug safety on international trade of medicinal herbs. Accordingly, an easy and accurate method for identification of medicinal plants has attracted greater attention (Leon et al., 2006).

In the present study, we employed eight candidate DNA barcodes to explore which barcode is better for the authentication of the family Polygonaceae described in Chinese pharmacopoeia. We are addressing a series of questions. For example, what are the levels of intraspecific variation between individuals and between populations, what are the levels of interspecific divergence? And could the DNA barcodes be able to classify the species of family Polygonaceae in Chinese pharmacopoeia and their adulterants?

2. Materials and methods

2.1. Plant materials

Thirty-eight specimens were collected which belong to eighteen species of seven genera in the family Polygonaceae. These include thirty samples of all ten species and five genera described in Chinese pharmacopoeia (Table 1). Five populations of *Rheum officinale*, three populations of *Rheum tanguticum* and one population of *Rheum palmatum* grown in eight counties of four provinces in China