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基于叶绿体基因组变异位点的葛属(豆科)植物资源遗传多样性的分子鉴定新方法

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摘 要

精准鉴定遗传多样性是植物资源利用和深入开展科学研究的基础。本文利用葛属3个种的叶绿体基因组序列中的物种特有的624个核苷酸变异位点作为分子性状，首次编制了分子鉴定检索表，供试样品得到成功鉴定。物种特有变异位点的数量和核苷酸构成存在种间差异。食用葛的特有变异位点的数量(576)最多，粉葛(38)和葛藤(10)的特有变异位点的数量明显较少。食用葛的特有变异位点中，4类核苷酸的比例由大到小依次为：T (26.91%)、A (26.04%)、C (24.13%)和G (22.92%)，差异不大。粉葛的特有变异位点中，T (39.47%)的比例最高，随后依次为C (26.32%)、A (18.42%)和G (15.79%)，T的比例是G的比例的约2.5倍。葛藤的特有变异位点数量相对稀少，其中，T的比例(50.00%)最高，是A或G的比例(20.00%)的2.5倍，是C的比例(10.00%)的5倍。结果显示，叶绿体基因组的单核苷酸变异位点信息可用于葛属植物资源遗传多样性的分子鉴定。本文调查了中国过去120多年来葛属植物标本的收集现状，讨论了存在的问题与对策。本研究对于葛属植物的分类修订、种质资源的保护和利用具有重要价值。

关键词

豆科，葛属，植物遗传多样性，叶绿体基因组，核苷酸变异位点，分子鉴定

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A Novel Method for Molecular Identification of Genetic Diversity of Plant Resources in *Pueraria* DC. (Fabaceae) Based on Variable Base Sites of Complete Chloroplast Genome

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Abstract

Accurate identification of genetic diversity is the basis for utilization of plant resources and further scientific research. In this paper, 624 taxon-specific variable nucleotide sites in the complete chloroplast genome of 3 species from the genus *Pueraria* DC. were used as molecular traits to identify successfully the genetic resources of this plant genus and compile a molecular classification key for the first time. There are differences in aspects of amount and base composition of taxon-specific variable nucleotide sites among the species. The amount of taxon-specific variable nucleotide sites in *Pueraria edulis* Pamp. (576) is the highest, those in *Pueraria montana* var. *thomsonii* (Benth.) M.R. Almeida (38) and *Pueraria montana* (Lour.) Merr. (10) are significantly fewer. The proportion of T (26.91%), A (26.04%), C (24.13%) and G (22.92%) is decreasing respectively but with small differences among taxon-specific variable nucleotide sites in *Pueraria edulis* Pamp. The proportion of T (39.47%) is the highest, followed by C (26.32%), A (18.42%) and G (15.79%) in taxon-specific variable nucleotide sites in *Pueraria montana* var. *thomsonii*, the proportion of T is 2.5 times that of G. Relatively, the amount of taxon-specific variable nucleotide sites is fewer in *Pueraria montana*, where the proportion of T (50.00%) is the highest, being 2.5 times that (20.00%) of A or G, and 5 times that (10.00%) of C. Our results indicated that taxon-specific variable nucleotide sites from the chloroplast genomes could be used for the molecular classification of the genetic diversity in the genus *Pueraria* resources. In this paper, the status of *Pueraria* plant specimens collected in the past 120 years in China is investigated and problems and possible strategies are discussed. This study is valuable for taxonomic revision, conservation and utilization of *Pueraria* plant germplasm resources.

Keywords

Fabaceae, *Pueraria* DC., Plant Genetic Diversity, Chloroplast Genome, Variable Nucleotide Site, Molecular Identification

1. 引言

分类是为无序状态的单元提供一套鉴定单元的方法，并将单元排列出一个顺序。分子系统学已经证实可以解决分类和进化方面的各种问题。然而，分类结果不一定总能够反映自然关系。例如，豆科的葛属植物具有多样的遗传特征，在系统发育关系上是多系，该属植物的人为分类结果不反映自然关系。葛属的分类仍然是一个难题[1]-[18]。

200 年前，de Candolle (1825)发现块根葛藤 *Pueraria tuberosa* (Roxb. ex Willd.) DC.缺乏岩黄耆族 Hedysareae 大多数物种拥有的荚果特征，建立了新属：葛属 *Pueraria* DC.，其中包含块根葛藤和 *Pueraria wallichii* DC.。*Pueraria wallichii* 是 de Candolle 根据 Nathaniel Wallich 博士在尼泊尔采集的材料描述的一个种[1]-[12]。de Candolle 当时根据这两个种的共有形态特征将它们联系在一起，例如，攀爬和缠绕习性、木质的圆柱形茎、三出的阔叶、圆锥花序为细长形、腋生。后来的研究发现，*Pueraria wallichii* 是须弥葛 *Haymondia wallichii* (DC.) A. N. Egan & B. Pan 的异名，实际上隶属于豆科的另外一个属：须弥葛属 *Haymondia* A. N. Egan & B. Pan [1]-[12]。

目前，全球葛属植物已经识别出约 20 种，自然分布于东亚、南亚、东南亚和西太平洋地区(日本、朝鲜、中国、印度、越南、菲律宾、澳大利亚等地)，具有重要的经济价值，是纺织品、食物、造纸和建筑材料的原料。各地有引种栽培。例如，通过 1876 年的费城百周年工业博览会(Centennial Exposition in Philadelphia)，野葛 *Pueraria montana* var. *lobata* (Willd.) Maesen & S. M. Almeida ex Sanjappa & Predeep (Kudzu)从亚洲引种到美国，用于控制水土流失和牲畜饲料，现在野葛在当地已经变成了一种高危的入侵植物[1]-[18]。*Pueraria phaseoloides* (Roxb.) Benth.在热带地区广泛引种，用于饲料和肥田的农作物(Cover crop)。后来的分类研究发现，*Pueraria phaseoloides* (Roxb.) Benth.是草葛 *Neustanthus phaseoloides* (Roxb.) Benth.的异名，应该归类在豆科的另外一个属：草葛属 *Neustanthus* Benth. [1]-[18]。

早期的几项分类处理已经意识到葛属植物不体现自然类群[1]-[12]。根据形态特征(例如，每节上的花朵数目、托叶类型、花萼的类型、荚果类型、种子形状)，Lackey (1977)将葛属植物暂时大致分为 4 组(A~D)，认为 A、B 和 C 三个组的物种应该位于葛属内，他同时自相矛盾地提到，C 组内的物种与长序大豆属 *Neonotonia* J.A. Lackey 的亲缘关系较近，可以从葛属删除[1]-[12]。Lackey (1977)认为，B 组内的物种明显不同，足以建立一个新属；根据形态特征以及含有化学成分刀豆氨酸(canavanine)，D 组内的物种应该从葛属中排除[3] [4]。在 Lackey 工作的基础上，van der Maesen 对葛属植物进行了深入研究，识别出 15 个种和几个变种[2]-[12]，建立了 3 个组：1) 葛组 *Pueraria* section *Pueraria* 的形态特征定义是每节上的花的数目为 2~3 朵、具有盾状托叶、种子为扁圆形；2) 多糖块根组 *Pueraria* section *Schizophyllon* Baker，仅包含 *Pueraria phaseoloides* (Roxb.) Benth.，形态特征定义是每节上的花的数目为 4~6 朵，基部着生有托叶，荚果为圆筒状，种子为桶状。后来的研究发现，*Pueraria phaseoloides* (Roxb.) Benth.是豆科草葛属的草葛的异名，即隶属于豆科的另外一个属；3) *Pueraria* section *Breviramulae* van der Maesen 组，形态特征的定义是短枝上每节的花的数目为 4 朵或以上、基部着生有托叶、荚果为扁平状、种子为扁平的卵球形[2]-[12]。

Egan 等(2016) [12]的取样覆盖了葛属内近 80%的现存物种，利用三个 DNA 片段：1 个细胞核 DNA 标记(*AS*2)和 2 个叶绿体 DNA 标记(*mat*K 和 *trn*D-*trn*T)，系统发育关系分析结果显示，葛属内的物种是多

系(存在 5 个谱系)，认为应该承认草葛属的分类地位，应该将 *Pueraria stricta* Kurz 移到琼豆属 *Teyleria* Backer 内，*Pueraria stricta* 是豆科琼豆属的紫花琼豆 *Teyleria stricta* (Kurz) A. N. Egan & B. Pan 的异名，提出建立两个新属：须弥葛属和苦葛属 *Toxicopueraria* A. N. Egan & B. Pan。

葛属植物的种类鉴定研究经历了形态学、细胞学、化学成分、同工酶、RAPD、SRAP 标记、SSR 标记、DNA 条形码技术等阶段[1]-[18]，但是都存在稳定性差、信息量和分辨力有限等问题。实际中，同名异物和同物异名现象导致资源名称混乱使用，也是制约着相关科研和产业发展的重要因素。近年来，叶绿体全基因组序列数据提供的遗传变异位点稳定，信息量较大，实验成本较低，性价比好，方法较简单，已广泛应用于植物的物种鉴定和系统发育研究[15]-[32]。为促进葛属植物的资源保护和可持续利用，本文报道在分子水平上鉴定葛属植物资源遗传多样性的一种新方法。

Table 1. Samples and Genbank accession numbers of chloroplast genome used in this study
表 1. 供试样品及叶绿体基因组序列号

	拉丁学名	中文名称	序列号
1	*Pueraria edulis* Pamp.	食用葛	OM686893.1
2	*Pueraria edulis* Pamp.	食用葛	OM048895.1
3	*Pueraria montana* (Lour.) Merr.	葛藤	OM156458.1
4	*Pueraria montana* (Lour.) Merr.	葛藤	OP963905.1
5	*Pueraria montana* (Lour.) Merr.	葛藤	OP963906.1
6	*Pueraria montana* (Lour.) Merr.	葛藤	OP963907.1
7	*Pueraria montana* (Lour.) Merr.	葛藤	OP963908.1
8	*Pueraria montana* var. *thomsonii* (Benth.) M.R. Almeida	粉葛	OM156440.1
9	*Pueraria montana* var. *thomsonii* (Benth.) M.R. Almeida	粉葛	OM156442.1
10	*Pueraria montana* var. *thomsonii* (Benth.) M.R. Almeida	粉葛	OM156453.1
11	*Amphicarpaea ferruginea* Benth.	锈毛两型豆*	ON050971.1
12	*Orbexilum onobrychis* (Nutt.) Rydb.	皱荚红豆*	

注：*外类群。

Table 2. The amount and base composition of variable nucleotide sites for classification of three species/varietas from the genus *Pueraria* DC. of the Fabaceae
表 2. 豆科葛属 3 种/变种的具有分类价值的核苷酸变异位点数目及碱基构成

*	A (%)**	T (%)**	C (%)**	G (%)**	合计(%)***
1a	150 (26.04)	155 (26.91)	139 (24.13)	132 (22.92)	576 (92.31/75.10)
1b	165 (28.65)	171 (29.69)	127 (22.05)	113 (19.62)	576 (92.31/75.10)
2a	2 (20.00)	5 (50.00)	1 (10.00)	2 (20.00)	10 (1.60/1.30)
2b	3 (30.00)	0 (0.00)	3 (30.00)	4 (40.00)	10 (1.60/1.30)
3a	7 (18.42)	15 (39.47)	10 (26.32)	6 (15.79)	38 (6.09/4.95)
3b	12 (31.58)	6 (15.79)	12 (31.58)	8 (21.05)	38 (6.09/4.95)
合计					624/767

注：*该列的序号与检索表内的序号对应；1a 为食用葛 *Pueraria edulis*，2a 为葛藤 *Pueraria montana*，3a 为粉葛 *Pueraria montana* var. *thomsonii*。**核苷酸变异位点数(在 4 种碱基中的占比)；***此列括号中，前一个数字是在物种特有变异位点总数(624)中所占的比例；后一个数字是在全部核苷酸变异位点总数(767)中所占的比例。

1a. Type $C_{115}A_{134}T_{253}T_{2008}G_{2077}A_{2084}T_{2333}C_{2664}T_{3033}T_{3059}C_{3281}C_{3451}A_{3715}A_{4096}A_{4404}C_{4494}A_{4524}G_{4836}$
$A_{4857}C_{4954}G_{5038}A_{5089}G_{5234}A_{5264}T_{5266}A_{5373}T_{5518}A_{5557}A_{5820}C_{5893}T_{6009}T_{6049}C_{6120}T_{6125}G_{6129}C_{6766}T_{6864}C_{8219}$
$G_{8378}C_{8567}T_{9196}G_{9563}A_{10026}C_{10201}T_{10263}G_{10269}T_{10627}G_{10763}C_{11211}C_{11266}A_{12230}G_{12243}C_{12638}T_{13136}A_{13276}C_{13631}$
$C_{13906}A_{13967}A_{14061}A_{14253}C_{14460}G_{14486}C_{14886}G_{14896}T_{14943}T_{15281}G_{15289}C_{15829}G_{16071}A_{16098}C_{16303}C_{16486}G_{16527}$
$C_{16529}T_{16581}A_{16637}A_{17215}C_{17274}A_{17284}G_{17477}G_{17786}T_{17802}C_{18649}A_{18745}A_{18925}T_{18971}C_{19079}G_{20407}G_{20609}G_{21212}$
$C_{22323}C_{22579}C_{22585}A_{22908}G_{24730}A_{24904}T_{24919}T_{25049}T_{25112}C_{25113}C_{25248}C_{25307}A_{25393}T_{25433}A_{25915}A_{26043}T_{26259}$
$A_{27267}G_{28399}T_{28913}T_{29058}G_{29123}A_{29289}T_{29291}T_{29294}G_{29340}T_{29505}C_{29605}A_{29778}T_{29787}T_{30137}C_{30276}G_{30314}A_{30353}$
$G_{30750}T_{30808}A_{31345}A_{31383}C_{31483}G_{31781}C_{31859}A_{32247}T_{32271}A_{32318}A_{32375}C_{32495}G_{32524}T_{32577}A_{32633}A_{32699}C_{32743}$
$C_{32801}C_{32891}T_{32919}A_{33059}A_{33086}C_{33409}G_{33717}C_{33749}A_{33974}G_{34295}A_{34296}T_{34479}G_{34926}G_{36037}A_{37817}A_{37934}C_{37939}$
$T_{37997}G_{38141}A_{39038}G_{39075}A_{39349}G_{39753}C_{40458}G_{40739}A_{40754}T_{40864}T_{41001}T_{41072}G_{41297}A_{41772}A_{41895}T_{42747}G_{43050}$
$A_{43412}A_{43581}A_{44404}T_{44411}T_{44415}T_{44417}A_{44418}C_{44419}C_{44420}G_{44421}T_{44423}A_{44425}A_{44428}C_{44430}T_{44431}C_{44432}A_{44433}$
$A_{44435}C_{44438}G_{44440}T_{44442}C_{44443}T_{44444}C_{44445}G_{44446}T_{44448}T_{44452}T_{44459}C_{45507}C_{45558}G_{45581}A_{45859}C_{45889}G_{46635}$
$C_{47553}G_{47691}G_{47792}T_{47940}T_{47995}G_{48019}A_{48114}A_{48278}T_{48319}G_{48371}G_{48417}A_{48495}T_{48556}G_{48833}G_{48896}C_{49025}G_{49107}$
$C_{49152}C_{49153}T_{49159}G_{49320}T_{49322}C_{49343}T_{49426}G_{50090}A_{50130}A_{50131}A_{50132}A_{50717}A_{50847}A_{51072}C_{51201}C_{51252}A_{51405}$
$A_{51423}C_{51489}A_{51930}T_{52329}T_{52671}T_{52673}A_{52683}C_{52732}A_{53087}T_{53153}C_{53301}A_{53307}C_{53765}G_{53843}G_{53997}G_{54139}C_{54234}$
$G_{54693}C_{54878}A_{54958}A_{55043}A_{55234}T_{55310}G_{55396}A_{55434}C_{55470}C_{55616}T_{55619}C_{55620}T_{55625}T_{55883}T_{55943}C_{55995}C_{56021}$
$A_{56087}A_{56162}G_{56194}G_{56245}G_{56720}A_{56883}G_{56899}G_{56924}A_{57009}T_{57047}C_{57440}T_{57650}C_{57834}T_{57899}G_{57944}G_{58074}T_{58101}$
$T_{58631}T_{58634}A_{58773}A_{59685}A_{59703}A_{59707}G_{60008}A_{60228}C_{60537}T_{60582}C_{60601}C_{60694}C_{60763}T_{60764}T_{60765}T_{60766}T_{60767}$
$G_{60970}A_{60975}A_{60976}C_{61018}C_{61882}A_{61914}A_{61944}C_{62041}G_{62070}C_{62403}A_{63016}T_{63389}G_{63644}A_{63685}C_{63825}C_{64004}C_{64496}$
$A_{64523}T_{65117}A_{65118}A_{65147}C_{65330}A_{65575}T_{65613}T_{65743}C_{65786}T_{65827}G_{65878}C_{66282}A_{66367}G_{66433}A_{66799}G_{66824}A_{66826}$
$C_{67016}A_{67203}C_{67445}G_{67848}G_{67876}C_{68278}G_{68461}T_{68464}G_{68465}A_{68655}C_{68781}A_{68963}A_{69059}T_{69105}T_{69391}C_{69961}T_{69962}$
$T_{69968}C_{70028}C_{70141}A_{70286}G_{70455}A_{70686}T_{70893}C_{70958}A_{71034}T_{71283}A_{71401}A_{71402}G_{71464}T_{71539}C_{72306}T_{72730}T_{73000}$
$T_{74212}G_{74366}C_{74644}T_{74869}T_{75270}T_{75274}T_{75612}C_{75678}C_{75753}G_{75794}A_{76588}A_{76913}T_{76971}C_{77245}T_{77308}A_{77781}A_{77964}$
$G_{78434}T_{78491}C_{78554}C_{78841}C_{78870}C_{79188}C_{79215}G_{79267}A_{79533}G_{79833}C_{80168}C_{80262}T_{80466}G_{80686}A_{80962}A_{81001}C_{81003}$
$C_{81081}G_{81205}G_{81568}T_{81629}C_{81977}A_{82174}A_{82198}G_{82231}G_{82294}G_{82342}G_{82417}C_{82457}G_{82540}A_{82731}G_{82899}C_{82922}T_{83127}$
$T_{83149}C_{83307}G_{83352}T_{83404}C_{83741}A_{84153}T_{84315}T_{84374}A_{85350}G_{87710}G_{91077}G_{92364}T_{94807}G_{94809}T_{98103}T_{100711}G_{108663}$
$T_{108942}C_{108944}T_{108945}G_{108946}A_{109712}G_{110361}C_{110458}T_{110696}G_{110751}T_{110800}G_{111026}T_{111215}G_{111288}G_{111317}G_{111417}$
$G_{111728}T_{111748}G_{111890}A_{112458}G_{112750}T_{112777}T_{112778}T_{112779}G_{112828}C_{113156}C_{113307}T_{113308}A_{113314}T_{113352}A_{113441}$

$A_{113533}A_{114024}G_{114165}C_{114239}T_{114266}G_{114327}A_{114336}G_{114648}C_{114706}T_{114825}T_{114916}G_{114960}C_{115941}A_{116543}T_{116546}$
$T_{116547}A_{116750}A_{116943}T_{116946}G_{116991}G_{117369}A_{117508}G_{117514}G_{117682}T_{118366}G_{118516}T_{119733}T_{119882}T_{119935}C_{119941}$
$T_{120035}G_{120191}T_{120351}G_{120653}A_{120750}G_{120768}G_{120832}G_{120983}T_{121384}C_{121692}T_{121755}C_{121953}A_{122348}T_{123009}C_{123030}$
$T_{123062}A_{123136}T_{123229}T_{123249}A_{123681}A_{123689}T_{123741}T_{123855}T_{123880}G_{124306}T_{124403}T_{124622}A_{124907}A_{124917}C_{124924}$
$C_{124925}C_{124926}A_{125067}A_{125276}T_{125341}A_{125393}G_{125398}G_{125441}C_{125927}C_{126112}T_{126430}A_{126583}C_{127036}T_{127122}G_{127136}$
$T_{127411}G_{127439}C_{127670}T_{127684}C_{127734}A_{127755}T_{127990}A_{128057}C_{128083}G_{128093}G_{128230}A_{128307}T_{128315}G_{128350}T_{129002}$
$C_{130051}A_{138003}A_{140611}A_{143917}G_{143918}C_{146350}C_{147637}C_{151004}T_{153368}$ .... **食用葛 *Pueraria edulis***

1b. Type $T_{115}T_{134}A_{253}G_{2008}C_{2077}G_{2084}A_{2333}T_{2664}G_{3033}G_{3059}T_{3281}T_{3451}C_{3715}C_{4096}C_{4404}A_{4494}C_{4524}A_{4836}$
$C_{4857}A_{4954}T_{5038}C_{5089}A_{5234}T_{5264}A_{5266}C_{5373}C_{5518}T_{5557}C_{5820}T_{5893}C_{6009}C_{6049}A_{6120}A_{6125}T_{6129}T_{6766}C_{6864}T_{8219}$
$C_{8378}T_{8567}C_{9196}T_{9563}C_{10026}A_{10201}A_{10263}T_{10269}C_{10627}T_{10763}G_{11211}G_{11266}C_{12230}A_{12243}T_{12638}C_{13136}C_{13276}T_{13631}$
$T_{13906}C_{13967}C_{14061}C_{14253}T_{14460}A_{14486}A_{14886}T_{14896}G_{14943}A_{15281}T_{15289}G_{15829}C_{16071}T_{16098}T_{16303}G_{16486}A_{16527}$
$A_{16529}G_{16581}C_{16637}G_{17215}A_{17274}T_{17284}A_{17477}T_{17786}G_{17802}A_{18649}T_{18745}T_{18925}G_{18971}T_{19079}A_{20407}A_{20609}T_{21212}$
$T_{22323}A_{22579}T_{22585}C_{22908}A_{24730}C_{24904}G_{24919}C_{25049}A_{25112}A_{25113}A_{25248}A_{25307}G_{25393}C_{25433}G_{25915}C_{26043}C_{26259}$
$T_{27267}A_{28399}A_{28913}A_{29058}A_{29123}T_{29289}A_{29291}A_{29294}A_{29340}G_{29505}A_{29605}C_{29778}A_{29787}G_{30137}T_{30276}T_{30314}C_{30353}$
$A_{30750}C_{30808}T_{31345}C_{31383}T_{31483}A_{31781}T_{31859}C_{32247}G_{32271}G_{32318}T_{32375}T_{32495}T_{32524}C_{32577}T_{32633}T_{32699}A_{32743}$
$A_{32801}G_{32891}G_{32919}T_{33059}G_{33086}T_{33409}T_{33717}T_{33749}C_{33974}T_{34295}C_{34296}C_{34479}A_{34926}T_{36037}G_{37817}G_{37934}T_{37939}$
$C_{37997}T_{38141}C_{39038}A_{39075}G_{39349}A_{39753}A_{40458}T_{40739}G_{40754}G_{40864}C_{41001}G_{41072}A_{41297}T_{41772}G_{41895}G_{42747}T_{43050}$
$C_{43412}C_{43581}T_{44404}A_{44411}A_{44415}C_{44417}G_{44418}A_{44419}G_{44420}A_{44421}C_{44423}G_{44425}T_{44428}T_{44430}G_{44431}A_{44432}G_{44433}$
$T_{44435}T_{44438}A_{44440}C_{44442}G_{44443}G_{44444}T_{44445}A_{44446}A_{44448}C_{44452}A_{44459}A_{45507}A_{45558}A_{45581}C_{45859}T_{45889}C_{46635}$
$T_{47553}C_{47691}T_{47792}A_{47940}A_{47995}A_{48019}C_{48114}G_{48278}G_{48319}A_{48371}C_{48417}G_{48495}G_{48556}A_{48833}A_{48896}A_{49025}A_{49107}$
$T_{49152}T_{49153}A_{49159}T_{49320}G_{49322}A_{49343}A_{49426}A_{50090}T_{50130}T_{50131}T_{50132}C_{50717}C_{50847}G_{51072}T_{51201}T_{51252}G_{51405}$
$G_{51423}T_{51489}G_{51930}G_{52329}A_{52671}A_{52673}T_{52683}A_{52732}T_{53087}G_{53153}T_{53301}C_{53307}T_{53765}T_{53843}C_{53997}T_{54139}A_{54234}$
$C_{54693}T_{54878}T_{54958}C_{55043}G_{55234}A_{55310}T_{55396}C_{55434}T_{55470}A_{55616}G_{55619}A_{55620}G_{55625}A_{55883}G_{55943}T_{55995}A_{56021}$
$T_{56087}T_{56162}C_{56194}A_{56245}T_{56720}C_{56883}T_{56899}A_{56924}T_{57009}A_{57047}T_{57440}A_{57650}A_{57834}A_{57899}A_{57944}A_{58074}G_{58101}$
$A_{58631}C_{58634}C_{58773}G_{59685}T_{59703}T_{59707}C_{60008}T_{60228}A_{60537}C_{60582}G_{60601}A_{60694}A_{60763}A_{60764}A_{60765}A_{60766}G_{60767}$
$C_{60970}C_{60975}T_{60976}A_{61018}A_{61882}C_{61914}T_{61944}A_{62041}A_{62070}A_{62403}T_{63016}G_{63389}A_{63644}T_{63685}T_{63825}A_{64004}T_{64496}$
$G_{64523}A_{65117}T_{65118}C_{65147}A_{65330}C_{65575}C_{65613}C_{65743}A_{65786}C_{65827}C_{65878}A_{66282}C_{66367}T_{66433}T_{66799}T_{66824}T_{66826}$
$A_{67016}T_{67203}A_{67445}T_{67848}T_{67876}T_{68278}A_{68461}C_{68464}A_{68465}T_{68655}A_{68781}G_{68963}T_{69059}C_{69105}C_{69391}A_{69961}C_{69962}$

$A_{69968}A_{70028}T_{70141}G_{70286}T_{70455}C_{70686}G_{70893}T_{70958}T_{71034}A_{71283}C_{71401}C_{71402}T_{71464}G_{71539}A_{72306}C_{72730}C_{73000}$
$C_{74212}T_{74366}G_{74644}A_{74869}G_{75270}C_{75274}G_{75612}T_{75678}A_{75753}A_{75794}T_{76588}C_{76913}G_{76971}A_{77245}G_{77308}G_{77781}C_{77964}$
$A_{78434}A_{78491}T_{78554}G_{78841}G_{78870}T_{79188}A_{79215}T_{79267}C_{79533}T_{79833}A_{80168}T_{80262}C_{80466}T_{80686}T_{80962}C_{81001}A_{81003}$
$A_{81081}A_{81205}T_{81568}C_{81629}T_{81977}C_{82174}T_{82198}T_{82231}T_{82294}T_{82342}A_{82417}G_{82457}T_{82540}G_{82731}T_{82899}A_{82922}G_{83127}$
$C_{83149}A_{83307}T_{83352}C_{83404}T_{83741}T_{84153}G_{84315}G_{84374}C_{85350}A_{87710}C_{91077}A_{92364}G_{94807}T_{94809}G_{98103}C_{100711}T_{108663}$
$A_{108942}A_{108944}A_{108945}A_{108946}G_{109712}A_{110361}T_{110458}G_{110696}A_{110751}C_{110800}T_{111026}C_{111215}C_{111288}C_{111317}C_{111417}$
$A_{111728}C_{111748}A_{111890}G_{112458}A_{112750}C_{112777}A_{112778}A_{112779}T_{112828}A_{113156}A_{113307}G_{113308}T_{113314}C_{113352}G_{113441}$
$C_{113533}C_{114024}A_{114165}A_{114239}A_{114266}T_{114327}C_{114336}T_{114648}A_{114706}G_{114825}G_{114916}T_{114960}T_{115941}G_{116543}A_{116546}$
$A_{116547}G_{116750}T_{116943}A_{116946}A_{116991}A_{117369}T_{117508}T_{117514}T_{117682}C_{118366}T_{118516}G_{119733}G_{119882}A_{119935}A_{119941}$
$G_{120035}T_{120191}A_{120351}C_{120653}T_{120750}T_{120768}A_{120832}T_{120983}G_{121384}T_{121692}G_{121755}T_{121953}G_{122348}C_{123009}T_{123030}$
$A_{123062}C_{123136}G_{123229}C_{123249}C_{123681}C_{123689}G_{123741}C_{123855}G_{123880}T_{124306}A_{124403}C_{124622}G_{124907}T_{124917}A_{124924}$
$A_{124925}T_{124926}C_{125067}G_{125276}G_{125341}G_{125393}T_{125398}C_{125441}G_{125927}T_{126112}G_{126430}C_{126583}G_{127036}G_{127122}T_{127136}$
$G_{127411}A_{127439}T_{127670}A_{127684}G_{127734}G_{127755}G_{127990}G_{128057}T_{128083}T_{128093}C_{128230}G_{128307}A_{128315}T_{128350}C_{129002}$
$A_{130051}G_{138003}C_{140611}C_{143917}T_{143918}T_{146350}G_{147637}T_{151004}G_{153368}$

.... 食用葛之外的 2 个种和变种 The two species/varietas other than *Pueraria edulis*

2a. Type $T_{4400}T_{6328}C_{33518}T_{33590}T_{59677}A_{67115}G_{68673}A_{114837}T_{120487}G_{124566}$

.. 葛藤 *Pueraria montana*

2b. Type $C_{4400}C_{6328}A_{33518}G_{33590}G_{59677}G_{67115}A_{68673}G_{114837}C_{120487}A_{124566}$

.... 葛藤之外的 2 个种和变种 The two species/varietas other than *Pueraria montana*

3a. Type $G_{5095}G_{8222}A_{10128}T_{12131}C_{14863}A_{15631}C_{18703}C_{19231}C_{23619}C_{24827}T_{25110}C_{26000}T_{29166}T_{30204}C_{31980}$
$G_{35104}T_{38329}A_{45840}T_{50189}T_{54934}T_{55839}G_{57156}A_{57844}T_{58395}T_{66828}A_{67960}G_{70370}T_{81042}C_{83833}A_{94433}G_{111719}C_{112998}$
$T_{114949}C_{115047}T_{119988}T_{124120}A_{125504}T_{144281}$ **......................... 粉葛 *Pueraria montana* var. *thomsonii***

3b. Type $T_{5095}A_{8222}G_{10128}C_{12131}A_{14863}G_{15631}A_{18703}G_{19231}A_{23619}G_{24827}A_{25110}A_{26000}C_{29166}C_{30204}A_{31980}$
$T_{35104}C_{38329}G_{45840}C_{50189}G_{54934}C_{55839}T_{57156}C_{57844}G_{58395}A_{66828}C_{67960}T_{70370}C_{81042}T_{83833}G_{94433}A_{111719}A_{112998}$
$A_{114949}A_{115047}C_{119988}C_{124120}T_{125504}C_{144281}$

.... 粉葛之外的 2 个种和变种 The two species other than *Pueraria montana* var. *thomsonii*

Figure 1. Molecular classification key to three species/varietas of the genus *Pueraria* DC. of the Fabaceae based on the taxon-specific variable nucleotide sites from the complete chloroplast genome
图 1. 基于叶绿体基因组的物种特有核苷酸变异位点的豆科葛属 3 个种/变种的分子分类检索表

2. 材料与方法

选取 10 份样品代表葛属的 3 个种。供试样品名称及其叶绿体基因组的序列号见表 1 (https://www.ncbi.nlm.nih.gov)。每个种/变种取样 2~5 个体。利用 MAFFT v7.055b 软件[33] (http://mafft.cbrc.jp/alignment/software)获得比对序列矩阵。比对后的序列矩阵的长度为 154,037 个核苷酸，由左向右，左端(5'-端)起的第 1 个核苷酸字母的位置编号为 1，最右端的核苷酸字母的位置编号为 154,037。用 MEGA 7.0 [34]和 DnaSP v6 软件[35]检测核苷酸变异位点(见表 2 和图 2)。仅保留种/变种间有差异的、种/变种内个体间一致的变异位点代表物种/变种的特有核苷酸变异位点，作为分子分类性状，利用本团队研发的方法[25]-[32]，编制葛属供试样品的分子分类检索表(见图 1)。豆科两型豆属 *Amphicarpaea* Elliott ex Nutt.的锈毛两型豆 *Amphicarpaea ferruginea* Benth.以及皱荚豆属 *Orbexilum* Raf.的皱荚红豆 *Orbexilum onobrychis* (Nutt.) Rydb.为外类群(见表 1 和图 2)。用 MEGA 7.0 软件的 Tamura 3-parameter model 参数模型推断系统发生关系(见图 2)。

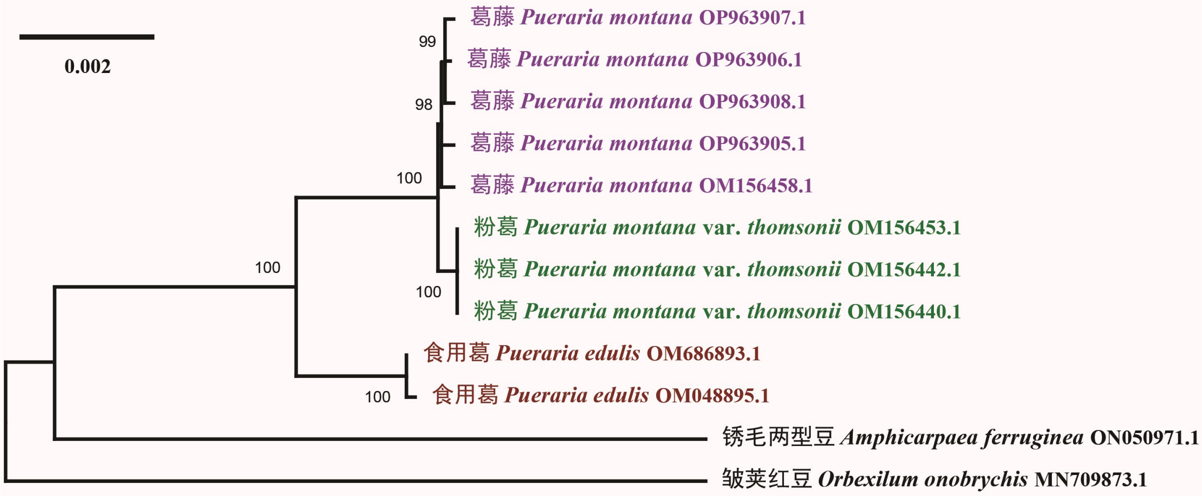

Figure 2. Phylogenetic tree of the three species/varietas of the genus *Pueraria* DC. based on complete chloroplast genome using the neighbour-joining method with the Tamura 3-parameter model (The numbers near the branches are bootstrap support values (%) of 1000 replications)

图 2. 利用邻接法和 Tamura 3 参数模型获得的基于叶绿体全基因组序列的葛属 3 个种/变种的系统发生关系(分支图中的数字为 1000 次重复抽样的自展支持率)

3. 结果

葛属供试样品的叶绿体基因组的长度为 153,368 (OM156458.1，葛藤 *Pueraria montana*)~153,551 (OM048895.1，食用葛 *Pueraria edulis*)个核苷酸。在比对序列中，共检测到 767 个核苷酸变异位点，占叶绿体基因组序列全长的约 0.50%。其中，各物种的特有核苷酸变异位点数目合计为 624 个核苷酸，占变异位点总数的 81.36%。食用葛的特有变异位点的数量(576)最多，粉葛(38)和葛藤(10)的特有变异位点的数量明显较少(见图 1 和表 2)。食用葛的特有变异位点中，4 类核苷酸的比例由大到小依次为：**T** (26.91%)、**A** (26.04%)、**C** (24.13%)和 **G** (22.92%)，差异不大。粉葛的特有变异位点中，**T** (39.47%)的比例最高，随后依次为 **C** (26.32%)、**A** (18.42%)和 **G** (15.79%)，**T** 的比例是 **G** 的比例的约 2.5 倍。葛藤的特有变异位点数量相对稀少，其中，**T** 的比例(50.00%)最高，是 **A** 或 **G** 的比例(20.00%)的 2.5 倍，是 **C** (10.00%)的比例的 5 倍(见表 2)。特有变异位点的数量和核苷酸构成存在种间差异。利用特有核苷酸变异位点，编制分子分类检索表，供试样品得到精准鉴定(见图 1)。系统发生关系树状图中，食用葛位于较基部位置(见图 2)。

Table 3. Specimens of *Pueraria* in the Chinese Virtual Herbarium
表 3. 中国数字植物标本库馆藏的葛属植物标本

	种/变种	中文名	标本份数
1	*Pueraria alopecuroides* Craib	密花葛	56
2	*Pueraria bella* Prain		1
3	*Pueraria boufffordii* H. Ohashi	贵州葛	2
4	*Pueraria calycina* Franch.	黄毛野葛	16
5	*Pueraria candollei* Wall. ex Benth.		2
6	*Pueraria edulis* Pamp.	食用葛	92
7	*Pueraria grandiflora* B. Pan bis & Bing Liu	大花葛	1
8	*Pueraria montana* (Lour.) Merr.	葛藤	724
9	*Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida ex Sanjappa & Predeep	野葛	3755
10	*Pueraria montana* (Lour.) Merr. var. *montana*	山葛藤	32
11	*Pueraria montana* var. *thomsonii* (Benth.) M.R. Almeida	粉葛	394
12	*Pueraria neocaledonica* Harms	新几内亚葛	1
13	*Pueraria pulcherrima* (Koord.) Koord.-Schum.		2
14	*Pueraria xyzhui* H. Ohashi & Iokawa		2
合计			5080

4. 讨论

中国数字植物标本库(http://www.cvh.ac.cn/)的数据显示，葛属标本的采集记录最早是 1900 年，经过 120 多年的积累，来自国内外的葛属植物标本共计 5080 份，14 个种/变种，含形态特征图片和文字描述，占葛属植物全球物种数量(20 种)的约 70% (表 3)。存在同物异名和同名异物现象(https://powo.science.kew.org/)，例如，*Pueraria forrestii* W.E. Evans 是黄毛野葛 *Pueraria calycina* Franch.的异名；*Pueraria bicalcarata* Gagnep.及 *Pueraria edulis* var. *likiangensis* P.C. Li 是食用葛 *Pueraria edulis* Pamp.的异名；*Pueraria lobata* var. *montana* (Lour.) Maesen 及 *Pueraria omeiensis* T. Tang & Wang 是葛藤 *Pueraria montana* (Lour.) Merr.的异名；*Pueraria hirsuta* (Thunb.) C.K. Schnei、*Pueraria koten* H. Lév. & Vaniot、*Pueraria lobata* (Willd.) Ohwi、*Pueraria pseudohirsuta* T. Tang & Wang 及 *Pueraria thunbergiana* (Siebold & Zucc.) Benth.是野葛 *Pueraria montana* var. *lobata* (Willd.) Maesen & S. M. Almeida ex Sanjappa & Predeep 的异名；*Pueraria thunbergiana* var. *formosana* Hosok.及 *Pueraria tonkinensis* Gagnep.是山葛藤 *Pueraria montana* var. *montana* 的异名；*Pueraria lobata* var. *thomsonii* (Benth.) Maesen、*Pueraria lobata* subsp. *thomsonii* (Benth.) H. Ohashi & Tateishi 及 *Pueraria thomsonii* Benth.是粉葛 *Pueraria montana* var. *thomsonii* (Benth.) M.R. Almeida 的异名。

如下 11 个名称已被分类处理为豆科其它 6 个属的物种的异名，例如，*Pueraria seguinii* H. Lév.是豆科木豆属 *Cajanus* Adans.的大花虫豆 *Cajanus grandiflorus* (Benth. ex Baker) Maesen 的异名；*Pueraria wallichii* DC.是豆科须弥葛属 *Haymondia* A. N. Egan & B. Pan 的须弥葛 *Haymondia wallichii* (DC.) A. N. Egan & B. Pan 的异名；*Pueraria phaseoloides* (Roxb.) Benth.及 *Pueraria phaseoloides* var. *phaseoloides* 是豆科草葛属 *Neustanthus* Benth.的草葛 *Neustanthus phaseoloides* (Roxb.) Benth.的异名；*Pueraria javanica* (Benth.)

Benth.及 *Pueraria phaseoloides* var. *javanica* (Benth.) Baker 是草葛属的爪哇葛 *Neustanthus phaseoloides* var. *javanicus* (Benth.) A. N. Egan & B. Pan 的异名；*Pueraria collettii* Prain 及 *Pueraria stricta* Kurz 是豆科琼豆属 *Teyleria* Backer 的紫花琼豆 *Teyleria stricta* (Kurz) A. N. Egan & B. Pan 的异名；*Pueraria peduncularis* (Benth.) Graham ex Benth.及 *Pueraria peduncularis* var. *violacea* Franch.是豆科苦葛属 *Toxicopueraria* A. N. Egan & B. Pan 的苦葛 *Toxicopueraria peduncularis* (Benth.) A. N. Egan & B. Pan 的异名；*Pueraria yunnanensis* Franch.是苦葛属的云南苦葛 *Toxicopueraria yunnanensis* (Franch.) A. N. Egan & B. Pan 的异名；*Pueraria chaneti* H. Lév 是豆科绿豆属 *Vigna* Savi 的绿豆 *Vigna radiata* (L.) R. Wilczek 的异名。

51%以上的葛属植物标本的采集日期为 50 年以前。葛属有 7 个种/变种拥有 3 份以上的标本，例如，野葛 *Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida ex Sanjappa & Predeep (3755 份)、葛藤 *Pueraria montana* (Lour.) Merr. (724 份)以及粉葛 *Pueraria montana* var. *thomsonii* (Benth.) M. R. Almeida (394 份)，即 50%的物种的标本的合计份数占到了该属的标本总数的 99.78%。50%的种/变种馆藏标本不足 3 份。现有的标本材料不足，严重制约了葛属的全球所有物种的分子鉴定和系统发生关系研究。在出现过的葛属物种名称(41)中，异名(27)占 65.85%。同名异物和同物异名造成了一定的混乱，制约着物种鉴定研究和相关领域的发展。对 21 国 40 个植物标本馆的 4500 份标本的取样调查结果显示，50%以上的热带植物标本存在名称鉴定错误[36]。叶绿体基因组序列使我们有机会在微观世界的约 15 万个核苷酸这样的大数据中筛选体现物种间遗传差异的分子性状，与依赖形态性状的物种鉴定相比，信息量增加了 100~1000 倍左右，提高了物种鉴定的分辨力和客观性。分子鉴定是对形态鉴定的辅助、补充和验证。利用形态鉴定可靠的标本或活植物作为研究材料，建立叶绿体基因组数据库，也有助于标本馆科尾存疑标本的清理、核准和鉴定。叶绿体基因组的物种标准数据库一旦成功建立，可用于未知样品的物种鉴定。全球范围内调查和补充采集葛属植物不同基因型的标本和活植物，有助于推动该属植物资源的分类修订和可持续利用，工作量仍然很大[37]-[39]。分子检索表中的具有分类价值的单核苷酸变异位点的形成机制，有待利用化学和量子科学领域的新技术深入研究。

5. 结论与展望

经历 120 多年的植物标本采集，中国标本库系统尚未完成对葛属植物的全球全部物种的标本收集，需要补充采集。目前，产业化利用的葛属植物的种类有限，在全球范围内挖掘资源潜力，筛选和培育更多优质的类型是未来的研究趋势。本研究从叶绿体基因组大数据中提取有物种分类价值的关键特征数据，在应用人工智能鉴定、管理和利用全球植物资源遗传多样性方面可达到节省算力、提高效率的目的。

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