

白及属药用植物 DNA 条形码的确立及其应用

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摘要: 为建立白及属药用植物适合的 DNA 条形码, 并以 DNA 条形码准确的鉴别白及属药用植物及其混伪品, 本研究对 41 份样品基因组 DNA 的 nrDNA ITS、*LFY* 同源基因内含子 2、叶绿体 *ycf1* 基因进行扩增、测序及序列分析; 比较物种内种间的变异, 并基于 K2P 距离分析 Barcoding Gap、构建 NJ 系统聚类树及 BLAST1 算法以评价不同序列的鉴定能力。结果显示, 白及属物种 nrDNA ITS 和 *ycf1* 序列的种间变异 (0.022~0.106 和 0.017~0.106) 明显大于种内变异 (0~0.012 和 0~0.015), NJ 树也能准确地区分 4 个物种; 而 *LFY* 同源基因内含子 2 序列未发现 Barcoding Gap, 不能有效的鉴别白及属物种。选用 nrDNA ITS 和 *ycf1* 构建 NJ 树成功鉴定了白及粉末状药材及其混伪品。结果表明, nrDNA ITS 和 *ycf1* 序列可以作为白及属及其混伪品鉴定用的 DNA 条形码序列。市场上“巨茎白及”的 nrDNA ITS 序列和 *ycf1* 序列与安徽六安白及亲缘关系最近, NJ 树均与白及聚为一支; 基于形态及分子数据可初步将安徽“巨茎白及”归为白及属白及植物。

关键词: 白及属; 巨茎白及; DNA 条形码; nrDNA ITS; *LFY* 同源基因内含子 2; *ycf1*

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DNA barcoding research and its application on medicinal plants of *Bletilla* H. G. Reichenbach

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Abstract: To identify adulterants from medicinal plants of *Bletilla* H. G. Reichenbach, the suitable candidate DNA barcoding of *Bletilla* was evaluated. In this study, the internal transcribed spacer (ITS) of nuclear ribosomal DNA, the *LFY* homologous gene intron 2 and chloroplast *ycf1* gene were amplified and sequenced from forty-one samples. The intra-specific and inter-specific divergences of *Bletilla* were calculated, and the identification efficiency was assessed using Barcoding Gap, NJ tree by K2P distance and BLAST1 method. The result showed the intra-specific divergence of nrDNA ITS and *ycf1* (0.022–0.106 and 0.017–0.106) were obviously higher than the inter-specific divergence (0–0.012 and 0–0.015), and four species of *Bletilla* were also accurately distinguished in NJ trees. Whereas, there was no Barcoding Gap on *LFY* homologous gene intron 2, thus it cannot effectively identify species of *Bletilla*. Using NJ tree of nrDNA ITS and *ycf1* gene, powdery medicine and the adulterants of *Bletilla* were successfully unidentified. In conclusion, nrDNA ITS and *ycf1* can be used as a potential DNA barcoding to identify the medicinal plants in *Bletilla* and its adulterants.

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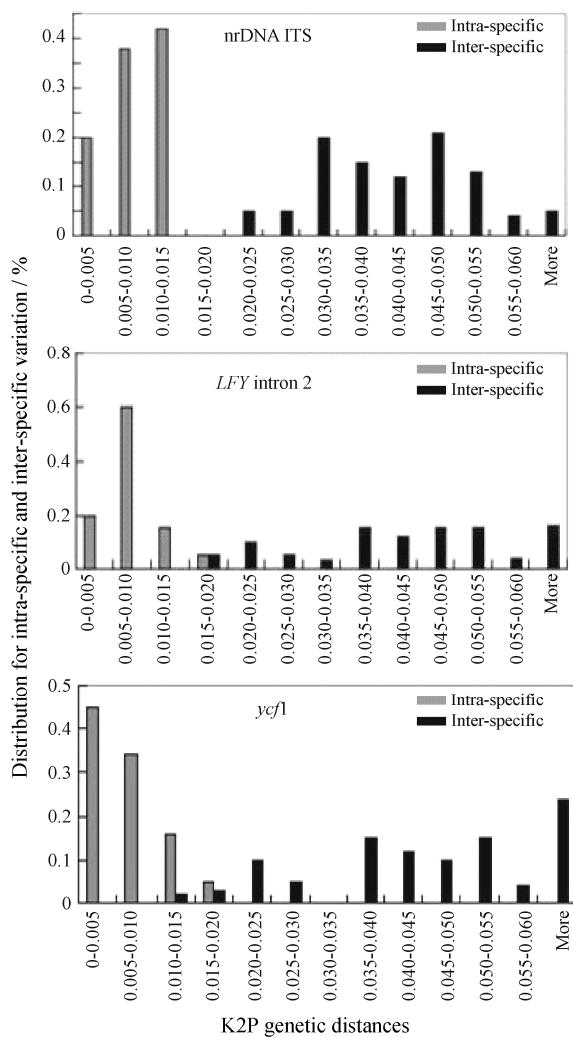


Figure 1 Distribution of intra- and inter-specific Kimura 2-parameter (K2P) distances for three candidate barcodes

有效的鉴别白及属种间植物样品。

为验证 nrDNA ITS 序列和 *ycf1* 序列在更多样本中的鉴定能力, 加入从 GenBank 下载兰科 40 个物种

65 条 nrDNA ITS 序列和 40 个物种的 42 条 *ycf1* 序列, 经 BLAST1 算法计算其鉴定成功率均为 100%。基于白及属植物 DNA 条形码的 Barcoding Gap、NJ 树及 BLAST1 算法鉴定效率分析, 本实验将 nrDNA ITS 和 *ycf1* 序列确定为鉴定白及属药用植物和混伪品的最适潜在条形码。

3 白及属药用植物待检品及混伪品的鉴定

为了有效的鉴定白及属药用植物药材及混伪品, 本文利用白及属 nrDNA ITS 和 *ycf1* 条形码分别构建 NJ 树 (图 3, 图 4)。其中 6 种黄精属混伪品, nrDNA ITS 片段共检测到了 4 个基因型, *ycf1* 基因共检测到了 4 个基因型。结果显示, NJ 树均分为两大支, 白及属植物聚为一支, 黄精属两个物种聚为另一支, 两个属植物样品得到了准确的区分。白及属 2 份待检品的 nrDNA ITS 和 *ycf1* 基因序列构建的 NJ 树与白及属白及聚为同一大枝, 同属白及植物; 粉末状待检品药材为白及属白及加工制成的。

另外, 8 份“巨茎白及”核基因及叶绿体基因序列均与安徽六安白及最为接近。在核基因方面, “巨茎白及”的 nrDNA ITS 拥有 3 个基因型, 其中, 亲代有 2 个基因型, 子一代有 3 个基因型。序列号为 KF763633 的基因型与白及样品 (KF680621) 的序列共有 3 个碱基的替换 (60 bp、121 bp、481 bp 位点), 序列号为 KF763634 的基因型与白及样品 (KF680621) 的序列有 2 个碱基的替换 (60 bp、481 bp 位点), 序列号为 KF763635 的基因型与白及样品 (KF680621) 的序列共有 4 个碱基的替换 (60 bp、120 bp、121 bp、481 bp 位点), 均是 G-C 之间的转换。在叶绿体基因方面, “巨茎白及”的 *ycf1* 序列 (KF763636) 与白及样品 (KF698981) 序列相同, 序列号为 KF763637 的基因型

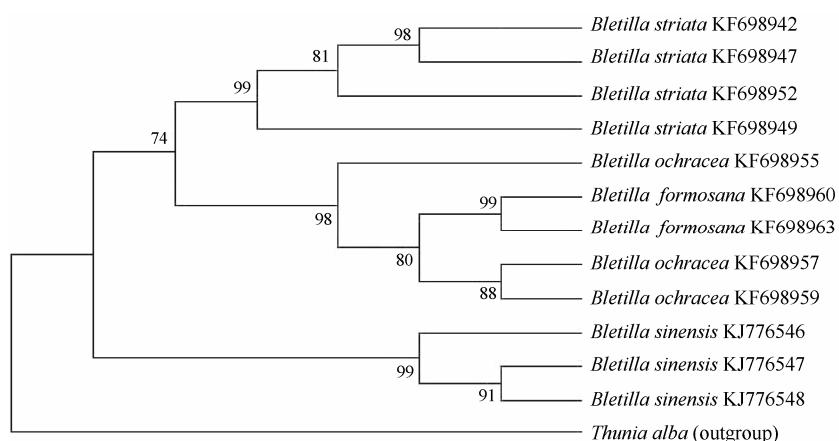


Figure 2 *LEAFY* intron 2 phylogenetic tree constructed using NJ method of *Bletilla*. Bootstrap values (>50%) are shown above the relevant branches

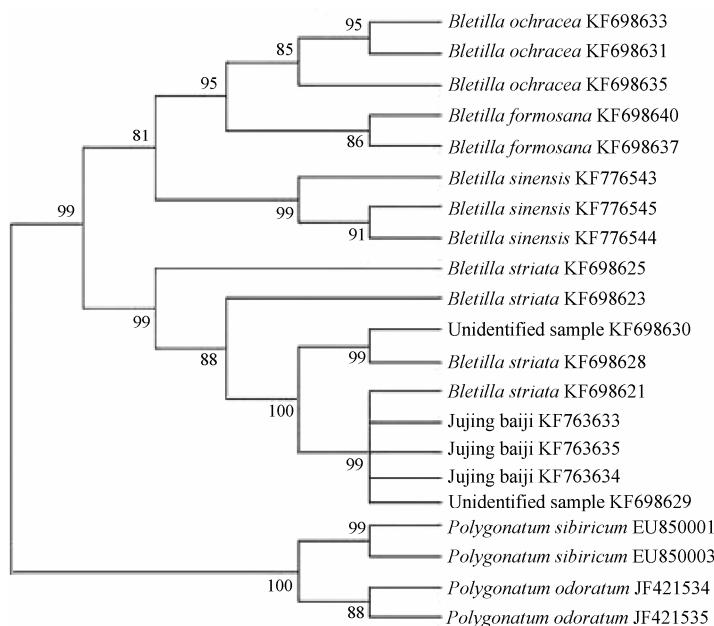


Figure 3 nrDNA ITS phylogenetic tree constructed using NJ method of *Bletilla* and its adulterants. Bootstrap values (>50%) are shown above the relevant branches

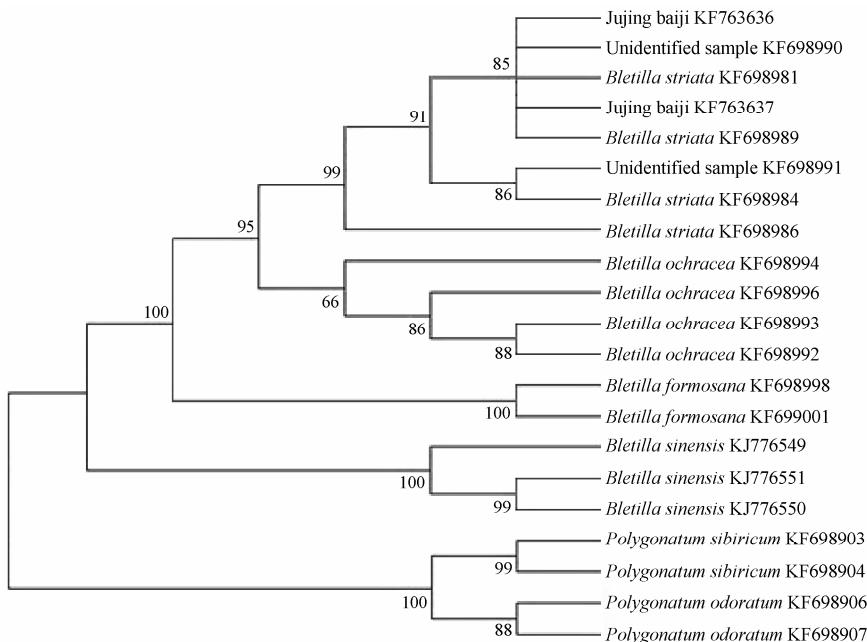


Figure 4 *ycf1* phylogenetic tree constructed using NJ method of *Bletilla* and its adulterants. Bootstrap values (>50%) are shown above the relevant branches

与白及样品 (KF698981) 的序列存在两个碱基的替换, 分别是在 121 bp 处 TA 转换与 898 bp 处 GC 的转换, 变异发生在子一代。nrDNA ITS 和 *ycf1* 基因序列构建的 NJ 树均与白及属白及聚为一支 (图 3, 图 4)。

讨论

1 DNA 条形码候选序列对白及属鉴定准确性评价

理想的 DNA 条形码通常需要满足以下 3 个标

准: ① 在物种水平具有显著的遗传变异和分化, 能够鉴别不同物种; ② 合适的长度, 便于 DNA 扩增、测序、拼接及排序时不需要手动调整; ③ 目的片段两端具有保守位点, 可用于通用引物设计^[6]。对于单拷贝核基因——*LEAFY* 同源基因, 在植物魔芋属 (*Amorphophallus* Blume ex Decaisne)、绣线梅属 (*Neillia* D.) 植物类群的系统发生分析研究中, *LFY* 基因序列相比 ITS 和 cpDNA 能提供更多的信息位

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