拟黑多刺蚁药材DNA分子鉴定研究

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摘要: 本研究以拟黑多刺蚁的*CO* I基因序列为基础设计特异性引物, 优化基因组DNA提取方式和扩增条件, 建立了一种高效、专属性强、准确性高的拟黑多刺蚁药材DNA分子鉴定方法。在该方法下拟黑多刺蚁药材扩增出长度为294～308 bp的目的片段, 其他伪品均无目的条带。本文建立的拟黑多刺蚁药材基原专属性鉴定方法可准确鉴定拟黑多刺蚁药材。

关键词: 拟黑多刺蚁; 双齿多刺蚁; DNA分子鉴定; 特异性引物; *CO* I基因

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DNA molecular identification of *Polyrhachis dives* medicinal materials

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Abstract: In the study, specific primers were designed based on the *CO* I gene sequence of *Polyrhachis dives*. By optimizing the genomic DNA extraction method and amplification conditions, we established an efficient, specific, and accurate DNA molecular identification method for *Polyrhachis dives*. In this method, the length of the target fragment was 294−308 bp, and the other counterfeits had no target bands. In this paper, the specific identification method of the origin of *Polyrhachis dives* established can be used to identify the medicinal materials of *Polyrhachis dives* accurately.

Key words: *Polyrhachis vicina* Roger; *Polyrhachis dives*; DNA molecular identification; specific PCR primers; *CO* I gene

蚂蚁是一种古老的社会性昆虫, 分布较广, 全世界约有260属16 000多种蚂蚁, 我国已知有2 000种[1], 其中已定名入药的蚂蚁品种有双齿多刺蚁 (*Polyrhachis dives*)、血红林蚁 (*Formica sanguinea*)、红褐林蚁 (*Formica rufa*)、黄猄蚁 (*Oecophylla smaragdina*)、叶形多刺蚁 (*Polyrhachis lamellidens*) 和丝光褐林蚁 (*Formica fusca*) 等[2-4]。其中拟黑多刺蚁 (*Polyrhachis vicina* Roger) 是双齿多刺蚁 (*Polyrhachis dives*) 的异名[5], 是唯一药食两用的蚂蚁[1,6], 亦是目前研究最多、运用最广的蚂蚁, 被多地中药材标准收录[7,8]。拟黑多刺蚁具有增强人体免疫功能、抗炎镇痛、抗衰老、调节血糖等药理作用, 在临床上主要用于治疗类风湿性关节炎、乙型肝炎、糖尿病、复发性葡萄膜炎等[9,10], 国内已有玄七通痹胶囊、复方黑蚂蚁胶囊、双蚁祛湿通络胶囊、黑蚂蚁降糖胶囊等蚂蚁制品[11-15]。

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蚂蚁种类繁多, 体型甚小, 性状鉴别较为困难, 市场上销售的药用蚂蚁饮片大多经过加工炮制, 故更加难以区分。而DNA分子鉴定方法具有特异性强, 灵敏度高等优势, 有望解决蚂蚁药材的基原物种鉴定问题。2020版《中华人民共和国药典》虽有发布“中药材DNA条形码分子鉴定法指导原则”, 但由于药用蚂蚁饮片多采用“烧烫的铁锅内小火焙干”等加工方式[16]以及其自身含有的蚁酸具有腐蚀性, 储存过程中极易发生DNA片段的降解和断裂, 不易提取出完整的DNA片段[17]。故无法根据通用方法获得药用蚂蚁饮片的*CO* I序列扩增产物, 为保障拟黑多刺蚁临床用药的安全, 亟待开发一种高效、专属性强、准确性高的拟黑多刺蚁药材DNA分子鉴定方法。基于线粒体DNA *CO* I基因的条形码技术的物种鉴定在新鲜蚂蚁样本和其他类昆虫的鉴定中已有广泛应用[18-20], 本研究以市面上较为常见的拟黑多刺蚁为基础, 开发了针对拟黑多刺蚁的DNA分子鉴别方法。

材料与方法

仪器 电子分析天平 (PB303-N Mettler Toledo); 高速冷冻离心机 (5415R 型 Eppendorf); PCR仪 (CFX96 Bio-rad); 水平电泳槽 (HE-120 Tanon); 显影仪 (Doc™ XR+1708195 Bio-rad)。

试剂 十二烷基磺酸钠 (SDS, 货号 3250GR500, 弗德生物科技有限公司); NaCl (批号 211023)、NaOH (批号 171118) (西陇科学股份有限公司); 二水合乙二胺四乙酸二钠 (EDTA, 货号 170426951D, 南京化学试剂股份有限公司); 蛋白酶K (20 mg·mL^{-1}, 货号 ST533)、TE溶液 (货号 ST725)、超光速Mix (货号 MF848) (碧云天生物技术有限公司); DNA提取酚试剂 (Tris饱和酚, 货号 T0250)、Tris-HCl (货号 T1150) (索莱宝科技有限公司); 氯仿 (批号 20220816)、异戊醇 (批号 04110) (上海凌峰化学试剂有限公司); 无水乙醇 (货号 01101143, General-reagent®); 异丙醇 (批号 20190524, 国药集团化学试剂有限公司); 琼脂糖 (货号 111860, Biowest®); 6× loading buffer (货号 9157, 宝日医生物技术有限公司); 50×TAE 电泳缓冲液 (货号 BL533, Biosharp®); Gelred (货号 BS354, Biosharp®); 细胞/组织基因组DNA提取 (货号 DC102, 诺唯赞生物科技股份有限公司); Insect DNA Kit (货号 D0926, OMEGA®)。

试药 拟黑多刺蚁S1～S24由南京中山制药有限公司收集, 其中S1～S22为中药饮片, S24、S25为野生 (活体), 经南京农业大学中药材研究所郭巧生教授鉴定为蚁科动物拟黑多刺蚁 (*Polyrhachis vicina* Roger) 的干燥虫体。S23、S24浸泡在纯乙醇溶液中, 置于4 ℃冰箱保存。S23为拟黑多刺蚁对照药材, 购于中国食品药品检定研究院, 室温保存。S26～S29为非拟黑多刺蚁饮片, 具体物种未知, 经初步性状鉴定推测S26～S28号样品为蚁科 (Formicidae) 蚁属 (*Formica*) 动物血红林蚁 (*Formica sanguinea*), S29号样品为蚁科 (Formicidae) 织叶蚁属 (*Oecophylla*) 动物黄猄蚁 (*Oecophylla smaragdina*), 作为本研究的阴性对照。样品信息详见表1。

基因组DNA提取 拟黑多刺蚁药材双蒸水漂洗后用无水乙醇稍浸泡取出晾干研磨备用, 对照药材直接使用。按“蛋白酶K-苯酚抽提法[21]”稍加改动提取基因组DNA, 此方法为“改良蛋白酶K-苯酚抽提法”: 称取药材约20 mg, 加入500 μL的裂解液和20 μL蛋白酶K混合均匀, 56 ℃水浴消化过夜; 其中裂解液配方为含1% SDS、30 mmol·L^{-1} Tris-HCl、200 mmol·L^{-1} NaCl及250 mmol·L^{-1} EDTA; 用等体积的饱和酚、饱和酚−氯仿−异戊醇 (25∶24∶1) 和氯仿−异戊醇 (24∶1) 抽提3次, 异丙醇沉淀, 75% 乙醇洗涤沉淀。采用Nanodrop 100型微量核酸定量分析仪测定DNA浓度和纯度。

Table 1 Sample information

Sample No.	Source area	Batch No.	Type	Sample No.	Source area	Batch No.	Type
S1	Tongren, Guizhou	210501	Decoction pieces	S16	Shaoguan, Guangdong	201209	Decoction pieces
S2	Tongren, Guizhou	210502	Decoction pieces	S17	Shaoguan, Guangdong	201210	Decoction pieces
S3	Tongren, Guizhou	210503	Decoction pieces	S18	Shaoguan, Guangdong	201211	Decoction pieces
S4	Yulin, Guangxi	210504	Decoction pieces	S19	Shaoguan, Guangdong	210101	Decoction pieces
S5	Yulin, Guangxi	210505	Decoction pieces	S20	Shaoguan, Guangdong	210304	Decoction pieces
S6	Yulin, Guangxi	210506	Decoction pieces	S21	Fujian	210308	Decoction pieces
S7	Jishou, Hunan	210507	Decoction pieces	S22	Yingde, Guangdong	211217	Decoction pieces
S8	Jishou, Hunan	210508	Decoction pieces	S23	NIFDC	121226-201102	Control sample
S9	Jishou, Hunan	210509	Decoction pieces				
S10	Mianyang, Sichuan	210510	Decoction pieces	S24	Guilin, Guangxi	220615	Fresh sample
S11	Mianyang, Sichuan	210511	Decoction pieces	S25	Yichun, Jiangxi	220802	Fresh sample
S12	Mianyang, Sichuan	210512	Decoction pieces	S26	Unknown	220914	Decoction pieces
S13	Kunming, Yunnan	210513	Decoction pieces	S27	Yichun, Heilongjiang	220927	Decoction pieces
S14	Kunming, Yunnan	210514	Decoction pieces	S28	Changbai Mountain	220928	Decoction pieces
S15	Kunming, Yunnan	210515	Decoction pieces	S29	Xizhang	220929	Decoction pieces

引物设计与筛选 在NCBI下载拟黑多刺蚁*CO* I基因序列 (OM420297.1), 使用引物设计软件Premier Primer 5.0设计拟黑多刺蚁的特异性鉴别引物PPD-1～PPD-8, 见表2, 由擎科生物科技有限公司合成。采用降落PCR扩增程序对引物进行筛选[22]: 95 ℃ 3 min; 95 ℃ 30 s, 65 ℃→−1 ℃/cycle 30 s, 72 ℃ 1 min×15; 95 ℃ 30 s, 50 ℃ 30 s, 72 ℃ 1 min×20; 72 ℃ 5 min。

Table 2 Specific primers used in the PCR process

Primer name		The sequence of primers (5′-3′)	Amplicon size/bp
PPD-1	F	TGAGCTGGAATACTAGGATCATCT	598
	R	CCGAAGGGTCAAAGAATGAAGTA	
PPD-2	F	GATTCTGACTTCTTCCACCTTCAA	383
	R	AGGATCACCACCTCCCGAA	
PPD-3	F	GATTCTGACTTCTTCCACCTTCAA	325
	R	AGTATAGTAATTGCTCCGGCTAGA	
PPD-4	F	ACAGGCTGAACCGTCTATCC	301
	R	CCGAAGGGTCAAAGAATGAAGTA	
PPD-5	F	GGAACAGGCTGAACCGTCTA	304
	R	CCGAAGGGTCAAAGAATGAAGTA	
PPD-6	F	GGAACAGGCTGAACCGTCTA	308
	R	CCTCCCGAAGGGTCAAAGAA	
PPD-7	F	ACAGGCTGAACCGTCTATCC	305
	R	CCTCCCGAAGGGTCAAAGAA	
PPD-8	F	TGAGCTGGAATACTAGGATCATCT	253
	R	TTGAAGGTGGAAGAAGTCAGAATC	

PCR扩增条件的确定 建立拟黑多刺蚁药材的鉴别方法, 考察其适用性。PCR反应体系为25 μL, 包含上游及下游引物各1 μL, 2×Mix 12.5 μL, 模板1 μL, 加ddH_2O至终体积为25 μL。扩增结束后在PCR产物中加入6×loading buffer, 1.5%琼脂糖凝胶电泳检测产物质量。

使用拟黑多刺蚁特异性引物对拟黑多刺蚁药材及常见伪品DNA进行扩增, 分别考察了① 退火温度 (55、52、50 ℃); ② 延伸时间 (25、20、15 s); ③ 循环次数 (34、35) 对扩增产物的影响。

方法适用性考察 采用“PCR扩增条件的确定”项下的所确定的反应体系和扩增程序, 进行拟黑多刺蚁DNA分子码鉴别的适用性考察, 对收集的25批拟黑多刺蚁药材和4批伪品进行检测鉴别, 验证该体系是否能稳定准确鉴别拟黑多刺蚁药材。

结果与分析

1 基因组DNA的提取及方法优化

虽有研究已开发出磁珠法应用于已降解的昆虫样本提取基因组DNA[23], 但其成本高昂不易于推广。本研究考察了基因组DNA提取的不同方法, 除改良蛋白酶K-苯酚抽提法外还包括直接用饱和酚−氯仿−异戊醇 (25∶24∶1) (蛋白酶K-苯酚抽提法) 重复抽提两次[21]和盐析法[24]提取; 改良裂解液配方, 考察EDTA浓度 (2、50和250 mmol·L^{-1})[25]; 调整裂解时间 (2、5、8、12 h); 用市售试剂盒作为对照, 检测S23号样品DNA的浓度和纯度, 优化提取方法。DNA纯度和浓度结果显示, 最优的提取方式为“改良蛋白酶K-苯抽提法”; 裂解液中EDTA浓度为250 mmol·L^{-1}; 裂解时间为12 h。不同提取方法的DNA提取结果见表3。根据本法提取所有样本的基因组DNA, DNA浓度和纯度结果见表4。

2 使用*CO* I基因通用引物进行PCR扩增

取S24按照“改良蛋白酶K-苯酚抽提法”提取基因组DNA, 运用*CO* I通用引物对目的基因进行扩增, 得到的PCR产物经琼脂糖凝胶检测后进行测序, 得到长度为684 bp的产物, 将测序结果在NCBI进行BLAST比对进行相似性检测。结果发现, 该拟黑多刺蚁 (野生活体) 与KM244657.1和OM420497.1序列相似度分别为99.71%和99.41%, 作为本实验的阳性对照。而*CO* I基因通用引物无法扩增出拟黑多刺蚁 (非活体) 基因组DNA, 结果见图1。

3 特异性PCR引物筛选

按照“PCR扩增条件的确定”项下, 配制PCR反应体系, 以S24、S23、S1、S22作为模板, 参照“引物设计与筛选”项下的降落PCR扩增程序筛选特异性引物。结果显示, PPD-4号引物均可扩增出对照药材S23、野生活体S24和拟黑多刺蚁药材S1和S23的目的条带, 且

Table 3 DNA extraction results of different extraction methods (n = 3)

Extraction method	Crack time/h	C_{EDTA} /mmol·L^{-1}	$OD_{A260/280}$/C / ng·μL^{-1}		
			A	B	C
Improved extraction of protease K- phenol	2	250	1.95/401.2	1.94/448.7	1.93/396.5
Improved extraction of protease K- phenol	5	250	1.87/442.0	1.92/466.1	2.00/465.6
Improved extraction of protease K- phenol	8	250	1.81/501.6	1.92/587.7	1.88/454.3
Improved extraction of protease K- phenol	12	250	2.00/654.5	1.99/615.6	1.97/664.8
Improved extraction of protease K- phenol	12	50	1.59/444.3	1.60/489.4	1.58/478.3
Improved extraction of protease K- phenol	12	2	1.62/445.9	1.66/413.8	1.65/403.0
Extraction of protease K- phenol	12	250	1.63/758.0	1.68/934.1	1.64/911.5
Salt out method	12	2	1.56/354.6	1.56/370.7	1.54/334.1
Cell/Tissue DNA isolation mini kit	4	Unknown	1.09/1.77	1.16/2.159	1.06/1.736
Insect DNA Kit	4	Unknown	1.88/8.89	1.94/8.77	1.90/7.83

Table 4 DNA extraction results (n = 3)

Sample No.	$OD_{A260/280}$/C / ng·μL^{-1}		
	A	B	C
S1	1.47/216.3	1.48/238.6	1.44/145.6
S2	1.52/193.6	1.51/179.2	1.52/178.5
S3	1.53/183.1	1.50/152.4	1.49/178.8
S4	1.69/120.1	1.63/119.1	1.69/106.3
S5	1.59/130.8	1.67/125.5	1.58/135.2
S6	1.69/104.1	1.66/80.6	1.73/105.8
S7	1.64/113.7	1.63/104.5	1.63/96.4
S8	1.75/214.5	1.69/222.8	1.80/222.9
S9	1.87/231.3	1.85/250.9	1.86/241.4
S10	1.81/276.0	1.87/308.1	1.90/315.4
S11	1.76/301.3	1.72/348.0	1.73/304.7
S12	1.81/227.8	1.74/213.4	1.71/220.2
S13	1.47/245.9	1.55/341.6	1.61/336.7
S14	1.61/353.3	1.75/312.3	1.63/260.3
S15	1.71/271.8	1.53/227.7	1.67/363.0
S16	1.76/303.9	1.78/291.4	1.83/283.9
S17	1.79/415.2	1.78/443.1	1.87/338.2
S18	1.96/408.6	1.9/348.2	1.96/406.0
S19	1.58/318.3	1.6/332.4	1.64/381.1
S20	1.75/493.6	1.82/475.3	1.82/483.0
S21	1.88/553.2	1.86/556.9	1.96/603.9
S22	1.88/272.0	1.96/240.6	1.82/270.7
S23	1.99/261.2	2.14/232.4	2.06/286.0
S24	2.06/213.1	2.07/131.3	2.19/204.7
S25	2.00/654.5	1.99/615.6	1.97/664.8
S26	1.77/178.8	2.13/223.7	2.09/252.7
S27	2.3/199.1	2.18/362.1	2.29/196.9
S28	1.66/230.2	1.77/270.7	1.75/297.9
S29	1.86/167.0	1.9/115.1	2.04/168.0

条带明亮清晰; PPD-1号、PPD-2号和PPD-3号引物只能扩增出野生活体S24的目的条带; PPD-5号引物扩增出的拟黑多刺蚁药材S22目的条带不清晰; PPD-6号引物扩增出的对照药材S23和拟黑多刺蚁药材S1目的条带弥散且S22不清晰; PPD-7号引物扩增出的拟黑多刺蚁药材S22目的条带弥散; PPD-8引物未扩增出对照药材S23的目的条带且拟黑多刺蚁药材S22的目的条带不清晰。故选择PPD-4引物作为拟黑多刺蚁的特征性引物。结果如图2。

4 PCR扩增条件优化

按照“PCR扩增条件的确定”项下, 配制PCR反应体系, 以所有批次饮片 (S1～S22)、对照药材 (S23) 及野生活体 (S24、S25) 的基因组DNA为模板, 参照“引物设计与筛选”项下的降落PCR扩增程序进行PCR扩增。结果发现, 虽然目的条带清晰, 但大部分都有条带, 均有弥散, 故此PCR扩增程序似乎并不适用于所有批次的药材 (图3)。

接下来继续考察运用常规PCR扩增方法进一步优化扩增程序。结果发现, 退火温度对目的条带影响不大, 但50 ℃下非特异性扩增最为明显, 加之对扩增效率的综合考虑, 选择退火温度为52 ℃。具体见图4。

为了消除引物的非特异性扩增, 又考察了延伸时间 (25、20、15 s) 对目的条带的影响。结果发现, 随着延伸时间的减少非特异性扩增也相应减少, 延伸时间为15 s时目的条带特异性扩增, 大部分条带清晰明亮。同时减少循环次数引物二聚体的表达也会相应减少, 结果见图5。

至此, 确定了拟黑多刺蚁药材DNA分子鉴别的特异性引物为PPD-4, PCR扩增程序为: 95 ℃ 3 min; 95 ℃ 25 s, 52 ℃ 25 s, 72 ℃ 15 s × 34; 72 ℃ 5 min。

5 PCR灵敏度考察

将S23号样品基因组DNA分别稀释10、20、50、100、200倍后作为模板进行DNA扩增, 结果发现, 稀释倍数对目的条带的影响不大。但DNA模板未稀释时不能扩增出目的条带, 推测可能是高浓度抑制了PCR的扩增反应, 结果见图6。

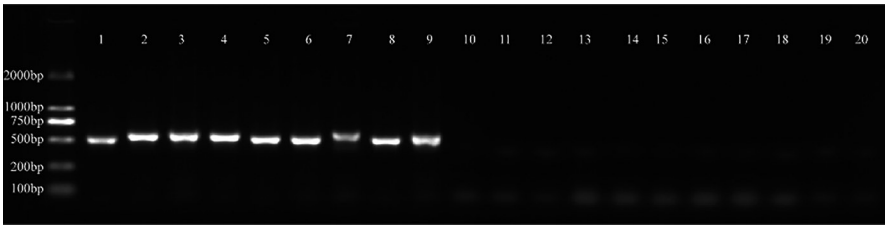

Figure 1 PCR amplification by *CO* I universal primers. Lanes 1 to 9 are wild live animals; Lanes 10 to 18 are *Polyrhachis dives* medicinal materials; Lanes 19 to 20 are blank control

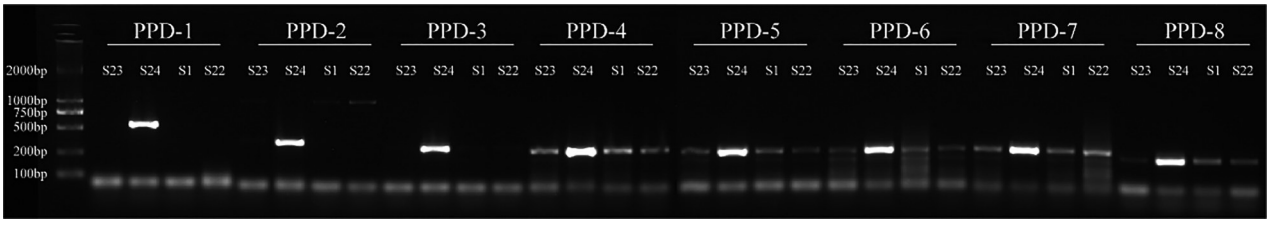

Figure 2 Screening of specific PCR primers

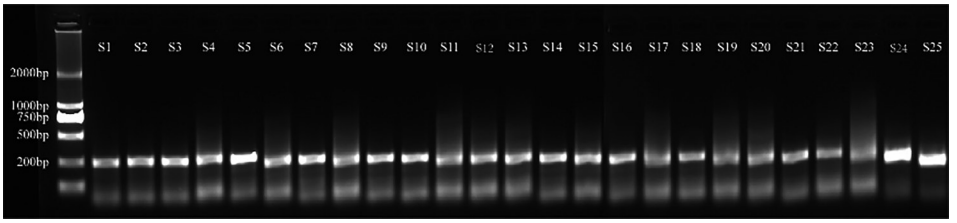

Figure 3 Agarose gel electrophoresis of touchdown PCR

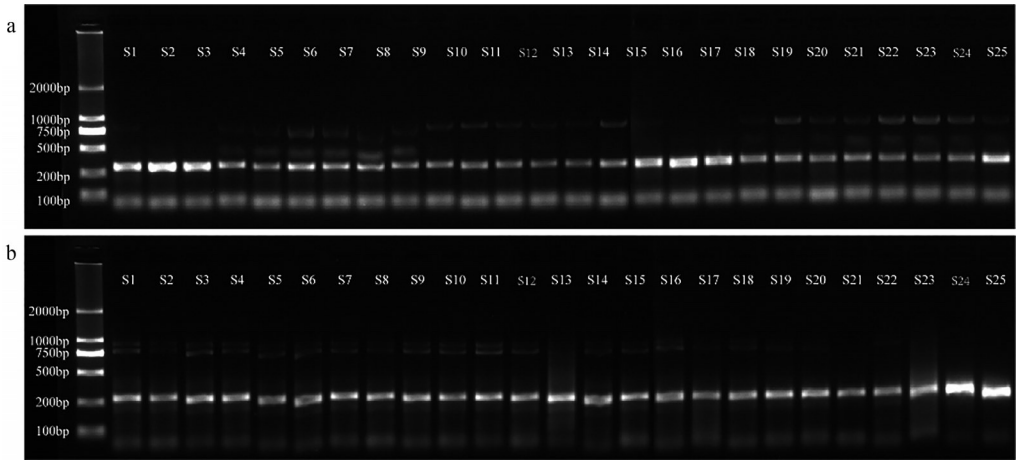

Figure 4 Effect of annealing temperature on PCR identification. a: Annealing temperature 50 ℃, elongation time 20 s, 35 cycle; b: Annealing temperature 55 ℃, elongation time 20 s, 35 cycle

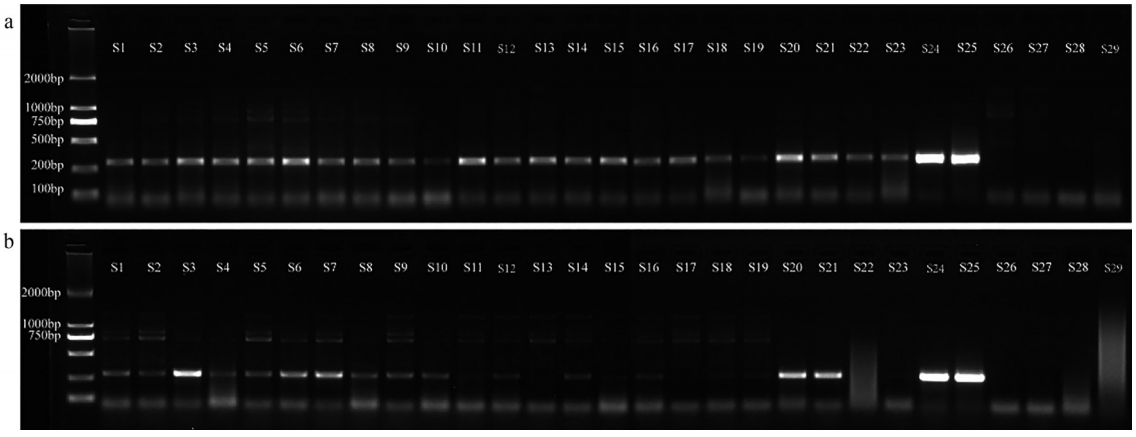

Figure 5 Effect of elongation time on PCR identification. a: Annealing temperature 52 ℃, elongation time 20 s, 34 cycle; b: Annealing temperature 52 ℃, elongation time 25 s, 34 cycle

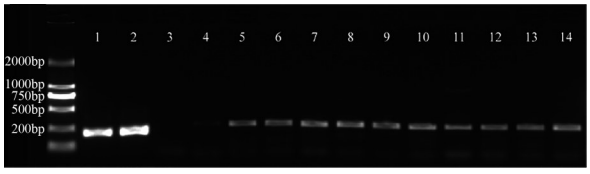

Figure 6 Effect of dilution ratio on PCR amplification. Lane 1 and 2 are S24 undiluted DNA templates; Lane 3 and 4 are S23 undiluted DNA templates; Lane 5 and 6 are S23 DNA templates diluted 10 times; Lane 7 and 8 are S23 DNA templates diluted 20 times; Lane 9 and 10 are S23 DNA templates diluted 50 times; Lane 11 and 12 are S23 DNA templates diluted 100 times; Lane 13 and 14 are S23 DNA templates diluted 200 times

6 市售拟黑多刺蚁药材的DNA分子鉴定

市售拟黑多刺蚁药材的检测结果见图7, 所有批次样品经过以上方法检测后, 目的条带清晰, 25批拟黑多刺蚁样品均可与PPD-4引物发生扩增反应, 4批伪品均未出现目的条带。

将PCR产物经琼脂糖凝胶检测后进行双向测序拼接后, 得到目的条带长度约为294～308 bp。测序结果在NCBI进行BLAST检索发现该药材与KM244657.1相似度达99.01%～99.67%, 显示与蚁科 (Formicidae) 多刺蚁属 (*Polyrhachis*) 动物拟黑多刺蚁 (*Polyrhachis*

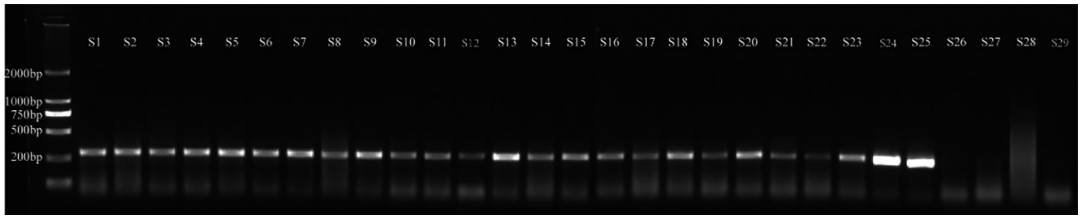

Figure 7 Agarose gel electrophoresis of experimental variety. Annealing temperature 52 ℃, elongation time 15 s, 34 cycle

dives) 具有最大的相似性, 鉴定为该批次药材品种基原为拟黑多刺蚁。各批次的鉴定结果见表5。

讨论

据2020版《中华人民共和国药典》记载, DNA分子鉴定技术已广泛应用于多种植物和动物类药材及基原物种鉴定。线粒体是动物细胞核外唯一含有DNA的细胞器。线粒体DNA分子为闭合双链环状分子, 裸露不与组蛋白结合, 分散在线粒体基质中, 具有遗传上的半自主性, 其在真核生物中具有高保守性、分子量小、结构简单、母性遗传和进化速度快等特点[21]。大多数动物群体中线粒体细胞色素C氧化酶亚基 (mt*CO* I) 基因具有高突变率, 对长度为648 bp的*CO* I基因序列进行分析, 发现即使近亲物种之间*CO* I基因也能表现出超过2%的差异[26]。但即使存在杂交/基因深入、新近起源物种和分子进化速率差异等问题, mt*CO* I基因也不会影响一般水平的物种鉴定。

近年来已有研究对拟黑多刺蚁的线粒体基因进行序列分析, 但都仅限于新鲜样本[27,28], 对于拟黑多刺蚁药材的基原鉴定一直存在空缺。本研究以动物类药材通用*CO* I基因为基础, 筛选特异性引物, 经PCR扩增、琼脂糖凝胶电泳检测后测序比对, 对拟黑多刺蚁药材进行基原物种鉴定。本研究尚存在一些不足之处, 比如前期基因组DNA提取的方法较复杂, 使用到的较多有机试剂且具有毒性; 提取的DNA易降解, 不易留样保存; 每次鉴定都需进行测序后比对, 操作不简便, 若能收集大量近亲物种基因片段设计特异性鉴别引物, 仅凭高特异条带即可进行物种鉴别。本方法填补了拟黑多刺蚁药材的基原鉴别的空白, 该DNA分子鉴定技术可准确、有效鉴别市售拟黑多刺蚁药材, 保证了临床的用药安全。

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Table 5 DNA molecular identification results of *Polyrhachis dives* medicinal materials

Sample No.	Type	Product length	Best-match species	NCBI accession No.	Sequence similarity	E value
S1	Decoction pieces	303	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S2	Decoction pieces	304	*Polyrhachis dives*	KM244657.1	99.34%	1e-150
S3	Decoction pieces	305	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S4	Decoction pieces	303	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S5	Decoction pieces	305	*Polyrhachis dives*	KM244657.1	99.01%	2e-149
S6	Decoction pieces	302	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S7	Decoction pieces	304	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S8	Decoction pieces	308	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S9	Decoction pieces	302	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S10	Decoction pieces	290	*Polyrhachis dives*	KM244657.1	99.65%	5e-145
S11	Decoction pieces	302	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S12	Decoction pieces	304	*Polyrhachis dives*	KM244657.1	99.34%	5e-150
S13	Decoction pieces	303	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S14	Decoction pieces	303	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S15	Decoction pieces	300	*Polyrhachis dives*	KM244657.1	99.67%	1e-151
S16	Decoction pieces	305	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S17	Decoction pieces	304	*Polyrhachis dives*	KM244657.1	99.34%	5e-150
S18	Decoction pieces	291	*Polyrhachis dives*	KM244657.1	99.66%	3e-146
S19	Decoction pieces	305	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S20	Decoction pieces	307	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S21	Decoction pieces	307	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S22	Decoction pieces	302	*Polyrhachis dives*	KM244657.1	99.34%	5e-150
S23	Control sample	302	*Polyrhachis dives*	KM244657.1	99.67%	3e-152
S24	Fresh sample	299	*Polyrhachis dives*	KM244657.1	99.66%	1e-150
S25	Fresh sample	299	*Polyrhachis dives*	KM244657.1	99.66%	1e-150

与实验及数据处理。

利益冲突: 无相关利益冲突。

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