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两栖蓼的分子系统学研究

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摘 要: 两栖蓼是一种水陆两栖植物,植株在不同生态环境下外部形态差异较大,同时两栖蓼的系统位置存在争议,被归入春蓼组(sect. *Persicaria*)或提升为两栖蓼组(sect. *Amphibium*)。该文选取两栖蓼及春蓼组植物12种,以及刺蓼组、头状蓼组、神血宁组、拳参组、萹蓄组和外类群掌叶大黄共23种植物进行研究。植物总DNA的提取采用改进的CTAB法,所测序列以及从Genbank数据库下载的序列,以掌叶大黄为外类群,采用最大简约法和贝叶斯法对核糖体ITS序列和叶绿体*trn*L-F序列进行了系统发育分析。ITS序列对位排列的长度为735 bp,包括489个可变位点,272个位点是信息位点。简约法得到9个简约树,步长为1 084,CI指数为0.680,RI指数为0.614。*trn*L-F序列对位排列的长度为1 121 bp,包括427个可变位点,239个位点是信息位点。简约法寻找到9个简约树,步长为551(CI=0.911,RI=0.910)。贝叶斯法和简约法得到的树基本一致。分子序列分析结果显示,*trn*L-F序列树类似于ITS序列树。ITS序列构建的发育树上,两栖蓼与刺蓼组植物、春蓼组其他植物形成3个并列的分支;在*trn*L-F序列树上,两栖蓼则与其他春蓼组植物形成两个并列的分支。由此可见,两栖蓼与春蓼组其他植物的亲缘关系较远,成一独立的分支。两个分子证据支持将两栖蓼提升为两栖蓼组的处理意见。此外,两栖蓼的花粉具散沟,与典型的春蓼组的具散孔花粉不一致。再加上两栖蓼水陆两栖的特性,因此支持把两栖蓼提升为两栖蓼组的观点。两栖蓼组的界定为多年生草本,水陆两栖,根状茎横生,生于水中茎漂浮,叶长圆形或椭圆形,生于陆地茎直立,叶披针形或长圆状披针形,托叶鞘为筒状、薄膜质,总状花序穗状,瘦果近圆形,花粉具散沟。

关键词: 两栖蓼; ITS序列; *trn*L-F序列; 春蓼组
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Phylogeny of *Polygonum amphibium* inferred from molecular sequences

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Abstract: *Polygonum amphibium* is an amphibious plant which external morphology is distinct under different ecological environment. Terrestrial plants can be converted to aquatic plants, aquatic plants could also be converted to terrestrial plants under appropriate ecological condition. Meanwhile, the systematic position of *P. amphibium* is in dispute, and the species had been placed within sect. *Persicaria* or treated as sect. *Amphibium*. The ribosomal ITS sequences and chloroplast *trn*L-F sequences are very widely used in plants of phylogenetic investigations, including for the genus *Polygonum*, and which has provided many new phylogenetic evidences for *Polygonum*. In this paper, a total of 23 kinds of plants including 12 representatives of sect. *Persicaria* (*P. amphibium*), 10 representatives of other sections of *Polygoum* (sect. *Echinocaulon*, sect. *Bistorta*, sect. *Cephalophilon*, sect. *Aconogonon*, sect.

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Avicularia) and one outgroup *Rheum palmatum* were included in the survey. The total DNA was isolated by improved CTAB methods. The ITS region (ITS1-5.8S-ITS2) was amplified with primers ITS1 and ITS4 described by White *et al*.(1990). The plastid *trn*L-F sequences was achieved using primers c and f described by Taberlet *et al*.(1991). All PCR products were purified using a QIA quick gel extraction kit and then sequenced directly on an ABI3770 automated sequencer. The sequenced sequences and the sequence download from Genbank were analysized. The ITS and *trn*L-F sequences were aligned separately using the program CLUSTALX1.83. *Rheum palmatum* was designed as outgroup. Bayesian and maximum parsimony analyses were made on sequences of nuclear ITS and plastid *trn*L-F data sets using the program MrBayes 3.0b4 and PAUP * 4.0b respectively. The sequenced nuclear ITS encompassed 735 bp aligned nucleotide positions, of which 489 sites were variable and 272 sites were parsimony informative. The heuristic search yielded 9 trees that were 1 084 steps long (CI=0.680, RI=0.614). The plastid *trn*L-F region comprised 1 121 bp nucleotide positions. A total of 427 positions were variable, and 239 sites were potentially parsimony informative. Parsimony-based analysis yielded 9 equally parsimonious trees of 551 steps with CI of 0. 911 and RI of 0.910. The trees constructed by Bayesian and MP analyses were basically congruent. Molecular analyses displayed that the ITS tree was similar to the *trn*L-F tree. In the ITS tree, *Polygonum amphibium* and other plants of sect. *Persicaria* plus sect. *Echinocaulon* formed three parallel clades; In the *trn*L-F tree, *P. amphibium* was sister to the remainder of sect. *Persicaria* with high bootstrap value. Molecular results could be achieved as follows: *P. amphibium* had a distant relationship to other plants of sect. *Persicaria*. In addition, the pollen morphology of *P. amphibium* was scattered ditch, the pollen morphology of the remaining species of sect. *Persicaria* were scattered hole. Combined with amphibious characteristics of *P. amphibium*, we were in agreement with Zhang's view that it was necessary to accord *P. amphibium* as section rank. sect. *Amphibium* was defined as herbs perennial, amphibious, rhizomes horizontal, aquatic plants: stems floating,leave blade oblong or elliptic, terrestrial plants: stems erect, leaf blade lanceolate or oblong-lanceolate, ocrea tubular, thinly membranous, inflorescence spicate, achene suborbicular, pollen with scattered ditch.

Key words: *Polygonum amphibium*; ITS sequences; *trn*L-F sequences; sect. *Persicaria*

两栖蓼(*Polygonum amphibium*)是林奈在1753年的植物种志命名的,是一种水陆两栖植物,其植株在不同生态环境下差异较大。曾宪峰(1992)观察到两栖蓼在一定水条件下,可以在很短的时间内由陆生直立草本转变成浮水草本或挺水草本;浮水草本和挺水草本也能转变成陆生直立草本。Steward(1930)根据两栖蓼托叶鞘为筒状、膜质,花序为穗状等特征将其放入春蓼组(sect. *Persicaria*)。Hara(1966)、Haraldson(1978)、Ronse Decraene *et al*.(1988)、李安仁等(1998)、吴征镒等(2003)、Li *et al*.(2003)、Freeman *et al*.(2005)都采用他的意见。张小平等(1998)则根据两栖蓼的花粉具散沟,与典型的春蓼组具散孔花粉不一致,把两栖蓼提升为两栖蓼组(sect. *Amphibium*)。由此可见,两栖蓼的系统位置存在分歧,需要进一步地研究。

核糖体ITS序列和叶绿体*trn*L-F序列,已被广泛用于植物属间、组间系统发育关系和遗传多样性的分析。在探讨蓼科植物系统发育中,ITS序列和*trn*L-F序列也得到广泛应用。许崇梅等(2009)依据*trn*L-F序列把大铜钱叶蓼(*Polygonum forrestii*)归到冰岛蓼属(*Koenigia*),依据ITS序列认为金线草(*Antenoron filiforme*)不应独立成属,应成立金线草组(sect. *Tovara*)(许崇梅等, 2011)。赵大鹏等(2012)基于叶绿体*trn*L-F、rbcL序列和核糖体ITS序列探讨蓼属(蓼科)头状蓼组的系统发育。但有关两栖蓼的研究报道较少,两栖蓼的分子系统学研究更未见报道。本文测定了两栖蓼及相关类群的ITS序列和*trn*L-F序列,以期解决两栖蓼的归属,为蓼属的系统发育关系提供新的分子证据。

1　材料与方法

1.1 材料

所选材料的分类依据"Flora of China"(Li *et al*.,2003)(表1)。凭证标本保存在山东师范大学植物标本室(SDNU)。部分序列来源于GenBank数据库。

1.2 DNA提取及PCR扩增和测序

所用植物材料均为硅胶干燥的叶片,植物总DNA

表 1 实验材料及其来源

Table 1 Experimental materials and their sources

类群 Taxa	采集地 Locality	凭证标本 Voucher	GenBank *trn*L-F Accession No.	GenBank ITS Accession No.
蓼属春蓼组 *Polygonum* sect. *Persicaria*				
两栖蓼 *P. amphibium*	山东章丘 Zhangqiu, Shandong	200210(SDNU)	KM435279	KM435280
蓼 *P. persicaria*	山东艾山 Mt. Aishan, Shandong	1156(SDNU)	EU024781	JF922105
辣蓼 *P. hydropiper*	四川成都 Chengdu, Sichuan	200331(SDNU)	FJ627267	EF653702
丛枝蓼 *P. caespitosum*	Genbank		JN235044	JN235084
酸模叶蓼 *P. lapathifolium*	Genbank		HQ843157	HM357991
红蓼 *P. orientale*	GenBank		HQ843132	DQ406631
粘蓼 *P. viscoferum*	GenBank		HQ843138	EU196921
蚕茧草 *P. japonicum*	GenBank		EU197024	EU196885
毛蓼 *P. barbata*	GenBank		EU197010	EU196871
光蓼 *P. glabrum*	GenBank		EU197017	JX144667
细叶蓼 *P. taquetii*	GenBank		EU197047	EU196918
长鬃蓼 *P. longiseta*	GenBank		EU109597	HQ843135
蓼属刺参组 *Polygonum* sect. *Echinocaulon*				
戟叶蓼 *P. thunbergii*	山东崂山 Mt. Laoshan, Shandong	1892(SDNU)	FJ627265	KM435282
箭头蓼 *P. sagittatum*	山东崂山 Mt. Laoshan, Shandong	0509 (SDNU)	FJ627266	KM435283
蓼属拳参组 *Polygonum* sect. *Bistorta*				
珠芽蓼 *P. viviparum*	新疆天山 Mt. Tianshan, Xinjiang	03005(SDNU)	EU024776	JF922101
倒根蓼 *P. ochotense*	吉林长白山 Mt. Changbaishan, Jilin	001(SDNU)	KM435281	JF922109
蓼属头状蓼组 *Polygonum* sect. *Cephalophilon*				
尼泊尔蓼 *P. nepalense*	山东泰山 Mt. Taishan, Shandong	0203(SDNU)	FJ627269	JF922100
头花蓼 *P. capitatum*	云南昆明 Kunming, Yunnan	087(SDNU)	FJ627270	KM435284
蓼属神血宁组 *Polygonum* sect. *Aconogonon*				
准噶尔神血宁 *P. songaricum*	GenBank		EU024788	JF922102
白花神血宁 *P. coriarium*	GenBank		EU024774	JF922103
蓼属萹蓄组 *Polygonum* sect. *Avicularia*				
萹蓄 *P. aviculare*	山东艾山 Mt. Aishan, Shandong	2013(SDNU)	FJ627271	EF653684
岩萹蓄 *P. cognatum*	新疆 Xinjiang	03003(SDNU)	FJ627272	JQ288766
大黄属 *Rheum*				
掌叶大黄 *R. palmatum*	GenBank		AY566451	AY207370

提取采用改进的 CTAB 法(Doyle *et al*., 1987)。*trn*L-F 序列扩增采用通用引物 c (CGAAATCGG-TAGACGCTACG) 和 f(ATTTGAACTGGTGA-CACGAG)(Taberlet *et al*.1991),PCR 扩增程序为 95 ℃预变性 5 min, 95 ℃ 变性 1 min,56 ℃ 退火 30 s,72 ℃ 延伸 1 min,30 个循环,最后 72 ℃ 延伸 5 min。ITS 序列扩增采用 White *et al*.(1990)的引物 ITS1 (5′-AGAAGTCGTAACAAGGTTTCCG-TAGG-3′) 和 ITS4 (5′-TCCTCCGCTT ATT-GATAT GC-3′),PCR 扩增程序为 94 ℃ 变性 1 min,50 ℃退火 1 min,72 ℃延伸 1.5 min,30 个循环,最后 72 ℃延伸 4 min。*trn*L-F 序列和 ITS 序列的 PCR 扩增产物纯化后直接用于测序反应(上海英骏公司的 ABI 3770 自动测序仪)。为保证序列的准确性,各样品均采用正向和反向测序。

1.3 序列的分析

所测的序列和从 GenBank 数据库中下载的序列,用 ClustalX1.83 软件进行对位排列,排好的序列用 PAUP * 4.0b 软件构建系统发育树(Swofford, 2003)。空位(gap)始终作为缺失状态。在简约法中采用启发式搜索 (heuristic search)方法, 1 000 次随机加入,TBR(tree bisection-reconnection) 枝长交换,进行启发式搜索,寻找最简约树。系统树的可靠性评价采用自展检验(Bootstrap)来检验,启发式搜索1 000次重复取样,计算各分支的支持率。

用 MrBayes3.0b4 软件(Ronquist *et al*.,2003)构建贝叶斯推论 Bayesian inference BI 分子系统树,选用 ModelTestver.3.6(Posada *et al*.,1998)选用最适合于拟合该数据集的 DNA 进化模型 ITS 为 GTR+I+G; *trn*L-F 为 GTR+G;同时建立 4 个马尔可夫链,共运行 200 万代,每 100 代抽样 1 次,忽略全部动态抽样 2 000 次,对剩余的 18 000 次静态抽样计算合意树,以后检验概率(Posterior probability)PP 作为评估参数。

2　结果与分析

2.1 ITS 序列

对位排列的长度为 735 bp,包括 489 个可变位点,272 个位点是信息位点。采用掌叶大黄作为外类群,用简约法寻找到 9 个简约树,步长为 1 084,CI 指数为 0.680,RI 指数为 0.614。贝叶斯构建的多数一致树与最大简约法基本一致,最大简约法得到的严格一致树和贝叶斯多数一致树见图 1。分支上的数字分别代表支持率和后验概率。

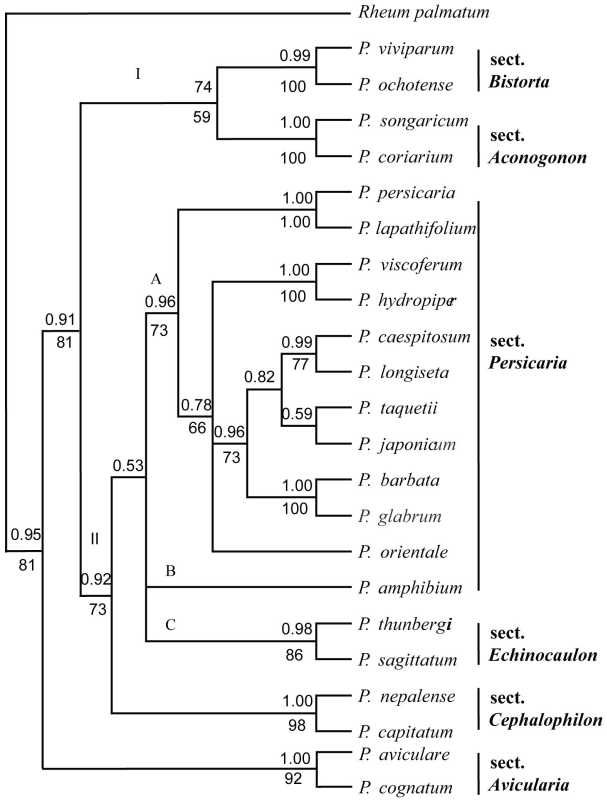

图 1　ITS 树　后验概率和支持率标记在各分支上,下同。

Fig. 1　The ITS tree　Posterior probabilities and bootstrap are indicated on the corresponding branches, the same below.

在 ITS 树上,葎蓄组植物首先分支出来,其他蓼属植物则分成 2 支(分支Ⅰ和分支Ⅱ)。分支Ⅰ由拳参组和分叉蓼组植物组成;分支Ⅱ由春蓼组、刺蓼组和头状蓼组植物组成。在分支Ⅱ上,头状蓼组植物又首先分支出来,另一分支由 3 个并列的亚支 A、B 和 C 组成。春蓼组植物除了两栖蓼外属于亚支 A,两栖蓼位于亚支 B 上,刺蓼组植物属于亚支 C。

2.2 *trn*L-F 序列

*trn*L-F 序列的范围根据已发表的蓼科植物确定。对位排列的长度为 1 121 bp,包括 427 个可变位点,239 个位点是信息位点。采用掌叶大黄作为外类群,用简约法寻找到 9 个简约树,步长为 551,CI 指数为 0.911,RI 指数为 0.910。最大简约法得到的严格一致树和贝叶斯多数一致树见图 2。分支上的数字分别代表支持率和后验概率。

*trn*L-F 序列树类似于 ITS 序列树,只是两栖蓼与其他春蓼组植物形成两个并列的分支,后与刺蓼组植物聚在一起。

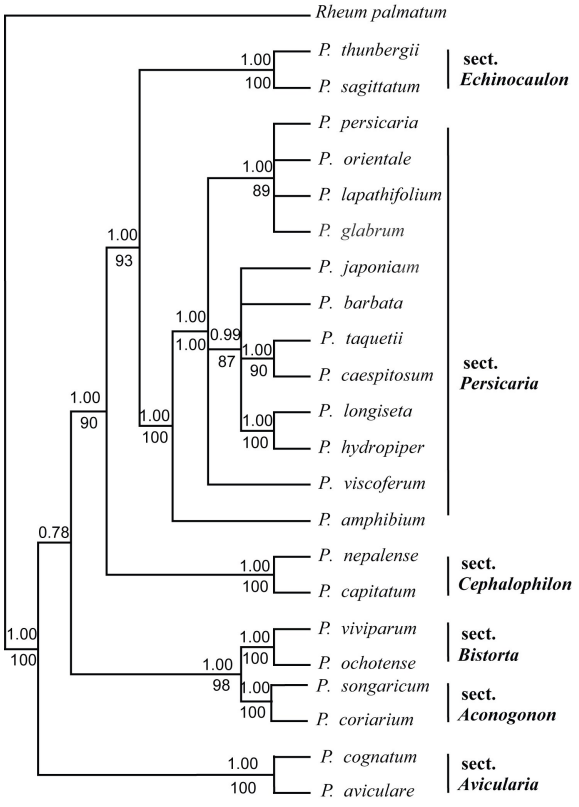

图 2 *trn*L-F 树

Fig. 2 The *trn*L-F tree

3 讨论

在 ITS 序列树上，两栖蓼与春蓼组其他植物、刺蓼组植物形成 3 个并列的分支；在 *trn*L-F 序列树上，两栖蓼则与其他春蓼组植物形成两个并列的分支，并得到 100%的支持率。由此可见，两栖蓼与春蓼组其他植物的亲缘关系较远，已达到单立组的水平。此外，两栖蓼的花粉具散沟，与典型的春蓼组的具散孔花粉不一致(张小平等，1998)。再加上两栖蓼水陆两栖的特性，因此，我们支持张小平等(1998)把两栖蓼提升为两栖蓼组的观点。两栖蓼组的界定为多年生草本，水陆两栖，根状茎横生，生于水中茎漂浮，叶长圆形或椭圆形，生于陆地茎直立，叶披针形或长圆状披针形，托叶鞘为筒状、薄膜质，总状花序穗状，瘦果近圆形，花粉具散沟。

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