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博士学位论文

东风螺的系统发育与群体遗传学研究

Research on Phylogeny and Population Genetics of
the Genus *Babylonia*

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摘要

东风螺属 (*Babylonia* Schluter, 1838) 隶属于软体动物门 (Mollusca)、腹足纲 (Gastropoda)、前鳃亚纲 (Prosobranchia)、新腹足目 (Nerobranchia)、蛾螺科 (Buccinidae), 现生种全部分布于印度-太平洋地区。中国大陆沿岸分布的东风螺为方斑东风螺和泥东风螺, 目前它们已在我国形成了规模化养殖产业。随着东风螺人工养殖的开展, 人们对东风螺亲螺的需求量猛增, 同时也开展了从国外引种的工作, 由此可能影响到东风螺的遗传结构和遗传多样性以及出现潜在的生物入侵风险。目前东风螺属各物种的亲缘关系及系统发生方面的研究非常有限, 其分类学也存在一些疑义。此外, 基于 DNA 分子标记的东风螺群体遗传结构及遗传多样性也缺少系统研究, 大时间尺度的个体扩散机制尚不明了。基于此, 本文从外部形态、染色体、线粒体基因组及核 DNA 多个水平对产自太平洋和印度洋的方斑东风螺、南洋象牙风螺、泥东风螺、台湾东风螺、锡兰东风螺和深沟东风螺等东风螺属的六个种的分类学、系统进化和分子生态学方面进行了研究, 揭示了它们的系统进化关系和种群遗传结构与遗传多样性, 澄清了若干分类学问题和动物地理学问题。主要研究结果如下:

对东风螺六个种的6个形态特征比例参数的分析表明, 东风螺属六个种之间特征参数差异极显著 ($P < 0.01$), 而方斑东风螺和南洋象牙风螺特征参数仅在壳口宽/壳口高 (EF/BH) 上发现差异极显著 ($P < 0.01$), 可作为区分两者的形态学指标。对东风螺六个种的齿舌形态结构比较中发现, 东风螺齿舌形态基本相似, 齿式均为 $0 \cdot 1 \cdot 1 \cdot 1 \cdot 0$, 然而齿舌横列数在个体间存在较大变异, 与壳高呈一定的相关性, 贝壳大小与齿舌大小呈正相关。对东风螺六个种齿舌的11个形态特征比例参数的分析表明, 壳长/齿舌宽 (SL/RW)、相邻两排中央齿间距/中央齿宽 (DE/AB)、侧齿第二突起/侧齿第一突起 (LM/JK)、侧齿宽/中央齿宽 (KL/AB) 指标在种间存在极显著差异 ($P < 0.01$)。南洋象牙风螺与方斑东风螺仅有KL/AB指标极显著差异 ($P < 0.01$)。对东风螺属的齿舌能谱分析发现齿舌的元素成分主要有C、O、Na、Ca、Mg和K, 其中C、O和K在种间存在显著差异 ($P < 0.05$), C和Na在齿舌不同部位间具有显著差异 ($P < 0.05$)。

方斑东风螺和泥东风螺的染色体核型研究结果发现两种螺的染色体数目均

为 $2n = 66$ ，此数目与蛾螺科大部分动物染色体数目接近，反映了其较高等的进化地位。两种螺的核型略有不同，方斑东风螺核型公式为 $2n = 66 = 32m + 20sm + 8st + 6t$ ，染色体总臂数 $NF = 118$ ，而泥东风螺核型公式为 $2n = 66 = 30m + 22sm + 8st + 6t$ ，总臂数 $NF = 118$ 。两种螺的全套染色体中均未发现次缢痕、随体及性染色体。

对方斑东风螺、南洋象牙凤螺和泥东风螺的线粒体全基因组序列的比较分析中得出，三种螺的基因排列顺序基本一致，与其它新腹足目动物相似，体现了近缘物种间基因顺序的保守性。三种螺均含有13个蛋白质编码基因、2个rRNA基因，不同的是方斑东风螺和泥东风螺均有22个tRNA，而南洋象牙凤螺仅有21个tRNA，位于tRNA^{Phe}和tRNA^{Ala}之间的tRNA^{Lys}在此种中缺失。三种螺均出现了较大片段的基因间隔区和基因重叠区，通过与其它腹足类动物的线粒体基因组的比对发现可能的复制起始区（Potential origin of replication, POR）位于trnF与COIII之间的这个区域。对三个东风螺13个蛋白编码基因的起始密码子和终止密码子的使用研究中发现，大部分的起始密码子为ATG，终止密码子为TAA，仅有ND2、ND3、ND4L、ND4和ATP6密码子出现变异。利用三种螺的线粒体全基因序列再结合Genebank上所有已发布的新腹足目动物的线粒体全基因组序列进行系统发育树的构建，结果显示同属蛾螺超科的织纹螺科动物没有与蛾螺科的东风螺聚在一起，东风螺反而与涡螺超科动物优先聚为一支。方斑东风螺和南洋象牙凤螺先聚为一支，暗示两者亲缘关系较近。

基于线粒体和28S rDNA片段序列对东风螺属六个种的系统进化关系研究均发现，东风螺属分为明显的两个大亚群，来自印度洋海域的锡兰东风螺和深沟东风螺组成一个亚群，来自太平洋海域的方斑东风螺、南洋象牙凤螺、泥东风螺和台湾东风螺组成另一个亚群，推测此进化结果与冰期时期海平面升降及始新世时期的板块运动导致的古地中海海域环境发生较大变化有关。线粒体片段序列结果还显示方斑东风螺和南洋象牙凤螺优先聚在一起，泥东风螺和台湾东风螺优先聚在一起，根据线粒体序列的遗传距离结果显示方斑东风螺和南洋象牙凤螺的遗传距离远小于东风螺不同种之间的遗传距离，据此认为两个种可能为亚种关系。28S rDNA由于较为保守，遗传变异不大，不能从遗传距离及系统发育树中得到上述结论，说明28S rDNA不适用于东风螺亚种及以下阶层的分类及系统进化研

究。对方斑东风螺和泥东风螺不同地理群体间个体的线粒体基因组序列的变异位点研究中均发现位于tRNA^{Phe}与COIII基因之间存在一个连续9个变异位点的高突变区,通过对方斑东风螺的突变区研究发现广西北海与广东硃洲岛个体间亲缘关系较近,而福建罗源和广东汕尾个体亲缘关系较近。

通过生物素—磁珠吸附微卫星富集法分别筛选出9对方斑东风螺和9对泥东风螺微卫星特异性分子标记,应用其中的5对方斑东风螺引物和8对泥东风螺引物对我国沿岸4个方斑东风螺野生群体(广西北海BH,海南临高LG,广东湛江ZJ,福建诏安ZA)和4个泥东风螺野生群体(海南临高LG,广东湛江ZJ,广东汕尾陆丰SW,福建诏安ZA)进行群体遗传多样性和群体遗传结构的研究。等位基因数目及杂合度值均显示两种东风螺群体的遗传多态性总体水平较高,种质资源状况良好。Nei's遗传距离值和AMOVA分子方差分析显示群体之间存在着一定的遗传分化,群体间的亲缘关系与地理距离有一定的相关性,群体间的基因流随着地理距离的增加而减少,遗传分化也会随之加剧。

关键词: 东风螺; 分类学; 系统进化; 群体遗传; 遗传标记; 线粒体全基因组

Abstract

The genus of *Babylonia* (Schluter, 1838) belongs to Mollusca, Gastropoda, Prosobranchia, Neogastropoda, Buccinidae, and all recent members of the genus are restricted to the Indo-Pacific region. In China, there are only *B. areolata* and *B. lutosa* distributing, and our country started to form industrial production cultivation of these two species. With the widely development of *Babylonia* artificial cultivation, the demand to the parent snails grows severely, and introduction from overseas develops. This would cause *Babylonia* genetic structure and genetic diversity being influenced as well as the latent biological invasion risk. At present, the study on *Babylonia* relationships and phylogeny is very limited. The taxonomy of *Babylonia* also has some divergences. Moreover, there is no systematic study on population genetic structure and genetic diversity based on DNA markers, so individual dispersing mechanics in large scale are not clear. For these reasons, this paper conducted the researches on taxonomy, phylogenetics and the molecular ecology in the *Babylonia* genus (including *B. areolata*, *B. lani* spec. nov., *B. lutosa*, *B. formosae formosae*, *B. zeylanica* and *B. spirata*) from the morphological level, the chromosomal level, the mitochondrial genome level and nuclear DNA level. This paper revealed phylogenetic relationships, population structure and genetic diversity in *Babylonia*, and classified some taxonomic and biogeographical problems. The main findings were as follows:

Morphological characteristic proportion parameters' analysis of six species of *Babylonia* indicated that the morphological characteristic parameters difference among these six species of *Babylonia* was extremely remarkable ($P > 0.01$). But there was only one parameter (EF/BH) extremely remarkable ($P > 0.01$) between *B. areolata* and *B. lani* spec. nov., and this parameter could be a morphological index differentiating these two species. Comparison of radula shape and structure of six species of *Babylonia* revealed that the basic shape of radula among the six species of *Babylonia* was similar and the teeth-formula $0 \cdot 1 \cdot 1 \cdot 1 \cdot 0$. However, the radula row number had big variation among different individuals, assumed certain relevance with the shell height. The shell size and the radula size were positive correlation. Eleven

morphological characteristic proportion parameters' analysis of six species of *Babylonia*' radula indicated that SL/RW, DE/AB, LM/JK and KL/AB parameters were extremely inter-species remarkable difference ($P>0.01$). But there is only one parameter (KL/AB) extremely remarkable ($P>0.01$) between *B. areolata* and *B. lani* spec. nov.. The EDS analysis discovered that radula's elemental composition mainly had C, O, Na, Ca, Mg and K. C, O and K had remarkable inter-species difference ($P<0.05$). C and Na had remarkable difference among different areas ($P<0.05$).

Research on the karyotypes of the chromosomes of *B. areolata* and *B. lutosa* found that the chromosome number in these two species was both $2n=66$. The chromosome number was close to most Buccinidae animals, and this result reflected *Babylonia* possessed high evolution status. The karyotypes between these two *Babylonia* species were a little different. The karyotype formulae of *B. areolata* was $2n = 66 = 32m + 20sm + 8st + 6t$, and NF was found to be 118. The karyotype formulae of *B. lutosa* was $2n = 66 = 30m + 22sm + 8st + 6t$, and NF was found to be 118. The secondary constriction, satellites and sex chromosomes were not observed.

The comparative analysis of complete mitochondrial genome in three *Babylonia* species (*B. areolata*, *B. lani* spec. nov. and *B. lutosa*) revealed that mitochondrial gene order in three species was similar to other Neogastropod animals, and this suggested close species had conservative gene orders. Three *Babylonia* species all possessed 13 protein-coding genes, 2 rRNA genes, and what was different was that *B. areolata* and *B. lutosa* both had 22 tRNA, but *B. lani* spec. nov. only had 21 tRNA, with tRNA^{Lys} (located between tRNA^{Phe} and tRNA^{Ala}) absent. Three species all had large fragmental gene gap and overlap regions. When compared with other gastropod animals' mitochondrial genome, we found the potential origin of replication (POR) located between trnF and COIII. Researches on the start codon and stop codon usage in the 13 protein-coding genes of the three *Babylonia* species revealed that most start codons were ATG, the termination codons were TAA, and only ND2, ND3, ND4L, ND4 and the ATP6 codons had variations. Using the complete mitochondrial genome sequences of the three *Babylonia* species and all Neogastropod animals submitted in the Genebank database, we constructed phylogenetic tree and found Nassariidae which

was also from Buccinoidea did not cluster with *Babylonia*. Moreover, *B. areolata* and *B. lani* spec. nov. clustered firstly, and it suggested the two species had closer relationship.

Phylogenetic relationship analysis based on mitochondrial sequence and 28S rDNA partial sequence both revealed that six species of *Babylonia* divided into two big clusters obviously. *B. zeylanica* and *B. spirata* both from Indian ocean formed one cluster and *B. areolata*, *B. lani* spec. nov., *B. lutosa* and *B. formosae formosae* all from Pacific sea formed the other cluster. The result was related to the Tethys Sea's environmental change caused by the sea level variation in the ice age and plate movements in the Eocene. Based on the partial mitochondrial sequence, *B. areolata* and *B. lani* spec. nov. clustered together, and *B. lutosa* and *B. formosae formosae* clustered together. The genetic distance between *B. areolata* and *B. lani* spec. nov. is far smaller than among other *Babylonia* species. According to this, we can infer that *B. areolata* and *B. lani* spec. nov. were subspecies relationship. Because 28S rDNA was more conservative and had less genetic variation, it could not deduce above conclusion. For this reason, 28S rDNA is not suitable for the taxonomic study among subspecies and below levels. We conducted SNP research in mitochondrial sequence among different geographical individuals of *B. areolata* and *B. lutosa* and found there was a high variation region including nine consecutive SNPs between tRNA^{Phe} and COIII gene. The high variation region in *B. areolata* revealed that individuals from Beihai and Naozhou Island were more relevant, and individuals from Luoyuan and Shanwei more relevant.

Nine microsatellite markers were developed respectively in *B. areolata* and *B. lutosa* using the biotin-magnetic-beads-enrichment method. From these markers, we choosed five microsatellite markers applied in four wild *B. areolata* populations (Hainan Lingao, LG; Guangxi Beihai, BH; Guangdong Zhanjiang, ZJ; Fujian Zhaoan, ZA) and eight markers in four wild *B. lutosa* populations (Hainan Lingao, LG; Guangdong Zhanjiang, ZJ; Guangdong Shanwei, SW; Fujian Zhaoan, ZA) to study the genetic diversity and population structure. Allele number and heterozygosity indicated the two *Babylonia* species possessed high genetic diversity and the wild

resource were both in good condition. Nei's genetic distance and AMOVA molecular variance analysis revealed a little genetic differentiation. In addition, the genetic relationship among different geographical population was positive correlated with the geographical distance. When the geographical distance increased, the gene flow decreased, and the genetic differentiation would also grow.

Key words: *Babylonia*; taxonomy; phylogeny; population genetics; genetic marker; mitochondrial genome

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