动 物 学 研 究 2007，Apr．28（2）：113－118 CN 53－1040/Q ISSN 0254－5853
Zoological Research

Genetic Structure of the Oriental River Prawn (*Macrobrachium nipponense*) from the Yangtze and Lancang Rivers, Inferred from COI Gene Sequence

YANG Pin[1], ZHANG Hao[1], CHEN Li-qiao[1,*], YE Jin-yun[2],
YU Na[1], GU Zhi-min[2], SONG Da-xiang[1,*]

(1. *School of Life Science, East China Normal University, Shanghai* 200062, *China*;
2. *Institute of Freshwater Fisheries, Zhejiang Province, Huzhou* 313001, *China*)

Abstract: This study analyzed nucleotide sequences from the mitochondrial cytochrome oxidase submit (COI) gene region (450 bp) to investigate the genetic structure of the oriental river prawn (*Macrobrachium nipponense*) among nine populations from the Yangtze and Lancang Rivers. A total of 79 individuals were collected for this work. Eighty-nine nucleotides were found to be variable, resulting in 46 haplotypes. Among the nine populations, the population from Kunming shows the greatest level of variability ($h = 1.000$, $\pi = 0.028$), whereas the population from Chongqing exhibits the lowest level of variability ($h = 0.700$, $\pi = 0.008$). Analysis of molecular variance suggested that of the total genetic diversity, 9.66% was attributable to inter-population diversity and the remainder (90.34%) to differences within populations. A molecular phylogenetic tree constructed using the Neighbor-joining (NJ) method showed that the 46 haplotypes were assigned to two clades associated with geographic regions. These results provide basic information for the conservation and sustainable exploitation of this species.

Key words: *Macrobrachium nipponense*; COI gene; Genetic structure; Genetic variation; Haplotype

利用 COI 基因序列分析长江与澜沧江水系日本沼虾群体的遗传结构

杨 频[1]，张 浩[1]，陈立侨[1,*]，叶金云[2]，禹 娜[1]，顾志敏[2]，宋大祥[1,*]

（1. 华东师范大学 生命科学学院，上海 200062；2. 浙江省淡水水产研究所，浙江 湖州 313001）

摘要：测定了我国长江水系和澜沧江水系的日本沼虾 9 个群体，共 79 个个体的线粒体 COI 基因序列片段（约 450 bp），结果发现有 89 个变异位点，共计有 46 个单倍型。其中云南昆明（KM）群体具有较丰富的遗传多样性（$h = 1.000$，$\pi = 0.028$），而重庆（CQ）群体的遗传多样性最小（$h = 0.700$，$\pi = 0.008$）。AMOVA 分析表明，群体间的遗传变异占总遗传变异的 9.66%，而 90.34% 的遗传变异源于群体内。采用邻接法（NJ）构建的分子系统树显示，46 个单倍型明显地聚为长江中下游和长江上游与澜沧江两个族群。其结果可以为合理开发和利用日本沼虾自然野生资源，以及建立和保护日本沼虾种质资源库及基因库提供必要的参考。

关键词：日本沼虾；COI 基因；遗传结构；遗传变异；单倍型
中图分类号：Q959.223；Q31 **文献标识码**：A **文章编号**：0254－5853（2007）02－0113－06

The oriental river prawn (*Macrobrachium nipponense*) is one of the few *Macrobrachium* species that has a temperate distribution and is capable of reproduction naturally in most regions of China. These facts, combined with its popularity with Chinese consumers, make it an attractive aquaculture species (New,

* Received date: 2006－12－28; Accepted date: 2007－02－06
Foundation item: This project was supported partly by Key Research Program of Zhejiang (2005C12006-01, 2006C12005)
* Corresponding authors (通讯作者): CHEN Li-qiao, E-mail: lqchen@bio.ecnu.edu.cn; SONG Da-xiang, E-mail: dxsong@mail.hbu.edu.cn
收稿日期：2006－12－28；接受日期：2007－02－06

2006). Over the past 20 years *M. nipponense* has become a commercially important aquaculture species. However, *M. nipponense* farming has recently substantially declined due to disease and the small size of the cultured animals (Fu et al, 2004a). The wild population has undergone a steady decline at the same time, mainly because of overexploitation, pollution, and construction of hydropower projects. However, the genetic variation among *M. nipponense* populations is largely unknown, and there is little information on population differentiation. Information on genetic variation is vital for the design and implementation of adequate management strategies for the species. Furthermore, in order to relieve pressure on wild stock, and to improve industry products through selective breeding, development of a domestication program for *M. nipponense* is important, requiring baseline information concerning genetic background information and genetic variations.

Until recently, all major genetic studies on this prawn have been based on morphological characteristics, cytogenetics, interspecific hybridization and polyploidy breeding (Mashiko, 1983, 1990, 1992; Mashiko & Numachi, 1993; Qiu et al, 1994, 1997; Mashiko & Numachib, 2000; Fu et al, 2004a, b; Zhao, 2006a), with the exception of Ni (2003) and Zhao et al (2006b), who simply studied genetic structure of this prawn. There has been no research on population variation in addition to the studies mentioned above. Therefore, it is necessary to study the genetic diversity of this species, especially using DNA tools.

As a genetic marker, mitchondrial DNA (mtDNA) has a few advantages over other markers. This includes the virtue of its maternal, nonrecombining mode of inheritance, its rapid pace of evolution, and extensive intraspecific polymorphism (Avise et al, 1987). mtDNA is widely used in the study of genetic variation in organisms, including some crustaceans. The object of this study is to investigate the genetic structure of the oriental river prawn (*M. nipponense*) within nine populations from southwest China, using nucleotide sequences from the mitochondrial cytochrome oxidase submit I (COI) gene region, and to provide baseline information for the conservation and sustainable utilization of the wild genetic resources of the oriental river prawn.

1 Material and Methods

1.1 Sample and DNA extraction

A total of 79 individuals of *Macrobrachium nipponense* were collected from nine localities. Details of each locality are shown in Tab 1. Samples were preserved in 75% ethanol until DNA was isolated for genetic analyses.

Ventral muscle tissue was dissected and incubated in a standard buffer [0.06 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.1 mmol/L Tris (pH 8.6) 0.5% sodium dodecyl sulphate (SDS)] overnight at 37℃ in the presence of Proteinase-K. After buffer incubation, DNA was extracted using standard phenol/chloroform extraction protocols and concentrated using column purification. The purified DNA was dried, dissolved in TE buffer and stored at −20℃.

1.2 DNA amplification

Primers were designed using Primer Premier 5.00 (Singh et al, 1998) based on the complete mtDNA of *M. rosenbergii* (Miller et al, 2005). Segments of the COI gene for each individual were amplified using PCR of COI F (5′- TTT ATC TTC GGA GCG TGA GC -3′) and COI R (5′- AGT TAT TCC TGG GGC TCG TAT G -3′). The PCR reaction mixture of 30 μl containing 1.2 units of *Taq* DNA Polymerase, 1 × reaction buffer, 5 mmol/L $MgCl_2$, 0.4 μmol/L primer, 250 μmol/L of each dNTP, and up to 50 ng of genomic DNA. The PCR cycling included pre-denaturing for 3 min at 94℃ and 30 cycles of 1 min at 94℃, 1 min at 50℃, and 1 min at 72℃, followed by final extension for 5 min at 72℃.

Tab. 1 Population codes, locations, sample size, and genetic diversity of the nine populations

Population	Locality	Sample size	Latitude	Longitude	Haplotype diversity (h)	Nucleotide diversity (π)
SZ	Suzhou, Jiangsu	9	31°10′N	120°38′E	0.917	0.013
NC	Nanchang, Jiangsu	9	29°22′N	116°20′E	0.806	0.016
HZ	Huzhou, Zhejiang	10	30°40′N	120°29′E	0.889	0.016
CQ	Chongqing	5	29°32′N	105°45′E	0.700	0.008
YD	Yidu, Hubei	10	30°38′N	111°45′E	0.800	0.016
WH	Wuhan, Hubei	9	30°33′N	114°19′E	1.000	0.016
SM	Simao, Yunnan	10	22°40′N	101°27′E	0.933	0.017
KM	Kunming, Yunan	8	25°00′N	102°40′E	1.000	0.028
XSBN	Xishuangbanna, Yunan	9	21°10′N	100°40′E	0.861	0.027

Samples wese sent to Shanghai DNA BioTchnologies Co，Ltd. for sequencing. For each sample，sequencing was performed in both directions.

1.3 Data analysis

Sequence chromatograms were viewed and edited manually using Chromas 2.13. Once edited，multiple alignments were performed using Clustal X 1.81 (Thompson et al，1997). Haplotype diversity (h) (Nei & Tajima，1981) and nucleotide diversity (π) (Nei，1987) were calculated using Dnasp 4.0 (Rozas et al，2003). An analysis of molecular variance (AMOVA；Excoffier et al，1992) was performed to partition the total phenotypic variance into intra- and inter-population variance using ARLEQUIN 3.01 (Excoffier et al，2005). The neighbor-joining (NJ) method was used to reconstruct the phylogentic relationships among haplotypes with MEGA (version 2.1，Kumar et al，2001).

2 Results

2.1 Sequence variation and haplotype distribution

A total of 450 bp mtDNA COI sequences were amplified successfully from 79 individuals of nine populations，resulting in the identification of 46 unique haplotypes defined by 89 variable sites (GenBank accession No：EF102435 – 102480). The mean total nucleotide composition was A = 30.7%，C = 21.2%，G = 19.2%，T = 28.9%. Among the 46 haplotypes，seven (15.2%) were shared by different populations；other haplotypes were unique to the corresponding population. Four haplotypes (hap5，hap8，hap11 and hap15) were shared between two of the populations，and three other haplotypes (hap4，hap10 and hap21) were shared by another three of the populations (Tab. 2).

2.2 Population genetic diversity and genetic divergence

The amount of COI gene sequence variation among the nine populations is summarized in Tab. 1. The Kunming (KM) population exhibits the greatest level of variability (h = 1.000，π = 0.028)，whereas the Chongqing (CQ) population exhibits the lowest level of variability (h = 0.800，π = 0.008). AMOVA identified that a high proportion of the total genetic variance was attributable to variations among populations (90.34%)，whereas only 9.66% occurred within populations (Tab. 3).

The fixation index (F_{ST}) and genetic distance (Nei's measure) between pairs of populations are shown in Tab. 4. These data suggested that the Huzhou (HZ) and Suzhou (SZ) populations were genetically most similar (F_{ST} = – 0.055)，while the Yidu (YD) and Chongqing (CQ) populations were most dissimilar (F_{ST} = 0.241).

2.3 Phylogeny reconstruction

The neighbour-joining tree (Fig. 1) was constructed

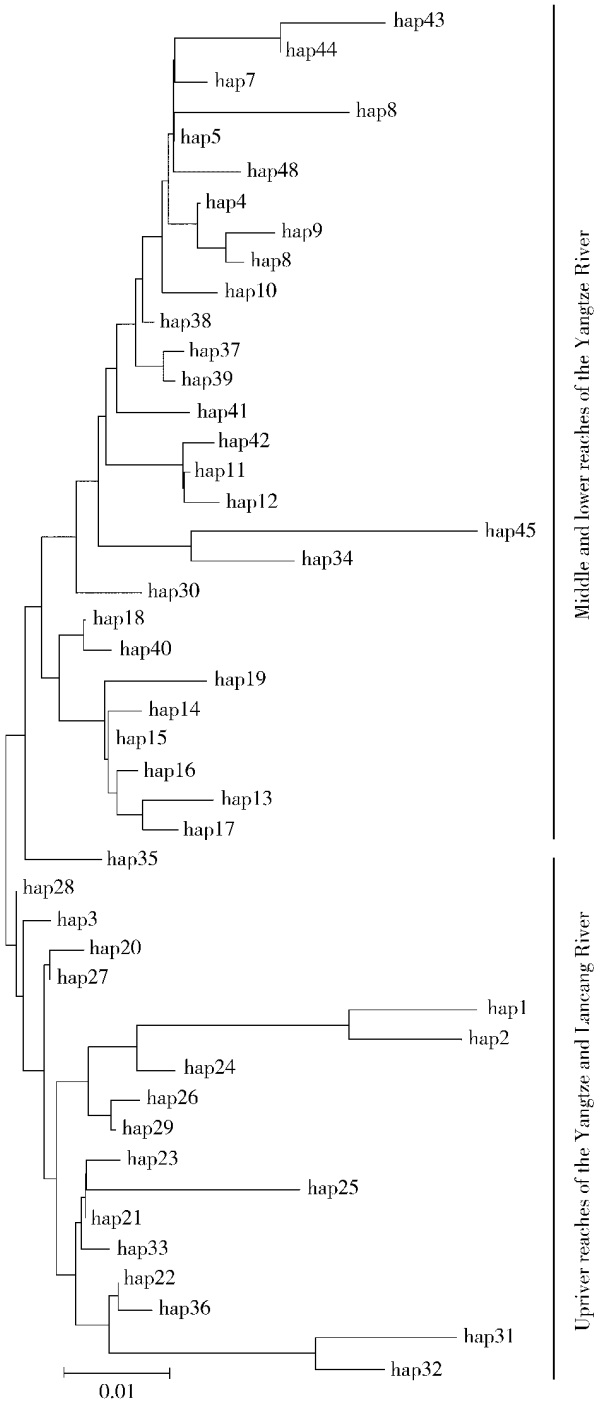

Fig. 1　Neighbor-joining tree of COI haplotypes of *Macrobrahcium nipponense*

Tab. 2 Distribution of the 46 haplotypes in *Macrobrchium nipponense* populations

Population	Haplotype																																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Suzhou				1	3	1	1			1																																	1	1		
Nanchang										4					2		1																		1							1				
Huzhou				2	3					2									1																										1	1
Chongqing															1					1							3																			
Yidu								1			4	1				3													1																	
Wuhan				1									1	1				1																			1	1	1	1	1					
Xishuangbanna								2	2												3				1							1														
Simao											1										3	1		1		1					1		1			1										
Kunming	1	1	1																		1		1					1		1				1												

with the complete data set of 46 haplotypes. Two main clades were identified. Samples from the Wuhan (WH), Yidu (YD), Nanchang (NC), Huzhou (HZ) and Suzhou (SZ) populations formed one cluster (middle and lower reaches of Yangtze River). The other clade was composed of the Chongqing (CQ), Kunming (KM), Simao (SM) and Xishuangbanna (XSBN) populations, which were collected from upriver reaches of Yangtze River and Lancang River.

Tab. 3 Analysis of molecular variance (AMOVA) for the *Macrobrachium nipponense* populations

Source of variation	Degree of freedom	Variance components	Percentage of variation
Among populations	8	0.047	9.66
Within populations	70	0.443	90.34
Total	78	0.490	100

F_{ST}: 0.097

Tab. 4 Fixation index (F_{ST}) (below) and genetic distance (above) in nine populations of *Macrobrachium nipponense*

	Kunming	Suzhou	Nanchang	Huzhou	Chongqing	Yidu	Wuhan	Xishuangbanna	Simao
Kunming	—	0.041	0.033	0.040	0.019	0.030	0.031	0.031	0.025
Suzhou	0.042	—	0.020	0.015	0.029	0.024	0.021	0.031	0.038
Nanchang	0.099	0.094	—	0.019	0.020	0.018	0.017	0.027	0.030
Huzhou	0.057	-0.055	0.069	—	0.028	0.023	0.020	0.031	0.037
Chongqing	0.134	0.177	0.204	0.190	—	0.018	0.018	0.021	0.015
Yidu	0.104	0.143	0.197	0.156	0.241	—	0.020	0.027	0.026
Wuhan	0.000	0.030	0.097	0.035	0.131	0.102	—	0.027	0.029
Xishuangbanna	0.030	0.111	0.167	0.125	0.208	0.151	0.069	—	0.025
Simao	-0.003	0.075	0.120	0.089	0.165	0.097	0.034	0.003	—

3 Discussion

This study used nucleotide sequences from the COI gene region to investigate the genetic structure of *Macrobrachium nipponense* among nine populations with a total of 79 individuals. The percentage of A + T base composition (59.6%) was much higher than C + G, which coincides with other crustacean protein-coding genes (Miller et al, 2005).

The present study of sequencing an approximately 450 bp fragment of this gene region revealed 46 haplotypes based on polymorphisms at 89 nucleotide sites. Cook et al (2002) investigated the genetic structure of *M. australiense* throughout the rivers of western Queensland in Australia. Their study revealed that sequences of a 607 bp fragment of the COI gene revealed 17 haplotypes in 28 individuals from six sites, based on polymorphisms at 36 nucleotide sites. Bruyn et al (2004) studied the phylogeography of *M. rosenbergii* from the Lake Carpentaris region. They amplified the 602 bp COI gene for all samples taken from 378 individuals, resulting in 43 unique haplotypes defined by 59 variable sites. Compared with the above studies, the present research resulted in more variable sites and haplotypes due to the larger collection range and more informative characters of the COI gene.

Pairwise F_{ST} analysis and Nei's genetic distance analysis indicated that the genetic different of *M. nipponense* between the Huzhou (HZ) and Suzhou (SZ) populations is the smallest, which corresponds

with the geographic distance. In this study, the Kunming (KM) population presents the greatest level of variability ($h = 1.000$, $\pi = 0.028$), while the Chongqing (CQ) population exhibits the lowest level of variability ($h = 0.800$, $\pi = 0.008$). The smaller (5 individuals) sample size might be part of the reason for the lower variability observed in the Chongqing (CQ) population. Furthermore, the development of small hydropower stations in this region have had adverse ecological effects on the aquatic system of the dammed streams, and have inevitably affected lower reaches, causing habitat fragmentation within dammed rivers and significant habitat changes downstream (Zhou, 2004). This would result in a decrease of effective population size and a decline in genetic diversity in the Chongqing (CQ) population.

The AMOVA analysis provided corroborating evidence for the genetic structure obtained from Nei's genetic diversity statistics. Of the total genetic diversity, 9.66% was attributable to inter-population diversity and the remainder (90.34%) to differences within populations. This indicates that there is a small differentiation among the nine populations, due to the high dispersal ability of this prawn and sample locality of this study. The F_{ST} value (0.097) suggested that although the differentiation between the nine populations is small, there is moderate genetic divergence among populations.

The mtDNA data resolved two divergent monophyletic clades, with the first representing the middle and lower reaches of Yangtze River, and the second corresponding to the upriver reaches of the Yangtze and Lancang Rivers. The present study showed a significant correlation between genetic differentiation and geographical distance. This coincides with results from morphological studies undertaken on this species (Fu et al, 2004b). Compared with other sample sites, upriver reaches of the Yangtze and Lancang Rivers are relative close. The high dispersal ability and passive dispersal of fry would contribute to the close relationship of the upriver reaches of these rivers. Although the results of this study may be affected by relatively small sample size and collection areas, they can be used as foundation for further nuclear DNA-based studies and baseline information for protection and management of this species.

The maintenance of genetic polymorphism in natural populations can reflect the process of adaptation to environmental heterogeneity. The reduction of genetic variation of a species could have serious consequences, such as reducing survival, growth and reproduction, and suppressing the ability of individuals in the population to adapt to a changing environment. In breeding, hatchery-reared individuals may escape or be released into the wild, which would reduce the genetic variability of wild stock. Although this study has shown high genetic polymorphism in wild populations, the genetic resources of *M. nipponense* is likely to decline with ongoing human activity. Therefore, it is necessary to design and implement adequate management strategies for the species to continue to exploit natural sources of *M. nipponense*.

Reference:

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics [J]. *Annu Rev Ecol Res*, **18**: 489-522.

Bruyn MD, Wilson JC, Mather PB. 2004. Reconciling geography and genealogy: Phylogeography of giant freshwater prawns from the Lake Carpentaria region [J]. *Mol Ecol*, **13** (11): 3515-3526.

Cook BD, Bunn SE, Hughes JM. 2002. Genetic structure and dispersal of *Macrobrachium austrsaliense* (Decapoda: Palaemonidae) in west Queensland, Australia [J]. *Freshwater Biol*, **47**: 2098-2112.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis [J]. *Evolutionary Bioinformatics Online*, **1**: 47-50.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data [J]. *Genetics*, **131** (2): 479-491.

Fu HT, Gong YS, Wu Y, Xu P, Wu CJ. 2004a. Artificial interspecific hybridization between *Macrobrachium* species [J]. *Aquaculture*, **232**: 215-223.

Fu HT, Gong YS, Wu Y, He XL, Wen HB. 2004b. Studies of polymorphisms in *Macrobrahcium nipponense* [A]. In: 2003 Forum on Fishery Science and Technology [C]. Beijing: Ocean Press, 33-40.

Kumar S, Tamura K, Jakobsen IB, Nei M. 2001. MEGA2: Molecular evolutionary genetics analysis software [J]. *Bioinformatics*, **17** (12): 1244-1245.

Mashiko K, Numachi K. 1993. Genetic evidence for the presence of distinct freshwater prawn (*Macrobrachium nipponense*) populations in a single river system [J]. *Zoo Sci*, **10** (1): 161-167.

Mashiko K. 1983. Evidence of differentiation between the estuarine and upper freshwater population inhabiting the same water system in the long-armed prawn *Macrobrachium nipponense* (de Hann) [J]. *Zool Mag Zool Soc Jap*, **92** (22): 180-185.

Mashiko K. 1990. Diversified egg and clutch sizes among local populations of the freshwater prawn *Macrobrachium nipponense* [J]. *J Crust Biol*, **10** (2): 306-314.

Mashiko K. 1992. Genetic egg and clutch size variations in freshwater

prawn populations [J]. *Oikos*, **63** (33): 454–458.
Mashiko K, Numachib K. 2000. Derivation of populations with different-sized eggs in the palaemonid prawn *Macrobrachium nipponense* [J]. *J Crust Biol*, **20** (1): 118–127.
Miller AD, Murphy NP, Burridge CP, Austin CM. 2005. Complete mitochondrial DNA sequences of the decapod crustaceans *Pseudocarcinus gigas* (Menippidae) and *Macrobrachium rosenbergii* (Palaemonidae) [J]. *Mar Biotechnol*, **7** (4): 339–349.
Nei M, Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases [J]. *Genetics*, **97** (1): 145–163.
Nei M. 1987. Molecular Evolutionary Genetics [M]. New York: Columbia University Press.
New MB. 2006. Freshwater prawn farming: Global status, recent research and a glance at the future [J]. *Aquac Res*, **36**: 210–230.
Ni J. 2003. Primary Studies on Nutritional Quality and Population Characteristics Among Four Populations of *Macrobrachium nipponense* [D]. M. Sc. Thesis, East China Normal University, Shanghai.
Qiu GF, Du NS, Lai W. 1994. Chromosomal and karyological studies on the freshwater prawn *Macrobrachium nipponense* (Crustacea, Decapoda) [J]. *Oceanologia et Limnologia Sinica*, **25** (5): 493–498.
Qiu GF, Du NS, Lai W. 1997. A preliminary study on induction of tetraploidy in the freshwater prawn *Macrobrachium nipponense* by heat shock [J]. *Chin J Fisheries*, **21** (1): 13–18.
Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods [J]. *Bioinformatics*, **19** (18): 2496–2497.
Singh VK, Mangalam AK, Dwivedi S, Naik S. 1998. Primer premier: Program for design of degenerate primers from a protein sequence [J]. *Biotechniques*, **24**: 318–319.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The clustal X windows interface: Flexible strategies for multiple sequences alignment aided by quality analysis tool [J]. *Nucleic Acids Res*, **25** (24): 4876–4882.
Zhao XQ, Ni J, Chen LQ, Gu ZM, Zhou ZM. 2006a. Analysis of morphological variations among four populations of *Macrobrachium nipponense* [J]. *J Fish Sci China*, **13** (2): 224–229.
Zhao XQ, Ni J, Chen LQ, Li EC, Gu ZM, Zhou ZM. 2006b. Genetic structure study of wild and cultured populations of shrimp *Macrobrachium nipponense* (Crustacea, Decapoda) [A]. In: Promotion of Fisheries by Biotechnology: 2005 Forum on Fishery Science and Technology [C]. Beijing: Ocean Press, 213–225.
Zhou DK. 2004. Impacts of small hydropower development on ecological environment should be hold [J]. *Decision-Making & Consultancy Newsletter*, **15** (3): 79–81.

本刊主编张亚平院士领导的“线粒体基因组多样性与东亚人群历史的研究”成果荣获国家自然科学二等奖

2007 年 2 月 27 日，党中央、国务院在人民大会堂隆重召开 2006 年度国家科技奖励大会，向获得 2006 年度国家最高科技奖、自然科学奖、技术发明奖和科技进步奖的获奖个人和单位颁奖。党和国家领导人胡锦涛、温家宝、曾庆红、李长春出席大会并为获奖代表颁奖。

全国授奖项目 308 项，其中中科院获奖 30 项。经云南省推荐，中国科学院昆明动物研究所所长、本刊主编张亚平院士及姚永刚、孔庆鹏、丁远春和孙昌四位博士共同完成的“线粒体基因组多样性与东亚人群历史的研究”成果荣获自然科学二等奖。这是中国科学院昆明动物研究所建所以来获得的最高级别的国家奖励。

云南省人民政府对此十分重视，在张亚平院士及其他几位获奖者 28 日载誉归来之际，云南省省长助理杨建昆、云南省科技厅厅长龙江等领导专程前往机场迎接，并举行了热烈的欢迎仪式。

获奖同日，中国科学院路甬祥院长发来贺信，对获得此项殊荣的昆明动物研究所及广大科技人员致以热烈的祝贺，并对科技人员为此付出的辛勤劳动表示崇高的敬意。路甬祥院长在贺信中指出：“希望你们与时俱进，再接再厉，大力协作，开拓进取，不断创新，高举邓小平理论和“三个代表”重要思想的伟大旗帜，全面贯彻落实科学发展观，投身于建设和谐社会和创新型国家的伟大实践中，勇攀世界科技高峰，不断为我国经济建设、国家安全和社会可持续发展做出基础性、战略性和前瞻性的重大创新贡献。”