Korean J. Genetics 28 (2) : 185-192 (June 2006)

Phylogeny of Northern Hemisphere Freshwater Crayfishes Based on 16S rRNA Gene Analysis

Dong-Ha Ahn[1], Tadashi Kawai[2], Se-Joo Kim[1], Hyun Soo Rho[3], Jong Woo Jung[3], Won Kim[3], Byung Jin Lim[1], Min Seop Kim[1] and Gi-Sik Min[1*]

[1]Department of Biological Sciences, Inha University, Incheon 402-751, Korea, [2]Hokkaido Nuclear Environmental Research Center, Hokkaido, 045-0123, Japan, [3]Department of Biological Sciences, Seoul National University, Seoul 151-742, Korea

Received March 20, 2006; accepted June 1, 2006

ABSTRACT

Freshwater crayfishes are divided into two superfamilies, and one of which exists only in the Southern Hemisphere (Parastacoidea), while another has been found only in parts of the Northern Hemisphere (Astacoidea). Although several conflict opinions have been revealed, monophyly of freshwater crayfishes, including the monophyly of crayfish superfamilies, are commonly accepted. The phylogenetic relationships among crayfish subgroups of the Northern Hemisphere, however, are rather controversial due to the disjunct zoogeographic distributions of two families, Astacidae and Cambaridae, and the enigmatic morphological affinities of eastern Asian crayfish genus *Cambaroides* to two families. In our 16S rDNA analysis, *Cambaroides* occupied the basal position of Astacoidea as a third group, and showed sister group relationships with the Cambaridae and Astacidae clades. Our results conflict with traditional taxonomy because the *Cambaroides* genus has been widely accepted as a member of the Cambaridae. However, they are in good agreement with recent molecular studies of crayfishes, and to a large degree with recent explanations of floristic exchanges among holarctic plant groups without enigmatic disjunction. Because many questions remain to be answered, it is desirable to note that, to obtain a reliable phylogeny of Northern Hemisphere crayfishes, more evidence must be collected from fossil records, biogeography of other freshwater animal groups, and multiple molecular data from both nuclear and mitochondrial genes.

Key words: 16S rDNA, *Cambaroides*, Northern Hemisphere crayfish, phylogeny.

*To whom correspondence should be addressed.
E-mail: mingisik@inha.ac.kr. This work was supported partially by grants of the Korea Research Foundation Grant (KRF-2004-041-C00266), the Regional Research Center for Costal Environments of Yellow Sea at Inha University, and Korea Research Foundation For the 21st Century.

INTRODUCTION

Freshwater crayfishes are well suited for studying the conservation biology of freshwater systems because they often act as indicator species for stream habitat quality. Furthermore, in many countries, freshwater crayfishes have suffered from range reductions and habitat degradation caused by environmental changes and water pollution, and they are believed to be deserving of conservation efforts. With about 540 living species worldwide, crayfishes (Decapoda, Astacidea) are native to every continent except Antarctica and Africa (although six species are native to Madagascar) (Villalobos, 1983; Hobbs, 1988; Lodge et al., 2000).

Small freshwater animals, such as planktonic crustaceans and tiny mollusks, can be dispersed rather easily by wind or bird-mediated transportation (Holland and Hadfield, 2004). Therefore, the distributions of these small animals are rather widespread, and the bounds among comparable groups are obscure. While freshwater animals with larger body size, especially benthic animals like crayfishes, are highly restricted in their habitats and the ranges and bounds of distribution are rather conspicuous without mixed zones, they may be ideal for the tracing of biogeographical dispersal routes and for uncovering of the history of freshwater animal evolution. As shown by a recent finding of crayfish fossils and burrows, crayfishes may have lived in freshwater for more than 300 million years prior to the separation of the Pangean super-continent. Taking this into consideration, the phylogenetic study of crayfishes can give important clues to disclose the dispersal history of freshwater animals related to continental drift theory.

Freshwater crayfishes are currently divided into two superfamilies, and the geographical separation between the two groups is fairly clear, as one of the superfamilies is present only in the Southern Hemisphere (superfamily Parastacoidea), while the other has been found only in the Northern Hemisphere (superfamily Astacoidea). In the tropical zone, the broad area between the two hemispheres, however, no representative crayfish species have been found (Cukerzis, 1988; Hobbs, 1974, 1988). The Parastacoidea contains only one family with 14 genera and around 180 species. They are distributed in Australia, New Zealand, South America, and Madagascar. Currently, Astacoidea has been recognized as two families, Astacidae and Cambaridae. The representatives of Astacidae live in Europe, Asia Minor, and western North America; Cambaridae is present in eastern Asia (Korea, Japan, and eastern parts of China and Russia), North America east of the Rocky Mountains, Central America, and Cuba (Hobbs, 1988; Scholtz, 2002) (Fig. 1). Family Cambaridae has the greatest diversity among the three families of crayfishes,

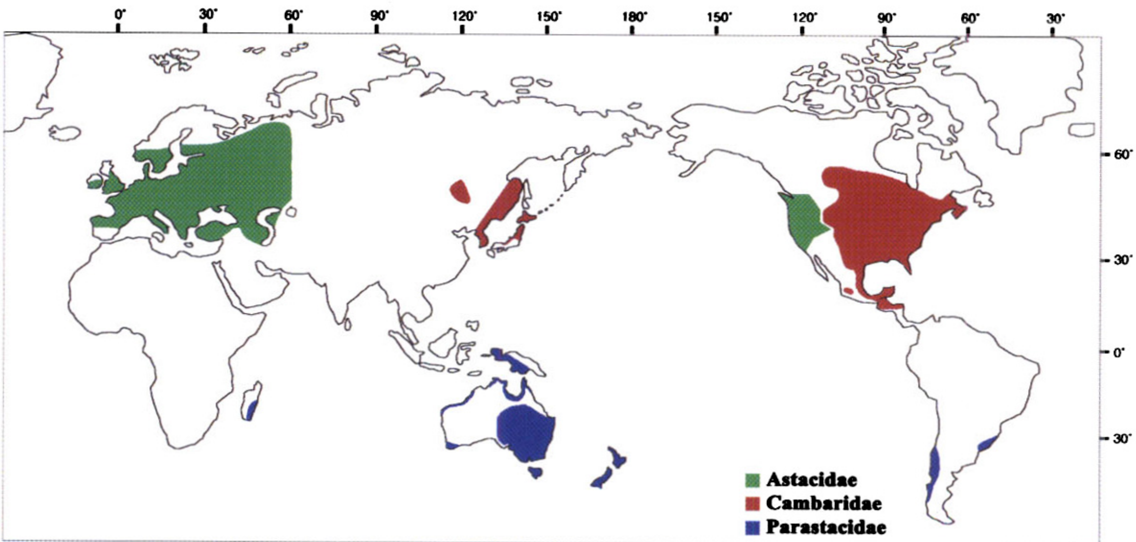

Figure 1. Geographical distribution of freshwater crayfishes (modified from http://crayfish.byu.edu/).

containing over 70% of all described species (Crandall et al., 2000).

According to Hobbs' (1974; 1988) taxonomical system, the Cambaridae can be divided into three subfamilies (Cambaroidinae, Cambarellinae and Combarinae). The subfamily Cambaroidinae contains only a single genus, *Cambaroides*. *Cambaroides* includes four species; *C. dauricus* (Pallas, 1773), *C. schrenckii* (Kessler, 1874), *C. japonicus* (De Haan, 1841), and *C. similis* (Koelbel, 1892), and all are distributed only in far eastern Asia. Although Hobbs cataloged the Cambaroidinae as a subfamily in the Cambaridae, he noted that the phylogenetic position of the Cambaroidinae is uncertain between the Cambaridae and Astacidae families because of their unusual morphological characteristics and due to the lack of study of this subgroup species, and the subfamily would be accorded to familial rank.

In the present study, the 16S rDNA sequences from the four *Cambaroides* species, which include all of the representative species of this genus, were determined in order to elucidate the phylogenetic position and taxonomical status of eastern Asian crayfishes based on mitochondrial 16S rDNA sequence analysis.

MATERIALS AND METHODS

Crayfish sampling

Korean (*Cambaroides similis*) and Japanese (*C. japonicus*) crayfishes were collected by hand and dipnet from the Republic of Korea and from Japan, preserved in 95% ethanol, and then delivered to the laboratory at room temperature. Chinese (*C. dauricus*) crayfishes were provided by Dr. Yong-Woo Lee. *C. schrenckii* was purchased from a pet store in Russia. Specimens of red swamp crayfish (*Procambarus clarkii*) and lobster (*Homarus americanus*) were bought from fish markets in Incheon, Korea.

DNA extraction, PCR, and Sequencing

One side of the carapace of each specimen (right or left side) was punctured and a tiny amount of muscle tissue was removed for DNA extraction. The remaining specimens were preserved in 95% EtOH and are housed at -70°C in a deep freezer. Genomic DNA was extracted using a DNeasy Tissue kit (Qiagen) or as described in Lee (2003) and Jung et al (2006). Polymerase chain reaction (PCR) amplification was carried out using previously published primers and PCR conditions. The

Table 1. Taxonomical list and GenBank accession numbers.

Species	Acc. No.	Source
Order Decapoda		
Infraorder Astacidea		
Superfamily Astacoidea		
Family Astacidae		
Astacus astacus	AF235983	GenBank
Austropotamobius italicus	AY611195	GenBank
Austropotamobius pallipes	AF237610	GenBank
Austropotamobius torrentium	AF235984	GenBank
Pacifalacus leniusculus	AF235985	GenBank
Family Cambaridae		
Subfamily Cabaroidinae		
Cabaroides dauricus 1	**DQ666837**	**This study**
Cabaroides dauricus 2	**DQ666838**	**This study**
Cambaroides japonicus 1	**DQ666839**	**This study**
Cambaroides japonicus 2	**DQ666840**	**This study**
Cambaroides schrencki 1	**DQ666835**	**This study**
Cambaroides schrencki 2	**DQ666836**	**This study**
Cabaroides similisK	**DQ666841**	**This study**
Cabaroides similisS	**DQ666842**	**This study**
Subfamily Cambarellinae		
Cambarellus shufeldtii	AF235986	GenBank
Subfamily Cambarinae		
Cambarus maculatus	AF235988	GenBank
Cambarus monongalensis	AY590472	GenBank
Orconectes luteus	AF376521	GenBank
Orconectes punctimanus	AY485442	GenBank
Orconectes placidus	AY609338	GenBank
Orconectes virilis	AF235989	GenBank
Procambarus sp.	AY214437	GenBank
Procambarus clakii	**DQ666844**	**This study**
Procambarus toltecae	AY214438	GenBank
Superfamily Parastacoidea		
Family Parastacidae		
Astacopsis franklinii	AF044240	GenBank
Astacopsis gouldi	AF135969	GenBank
Cherax cairnsensis	AY191763	GenBank
Cherax depressus	AY191760	GenBank
Engaeus merosetosus	AY223712	GenBank
Engaeus sericatus	AY223713	GenBank
Engaewa similis	AF135982	GenBank
Euastacus bispinosus	AF235991	GenBank
Euastacus rieki	AF135984	GenBank
Geocharax gracilis	AF235992	GenBank
Geocharax insolitus	AF135991	GenBank
Gramastacu sp.	AY223717	GenBank
Gramastacus insolitus	AY223715	GenBank
Ombrastacoides asperri	AY156061	GenBank
Ombrastacoides professorum	AY156058	GenBank
Paranephrops planifrons	AF135995	GenBank
Parastacoides insignis	AF135996	GenBank
Parastacoides pulcher	AF135997	GenBank
Prastacus brasiliensis	AF175245	GenBank
Prastacus pugnax	AF175237	GenBank
Samastacus spinifrons	AF175241	GenBank
Spinastacoides catinipalmus	AY156055	GenBank
Spinastacoides insignis	AY156057	GenBank
Tenuibranchiurus glypticus	AF135998	GenBank
Virilastacus araucanius	AF175236	GenBank
Superfamily Nephropoidea		
Family Nephropidae		
Homarus americanus	**DQ666843**	**This study**
Infraorder Dendrobranchiata		
Superfamily Penaeoidea		
Family Penaeidae		
Litopenaeu svannamei	AY264914	GenBank

PCR fragments were gel-purified with a GeneClean III kit (Q • BIOgene). The purified samples were sequenced in an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems) using a Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems).

Phylogenetic analysis

Both strands were sequenced and aligned using the ClustalX multiple alignment program (Thompson et al., 1997) and adjusted by visual recognition. A list of species and GenBank sequence accession numbers is given in Table 1.

The aligned data were analyzed by maximum parsimony (MP) (Fitch, 1971), maximum-likelihood (ML) (Felsenstein, 1981), neighbor-joining (NJ) (Saitou and Nei, 1987), and Bayesian methods of phylogenetic inference.

MP, ML, and NJ analyses were conducted using the PAUP 4.0b10 computer program (Swofford, 2003). Gaps were treated as missing data. MP and ML analyses were carried out using heuristic searching with ten random stepwise additions and tree bisection-reconnection (TBR) branch-swapping. NJ analyses were carried out using the Kimura two-parameter distance method (Kimura, 1980). For determining the appropriate DNA substitution model for the phylogenetic analysis of NJ, ML, and Bayesian inference, the Akaike information criterion (AIC) method was performed to find the best model of evolution that fit our data using the Modeltest computer program (Posada and Crandall, 1998), which was implemented within the PAUP program package. Confidence in the resulting relationships of MP, ML, and NJ trees was assessed using the bootstrap procedure (Felsenstein, 1985) with 1,000 replications for MP and NJ, and 100 replications for ML. Bayesian inference was performed using MrBayes 3.0 to simulate a Markov chain for 1,500,000 cycles, 300,000 of which were discarded as burn-in.

RESULTS AND DISCUSSION

Although several controversial opinions have been revealed, monophyly of freshwater crayfishes, including the monophyly of crayfish superfamilies, are commonly and categorically accepted by most modern astacologists because much supporting evidence of their monophyly has been found from recent studies of both molecular biology and morphology (Huxely, 1880; Ortmann, 1902; Scholtz and Richter, 1995; Hobbs, 1974; Crandall et al., 2000). Because all crayfishes of the Southern Hemisphere share several apomorphic characteristics, the monophyly of Parastacoida has not been seriously doubted (Scholtz, 2002). Because considerable amounts of supporting evidence have been found from recent molecular and morphological studies, the monophyly of Northern Hemisphere crayfishes is also commonly accepted by most modern astacologists (Huxely, 1880; Ortmann, 1902; Hobbs, 1974; Scholtz and Richter, 1995; Crandall et al., 2000; Scholtz, 2002). However, the phylogenetic relationships among Northern Hemisphere crayfish subgroups remain somewhat controversial due to the disjunct zoogeographic distributions of the two families, Astacidae and Cambaridae (Fig. 1), and because of the enigmatic morphological affinities of eastern Asian crayfish genus, *Cambaroides*, to the two families. Therefore, elucidation of the phylogenetic position of genus *Cambaroides* in superfamily Astacoidea is most crucial for tracing the phylogenetic history of Northern Hemisphere crayfishes.

Mitochondrial 16S rDNA regions have been used most popularly in the phylogenetic studies of crayfishes because many sequences comprising almost all the genera of crayfishes already have been identified and showed rather consistent and meaningful crayfish phylogenies (Crandall and Fitzpatrick, 1996; Crandall et al., 1999, 2000; Grandjean et al., 200; Munasinghe et al., 2004).

For investigating the phylogenetic relationships among Northern Hemisphere crayfishes, we identified partial sequences of 16S rDNA from all representatives of the four described species of eastern Asian *Cambaroides* crayfishes, and those of *Procambarus clarkii* and *Homarus americanus* were also obtained. Determined sequences were deposited to GenBank under accession numbers DQ666835-DQ666844. The known sequences were included and retrieved from the GenBank database, and alignment datasets were prepared.

The alignment, which includes the 16S rDNA sequences of 49 crayfishes and two outgroups (*Homarus americanus* and *Litopenaeus vannamei*) (Table 1), contains 536 base pairs (bp): 306 (57.1%) characters are constant, 85 (15.9%) are parsimony non-informative, and 145 (27.0%) are parsimony informative sites. The base composition appeared biased with a high AT (70.49%) ratio: A=36.23%, C=9.36%, G=20.16%, and T=34.26%.

From the likelihood ratio test and the AIC test

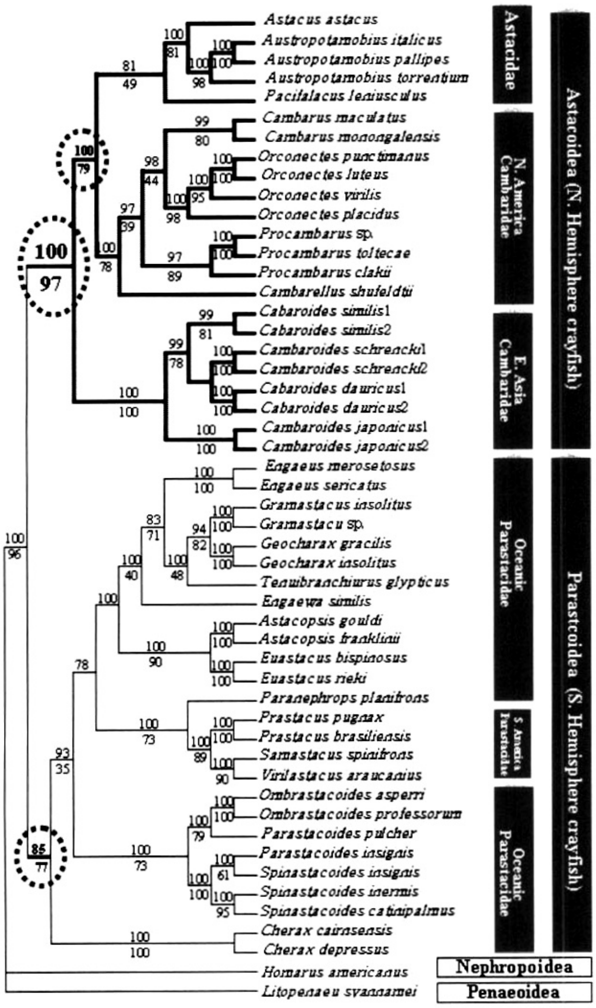

Figure 2. Phylogenetic relationship of freshwater crayfishes based on the mitochondrial 16S rDNA sequences. The trees presented were constructed by neighbor-joining distance analyses. Numbers at branches indicate the Bayesian posterior probabilities (above) and bootstrap percentage of neighbor-joining analysis (below).

implemented in the Modeltest program, GTR+G was selected as the best model that fit the dataset, and six rate classes were used to estimate the shape parameter of the gamma distribution (=0.3472). Using this model, NJ and Bayesian approaches were performed, and the two methods showed largely identical tree topologies. From these analyses, monophyly of each astacid superfamily, Astacoidea and Parastacoidea, was strongly supported by high bootstrap values and high posterior probabilities. In our analysis, however, the Asiatic crayfishes (*Cambaroides*) occupied the basal position of Astacoidea as a third group, and showed sister relationships with the Cambaridae and Astacidae clades (Fig. 1). Therefore, the superfamily Astacoidea consisted of three distinct monophyletic groups, Astacidae, Cambaridae minus *Cambaroides,* and genus *Cambaroides* groups.

A focus on the Astacoidean ingroup radiation, distance (NJ), parsimony (MP), likelihood (ML), and posterior probability test (Bayesian) yielded congruent topologies with high bootstrap values and high posterior probabilities, identical with total dataset analyses (Fig. 2).

It is very interesting that the genus *Cambaroides* was not included in family Cambaridae, as supported by recent molecular phylogenetic analysis (Crandall et al., 2000). Our results, however, conflict with traditional taxonomy because, in terms of morphological criteria, *Cambaroides* has been widely accepted as a member of Cambaridae (Hobbs, 1974, 1988). Meanwhile, other studies supported the close relationships of *Cambaroides* to Astacidae rather than to Cambaridae; no cyclic dimorphism, considered the most important characteristic in distinguishing between the two Astacoidea families, was found from male *Cambaroides* as Astacidae (Kawai and Saito, 1998); embryonic characters of the maxillae of *C. similis* in juvenile stage 1 and the antenna, maxilla, and pleopods in juvenile stage 2 are more similar to those of Astacidae than to Cambaridae (Ko and Kawai, 2001). Therefore, the phylogenetic position of *Cambaroides* is quite questionable, even in regard to morphological criteria.

Based on molecular analysis, however, the phylogenetic relationship of Northern Hemisphere crayfishes is well congruent with the biogeographic relationships of plant taxa of eastern Asia, and western and eastern North America. The disjunct distribution of morphologically similar plants between eastern Asia and eastern North America is also a classical topic in plant biogeography (Wen, 1998; Wen et al., 1998; Tiffney and Manchester, 2001). In recent molecular phylogenetic studies, although a few exceptions exist (eg., *Ligudambar* and *Staphylea*), many plant groups showed close relationships among taxa from eastern North America and western North America, with eastern Asia basal to the North American clades. The morphological similarity in these disjunct plant groups might be attributable to evolutionary convergence by the general similarity of the habitats of the eastern Asian and eastern North American taxa (Qiu et al., 1995).

Our data used in this paper are not sufficient for discussing the geographical dispersal routes and/or the

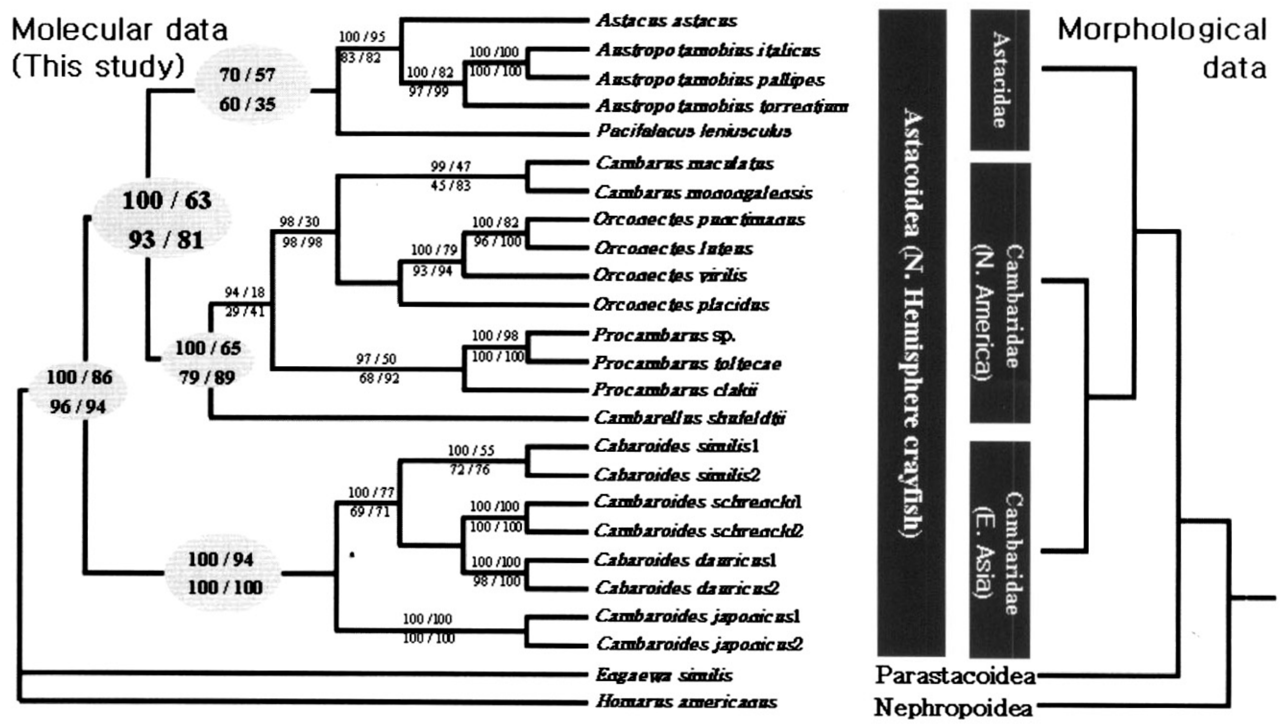

Figure 3. Phylogenetic relationship of Northern Hemisphere crayfishes based on the mitochondrial 16S rDNA sequences. The alignment includes only astacoid crayfishes and two outgroup species to determine more detailed phylogenetic relationships within Northern Hemisphere crayfishes. The trees presented were constructed by neighbor-joining distance analyses. Numbers at branches indicate the posterior probabilities and bootstrap percentage of Bayesian/maximum-likelihood (above) and bootstrap percentages of maximum parsimony/neighbor-joining analysis (below).

origins of Northern Hemisphere crayfishes in detail. Nevertheless, the following inferences, based on the combined data of recent crayfish phylogeny and plant biogeography, can be addressed as the most plausible hypothesis for the pattern of distribution of the Northern Hemisphere crayfishes. Firstly, the ancestral astacid (family Astacidae) arose from the astacuran prototype (ancestor of superfamily Astacoidea, and the modern *Cambaroides* may contain the most direct descendants of this primitive crayfish ancestor) around eastern Asia. Secondly, the ancestral groups of astacids, crossed Central Asia and the Bering Straight, and emigrated to Europe and western North America. Finally, the astacids of western North America gave rise to the North American cambarid type.

The strength of our idea is that the distribution patterns of holarctic (Asian European and North American) crayfishes can be explained rather comprehensively without enigmatic disjunction (Enghoff, 1995), and appear to be largely concordant with recent plant biogeography. However, it is important to note that our assumption is standing on very weak fundamentals with many unexplained parts due to the paucity of fossil records and living intermediates. The distribution areas of Asiatic crayfishes are highly restricted and are found only western Asia, at areas bordering Europe, and in a small part of eastern Asia. No living crayfish has been collected across the hypothetical astacid migration routes (Fig. 1). Therefore, to reconstruct a reliable phylogeny of crayfishes of the Northern Hemisphere, we must collect more evidence from fossil records, biogeography of other freshwater animal groups, and multiple molecular data from both nuclear and mitochondrial genes.

ACKNOWLEDGMENTS

We thank Dr. Yong-Woo Lee of Yanbian University of Science and Technology for his collection and provision of specimens of *Cambaroides dauricus*. We are thankful to Dr. Byung-Hee Choi for his comments

on plant biogeography.

REFERENCES

Crandall KA and Fitzpatrick JF Jr (1996) Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. Syst. Biol. 45: 1-26.

Crandall KA, Fetzner JW Jr, Jara CG and Buckup L (2000) On the phylogenetic positioning of the South American freshwater crayfish genera (Decapoda: Parastacidae). J. Crust. Biol. 20: 530-540.

Crandall KA, Fetzner JW Jr, Lawler SH, Kinnersley M and Austin CM (1999) Phylogenetic relationships among the Australian and New Zealand genera of freshwater crayfishes (Decapoda: Parastacidae). Aust. J. Zool. 47: 199-214.

Crandall KA, Harris DJ and Fetzner JW Jr (2000) The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. Proc. R. Soc. Lond. 267: 1679-1686.

Cukerzis JM (1988) On the origin of freshwater crayfish (*Astacura*). Freshwater Crayfish 7: 343-349.

Enghoff H (1995) Historical biogeography of the Holarctic: area relationships, ancestral areas, and dispersal of non-marine animals. Cladistics 11: 223-263.

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17: 368-376.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Fitch WM (1971) Towards defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. 20: 406-416.

Grandjean F, Harris DJ, Souty-Grosset C and Grandjean KA (2000) Systematics of the European endangered crayfish species *Austropotamobius pallipes* (Decapoda: Astacidae). J. Crust. Biol. 20: 522-529.

Hobbs HH Jr (1974) Synopsis of the families and genera of crayfishes Crustacea: Decapoda. Smith. Contr. Zool. 164: 1-32.

Hobbs HH Jr (1988) Crayfish distribution, adaptive radiation and evolution. In *Freshwater crayfish: biology, management and exploitation*, D.M. Holdich and R.S. Lowery, eds., Timber press, Portland, pp. 52-82.

Jung S-O, Kum BG, Yoon YD and Lee J-S (2006) Complete mitochondrial genome of the Korean fire-bellied frog *Bomina orientalis* from Korea (Anura, Bombinatoridae) and difference between biogrographically different individual from China. Korean J. Genet. 28: 17-26.

Kawai T and Saito K (1998) Taxonomic implication of the "form" and further morphological characters for the crayfish genus *Cambaroides* (Cambaridae). In *Freshwater Crayfish 12*, M. Keller, M.M. Keller, B. Oidtman, R. Hoffmann and G. Vogt, eds., International Association of Astacology, Augsburg, Germany, pp. 82-89.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

Ko HS and Kawai T (2001) Postembryonic development of the Korean crayfish, *Cambaroides* similes (Decapoda, Cambaridae) reared in the laboratory. Korean J. Syst. Zool. 17: 35-47.

Lee J-S (2003) cDNA cloning of *rho*A gene from the intertidal harpacticoid copepod *Tigropus japonicus* (Crustacea, Copepoda). Korean J. Genet. 25: 403-408.

Lodge DM, Taylor CA, Holdich DM and Skurdal M (2000) Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries 25: 7-20.

Munasinghe DHN, Burridge CP and Austin CM (2004) Molecular phylogeny and zoogeography of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in Australia. Biol. J. Linn. Soc. London 81: 553-563.

Ortmann AE (1902) The geographical distribution of freshwater decapods and its bearing upon ancient geography. Proc. Am. Phil. Soc. 41: 267-400.

Posada D and Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Qiu YL, Chase MW and Parks CR (1995) A chloroplast DNA phylogenetic study of the eastern Asia-eastern North America disjuct section *Rytidospermum* of *Magnolia* (Magnoliaceae). American J. Botany 82: 1582-1588.

Saitou N and Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

Scholtz G (2002) Phylogeny and Evolution. In *Biology of freshwater crayfish*, D.M. Holdich, ed., Blackwell Science, London, pp. 30-52.

Scholtz G and Richter S (1995) Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). Zool. J. Linn. Soc. 113: 289-328.

Swofford DL (2003) PAUP, Phylogenetic analysis using parsimony, and other Methods, ver. 4.0 (Software).

Sinauer Associates, Sunderland, MA.

Thompson JD, Gibson TJ, Plewniak DE, Jeanmougin F and Higgins DG (1997) The CLUSTAL X-windows interface: flexible strategies for multiple sequence alignment aided by quality analysis. Nucleic Acids Res. 25: 4876-4882.

Tiffney B and Manchester S (2001) The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere tertiary. Int. J. Plant Sci. 162 (Suppl.): S3-S17.

Villalobos A (1983) Crayfishes of Mexico (Crustacea: Decapoda). Amerind Publishing Co., New Dhli, 276pp. (translated from Spanish)

Wen J (1998) Evolution of the eastern Asian and eastern North American disjuct pattern: insights from phylogenetic studies. Kor. J. Plant Tax. 28: 63-81.

Wen J, Shi S, Jansen RK and Zimmer EA (1998) Phylogeny and biogeography of *Aralia* sect. *Aralia* (Araliaceae). American J. Botany 85: 866-875.