

Systematic Relationships of Korean Freshwater Snails of *Semisulcospira*, *Koreanomelania*, and *Koreoleptoxis* (Cerithioidae; Pleuroceridae) revealed by Mitochondrial Cytochrome Oxidase I Sequences

Woo-Jin Kim¹, Dae-Hee Kim⁴, Jun Sang Lee², In-Chul Bang³, Wan-Ok Lee⁴ and Hyungtaek Jung¹

¹Biotechnology Research Division, National Fisheries Research and Development Institute, Busan 619-902, Korea

²Institute of Environmental Research, Kangwon National University, Chunchon 200-701, Korea;

³Department of Marine Biotechnology, Soonchunhyang University, Asan 336-745, Korea

⁴Central Regional Inland Fisheries Research, National Fisheries Research and Development Institute, Cheongpyeong, Gyeonggi-do 477-815, Korea

ABSTRACT

Many freshwater snail taxa are difficult to identify using morphological traits due to phenotypic plasticity. However, using of molecular DNA marker in combination with morphological traits can provide a reliable means for discriminating among freshwater snail taxa including cryptic species. To discriminate among Korean freshwater snail taxa and resolve their systematic relationships, wesequenced a fragment of mtDNA cytochrome oxidase I (COI) gene from 82 specimens collected from ten different sites distributed along the Korean peninsula. We identified more than seven freshwater snail taxa including cryptic species in Korea. Whereas traditional shell morphology of freshwater snails offers only weak discriminatory power for recognizing 'good' taxa, DNA sequence data provided positive and reliable identification. In addition, a major *Semisulcospira* clade was clearly separated from the remaining lineages observed including cryptic species. However, a phylogenetic tree inferred from the COI gene data did not fully resolve systematic relationships among pleurocerid taxa in Korea. Establishing more robust shell characteristics for identifying taxa unambiguously and hence improving traditional key shell morphology characters for freshwater snail species is an urgent requirement and will require more rigorous examination of all nominal taxa. While molecular data generated here will be useful for species identification and for describing the systematic relationships among Korean freshwater snails, further analysis will be required.

Key words: freshwater snail, *Semisulcospira*, *Koreanomelania*, *Koreoleptoxis*, mitochondrial cytochrome oxidase I, systematic relationships

INTRODUCTION

Freshwater snails in the genera *Semisulcospira*, *Koreanomelania*, and *Koreoleptoxis* are widely distributed across East Asia (Davis, 1969). While

snails have long been used for human consumption, over-exploitation, habitat degradation, and water pollution by insecticides and heavy metals have resulted in population declines and loss of species richness and diversity. Restoration efforts based on cytological and reproductive studies have been conducted (Kim *et al.*, 1987; Chang *et al.*, 2000) to address declining snail populations. However, accurate species identification remains problematic and can compromise restoration programs. As an initial step, understanding species delimitation is an important component in developing successful and sustainable

Received September 27, 2010; Revised October 21, 2010;
Accepted November 1, 2010

Corresponding author: Kim, Woo-Jin and Jung, Hyungtaek
Tel: +82 (51) 720-2451

e-mail: wjkim@nfrdi.go.kr, chunght77@gmail.com
1225-3480/24364

Table 1. Freshwater snail species used in this study, their sampling locations, abbreviations and GenBank accession numbers

Taxa	Sampling locations	Abbreviation	Sample number	GenBank accession no.
<i>S. libertina</i>	Jeju-si, Jeju-do, Korea	JJJ	10	HM991871
<i>S. gottschei</i>	Inje-gun, Gangwon-do, Korea	GJJ	9	HM991872-5
<i>S. gottschei</i>	Yangpyeong-gun, Gyeonggi-do, Korea	GYP	7	HM991876
<i>S. forticosta</i>	Samcheok-si, Gangwon-do, Korea	GSC	8	HM991877-8
<i>S. tegulata</i>	Damyang-gun, Jeollanam-do, Korea	JDY	4	HM991879
<i>S. coreana</i>	Yanggu-gun, Gangwon-do, Korea	GYG	7	HM991880-1
<i>S. coreana</i>	Goseong-gun, Gangwon-do, Korea	GGS	10	HM991882-3
<i>S. coreana</i>	Yeongdong-gun, Chungcheongbuk-do, Korea	CYD	7	HM991884-7
<i>Koreanomelania nodifila</i>	Pyeongchang-gun, Gangwon-do, Korea	GPN	10	HM991888-9
<i>Koreoleptoxis globus ovalis</i>	Pyeongchang-gun, Gangwon-do, Korea	GPG	10	HM991890-3
* <i>Juga nigrina</i>	U.S.A.		1	EF586984

*Designated outgroup for our phylogenetic analyses.

(Applied Biosystems).

3. Statistical analysis

Nucleotide sequence data analyzed in this study were deposited in GenBank under accession numbers HM991871-HM991893. The resulting sequences were edited and aligned using SeqMan II (DNASTAR). For clarity and convenience, all sequences were trimmed to the same length after removal of primer sites. Estimates of nucleotide and haplotype diversity were obtained using DnaSP v5.10 (Rozas *et al.*, 2003). The best-fitting model (HKY + I + G a = 1.1362 and I = 0.5975) was selected through a hierarchical likelihood ratio test in Modeltest 3.7 (Posada and Crandall, 1998). A systematic relationship tree was reconstructed using the neighbor-joining (NJ) method in Phylip v3.67 (Felsenstein, 1989), the maximum likelihood (ML) method in PhyML v3.0 (Guindon and Gascuel, 2003), and Bayesian inference (BI) analysis in MrBayes v3.1.1 (Huesenbeck and Ronquist, 2001). Tree manipulation was performed using TreeDyn (Chevenet *et al.*, 2006). Non-parametric bootstrapping with 1000 pseudo-replications was performed to estimate the confidence of all tree topologies. For Bayesian analysis, the same model used for the ML analysis was adapted, and Markov chain Monte Carlo runs of 10^6 generations long in four chains were conducted, sampling trees every 100 generations.

Burn-in was determined by plotting parameters across all runs for a given analysis. North American taxa *Juga nigrina* (EF586984) were employed as outgroups in all phylogenetic analyses.

RESULTS

1. DNA sequence analysis

The alignment of 82 Korean freshwater snail sequences resulted in 74 putatively positive species after identification following Martens' (1905) diagnostic shell characters. COI gene sequences revealed a total of 27 haplotypes including four haplotypes from eight ambiguous sequences in *Semisulcospira*, *Koreanomelania* and *Koreoleptoxis* taxa. The COI fragments were composed of 658 sites after alignment. All observed mitochondrial single nucleotide polymorphisms (mtSNPs) within populations constituted silent mutations. Eight, five, one, and three silent mutations were detected in *S. gottschei* (Inje-gun in Gangwon-do and Yangpyeong-gun in Gyeonggi-do), *S. coreana*, *Koreanomelania nodifila*, and *Koreoleptoxis globus ovalis*, respectively.

Site-specific haplotype diversity ranged from a minimum of 0.286 ± 0.196 to a maximum of 0.905 ± 0.103 among sequences for the *S. coreana* group (Table 2). One major haplotype was present in all *S. coreana* collection sites regardless of geographical

Table 2. Genetic diversity of Korean freshwater snails inferred from mitochondrial cytochrome c oxidase I genes

Presumptive species identification ^a	Abbreviation	No. of individuals sequenced	No. of positive species ^b	No. of haplotypes	Clade affiliation in COI phylogenetic analysis	(H) ^c	(π) ^d
<i>S. libertina</i>	JJJ	10	10	1	<i>S. libertina</i>	0	0
<i>S. gottschei</i>	GIJ	9	9	4	<i>S. gottschei</i>	0.583 ± 0.183	0.00135 ± 0.00055
<i>S. gottschei</i>	GYP	7	7	1	<i>S. gottschei</i>	0	0
All <i>S. gottschei</i> [†]		16	16	5	<i>S. gottschei</i>	0.684 ± 0.081	0.00456 ± 0.00039
<i>S. forticosta</i>	GSC	8	3	2	<i>S. forticosta</i> and Cryptic or new species	0.667 ± 0.314	0.00101 ± 0.00048
<i>S. tegulata</i>	JDY	4	4	1	<i>S. tegulata</i>	0	0
<i>S. coreana</i>	GYG	7	7	2	<i>S. coreana</i>	0.286 ± 0.196	0.00217 ± 0.00149
<i>S. coreana</i>	GGG	10	10	2	<i>S. coreana</i>	0.356 ± 0.159	0.00270 ± 0.00121
<i>S. coreana</i>	CYD	7	7	4	<i>S. coreana</i>	0.905 ± 0.103	0.00926 ± 0.00221
All <i>S. coreana</i> [‡]		24	24	5	<i>S. coreana</i>	0.857 ± 0.108	0.00939 ± 0.00148
<i>Koreanomelania nodifila</i>	GPN	10	9	2	<i>Koreanomelania nodifila</i> and cryptic or new species	0.389 ± 0.614	0.00059 ± 0.00025
<i>Koreoleptoxis globus ovalis</i>	GPG	10	8	4	<i>Koreoleptoxis globus ovalis</i> and cryptic or new species	0.643 ± 0.184	0.00141 ± 0.00051

All statistical values were analyzed by DnaSP V 5.10 (Rozas et al., 2003).

[†] All *S. gottschei* meant samples collected from GIJ and GYP.

[‡] All *S. coreana* meant samples collected from GYG, GGS and CYD.

^a Initial species identification done by field observation. ^b Clade affiliation in COI phylogenetic analyses.

^c Haplotype diversity (H). ^d Nucleotide diversity (π)

region. Estimates of nucleotide diversity (π) were fairly similar across populations (Table 2). The average sequence diversity among *Semisulcospira* species was less than 9%. Sequence divergence also exceeded 14% in *Semisulcospira*, *Koreanomelania*, and *Koreoleptoxis*. Interestingly, *Koreoleptoxis* spp. GPG5, which was originally collected as *K. globus ovalis* from Pyeongchang-gun in Gangwon-do and *Semisulcospira* spp. GSC3 that was originally collected as *S. forticosta* from Samcheok-siin Gangwon-do were highly

divergent from the *Semisulcospira*, *Koreanomelania*, and *Koreoleptoxis* groups, with 15–19.5%, 17–18%, and 17–18% divergence, respectively. High sequence diversity (16.5%) was also observed between species (GPG5 and GSC3). Another interesting result was observed for *Koreoleptoxis* spp. GPG6, which was originally collected as *K. globus ovalis* from Pyeongchang-gun in Gangwon-do, and *Koreanomelania* spp. GPN3, which was originally collected as *K. nodifila* from Pyeongchang-gun in Gangwon-do.

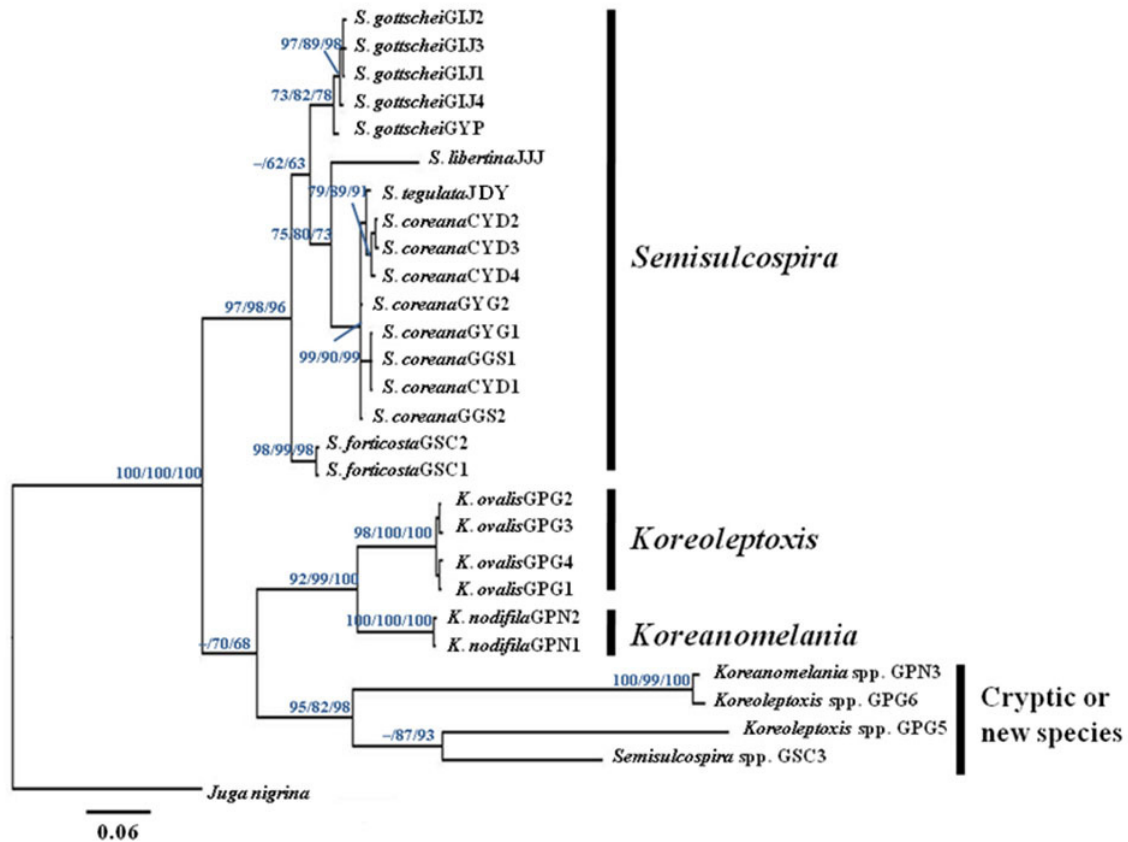


Fig. 2. Molecular phylogeny of Korean freshwater snails inferred from nucleotide sequences of mitochondrial cytochrome oxidase I gene. The numbers on branches indicate bootstrap support in percent based on 1000 pseudo-replications in the neighbor-joining, maximum likelihood analyses and the posterior probabilities in Bayesian analysis, respectively. The dashes (-) at a node indicate bootstrap support lower than 50%. *Juga nigrina* was used as a designated outgroup.

Although the two species were highly divergent from the *Semisulcospira*, *Koreanomelania*, and *Koreoleptoxis* groups (17% divergence), they were only 1.4% divergent (GPG6 and GPN3).

2. Systematic analysis

Given that Korean freshwater snails are well known for presenting taxonomic difficulties, phylogenetic trees were inferred from partial mitochondrial COI sequences to improve our understanding of the systematic relationships among them. Neighbor-joining (NJ), maximum likelihood (ML), and Bayesian inference (BI) analyses yielded monophyletic tree topologies (Fig. 2). Haplotypes from each *Semisulcospira* species collected from several locations were monophyletic and supported with high

bootstrap values. Although *S. coreana* clades showed a polytomy, all three phylogenetic approaches generated essentially the same tree topologies with significant statistical support. Assuming this tree to be accurate, *S. tegulata* JDY formed a sister clade with *S. coreana* groups, and *S. libertina* JJJ formed a basal clade with *S. tegulata* and *S. coreana*. The placement of *S. gottschei* among *Semisulcospira* taxa was, however, not clearly resolved here (low bootstrap support). The remaining *S. forficata* were recovered as a sister clade to *Semisulcospira* with high statistical support.

The remaining clade of *K. globus ovalis* and *K. nodifila* formed a monophyletic group and are clearly sister taxa. The remaining four species, *Koreanomelania* spp. GPN3, *Koreoleptoxis* spp. GPG5 and GPG6, and *Semisulcospira* spp. GSC3 formed an

additional monophyletic group clearly separated from *Semisulcospira* taxa. However, these cryptic taxa were not clearly separated from *K. globus ovalis* and *K. nodifila* (low bootstrap values). While these relationships were recovered partially here, the precise positioning of *Koreanomelania* spp GPN3, *Koreoleptoxis* spp GPG5 and GPG6, and *Semisulcospira* spp. GSC3 within the Korean freshwater snail clade remains ambiguous (Fig. 2). Further observation of the cryptic species and their clear identity within the COI tree could indicate that more than seven species are present in Korea but a more detailed study (molecular and morphological data) will be required to resolve these systematic relationships.

DISCUSSION

1. Species composition of *Semisulcospira*

Despite several attempts to characterize *Semisulcospira* and other species of Pleuroceridae using molecular markers (Minton and Lydeard, 2003; Lee *et al.*, 2006; Lee *et al.*, 2007), this is the first study to use a substantial number of partial COI sequences from Korean freshwater snails. The monophyly of all presumed *Semisulcospira* individuals in this study (excluding *Semisulcospira* spp. GSC3) suggests that the Korean *Semisulcospira* species form a single natural group. The tree-based species delimitation approach using NJ, ML, and BI analyses strongly indicates the presence of multiple species in Korea (at least seven taxa).

In ML and BI analyses, *S. tegulata* JDY was identified as sister to *S. coreana*, while *S. libertina* JJJ was identified as sister to both *S. coreana* and *S. tegulata* JDY with reasonable statistical support (NJ = 84, ML = 80, BI = 91). These results may imply that the endemic *S. coreana*, *S. tegulata*, and *S. libertina* all share a most recent common ancestor. Despite use of only small sample sizes, *S. coreana* was most closely related to *S. tegulata* and *S. libertina*, and this outcome from mtDNA was congruent with reported isozyme relationships (Kim, 1995).

The maximum sequenced divergence within a

putative species was 1.8% for *S. coreana* and 0.9% for *S. gottschei*. The genetic divergences observed for *Semisulcospira* were comparable to the largest published values for mitochondrial 16S rDNA (3.7%) and COI (4.27%) sequence variation in other Pleuroceridae (Lydeard *et al.*, 1997, 1998; Holznagel and Lydeard, 2000; Minton and Lydeard, 2003). While the maximum putative intra- and intergeneric divergence observed here was 19.5%, previously reported maximum intra- and intergeneric differences were 15% for 16S rDNA (Holznagel and Lydeard, 2000) and 15.5% for COI (Minton and Lydeard, 2003). Additional studies are required to understand the dispersal history of *S. coreana* across Korean rivers and drainages because the genetic distances among the observed haplotypes were small and haplotypes were shared among *S. coreana* sites (CYD, GGS, and GYG). A possible explanation is that insular *S. coreana* populations may have attained their unique morphological features rapidly following recent dispersal events. Alternatively, samples could have come from restocking program strains from aquaculture lines. If so, this suggests that potential gene flow by human-made activities could have produced the patterns of genetic mixing observed here. Unlike in *S. coreana*, the genetic distances among observed *S. gottschei* haplotypes (GIJ and GYP) were small, and no haplotypes were shared between the two collecting sites, suggesting limited gene flow.

The two species are known to co-exist in one site even though clear morphological differences exist between them. This observation led to speculation that reproductive isolation between them was limited, indicating possible crossbreeding in the wild (Kim, 1995; Minton and Lydeard, 2003). Cytological research on *S. forticosta* and *S. gottschei* found that karyotypes were different but the two taxa possessed the same chromosome numbers (Kim *et al.*, 1987). The close relationship among them was also denoted using isozyme markers, a result consistent with previous findings (Kim, 1995). Based on molecular data, it is possible that the *Semisulcospira* taxa studied here do constitute 'true' phylogenetic species however,

additional studies, including those of detailed ectopic placement, possible hybrids, and ecological and genetic comparisons of *Semisulcospira* species populations within the archipelago, are needed to further clarify the mechanisms underlying their morphological variations.

2. Species delineation of *Koreanomelania* and *Koreoleptoxis*

Melania nodifila and *M. globus* (Martens, 1886) were originally classified as *S. nodifila* and *S. globus*, respectively, by Kuroda (1929). They were placed into the genus *Koreanomelania* based on their oviparous LHTs (Kwon and Habe, 1979). Burch *et al.* (1987), however, recorded *K. nodifila* (Kwon and Habe, 1979) as *Hua nodifila* and Burch and Jung (1987) doubted that *M. globus* was present in Korea so they denoted a new subspecies, *K. globus ovalis*, which was separated from previous recorded species. In addition, its morphological features were identical to *Koreanomelania globus* (Kwon, 1990; Kwon *et al.*, 1993). Thus, *Semisulcospira globus* and *Koreanomelania globus* (Kwon *et al.*, 1993) were treated as a new subspecies as suggested by Burch and Jung (1987). In recent studies, nine species were identified in Korean freshwater ecosystems (Choi and Yoon, 1997), and seven species were classified by isozyme studies and external radulae analysis (Ko *et al.*, 2001; Lee *et al.*, 2001). In this study, systematic analysis of mtDNA COI sequence divergence recognized more than seven taxa in Korea. However, several previous studies indicated the presence of divergent mitochondrial haplotypes among the pleuroceridae, which has been attributed to undocumented cryptic species being present (Lydeard *et al.*, 1998; Minton and Lydeard, 2003, Lee *et al.*, 2007). This recognition needs to be treated cautiously, however, due to the limited genetic and morphological information available for these taxa. The remaining two focal taxa (*K. globus ovalis* and *K. nodifila*) were monophyletic in our analysis. However, according to previous isozyme variation studies (Lee *et al.*, 2001) and a study of external radulae (Ko *et al.*, 2001), three genera in the pleuroceridae are present in Korea, indicating a separate genus between

Koreoleptoxis and *Koreanomelania*.

Phylogenetic analysis can provide evidence for discrete lineages based on sequence diversity when morphological plasticity makes species delineation problematic (Wilke and Falinowski, 2000). This could be useful in pleurocerids, where cryptic species that are not closely related have been observed regularly (Chambers, 1978). Based on this assumption, potentially five (*Semisulcospira*) and six (*Koreoleptoxis* and *Koreanomelania* including cryptic species) phylogenetic species may be recognized based on mtDNA COI sequence divergence in Korean pleurocerids (Fig. 2). Further morphological and molecular work will be required to determine the identity and placement of the four cryptic species (*Koreanomelania* spp GPN3, *Koreoleptoxis* spp GPG5 and GPG6, and *Semisulcospira* spp. GSC3). The remaining taxa formed a well-supported clade that should be recognized as two lineages, *Semisulcospira* and a group of *Koreoleptoxis* and *Koreanomelania* (as a sister genus) here. It is, however, still difficult to say how many extant species are present in Korea. Although use of shell characters has led to many errors and confusion in freshwater snail taxonomy, the data here suggest that at least more than nine species could be present in Korea based on phylogenetic analysis inferred from the mitochondrial COI gene sequence. The results here suggest that cryptic taxa could also be present in other freshwater snail taxa in Korea and are awaiting recognition. Long-term secure protection for cryptic taxa will require both analyses of genetic (mitochondrial and nuclear markers) and morphological data, as well as redefinitions of key shell morphology characters for freshwater snails to improve recognition of natural biodiversity in the region.

ACKNOWLEDGEMENTS

The authors thank K.Y. Kim and Professor Peter Mather for providing helpful comments and suggestions on the manuscripts. This study was supported in part by National Fisheries Research and Development Institute (RP-2010-BT-025) and in part by Korea Ministry of Environment as "The

Eco-technopia 21 project".

REFERENCES

- Asahida, T., Kobayashi, T., Saitoh, K. and Nakayama, I. (1996) Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. *Fisheries Science*, **62**: 727-730.
- Burch, J.B., Chung, P.R. and Jung, Y. (1987) A guide to the fresh water snails of Korea. *Walkerana*, **2**: 195-232.
- Burch, J.B. and Jung, Y. (1987) A new freshwater prosobranch snail (Mesogastropoda: Pleuroceridae) from Korea. *Walkerana*, **2**: 287-293.
- Chambers, S.M. (1978) An electrophoretically detected sibling species of *Goniobasis floridensis*. *Malacologia*, **17**: 157-162.
- Chang, Y.J., Chang, H.J., Min, B.H. and Bang, I.C. (2000) Reproductive cycle of the melania snail, *Semisulcospira libertina libertina*. *Development and Reproduction*, **4**: 175-180.
- Chevenet, F., Brun, C., Banuls, A.L., Jacq, B. and Christen, R. (2006) TreeDyn: towards dynamic graphics and annotations for analyses of trees. *BMC Bioinformatics*, **7**: 439.
- Choi, B.R. and Yoon, S.H. (1997) Mollusca. In: List of animals in Korea. The Korean Society of Systematic Zoology, Seoul, Korea.
- Davis, G.M. (1969) A taxonomic study of some species of *Semisulcospira* in Japan (Mesogastropoda: Pleuroceridae). *Malacologia*, **7**: 211-294.
- Felsenstein, J. (1989) PHYLIP - Phylogeny Inference Package (Version 3.2). *Cladistics*, **5**: 164-166.
- Folmer, D., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrate. *Molecular Marine Biology and Biotechnology*, **3**: 294-299.
- Guindon, S. and Gascuel, O. (2003) PhyML - A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**: 696-704.
- Holznagel, W.E. and Lydeard, C. (2000) A molecular phylogeny of North American Pleuroceridae (Gastropoda: Cerithioidea) based on mitochondrial 16S rDNA sequences. *Journal of Molluscan Studies*, **66**: 233-257.
- Huesenbeck, J.P. and Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754-755.
- Jeong, Y., Park, G.M., Park, J., Kim, J.J., Min, D.Y. and Chung, P.R. (1999) Allozyme variability in three species of genus *Semisulcospira* (Prosobranchia: Pleuroceridae). *The Korean Journal of Malacology*, **15**: 13-20.
- Kim, S.S., Kim, D.C., Chung, P.L. and Chung, Y.J. (1987) A cytological study on two species of genus *Semisulcospira* (Gastropoda: Pleuroceridae) in Korea. *The Korean Journal of Malacology*, **3**: 24-34.
- Kim, J.J. (1995) Isozyme variation of the genus *Semisulcospira* (Pleuroceridae: Gastropoda) in Korea. *The Korean Journal of Malacology*, **11**: 171-179.
- Ko, J.H., Lee, J.S. and Kwon, O.K. (2001) Study on radulae of seven species of the Family Pleuroceridae in Korea. *The Korean Journal of Malacology*, **17**: 105-115.
- Kuroda, T. (1929) On the species of Japanese Kawina (*Semisulcospira*). *Venus*, **1**: 179-193.
- Kwon, O.K. and Habe, T. (1979) A list of non-marine molluscan fauna of Korea. *Korean Journal of Limnology*, **12**: 24-31.
- Kwon, O.K. (1990) Illustrated encyclopedia of fauna and flora of Korea Vol. 32. Mollusca (I). Ministry of Education. Seoul, Korea.
- Kwon, O.K., Park, G.M. and Lee, J.S. (1993) Colored shells of Korea. Academy publishing company. Seoul, Korea.
- Lee, J.S., Ko, J.H. and Kwon, O.K. (2001) Isozyme variation in two species of freshwater Pleurocerid snails in Korea: *Koreanomelania nodifila* and *Koreoleptoxis globus ovalis*. *The Korean Journal of Malacology*, **17**: 117-123.
- Lee, T., Hong, H.C., Kim, J.J. and Ó Foighil, D. (2006) Crossing the continental divide: the Columbia drainage species *Juga hemphilli* (Henderson 1935) is a cryptic member of the eastern North American genus *Elimia* (Cerithioidea: Pleuroceridae). *Journal of Molluscan Studies*, **72**: 314-317.
- Lee, T., Hong, H.C., Kim, J.J. and Ó Foighil, D. (2007) Phylogenetic and taxonomic incongruence involving nuclear and mitochondrial markers in Korean populations of the freshwater snail genus *Semisulcospira* (Cerithioidea: Pleuroceridae). *Molecular Phylogenetics and Evolution*, **43**: 386-397.
- Lydeard, C., Holznagel, W.E., Garner, J., Hartfield, P. and Pierson, M. (1997) A molecular phylogeny of Mobile River drainage pleurocerid snails (Caenogastropoda: Cerithioidea). *Molecular Phylogenetics and Evolution*, **7**: 117-128.
- Lydeard, C., Yoder, J.H., Holznagel, W.E., Thompson, F.G. and Hartfield, P. (1998) Phylogenetic utility of the 5'-half of mitochondrial 16S rDNA gene sequences for inferring relationships of *Elimia* (Cerithioidea: Pleuroceridae). *Malacologia*, **39**: 183-193.
- Martens, E. von (1886) In Japan und Korea gesammelten land-und Süßwasser-Mollusken. Sitzungs-Bericht der Gesellschaft naturforschender Freunde zu Berlin, **5**: 76-78.
- Martens E. von (1905) Koreanische Süßwasser-Mollusken. *Zoologische Jahrbücher Supplement*, **8**: 23-70.
- Milton, R.L. and Lydeard, C. (2003) Phylogeny, taxonomy, genetics and global heritage ranks of an imperiled, freshwater snails genus *Lithasia*

- (Pleuroceridae). *Molecular Ecology*, **12**: 75-87.
- Miyana, M. (1942) Kwwanina snails of the genus *Semisulcospira* from Korea. *Chosen Hakubutsu Zasshi. Korean Journal of Biology Science*, **9**: 114-130.
- Posada, D. and Crandall, K. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**: 817-818
- Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X. and Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**: 2496-2497.
- Wilke, T. and Falinowski, A. (2000) The genus *Adriohydrobia*: polytypic species or polymorphic populations? *Journal of Zoological Systematics and Evolutionary Research*, **39**: 227-234.