

## RESEARCH NOTE

### RARE, DIVERGENT KOREAN *SEMISULCOSPIRA* SPP. MITOCHONDRIAL HAPLOTYPES HAVE JAPANESE SISTER LINEAGES

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Cerithioidean gastropods have mounted two or three separate invasions of freshwater environments and comprise a substantial fraction of global freshwater snail diversity (Strong *et al.*, 2011). Freshwater cerithioideans have been the subject of considerable molecular phylogenetic study, especially using mitochondrial (mt) markers, and such studies typically document a complex pattern of genetic structuring involving pronounced among-species mt non-monophyly (Minton & Lydeard, 2003; Glaubrecht & Köhler, 2004; Köhler *et al.*, 2004; Köhler & Glaubrecht, 2006; Lee *et al.*, 2007; Glaubrecht & Rintelen, 2008; Dillon & Robinson, 2009; Kim *et al.*, 2010; Köhler, Panha & Glaubrecht, 2010; Glaubrecht, 2011). The resulting topological/taxonomic incongruencies have been attributed to a number of mechanisms: the presence of cryptic species (Minton & Lydeard, 2003; Kim *et al.*, 2010); incomplete lineage sorting in ancient lineages (Dillon & Robinson, 2009); incomplete lineage sorting in young radiations caused by either rapid speciation or by secondary introgression (Glaubrecht & Köhler, 2004; Köhler *et al.*, 2004; Köhler & Glaubrecht, 2006); and introgressive hybridization (Glaubrecht & Rintelen, 2008; Köhler *et al.*, 2010; Köhler & Deen, 2010).

One of the most perplexing cases of freshwater cerithioidean mt and nuclear genotype non-monophyly involved Korean populations of the Northeast Asian genus *Semisulcospira* (Lee *et al.*, 2007) and this present study focuses exclusively on the mt heterogeneity documented therein. *Semisulcospira libertina* (Gould, 1859) and multiple co-occurring nominal congeners [excluding the phylogenetically distinct *S. extensa* (Martens, 1886)] exhibited extraordinary within-population levels of mt diversity that resolved into two taxonomically heterogeneous clades differing markedly in phylogenetic structure (Lee *et al.*, 2007). A numerically predominant (modal) clade exhibited drainage-level structuring and was characterized by short internal branches. In contrast, a subset of rare, genetically divergent haplotypes formed a deeply branched sister clade that lacked drainage-level structuring. Extreme mt diversity was observed in the most heavily sampled Korean *S. libertina* population: snails from a single Geum River location, Muju (MJ), contained five of the seven highly divergent rare mt lineages detected in Korea, in addition to the modal clade (Lee *et al.*, 2007).

Lee *et al.* (2007) found that the available data did not support paralogous mt marker phenomena or the presence of cryptic species. However, they could not distinguish among retention of ancestral polymorphisms and dispersal events as causative mechanisms. The rare, deeply branched mt clade was particularly enigmatic: although it lacked drainage-level structuring, it was ostensibly a product of prolonged cladogenesis in Korean watersheds. Lee *et al.* (2007: 386) were of the view that "... it may require the genetic characterization of *Semisulcospira* lineages throughout much of their collective East Asian range to address this issue". If the rare divergent Korean genotypes stem from dispersal events, an expanded sampling of regional *Semisulcospira* spp. populations may uncover convincing sister lineages within putative source populations.

In addition to Korea, *S. libertina* ranges throughout Japan (Davis, 1972) and is regarded as the most common Japanese freshwater snail (Nakano, 1990). Two of us (OM and FK) independently sampled and genotyped Japanese *Semisulcospira* species, including *S. libertina*, from a combined total of 63 locations in Kyushu, Shikoku, Honshu and Hokkaido (detailed in Supplementary Material Table S1 and Fig. S1). A total of 136 of these Japanese snails were collectively genotyped for the same large mt ribosomal (16S) fragment utilized by Lee *et al.* (2007) in their Korean study (see Supplementary Material for details of the genotyping protocols). Combining these unpublished Japanese mt genotypes with the Korean dataset of Lee *et al.* (2007) allowed us to test the phylogenetic affinities of the two Korean mt clades (Lee *et al.*, 2007) within a regional biogeographic context.

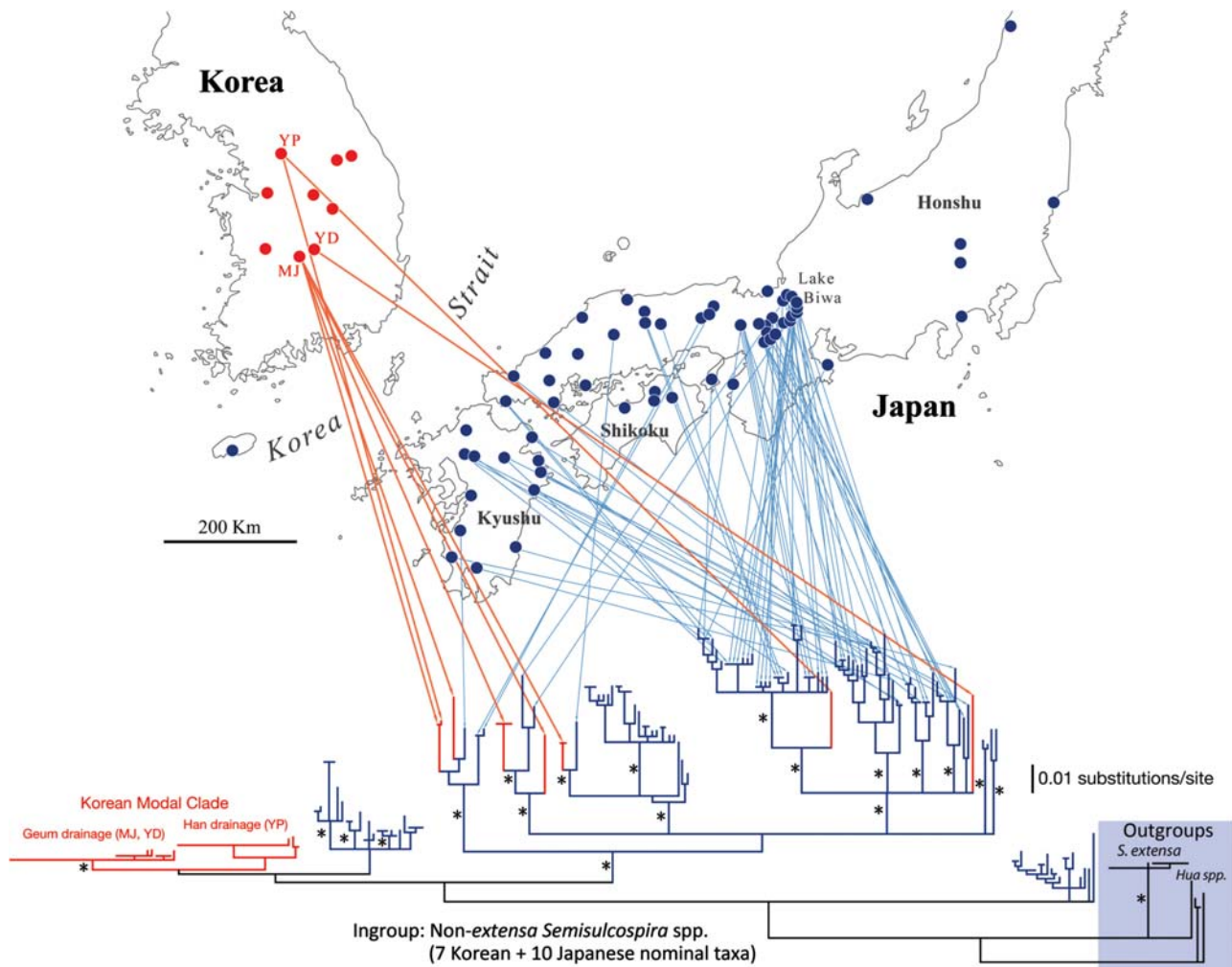
The three mt 16S datasets, one Korean and two Japanese, were aligned for a homologous 544 nucleotide fragment using Se-Al v.2.0a11 (Rambaut, 2002). Bayesian analyses were performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) set for a MrModeltest 3.7-selected GTR +  $\Gamma$  + I model (Posada & Crandall, 1998). Model parameters were treated as unknown and were estimated for each analysis. Four chains were run simultaneously for 1,000,000 generations and trees were sampled every 100 cycles. Posterior probability values were estimated by generating a 50% majority rule consensus tree, after discarding the first 3,000 sampled trees as burn-in, using PAUP\* v. 4.0b10 (Swofford, 2003).

Figure 1 shows, in abridged form, a Bayesian phylogenetic tree and sampling map incorporating Korean and Japanese *Semisulcospira* spp. mt genotypes (see Supplementary Material Figs S1, S2 and Table S1, for details). Korean mt lineages were polyphyletic, exhibiting multiple exclusive sister relationships with Japanese congeners, but the extent of these trans-Korea Strait relationships differed markedly among Lee *et al.*'s (2007) two Korean mt clades. The Korean modal clade remained intact and kept its drainage-level structure, but was now weakly sister to a primarily *S. libertina* Japanese sister clade recovered from Honshu drainages (also from offshore islands). In contrast, Lee *et al.*'s (2007) rare, deeply branched Korean mt clade disappeared. Its seven constituent mt lineages, detected in three Korean populations (Geum Drainage: MJ, YD; Han Drainage: YP), now nested robustly within the numerically predominant Japanese mt clade, each in a separate topological position. Although co-occurring within Korean drainages, they are not sister lineages. Instead, their sister lineages are widely distributed across south-central Japan, being heavily represented among Lake Biwa taxa, but

also among snails (primarily *S. libertina*) from Honshu, Kyushu and Shikoku drainages (see Supplementary Material Figs S1, S2 and Table S1 for details).

Our novel results are incongruent with the hypothesis that the exceptional within-population mt diversity exhibited by the Korean snails is a product of ancestral polymorphism phenomena. Because all seven rare divergent Korean mt lineages are short-branched members of the numerically predominant Japanese clade, they cannot be endemic products of long-term cladogenesis within Korean drainages, as originally suggested by the Korea-only analysis (Lee *et al.*, 2007). The presence of multiple latent trans-Korea Strait phylogenetic ties among the rare divergent Korean haplotypes is surprising because this 195-km-wide marine barrier to dispersal effectively separates Korean and Japanese freshwater snail populations (Fig. 1).

*Semisulcospira* species are part of the traditional cuisines of Korea, *daseulgi* (Lee & Pham, 2010), and of Japan, *kawanina* (Kihira, Matsuda & Uchiyama, 2003). Given this long history of exploitation, and of trans-Korea Strait trade (Park, 2002), undocumented anthropogenic introductions might conceivably



**Figure 1.** Phylogenetic relationships among Korean and Japanese members of the genus *Semisulcospira* inferred by Bayesian analysis of mitochondrial large subunit ribosomal DNA haplotypes. See Supplementary Material Figs S1, S2 and Table S1 for details of taxonomic identity, sampling locality and voucher information associated with each Japanese haplotype; Korean haplotypes were sourced from Lee *et al.* (2007). Mainland Korean sampling sites are depicted in red, Japanese sampling sites (plus Cheju Island) are in blue and this geographic color scheme is also applied to the phylogenetic tree ingroup topology. Geographic location and topological placement of Lee *et al.*'s (2007) seven rare/divergent Korean mt lineages, collectively sampled from three of nine sampling locations [one Han drainage location (Yangpyeong: YP) and two Geum drainage locations (Muju: MJ; Yeongdong: YD)], are indicated with red arrows; the geographical locations of their respective Japanese sister lineages are indicated with blue arrows. Nodes with Bayesian posterior probabilities >95 are labelled with an asterisk.

have resulted in the presence of mt haplotypes with Japanese sister lineages in Korean populations. However, the apparent absence of shared haplotypes in Korea and Japan undermines the case for human introduction, as do estimated divergence times for the seven pairs of Korean/Japanese sister lineages highlighted in Figure 1. We used BEAST v. 1.7.1 (Drummond *et al.*, 2012), and a broad range of estimated gastropod mt 16S sequence divergence rates, from 0.08% (Hausdorf, Röpstorff & Riedel, 2003) to 10% per million years (Ma) (Chiba, 1999) (equivalent to a substitution rate range of 0.04–5% Ma), as a molecular clock to calibrate the 16S phylogeny (see Supplementary Material for details). The seven Korean/Japanese sister lineage estimates ranged from 1.43 Ma [95% highest posterior density (HPD) age intervals: 0.3–3.5 Ma] to 0.76 Ma (95% HPD: 0.1–1.9 Ma) (see Supplementary Material Fig. S3). Although additional sampling is likely to uncover closer trans-Korea Strait sister relationships within this clade, all of our estimates greatly exceeded the 0.038 Ma earliest known record of humans in Japan (Takashi, 2012).

Coastal freshwater drainages may be profoundly rearranged by cyclic marine transgressions (Bănărescu, 1990) and extensive paleo-drainage connections were formed among now-disjunct drainages during Pleistocene Glacial Maxima, particularly in Asia (Voris, 2000). Palaeontological analyses of Japanese mega-faunal invasions have concluded that Japan was connected by land bridges to Southern Korea at least three times (*c.* 1.25, 0.63 and 0.43 Ma) during Early-Middle Pleistocene Glacial Maxima (Yoshikawa, Kawamura & Taruno, 2007), although a connection during the Last Glacial Maximum (*c.* 0.02 Ma) is unlikely (Kawamura, 2007). All three of these inferred Pleistocene Korea Strait closures (Yoshikawa *et al.*, 2007) fall within the 95% HPD age interval (1.9–0.1 Ma) of the youngest of the seven Korea/Japan sister relationships.

Cycles of trans-Korea Strait closure and opening offered potential opportunities for dispersal (presence of land bridge with freshwater drainage contiguity) and vicariance (presence of marine barrier) events to shape Korean–Japanese freshwater snail phylogenetic relationships. The regional literature emphasizes dispersal over vicariance for the non-marine biota, presumably due to the relatively short persistence of inferred land bridges (Konishi & Yoshikawa, 1999; Yoshikawa *et al.*, 2007). In principle, phylogenetic topologies can be used to distinguish among these two processes (Yoder & Nowak, 2006) and the topology observed—individual rare divergent Korean lineages deeply nested within the predominant Japanese clade (Fig. 1)—is consistent with dispersal expectations. In contrast, a vicariant origin is expected to result in a spectrum of exclusive sister relationships within the sundered populations, reflecting their long-term *in situ* persistence and cladogenesis. That characteristic is conspicuously missing for the rare divergent Korean mt haplotypes, but not for their Japanese sister lineages (Fig. 1). A vicariant genesis would require a very distinctive history for Korean members of this mt clade, involving retention of the primary pre-vicariance mt framework, despite severe differential lineage sorting of intermediate haplotypes that removed all evidence of significant within-Korea cladogenesis, and almost complete within-population replacement by modal clade haplotypes. This complex evolutionary scenario seems less than likely.

Based on available data, we conclude that inferred palaeo-dispersal events from Japanese source populations best explain the presence of rare divergent mt haplotypes in Korean *Semisulcospira* species. Many aspects of this inferred dispersal are enigmatic, most notably the unidirectional mt gene flow from Japanese to Korean drainages. It is difficult to envisage a specific dispersal mechanism that would yield such a result. Nevertheless, the trans-Korea Strait dispersal scenario explains many otherwise puzzling attributes of the pronounced Korean

*Semisulcospira* species within-population mt diversity noted by Lee *et al.* (2007). These include their collective non-clock-like topology and, for the rare and divergent Korean haplotypes, their lack of drainage-level structuring and their very long phylogenetic branches bereft of intermediate haplotypes.

Our inference that long-distance palaeo-migration, rather than the presence of cryptic species (Kim *et al.*, 2010), underlies the pronounced within-population mt heterogeneity of Korean *Semisulcospira* spp. populations should help clarify the very challenging taxonomic issues pertaining to this genus in Korea. This inference may also apply to the divergent mt COI haplotypes that Kim *et al.* (2010) documented in *Hua* (*Koreanomelania*) *nodifila* and *Koreoleptoxis globus* populations and ascribed to the presence of multiple cryptic species. For these Korean taxa, the challenge remains to find convincing non-mt characters that can be used as the basis for a corroborated taxonomy (Lee *et al.*, 2007).

The complex population genetic structuring that Lee *et al.* (2007) uncovered in multiple Korean *Semisulcospira* species led them to suggest that molecular phylogenetic characterization of freshwater cerithioidean lineages may require the use of both mt and nuclear markers together with population-level sampling of all nominal taxa within regional drainages. Our new data for this study system compound the scale of the challenge. They reveal that additional sampling of geographically distant Japanese populations is required to provide a full phylogenetic explanation for the divergent mt diversity present within at least some Korean populations. Our results are particularly relevant to other freshwater cerithioidean studies that have attributed heightened within-population mt diversity, in the form of long-branched basal lineages, solely to ancestral polymorphism processes (Dillon & Robinson, 2009). Among-drainage migration, and potentially also introgression, may prove to be major contributing factors in these cases also, if sampling is scaled to the potential dispersal capacity of these snail lineages, e.g. the >700 km range from Lake Biwa to South Korean drainages.

## SUPPLEMENTARY MATERIAL

Supplementary Material is available at *Journal of Molluscan Studies* online.

## ACKNOWLEDGEMENTS

This work received support from a Japan Society for the Promotion of Science Grant-in-Aid and a Smithsonian Marine Science Network Postdoctoral Fellowship (to O. Miura), a German Research Foundation award (DFG KO 3381 3/1 to F. Köhler) and a National Science Foundation award (NSF OCE-0850025 to D. Ó Foighil). M. Grygier, M. Matsuda and N. Katsuki provided logistic help and UMMZ museum artist J. Megahan assisted with Figure 1. The manuscript was improved by the constructive comments of two anonymous reviewers.

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