

Evolutionary history of *Schizocodon* (Diapensiaceae), an endemic genus in Japan

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Summary

Our current biodiversity developed over a long period of time with the accumulation of divergent evolutionary events (cladogenesis). The evolutionary history of organisms is a fascinating topic and research on evolutionary history based on molecular data has flourished. This study focused on *Schizocodon* (Diapensiaceae), a genus endemic to Japan, and elucidated its evolutionary history. This study considered three aspects of the evolutionary history of *Schizocodon*. First, a molecular phylogeny was constructed and any unstable taxonomic classification reexamined. Second, the demographic history of the genus was elucidated to estimate the evolutionary processes involved. Investigations on gene flow, divergence time, and range shifts are useful for unraveling speciation details, such as the mode of speciation, the drivers of speciation, and the effects of climate change. Finally, resolving questions about the role of ecogeographic (habitat) isolation at the gene-flow level is important in the evolution of *Schizocodon* as a model system. Despite recognizing the importance of ecogeographic isolation, whether greater ecogeographic isolation places greater limits on migration rates in the evolutionary process, or vice versa, is unclear. Knowledge of these three points will contribute to a better understanding of how the endemic genus *Schizocodon* evolved in Japan.

In Chapter 1, I describe the development of eight nuclear sequence markers and reconstruct molecular phylogenetic trees of Diapensiaceae to clarify the taxonomic status of the genus *Schizocodon*. The classification of the genus has long been disputed, and the genus has been treated either as an independent genus or included in *Shortia sensu lato* (*s.l.*). My phylogenetic analyses of *Shortia s.l.* and allied genera using maximum likelihood and Bayesian methods suggested that *Shortia s.l.* is paraphyletic, and the reciprocally monophyletic groups *Schizocodon* and *Shortia sensu stricto* should be recognized. In addition, *Schizocodon* is sister to the genus *Diapensia* despite their remarkable differences in morphology. These findings revealed that *Schizocodon* constitutes an independent genus.

In Chapter 2, I describe a multidisciplinary approach combining the molecular phylogeny, molecular phylogeography, and an ecological niche model (ENM) to investigate the phylogenetic relationships and demographic history of *Schizocodon*. A molecular phylogeny based on amplified length fragment polymorphisms (AFLPs) for 48 populations covering the entire range of *Schizocodon* supported the traditional taxonomic species classification that recognize *S. soldanelloides* and *S. ilicifolius*. Two varieties of *S. soldanelloides* (*S. soldanelloides* var. *soldanelloides* and *S. soldanelloides* var. *magnus*) were an admixture in one clade, indicating that the classification of the varieties is inappropriate. In contrast, *S. ilicifolius* var. *ilicifolius* and *S. ilicifolius* var. *intercedens* are reciprocally monophyletic, so each variety is appropriate. Individuals of *S. ilicifolius* var. *minimus* (endemic to Yakushima) were embedded in *S. soldanelloides*, suggesting that recombination should be considered.

However, both species shared plastid DNA haplotypes in each region (northeastern, north–central, south–central, and southwestern Japan). Due to the strong

geographical structure of the shared haplotypes across species and the different mutation rates between nuclear genes and plastid DNA, discordance between plastid DNA and the AFLP phylogeny indicated the occurrence of multiple introgression events in *Schizocodon* attributable to the Pleistocene climatic oscillation. Notably, the disjunct plastid haplotypes found only in *S. ilicifolius* var. *intercedens* suggested that introgression occurred unidirectionally from *S. soldanelloides* to *S. ilicifolius*, with complete plastid DNA replacement in local areas from *S. soldanelloides* to *S. ilicifolius* var. *ilicifolius*.

Furthermore, both the high degree of genetic diversity and the large potential distribution areas indicated that *S. soldanelloides* persisted in the mountains of central Japan during the Last Glacial Maximum (LGM). In addition, the limited potential distribution areas in the northeastern and southwestern regions during the LGM and mismatch distribution analyses suggested that the current widespread range resulted from postglacial range expansion. Therefore, *S. soldanelloides* range shifts could have been characterized by glacial range contraction into refugia in the mountains of central Japan and range expansion after evacuation to refugia. Unfortunately, the range shift history of *S. ilicifolius* could not be estimated due to serious introgression. Consequently, historical range shifts associated with the Pleistocene climatic oscillation would have favored multiple and regional introgression events in *Schizocodon*.

In Chapter 3, I revise the taxonomic treatment of *Schizocodon* on Yakushima according to the results of Chapter 2. The taxonomic status of the plants has been debated over the last century and the scientific name *Schizocodon ilicifolius* var. *minimus* has been accepted widely in the academic literature and in illustrated encyclopedias. However, the phylogenetic relationships of *Schizocodon* in Chapter 2

suggested that *S. ilicifolius* var. *minimus* should be reassigned to *S. soldanelloides*. I examined specimens of *Schizocodon* on Yakushima in the herbaria at Kyoto University (KYO), the University of Tokyo (TI), and the National Museum of Nature and Science (TNS), and concluded that they were morphologically identical to *S. soldanelloides*. Therefore, I accept the combination *Schizocodon soldanelloides* var. *minimus* (Makino) H.Hara for this Yakushima endemic.

In Chapter 4, I investigate the extent of ecogeographic isolation in the evolutionary process of *Schizocodon* in addition to a more detailed demographic analysis. First, ENMs were generated to determine suitable climate envelopes for each taxon using 19 climatic variables and the ENMs were compared reciprocally. The statistical comparison showed that the climatic niches of *Schizocodon* taxa were not identical ($P < 0.01$) and diverged from each other, indicating niche divergence and ecogeographic isolation. Both principal components analyses and comparisons of the climatic variables indicated that the environmental niches of the two species were characterized by precipitation, as all precipitation variables differed significantly among the *Schizocodon* species. Furthermore, the *Schizocodon* species occupied distinct climatic regions, the same as the predicted suitable areas: *S. soldanelloides* preferred the environment of the side facing the Sea of Japan, and *S. ilicifolius* was more suited to the side facing the Pacific Ocean. The winter snowfall differs distinctly between the Sea of Japan side, with high snowfall and humidity, and the Pacific Ocean side, which is relatively dry, suggesting that the *Schizocodon* species prefer and can adapt to their respective winter environments.

Second, *Schizocodon* individuals from 30 populations were genotyped using 14 nuclear sequences to estimate the demography under an isolation-with-migration model,

which assumed that *S. ilicifolius* var. *ilicifolius* and *S. ilicifolius* var. *intercedens* diverged more recently from each other after the divergence of *S. soldanelloides* and the common ancestor of *S. ilicifolius*. I found no gene exchange during the process of speciation between *S. soldanelloides* and the common ancestor of *S. ilicifolius*, providing strong support for allopatric speciation in *Schizocodon*. In addition, after the intraspecific divergence of *S. ilicifolius*, three instances of unidirectional gene flow occurred: from *S. soldanelloides* to *S. ilicifolius* var. *ilicifolius*, from *S. soldanelloides* to *S. ilicifolius* var. *intercedens*, and from *S. ilicifolius* var. *ilicifolius* to *S. ilicifolius* var. *intercedens*. These findings demonstrated unidirectional gene flow from *S. soldanelloides* to *S. ilicifolius*, as suggested in Chapter 2. The gene flow from *S. soldanelloides* to *S. ilicifolius* var. *ilicifolius* or to *S. ilicifolius* var. *intercedens* was statistically lower than the gene flow from *S. ilicifolius* var. *ilicifolius* to *S. ilicifolius* var. *intercedens*.

Finally, the strength of ecogeographic isolation and the degree of gene flow were compared. The interspecific migrations were statistically low, and the corresponding ecogeographic isolation value was relatively high. However, migration from *S. ilicifolius* var. *ilicifolius* to *S. ilicifolius* var. *intercedens* was higher than the interspecific migration, which had a small ecogeographic isolation value. Therefore, this study showed a conflicting relationship between the levels of ecogeographic isolation and the degree of gene flow, suggesting the importance of ecogeographic isolation as a reproductive barrier.