Development and Characterization of 14 Microsatellite Markers for the Antarctic Midge *Parochlus steinenii* (Diptera, Chironomidae) in Maritime Antarctic

Hanna Kim¹,², Seunghyun Kang¹,², Hanul Kim¹, Sanghee Kim¹, Jongwoo Jung¹,²,*(*)

¹The Division of EcoCreative, Ewha Womans University, Seoul 03760, Korea
²Unit of Polar Genomics, Korea Polar Research Institute, Incheon 21900, Korea
³Division of Polar Life Sciences, Korea Polar Research Institute, Incheon 21900, Korea
⁴Department of Science Education, Ewha Womans University, Seoul 03760, Korea

*These authors contributed equally to this work.

**ABSTRACT**

A winged midge species, *Parochlus steinenii* is one of the most abundant species in Antarctica, which is distributed over a wide area from the South American continent to the South Shetland Islands in Antarctica. It was dispersed into islands in the South Shetland Islands from the South American continent, and it adapted to a variety of environments and settled. This species, therefore, is a good model organism to explain the evolutionary process of Antarctic terrestrial fauna. Nevertheless, there are few genetic studies on this species, which are necessary for understanding the genetic diversity, population structure, etc. Here, we developed and characterized 14 polymorphic microsatellite markers. The number of alleles per locus ranged from 2 to 5. The observed and expected heterozygosities were in the range of 0.024 to 0.561 and 0.024 to 0.535, respectively. Identifying genetic differences between populations, they are suitable markers for researches investigating genetic diversity and population structure of *P. steinenii*, which provide us with clues to dispersion, evolution and ecology of this species.

**Keywords:** *Parochlus steinenii*, Antarctica winged midge, microsatellite marker, next generation sequencing, evolution, population genetics

**INTRODUCTION**

Compared with the Arctic that has more than 1,650 species of insects, Antarctica has no insects except two dipteran species, winged midge *Parochlus steinenii* (Gerke) and wingless midge *Belgica antarctica* Jacobs (Convey and Block, 1996). Of these, *P. steinenii* is distributed from the South Shetland Islands on the west coast of the Antarctic Peninsula to the Tierra del Fuego on the South American continent and it is known as one of the most abundant species in Antarctica (Brundin, 1970; Convey, 1996).

Unlike *B. antarctica*, which lives only in Maritime Antarctica, *P. steinenii* was dispersed and settled in several islands in the South Shetland Islands as well as continent of South America (Usher and Edwards, 1984; Edwards and Usher, 1985; Convey and Block, 1996). Several researchers have suggested that the speciation potential of this species which adapted to various environmental conditions may be considerable (Sublette and Wirth, 1980; Edwards and Usher, 1985). This species, therefore, can be a good model to explain the distribution and evolution of Antarctic insects. But previous studies on *P. steinenii* have focused mainly on morphology and ecology (Wirth and Gressitt, 1967; Edwards and Usher, 1985; Shimada et al., 1991; Convey and Block, 1996; Hahn and Reinhardt, 2006; Rico and Quesada, 2013). No suitable genetic markers have been developed for this species as well as research to understand the population genetic structure in order to investigate the evolution of this species.

Population genetics studies are needed to understand the adaptation, evolution and dispersion process of organisms. One of the most popular genetic markers for population genetics is a microsatellite due to its high polymorphism, co-dominant property, etc. (Tautz, 1989). Information obtained
using microsatellites is useful for establishing conservation measures as well as for investigating the population structure, evolution, dispersal and ecological properties (Chistia-kov et al., 2006; Selkoe and Toonen, 2006). Here, we firstly developed 14 microsatellite markers for *P. steinenii*.

Samples of *P. steinenii* were collected from King George Island, West Antarctica (62°14S, 58°47W) in 2015 (Fig. 1, site A). Genomic DNAs were extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). Of these, 41 individuals were used in this study.

Next generation sequencing was performed with the MiSeq platform (Illumina, San Diego, CA, USA) and shotgun libraries were prepared and sequenced according to the manufacturer’s instructions.

Microsatellite candidates were selected from genomic data using the QDD software (Meglécz et al., 2014). A total of 504 microsatellite loci were obtained, and for these loci 56 primers were designed using Primer3 program (http://bioinfo.ut.ee/primer3, Untergasser et al., 2012). PCR amplifications were performed in a total solution of 25 μL containing 1.0 μL of template DNA, 2.5 μL of 10× buffer, 0.7 μL of dNTP (10 mM), 1.5 μL of MgCl₂ (25 mM), 0.5 μL of each primer (10 μM), and 0.3 μL of Taq polymerase (Takara, Otsu, Japan). Cycling program conditions were follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 45–60°C for 60 sec, elongation at 72°C for 90 sec, and final elongation at 72°C for 7 min.

M13 tail (FAM: TTT CCC AGT CAC GAC GTT G, PET: GCG GAT AAC AAT TTC ACA CAG G, NED: TAA AAC GAC GGC CAG TGC) was attached to the 5’ end of each forward primer, and pig tail (GTG TCT T) was attached to the 5’ end of all reverse primers. For genetic diversity analysis, multiplex PCR amplification was performed in 16 μL. PCR mixture composed of 5 μL of 2× QIAGEN Multiplex PCR master mix (Qiagen), 0.08 μL of M13-tailed forward primer (10 μM), 0.8 μL of pig tailed reverse primer (10 μM), 0.3 μL of template DNA, and 0.16 μL of each fluorescence primer (FAM/PET/NED) (10 μM). PCR cycle conditions were as follows: initial denaturation at 95°C for 15 min, 90 sec of annealing at a specific temperature (14 cycles at 63°C, 7 cycles at 58°C, 20 cycles at 55°C), each cycle elongation at 72°C for 30 sec, and final elongation at 72°C for 20 min. Genotyping was performed using ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) and GeneMapper software v.3.7 (Applied Biosystems).

The number of observed alleles at each locus (*A*), observed heterozygosity (*H*<sub>O</sub>) and expected heterozygosity (*H*<sub>E</sub>) for each locus were calculated using the program ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested using GENEPOP 4.2.1 (Raymond and Rousset, 1995; Rousset, 2008). Program Micro-Checker v2.2.3 was used to confirm the existence of null alleles (Van Oosterhout et al., 2004).

**RESULTS AND DISCUSSION**

A total of 56 primer sets were selected from 504 candidates and we successfully developed 14 polymorphic markers (Table 1). The number of alleles per locus ranged from 2 to 5. *H*<sub>O</sub> and *H*<sub>E</sub> varied from 0.024 to 0.561 and 0.024 to 0.535, respectively. All loci, except 3 loci, showed significant deviation from HWE after Bonferroni correction (*p* = 0.0035). Evidence of null allele was confirmed in 5 loci, which was
presumably due to excess of homozygotes or scoring error. The LD of each pair of loci was not significant after Bonferroni correction.

The 14 newly developed markers showed low genetic diversity, but it was probably because the samples used for the analyses were collected from an isolated area. Although not presented in this paper, we performed Bayesian clustering of this population with the population of Deception Island (site B) in the South Shetland Islands using the STRUCTURE program (Pritchard et al., 2000). As a result, we confirmed that the two populations had a genetically distinct structure. These results confirmed that the newly developed markers show discernment to investigate the genetic structure of populations. These new markers could be useful for establishing a conservation management plan of indigenous species in Maritime Antarctica.

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REFERENCES


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