ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 33, No. 2: 140-143, April 2017
https://doi.org/10.5635/ASED.2017.33.2.010

Short communication

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

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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, codominant property, etc. (Tautz, 1989). Information obtained

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eISSN 2234-8190

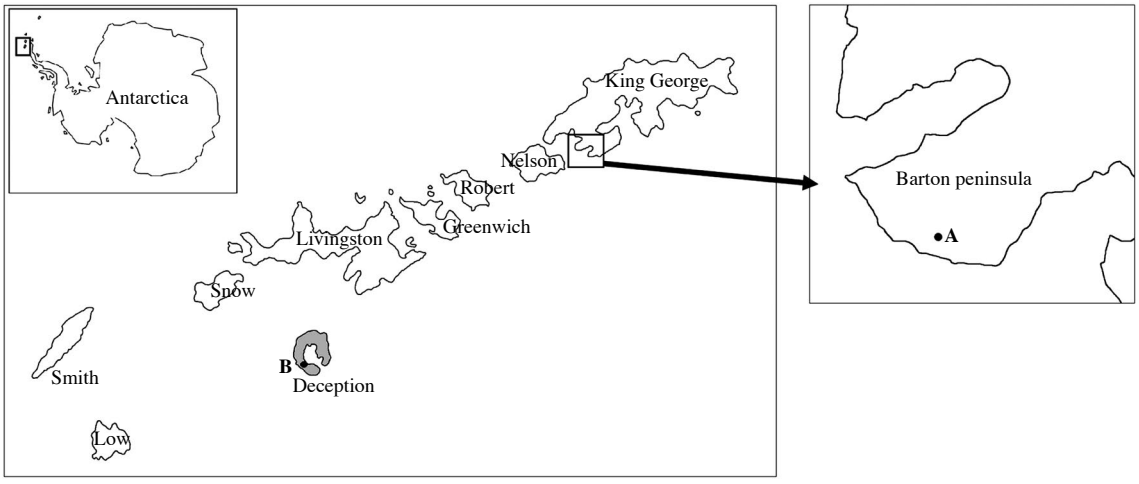

Fig. 1. Map showing the collection sites of *Parochlus steinenii* in Antarctica.

using microsatellites is useful for establishing conservation measures as well as for investigating the population structure, evolution, dispersal and ecological properties (Chistiakov 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 $MgCl_2$ (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 primers, and pig tail (GTT 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_O) and expected heterozygosity (H_E) 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_O and H_E 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

Table 1. Fourteen microsatellite loci developed from *Parochlus steinenii*

Locus	Repeat motif	Primer sequence (5′-3′)	Fluorescent dye	Size range (bp)	*A*	H_O	H_E	HWE
A-10	AAC	F: TTTCCCAGTCACGACGTTGGCCTTATTTAAAGAATTTAGCAATCG R: GTTTCTTGTCATGATGGCCTGACCAA	6FAM	120–123	2	0.079	0.169	0.018
A-11	AAC	F: TTTCCCAGTCACGACGTTGTTGTTGGTTAGTGACAACGTCC R: GTTTCTTAAATTCATAGATGGCTCGAATATC	6FAM	118–124	2	0.488	0.506	1.000
A-16	AG	F: TTTCCCAGTCACGACGTTGGGTTCCACCGCACTAACACT R: GTTTCTTGGGCGGAGCCTAAATTTGTA	6FAM	119–127	2	0.195	0.287	0.069
B-06	AAC	F: TAAAACGACGGCCAGTGCGAGGTGGATTTGTGGCATTC R: GTTTCTTTCATAGCCGGTGATTTATTCG	NED	276–279	3	0.075	0.074	1.000
B-11	AAC	F: TAAAACGACGGCCAGTGCAACTAACCTGAATTTCGCTAACCA R: GTTTCTTGCGCTCAGTTGCCTCAGT	NED	265–268	2	0.244	0.506	0.001[a]
B-13	AT	F: TAAAACGACGGCCAGTGCAAATAAGATGGTGGAGGCGA R: GTTTCTTGTAAGAAATGTGTATCGGCGG	NED	259–270	5	0.300	0.357	0.372
B-15	ACCT	F: TAAAACGACGGCCAGTGCTGGTGACATTGCTGGAGTTG R: GTTTCTTCCAACAATATTTGGGCGATT	NED	284–292	3	0.561	0.527	0.603
B-16	AAG	F: TAAAACGACGGCCAGTGCGGCCGTTGTATGACGAAAGT R: GTTTCTTTTCATTTCCTTTAATCTTTGAACCA	NED	173–183	4	0.128	0.535	0.000[a]
C-03	AAC	F: GCGGATAACAATTTCACACAGGGGAGAAGTGAGTATTTCGCAGG R: GTTTCTTCTGTTTGAGTGGTGAAGCTTGT	PET	315–318	2	0.450	0.444	1.000
C-07	AAG	F: GCGGATAACAATTTCACACAGGCAACACCAAATCTTCCTTTGC R: GTTTCTTTGCAAATGAATGGCAGAAAG	PET	330–336	4	0.300	0.531	0.001[a]
C-10	AAT	F: GCGGATAACAATTTCACACAGGACCGTTTGAGGATAAAGGAAGA R: GTTTCTTTTATCCGCTTGCCAATACG	PET	375–378	2	0.289	0.321	0.610
C-11	AAC	F: GCGGATAACAATTTCACACAGGAAATAAATACAGTATCAAGCAGGCA R: GTTTCTTAGCCCGCCAAGTACTCATT	PET	422–423	2	0.024	0.024	1.000
C-12	AAG	F: GCGGATAACAATTTCACACAGGAGACGCAAATGCTGTGAAAGT R: GTTTCTTATCTCACGCCATCACACTGA	PET	429	3	0.024	0.048	0.012
C-13	AAT	F: GCGGATAACAATTTCACACAGGGGAAATAGGAGTAGTGCAGTTGG R: GTTTCTTTCATCTGATCTGGTCAAGGAA	PET	437–439	2	0.171	0.270	0.042

A, number of allele; H_O, observed heterozygosity; H_E, expected heterozygosity; HWE, Hardy-Weinberg equilibrium.
[a]Significant after Bonferroni correction ($p < 0.0035$).

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 (Fig. 1, 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.

ACKNOWLEDGMENTS

This study was supported by the Korea Polar Research Institute (research grants PE17090).

REFERENCES

Brundin L, 1970. Diptera: Chironomidae of South Georgia. Pacific Insects Monographs, 23:276.

Chistiakov DA, Hellemans B, Volckaert FAM, 2006. Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture, 255:1-29. http://doi.org/10.1016/j.aquaculture.2005.11.031

Convey P, 1996. The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. Biological Reviews, 71:191-225. https://doi.org/10.1111/j.1469-185X.1996.tb00747.x

Convey P, Block W, 1996. Antarctic Diptera: ecology, physiology and distribution. European Journal of Entomology, 93:1-13.

Edwards M, Usher MB, 1985. The winged Antarctic midge *Parochlus steinenii* (Gerke) (Diptera: Chironomidae) in the South Shetland Islands. Biological Journal of the Linnean Society, 26:83-93. https://doi.org/10.1111/j.1095-8312.1985.tb01553.x

Excoffier L, Lischer HEL, 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources,

10:564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Hahn S, Reinhardt K, 2006. Habitat preference and reproductive traits in the Antarctic midge *Parochlus steinenii* (Diptera: Chironomidae). Antarctic Science, 18:175-181. https://doi.org/10.1017/S0954102006000204

Meglécz E, Pech N, Gilles A, Dubut V, Hingamp P, Trilles A, Grenier R, Martin JF, 2014. QDD version 3.1: a user-friendly computer program for microsatellite selection and primer design revisited: experimental validation of variables determining genotyping success rate. Molecular Ecology Resources, 14:1302-1313. https://doi.org/10.1111/1755-0998.12271

Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. Genetics, 155:945-959.

Raymond M, Rousset F, 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86:248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rico E, Quesada A, 2013. Distribution and ecology of chironomids (Diptera, Chironomidae) on Byers Peninsula, Maritime Antarctica. Antarctic Science, 25:288-291. https://doi.org/10.1017/S095410201200096X

Rousset F, 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources, 8:103-106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Selkoe KA, Toonen RJ, 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters, 9:615-629. https://doi.org/10.1111/j.1461-0248.2006.00889.x

Shimada K, Ohyama Y, Pan CX, 1991. Cold-hardiness of the Antarctic winged midge *Parochlus steinenii* during the active season at King George Island. Polar Biology, 11:311-314. https://doi.org/10.1007/BF00239023

Sublette JE, Wirth WW, 1980. The Chironomidae and Ceratopogonidae (Diptera) of New Zealand's subantarctic islands. New Zealand Journal of Zoology, 7:299-378. https://doi.org/10.1080/03014223.1980.10423791

Tautz D, 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research, 17:6463-6471. https://doi.org/10.1093/nar/17.16.6463

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG, 2012. Primer3-new capabilities and interfaces. Nucleic Acids Research, 40:e115. https://doi.org/10.1093/nar/gks596

Usher MB, Edwards M, 1984. A dipteran from south of the Antarctic Circle: *Belgica antarctica* (Chironomidae) with a description of its larva. Biological Journal of the Linnean Society, 23:19-31. https://doi.org/10.1111/j.1095-8312.1984.tb00803.x

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P, 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4:535-538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Wirth WW, Gressitt JL, 1967. Diptera: Chironomidae (Midges) 1. In: Entomology of Antarctica (Ed., Gressitt JL). American Geophysical Union, Washington, DC, pp. 197-203.

Received April 3, 2017
Accepted April 24, 2017