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Genome sequencing of the winged midge, Parochlus steinenii, from the Antarctic Peninsula

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Abstract:	Background In the Antarctic, only two species of Chironomidae occur naturally: the wingless midge, Belgica antarctica, and the winged midge, Parochlus steinenii. B. antarctica has unusual characteristics and it has adapted to an extreme environment. The larvae of B. antarctica are desiccation and freeze tolerant, and the adults lose their wings. Recently, a study suggested that the compact genome of B. antarctica could be the result of adaptation to an extreme environment. On the other hand, P. steinenii, is cold tolerant but not freeze tolerant at the larval stage, even though it occurs naturally in the Antarctic with B. antarctica. In addition, P. steinenii adults are winged. As a result, P. steinenii could be a good species for comparative analysis in order to understand the notable adaptations of B. antarctica. In this study, we sequenced the genome of P. steinenii. Results The draft genome of P. steinenii had a total size of 137 Mb, comprising 9,513 contigs with an N50 contig size of 34,110 bp, and a GC content of 32.2%. The assembled contig had a contig coverage of approximately 108.5×. In all, 13,468 genes were predicted using MAKER annotation pipeline and classified to functions for 10,801 (80.2%) predicted genes in gene ontology. Conclusions We present an annotated draft genome of the Antarctic midge, P. steinenii. The P. steinenii genome will help reveal the mechanism underlying freeze tolerance when compared with the genome of B. antarctica, as P. steinenii is cold tolerant but not freeze tolerant in the larval form.
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Genome sequencing of the winged midge, *Parochlus steinenii*, from the Antarctic Peninsula

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Keywords: *Parochlus steinenii*, complete mitochondrial genome, Antarctic winged midge

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Abstract

Background

In the Antarctic, only two species of Chironomidae occur naturally—the wingless midge, *Belgica antarctica*; and the winged midge, *Parochlus steinenii*. *B. antarctica* has unusual characteristics with a compact genome as a result of adaptation to an extreme environment. The larvae of *B. antarctica* are desiccation and freeze tolerant and the adults lose their wings. Even though they occur naturally in the Antarctic with *B. antarctica*, the larvae of *P. steinenii* are cold, but not freeze, tolerant and the adults are winged. Therefore, *P. steinenii* could be a good species for comparative analysis in order to understand the notable adaptations of *B. antarctica*. In this study, we sequenced the genome of *P. steinenii*.

Results

The draft genome of *P. steinenii* had a total size of 137 Mbp, comprising 9,513 contigs with an N50 contig size of 34,110 bp and a GC content of 32.2%. In all, 13,468 genes were predicted using MAKER annotation pipeline, and gene ontology classified 10,801 (80.2%) predicted genes to a function. As compared to genome architecture of *B. antarctica*, that of *P. steinenii* was 39 Mbp longer with 4-fold increased repeat sequences, whereas gene regions were similarly compact as *B. antarctica*.

Conclusions

We present an annotated draft genome of the Antarctic midge, *P. steinenii*. The *P. steinenii* genome will help reveal the mechanism underlying freeze tolerance when compared to the genome of *B. antarctica*, as *P. steinenii* is cold, but not freeze, tolerant in the larval form.

Keywords

Parochlus steinenii, cold tolerant, Antarctic midge

Data description

Sequencing

Specimen of *Parochlus steinenii* [1-3] was collected from King George Island, West Antarctica (62°14′S, 58°47′W) during 2014 and 2015. Genomic DNA was extracted using a DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). For genome sequencing and assembly using ALLPATHS-LG [4], two types of libraries were prepared. One was a fragment library, which was of paired-end type with an insert size of 400 bp, while the other was a jumping library, which was of mate-pair type with insert sizes of 3 kbp and 5 kbp. Paired-end libraries were sequenced with the MiSeq platform (Illumina, San Diego, CA, USA) using a read length configuration of 2 × 300 bp, and mate-pair libraries were sequenced with the Illumina HiSeq platform (Illumina, San Diego, CA, USA) using a read length configuration of 2 × 150 bp (see Table 1). Library preparation and sequencing were performed according to the manufacturer's instructions.

For gene annotation with expressed sequencing tags, RNA was extracted from whole body of *P. steinenii* using the Qiagen kit, according to the manufacturer's instructions. Paired-end libraries with the insert size of 300 bp were constructed and sequenced with the Illumina HiSeq platform (Illumina, San Diego, CA, USA), using a read length configuration of 2 × 150 bp (Table 1).

Before assembly using ALLPATH-LG, the paired-end reads resulting from the fragment library were trimmed using the FASTX-Toolkit (Ver. 0.0.11) (http://hannonlab.cshl.edu/fastx_toolkit) with the parameters -t 30, -l 200, and -Q 33. Paired sequences from the trimmed Illumina reads were then selected. Finally, data from paired-end trimmed reads with 14 gigabase pairs (Gbp) were obtained (Table 1).

Table 1. Sequencing statistics of *P. steinenii*

Library	Mode	Insert size	Library type	Reads	Read lengths	Source
PE400trim	2×300	400	paired-end	51 648 324	14 775 480 106	Genomic DNA
PE400	2×300	400	paired-end	51 892 430	15 567 729 000	Genomic DNA
MP3K	2×150	3 000	mate-pair	170 887 140	25 633 071 000	Genomic DNA
MP5K	2×150	5 000	mate-pair	157 622 418	23 643 362 700	Genomic DNA
PE300	2×150	300	paired-end	27 663 170	3 539 060 573	RNA
PE300	2×150	300	paired-end	27 782 288	3 483 157 066	RNA
PE300	2×150	300	paired-end	30 806 804	3 875 228 963	RNA

Genome assembly

Assembly was performed using ALLPATHS-LG for both, the fragment libraries (400 bp) and the jumping libraries (3 kbp and 5 kbp). These were performed on a 96-processor workstation with Intel Xeon X7460 2.66 GHz processors, 1 terabyte RAM, and default parameters. In ALLPATHS-LG, paired-end reads from the fragment library were merged to make longer reads, resulting in a better assembly and a larger k-mer size [4]. As a result, the fragment library should be designed to overlap, and the size of the paired-end library was slightly less than twice the read size [4]. In this assembly, 93.8% of the fragment library was full. The resulting assembly had a total size of 137 Mb, comprising 9,513 contigs, with an N50 contig size of 34,110 bp, and an N50 scaffold size of 168 kb (Table 2). The GC content was 32.2% and the assembly revealed contig coverage of approximately 108.5 ×.

Table 2. Global statistics of the *P. steinenii* genome assembly.

Assembly results	**Number**	**N50 (kb)** [*]	**Size (Mb)**
Contig	9 513	34.1	130.6
Scaffold	4 151	168.1	138.0

Annotation	**Number**	**Total length (kb)**	**Percentage of genome (%)**
Genes	13 468	36 239.1	26.3
Coding region (Coding regions in *B. antarctica*)	13 468 (13 517)	17 967.6 (18 964.3)	13.0 (19.4)
Introns (Introns in *B. antarctica*)	69 960 (43 577)	24 191.6 (15 495.0)	17.5 (15.7)
Repeats (Repeats in *B. antarctica*)	37 507 (10 084)	2 252.6 (429.7)	1.6 (0.49)

[*] Minimum sequence length in which half of the assembled bases were found. The statistics of gene annotation of *B. antarctica* are quoted from a previously reported paper [5].

Gene annotation

Gene annotation was accomplished using MAKER2 annotation pipeline [6]. RepeatMasker (Ver. 3.3.0) [7] was used to identify repetitive elements against a *de novo* repeat library, and the SNAP gene finder [8] was selected to perform *ab initio* gene prediction from the masked genome sequence in MAKER2. For proper gene annotation, RNA and protein evidence alignment were used. Alignment of expressed sequence tags with BLASTn and homologous protein information from tBLASTx were considered for evidence of alignment. Transcriptome assembly results were used for RNA evidence, and a CLC Genomics Workbench (Ver. 8.0.0) was used for assembly. In all, 68,392 contigs with an N50 contig size of 435 bp and an average contig size of 407 bp, were generated.

Protein sequences from six species, given in NCBI reference sequences, were used in the analysis—*Drosophila melanogaster* (Fruit fly, GCF_000001215.4), *Ceratitis capitata* (Mediterranean fruit fly, NC_000857.1), *Bactrocera dorsalis* (oriental fruit fly, NC_008748.1), *Anopheles gambiae* (African malaria mosquito, NZ_AAAB00000000.1), *Aedes aegypti* (yellow fever mosquito, AAGE00000000.2), and *Culex quinquefasciatus* (southern house mosquito, AAWU01000000). A total of 13,468 genes in the *P. steinenii* genome were predicted using the MAKER2 pipeline. This is similar to the number of genes in *B. antarctica* [5]. The compact genome of *B. antarctica* (99 Mbp) [5], which is endemic to Antarctica, notably comprises of low repeat density and a reduced intron length. Although *P. steinenii* showed a low repeat density (1.6%; Table 2), it was not as low as that of *B. antarctica*, but it does have a similar intron length in a percentage of genome [5].

Blast2Go (Ver. 2.6.0) assigned preliminary functions for 13,468 genes, and gene ontology (GO) classified 10,801 (80.2%) of the predicted genes to a function. This was annotated with the BLASTp results and InterproScan [9]. GO annotation described the classified proteins as those required for biological processes (7,434, 55.2%) and molecular functions (9,576, 71.1%), and as cellular components (4,871, 36.2%). Enzyme commission (EC) numbers were obtained for 987 proteins.

Gene annotation for *B. antarctica*

To investigate the difference in gene contents between *P. steinenii* and *B. antarctica*, we also annotated the genome of *B. antarctica* with the same methods used for *P. steinenii*. For RNA evidence alignment in MAKER2 annotation pipe lines [6], the reads in various experimental conditions with *B. antarctica* (SRR566981, SRR567289, SRR567164~7, SRR567169~71) were downloaded from SRA databases in NCBI and we assembled the reads into 38,017 contigs with an N50 contig size of 1,799 bp and an average contig size of 913 bp through CLC Genomics Workbench (Ver. 8.0.0).

We matched proteins from *P. steinenii* to those from six other species for protein evidence. From MAKER2, a total of 11,005 genes were predicted in the *B. antarctica* genome and were used for ortholog analysis.

Repeat analysis and Non-coding RNA

Interspersed repeats were predicted using RepeatMasker (Ver. 3.3.0) with a *de novo* repeat library [7]. A *de novo* repeat library was constructed using RepeatModeler (Ver. 1.0.3) [10], including the RECON (Ver. 1.07) [10] and RepeatScout (Ver. 1.0.5) [11] software, with default parameters, and tandem repeats including simple repeats, satellites, and low complexity repeats were predicted using TRF [12]. Putative tRNA genes were identified using tRNAscan-SE (Ver. 1.3.1) [13] with option -H. The total coverage of repeat sequences in *P. steinenii* were up to approximately four-fold from those of repeat sequences in *B. antarctica* (Table 2), and the percentage of genome was increased approximately three-fold as compared to that of *B. antarctica*. Most statistics of repeats were increased in the library of *P. steinenii* (Table 3). Through tRNAscan-SE, 186 tRNAs were predicted (Table 4).

Table 3. Repeat content in Antarctic midges

	P. steinenii		***B. antarctica***	
	Total coverage (bp)	**Number of sequences**	**Total coverage (bp)**	**Number of sequences**
Low complexity	404 490	8 661	276 261	8 536

Simple repeats	1 105 449	26 336	36 911	999
Transposon elements				
Class I/LTR	289 059	1 075	74 297	336
Class I/Non-LTR	169 298	675	26 554	128
Class II/DNA elements	216 807	649	8 536	64
Small RNA	67 503	111	7 165	36
Total	2 252 606	37 507	429 724	10 069

The statistics of repeats of *B. antarctica* are quoted from a previously reported paper [5].

Table 4. tRNA in *P. steinenii*

Anticodon	**number**
Ala	4
Arg	13
Asn	5
Asp	5
Cys	3
Gln	9
Glu	15
Gly	9
His	9
Ile	8

Leu	13
Lys	7
Met	7
Phe	5
Pro	7
Pseudo	15
SeC(e)	1
Ser	13
Thr	13
Trp	3
Tyr	9
Val	13
sum	**186**

Ortholog analysis.

Orthologous groups were identified using OrthoMCL (Ver. 2.0.5) [14]. We used the standard parameters and options of OrthoMCL for all steps. In this analysis, coding sequences (CDS) from six insects (*D. melanogaster*, *A. gambiae*, *A. aegypti, C. quinquefasciatus, B. antarctica, and P. steinenii*) were used. In this study, CDS from four genome assemblies (BDGP6 for *D. melanogaster*, AgamP4 for *A. gambiae*, AaegL3 for *A. aegypti*, and CpipJ2 for *C. quinquefasciatus*) were collected from Ensemble Metazoa (http://metazoa.ensembl.org/index.html) and the CDS from MAKER2 were used for *B. antarctica and P. steinenii*. Total proteins were categorized into 15,633 groups—4,814 orthologous groups were identified as common to all the six insects, 437 orthologous groups in *P. steinenii* genes were not identified in any other species, and 349 groups were identified only in the two Antarctic midges (Fig. 1A and Table 5).

Table 5. Shared orthologous gene clusters among six insects—*D. melanogaster, A. gambiae, A. aegypti, C. quinquefasciatus, B. antarctica,* and *P. steinenii.*

Group	Number	Group	Number
A	437	B	192
AB	349	BC	28
ABC	18	BCD	34
ABCD	46	BCDE	130
ABCDE	452	BCDEF	682
ABCDEF	4 814	BCDF	22
ABCDF	84	BCE	9
ABCE	24	BCEF	25

ABCEF	102
ABCF	8
ABD	9
ABDE	20
ABDEF	190
ABDF	8
ABE	11
ABEF	37
ABF	69
AC	71
ACD	65
ACDE	158
ACDEF	410
ACDF	32
ACE	15
ACEF	23
ACF	4
AD	18
ADE	12
ADEF	22
ADF	3
AE	15
AEF	9
AF	46
BCF	5
BD	10
BDE	6
BDEF	31
BDF	2
BE	6
BEF	6
BF	33
C	638
CD	1 196
CDE	1 258
CDEF	359
CDF	50
CE	105
CEF	20
CF	31
D	375
DE	114
DEF	17
DF	25
E	288
EF	25
F	2 330
Total	**15 633**

A: *P. steinenii*, B: *B. antarctica*, C: *C. quinquefasciatus*, D: *A. aegypti*, E: *A. gambiae*, and F: *D. melanogaster*

Gene structure of Orthologous groups

B. antarctica showed a reduction in intron length with very low repeat sequences [5], so we compared intron lengths of orthologous gene among six insects to identify whether the intron length of the gene in *P. steinenii* was reduced or not. We used the information of gene structures from four genome assemblies (BDGP6 for *D. melanogaster*, AgamP4 for *A. gambiae*, AaegL3 for *A. aegypti*, and CpipJ2 for *C. quinquefasciatus*) and the information of maker annotation of *B. antarctica* and *P. steinenii*. Among the six insects, the average intron length of *B. antarctica* (302 bp) was reported as the smallest, but that of *P. steinenii* (319 bp) was similar to that of *B. antarctica* (Fig. 1B). Despite 39 Mbp difference in genome size between *B. antarctica* and *P. steinenii*, the average length of gene regions and CDS was also similar, but the average intron number in orthologous genes was highest in *P. steinenii* (Fig. 1B).

GO enrichment test

We identified which GO terms of 437 orthologous groups were statistically represented versus GO terms of total genes of *P. steinenii* using AgriGO [15]. AgriGO is a web-based tool for GO analysis and we tested GO terms with significant levels of p = 0.05. Complete hierarchies of GO terms for each gene were examined. Eighteen GO terms in biological process, 5 GO terms in cellular component, and 18 GO terms were identified significantly by GO enrichment analysis (Table 6). Enriched GO terms in this test proposed that the proteins associated with unfolded protein response [16] under stress conditions were developed independently. Representative GO terms related with unfolded protein response were RNA splicing, via endonucleolytic cleavage and ligation (GO:0000394), response to unfolded protein (GO:0006986), and endoplasmic reticulum unfolded protein response (GO:0030968) in biological process.

Table 6. GO terms were statistically overrepresented only in *P. steinenii*.

GO ID	GO tree	Term	number of target genes in term	number of genes in terms	p-value	FDR
GO:0006508	P	proteolysis	106	632	8.60E-13	2.60E-10
GO:0006397	P	mRNA processing	32	120	6.80E-10	1.00E-07
GO:0070054	P	mRNA splicing, via endonucleolytic cleavage and ligation	8	8	1.40E-09	1.40E-07
GO:0016071	P	mRNA metabolic process	32	130	5.80E-09	4.50E-07
GO:0000394	P	RNA splicing, via endonucleolytic cleavage and ligation	8	11	1.90E-07	1.10E-05
GO:0006986	P	response to unfolded protein	6	7	1.50E-06	7.60E-05
GO:0019538	P	protein metabolic process	173	1 506	2.20E-06	9.40E-05
GO:0051789	P	response to protein stimulus	6	8	5.50E-06	0.00021
GO:0006950	P	response to stress	50	330	8.00E-06	0.00027
GO:0006468	P	protein amino acid phosphorylation	42	272	2.40E-05	0.00074
GO:0080135	P	regulation of cellular response to stress	9	24	4.80E-05	0.0013
GO:0006396	P	RNA processing	34	210	5.00E-05	0.0013
GO:0051347	P	positive regulation of transferase activity	8	22	0.00016	0.0031
GO:0033674	P	positive regulation of kinase activity	8	22	0.00016	0.0031
GO:0045860	P	positive regulation of protein kinase activity	8	22	0.00016	0.0031
GO:0034620	P	cellular response to unfolded protein	4	5	0.00017	0.0031
GO:0030968	P	endoplasmic reticulum unfolded protein response	4	5	0.00017	0.0031
GO:0042246	P	tissue regeneration	6	13	0.00024	0.0041
GO:0031463	C	Cul3-RING ubiquitin ligase complex	5	5	2.90E-06	0.00019

GO:0031461	C	cullin-RING ubiquitin ligase complex	5	12	0.0014	0.047
GO:0005789	C	endoplasmic reticulum membrane	11	55	0.0032	0.063
GO:0042175	C	nuclear envelope-endoplasmic reticulum network	11	57	0.0042	0.063
GO:0044432	C	endoplasmic reticulum part	11	58	0.0049	0.063
GO:0004252	F	serine-type endopeptidase activity	76	292	3.70E-20	5.50E-18
GO:0004540	F	ribonuclease activity	30	54	1.90E-19	1.40E-17
GO:0008236	F	serine-type peptidase activity	76	318	6.90E-18	2.50E-16
GO:0017171	F	serine hydrolase activity	76	318	6.90E-18	2.50E-16
GO:0004175	F	endopeptidase activity	84	416	5.70E-15	1.70E-13
GO:0070011	F	peptidase activity, acting on L-amino acid peptides	103	570	1.60E-14	4.00E-13
GO:0008233	F	peptidase activity	103	595	2.40E-13	5.10E-12
GO:0004518	F	nuclease activity	30	102	1.70E-10	3.10E-09
GO:0031072	F	heat shock protein binding	10	17	1.00E-07	1.60E-06
GO:0004672	F	protein kinase activity	47	300	5.90E-06	8.70E-05
GO:0008234	F	cysteine-type peptidase activity	15	59	3.70E-05	0.00049
GO:0016787	F	hydrolase activity	171	1 580	5.00E-05	0.00061
GO:0016773	F	phosphotransferase activity, alcohol group as acceptor	49	363	0.00018	0.002
GO:0042802	F	identical protein binding	10	38	0.00052	0.0055
GO:0031625	F	ubiquitin protein ligase binding	5	12	0.0014	0.014
GO:0005515	F	protein binding	229	2 357	0.0015	0.014
GO:0016301	F	kinase activity	48	405	0.0032	0.027
GO:0003676	F	nucleic acid binding	144	1 469	0.0055	0.045

Likelihood analysis of gene gain and loss

The size of gene families had been changed through evolution [17, 18]. To estimate the average gene expansion/contraction rate and to identify gene families that have undergone significant size changes, we estimated differences in the size of 15,633 orthologs using the program CAFE3.0 (www.bio.indiana.edu/~hahnlab/Software.html) [19]. The ultrametric tree of the species drawn through Timetree [20] was used for the analysis (Fig. 1C). We performed the program using $p < 0.05$, estimated birth (λ) and death (μ) rates were calculated by using the program LambdaMu with “–s” option. We calculate the number of gene gains and losses on each branch of the tree with “–t” option. Average expansion size of two Antarctic midges were relatively lower than other insects (Fig. 1C), and average expansion size of *D. melanogaster* showed the highest score among six insects. Using $p < 0.0001$ in family-wide p-values, we expect there to be approximately one significant result by chance and calculated the exact p-values for transitions over every branch. We called individual branches significant at $p < 0.005$ [21]. We could identify that 3 and 2 gene families were significantly expanded in *P. steinenii* and *B. antarctica*, respectively (Table 7).

Table 7. Gene families were significantly expanded in Antarctic midges

ID	Annotation	*P. steinenii*	*B. antarctica*	1*	2*	*C. quinquefasciatus*	*A. aegypti*	3*	*A. gambiae*	4*	*D. melanogaster*	Family-wide P-values	*P. steinenii*	*B. antarctica*	1*	2*	*C. quinquefasciatus*	*A. aegypti*	3*	*A. gambiae*	4*	*D. melanogaster*
PS0025	leucine rich membrane protein	42	0	0	0	0	0	0	0	1	0	0	0	0.625	0.073	0.161	0.5	0.5	0.5	0.5	0.509	0.14
PS0032	clip-domain serine protease	40	0	0	0	0	0	0	0	1	0	0	0	0.625	0.073	0.161	0.5	0.5	0.5	0.5	0.509	0.14
PS0098	zinc finger protein	26	1	0	0	0	0	0	0	1	0	0	0	0.875	0.073	0.161	0.5	0.5	0.5	0.5	0.509	0.14
PS0074	serine protease	0	29	0	0	0	0	1	0	1	0	0	0.625	0	0.073	0.161	0.5	0.5	0.5	0.5	0.509	0.14
PS0114	leucine rich repeat protein	0	26	0	0	0	0	1	0	1	0	0	0.625	0	0.073	0.161	0.5	0.5	0.5	0.5	0.509	0.14

Although *B. antarctica* is notable for being freeze tolerant in its larval stages [5], the anti-freezing protein has not yet been identified from the genome, and the mechanism is unclear. In this report, we present the draft genome and annotation of the Antarctic midge, *P. steinenii*. The genome of *P. steinenii*, which is only cold tolerant, rather than freeze tolerant, in their larval stage [1, 3], will help to clarify the mechanism for freeze tolerance.

Availability of supporting data

Supporting data are available in the GigaDB database, and the raw data were deposited in the PRJNA284858 (SRX1976250–5).

Declarations:

List of abbreviations

Gbp; giga base pairs, Mbp; mega base pairs; GO, gene ontology; EC, enzyme commission; CDS, coding sequence; SRA, short read archive

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Authors' Contributions

SHK, HGC, HP, and SCS designed the study. SHK, WSJ, HGC collected the samples and performed the experiments, S.C.S, H.P, and J.H.P analyzed the data. All authors participated in the writing of the manuscript.

Figure Legends

Fig. 1. Genome-wide analysis of protein-coding genes in *P. steinenii*. **(A)** Venn diagram displaying the overlap in orthologous genes in six insect species. **(B)** The statistics of gene structure of the six insects. **(C)** Lineage-specific gene gain and loss among the 6 insects. The numbers in boxes are identifiers for internal branches of the phylogeny. Numbers on each branch denote the number of expansion, remain, and decress. AE denotes average expansion.

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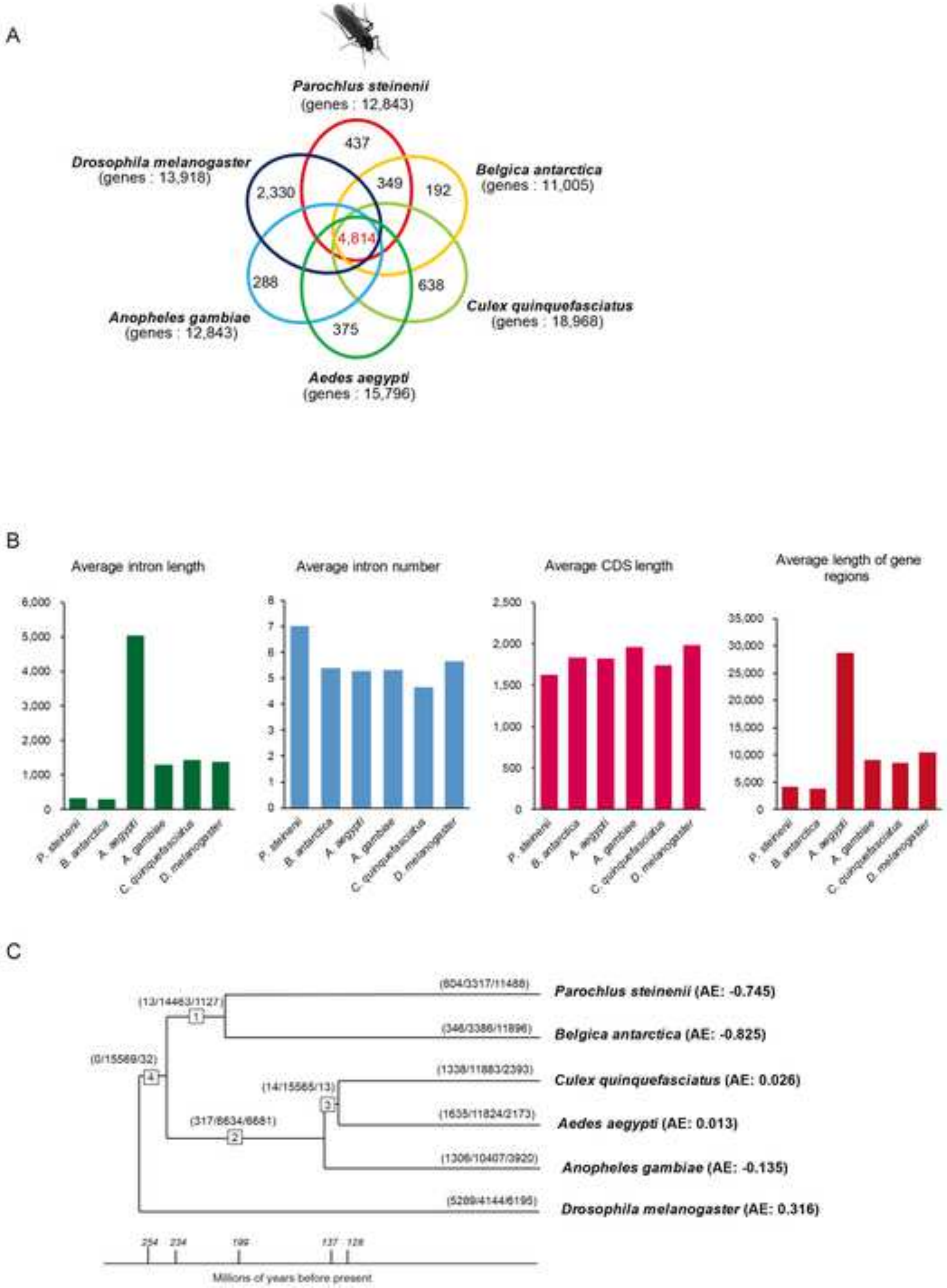
A
Parochlus steinenii
(genes : 12,843)
Drosophila melanogaster
(genes : 13,918)
Belgica antarctica
(genes : 11,005)
Culex quinquefasciatus
(genes : 18,968)
Anopheles gambiae
(genes : 12,843)
Aedes aegypti
(genes : 15,796)
437
2,330
349
192
4,814
288
638
375
B
Average intron length
6,000
5,000
4,000
3,000
2,000
1,000
0
Average intron number
8
7
6
5
4
3
2
1
0
Average CDS length
2,500
2,000
1,500
1,000
500
0
Average length of gene regions
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
P. steinenii
B. antarctica
A. aegypti
A. gambiae
C. quinquefasciatus
D. melanogaster
C
(604/3317/11488)
Parochlus steinenii (AE: -0.745)
(13/14463/1127)
1
(346/3386/11896)
Belgica antarctica (AE: -0.825)
(0/15569/32)
4
(1338/11883/2393)
Culex quinquefasciatus (AE: 0.026)
(14/15565/13)
3
(1635/11824/2173)
Aedes aegypti (AE: 0.013)
(317/8634/6681)
2
(1306/10407/3920)
Anopheles gambiae (AE: -0.135)
(5289/4144/6196)
Drosophila melanogaster (AE: 0.316)
254
234
199
137
128
Millions of years before present

July 29, 2016

Dear Editor:

We wish to submit a new manuscript entitled, “**Genome sequencing of the winged midge, *Parochlus steinenii*, from the Antarctic Peninsula**”, to be considered for publication in *GigaScience.*

In the Antarctic, only two species of Chironomidae occur naturally: the wingless midge *Belgica antarctica,* and the winged midge *Parochlus steinenii*. *B. antarctica* is notable for its tolerance to freezing, and its compact genome is thought to be the result of adaptation to an extreme environment. Despite this, an anti-freezing protein has not yet been identified in the genome, and the mechanism of freeze tolerance is unclear.

In this study, we present the annotated, draft genome of the Antarctic midge, *P. steinenii*. *P. steinenii* is cold tolerant but not freeze tolerant in the larval stage, so its genome will help to clarify the mechanism for freeze tolerance when compared with that of *B. antarctica*.

I confirm that all authors have approved the manuscript for submission, and the content of the manuscript has not been published, or submitted for publication, elsewhere.

Please address all correspondence concerning this manuscript to me, at ssc@kopri.re.kr.

Thank you for considering our manuscript.

Sincerely,

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