

Supplemental Tables

Supplemental Table S1. Library data statistics of SMRT sequencing by using the PacBio sequencing platform

Sample name	Total number	Total number (>2kb)	Total bases (Gb)	Max length (bp)	Mean length (bp)	N50 (bp)	N90 (bp)	GC Content (%)
<i>Leptobrachium boringii</i>	25,247,217	23,108,641	333.6	239,796	13,213	18,210	8,071	43.2

Supplemental Table S2. Assembly result of *L.boringii* genome

Mode	Total length (bp)	Total number	Total number (>= 2 kb)	max length(bp)	N50 (bp)	N90 (bp)	GC content (%)
assembly(canu+wtDBG)	3,766,027,940	5,431	5,431	40,219,274	5,679,619	505,185	44
assembly+arrow	3,766,064,883	5,431	5,431	40,227,961	5,682,372	504,975	44.1
assembly+arrow+pilon	3,764,520,168	5,431	5,431	40,210,739	5,679,266	504,721	44.1
assembly+arrow+pilon+Purge_haplotigs	3,377,877,096	1,570	1,570	40,210,739	6,410,543	1,223,751	43.9

Supplemental Table S3. Assembly result sequence statistics

Parameters	Contig Length(bp)	Contig Number	Scaffold Length(bp)	Scaffold Number
N90	1,223,751	611	1,223,751	611
N70	3,432,816	290	3,432,816	290
N60	4,660,696	206	4,660,696	206
N50	6,410,543	146	6,410,543	146
Total length	3,377,877,096	-	3,377,877,096	-
Number(>=100bp)	-	1,570	-	1,570
Number(>=2kb)	-	1,570	-	1,570
Max length	40,210,739	-	40,210,739	-

Supplemental Table S4. Assessment of the assembly quality of *L.boringii* using the BUSCO gene set metazoan_odb9 as reference.

Type	Proteins	Percentage(%)
Complete BUSCOs (C)	909	92.94
Complete and single-copy BUSCOs (S)	889	90.9
Complete and duplicated BUSCOs (D)	20	2.04
Fragmented BUSCOs (F)	16	1.64
Missing BUSCOs (M)	53	5.42
Total BUSCO groups searched	978	100

Supplemental Table S5. The classification results of repetitive sequences analyzed using three methods: RepBase TEs, TE Proteins, and De novo.

Category	RepBase TEs		TE Proteins		De novo		Combined TEs	
	Length (bp)	%in Genome	Length (bp)	%in Genome	Length (bp)	%in Genome	Length (bp)	%in Genome
DNA	245,453,156	7.27	98,648,187	2.92	311,275,083	9.21	402,910,342	11.93
LINE	215,736,445	6.39	171,859,388	5.09	235,938,667	6.98	371,593,803	11
SINE	2,750,268	0.08	0	0	1,836,234	0.05	4,323,969	0.13
LTR	156,375,646	4.63	159,674,731	4.73	1,045,835,251	30.96	1,181,417,726	34.97
Satellite	12,663,409	0.37	0	0	5,518,707	0.16	17,461,676	0.52
Simple_repeat	0	0	0	0	397,880	0.01	397,880	0.01
Other	1,973	0	0	0	0	0	1,973	0
Unknown	2,219,734	0.07	0	0	493,927,738	14.62	495,193,707	14.66
Total	624,224,757	18.48	430,082,614	12.73	2,019,387,243	59.78	2,345,356,867	69.43

"Total" represents the combined results obtained from these methods, excluding overlapping regions to obtain non-redundant results.

Supplemental Table S6. Functional annotation statistical results. Functional annotation results from seven different databases (NR, GO, KEGG, KOG, Pfam, SwissProt, and TrEMBL).

Category	Number	Percent (%)
Total	20,327	
Annotated	19,277	94.83
InterPro	17,822	87.68
GO	13,543	66.63
KEGG_ALL	18,560	91.31
KEGG_KO	11,698	57.55
Swissprot	16,844	82.87
TrEMBL	18,799	92.48
TF	3,002	14.77
Pfam	17,209	84.66
NR	18,914	93.05
KOG	15,341	75.47
Unannotated	1,050	5.17

Supplemental Table S7. BUSCO assessment of the annotation quality of *L. boringii* using the BUSCO gene set metazoan_odb9 as reference.

Type	Proteins	Percentage(%)
Complete BUSCOs (C)	933	95.4
Complete and single-copy BUSCOs (S)	904	92.4
Complete and duplicated BUSCOs (D)	29	3
Fragmented BUSCOs (F)	20	2
Missing BUSCOs (M)	25	2.6
Total BUSCO groups searched	978	100

Supplemental Table S8. Statistical results of the Hi-C assisted assembly for *L. boringii*, with a chromosome anchoring rate of 99.84%.

Category	Sequence Length (bp)	Sequence number	Contig N50 (bp)	Scaffold N50 (bp)
Assembly draft	3,377,877,096	1,570	6,410,543	6,410,543
Genome after Hi-C assisted assembly	3,378,025,596	237	6,383,561	406,887,571
Chromosomes after Hi-C assisted assembly	3,372,468,942	13	6,397,514	406,887,571
Free sequences after Hi-C assisted assembly	5,556,654	224	80,477	97,000

Supplemental Table S9. Summary of assembled pseudochromosomes.

Chr ID	Gene Number	Sequence Length (bp)
chr1	2759	575427445
chr2	2466	475785384
chr3	2306	414154962
chr4	1948	406887571
chr5	1570	355962951
chr6	1464	177778454
chr7	1325	174743545
chr8	1110	172850131
chr9	1315	148624053
chr10	1001	133087010
chr11	1075	126895693
chr12	1083	113301462
chr13	891	96970281

Supplemental Table S10. Comparison of the Relative Length of Chromosome (RL) of Chr1 in a male individual of *L.boringii* from karyotypic data.

	Mitosis1	Mitosis2	Mitosis3	Mitosis4	Mitosis5	Mitosis6	Mitosis7	Mitosis8
Long	14.63	13.83	13.85	14.93	17.15	16.70	14.53	14.46
short	13.49	13.50	13.60	14.86	16.50	14.20	14.43	16.38
Male	Mitosis9	Mitosis10	Mitosis11	Mitosis12	Mitosis13	Average	Standard Deviation	<i>p</i> value
Long	14.65	16.26	15.43	16.53	14.57	15.194	1.067	0.21
short	14.46	16.21	14.94	14.68	14.29	14.734	1.002	

Supplemental Table S11. Comparison of the Relative Length of Chromosome (RL) of Chr1 in a female individual of *L.boringii* from karyotypic data.

	Mitosis1	Mitosis2	Mitosis3	Mitosis4	Mitosis5	Mitosis6	Mitosis7
Long	16.326	15.451	13.717	14.736	15.959	13.773	15.955
short	15.170	14.310	13.689	14.260	15.956	13.684	14.916
Female	Mitosis9	Mitosis10	Mitosis11	Mitosis12	Average	Standard Deviation	<i>p</i> value
Long	14.230	14.721	14.427	14.419	14.883	0.86	0.13
short	13.470	14.553	14.333	13.269	14.328	0.76	

Supplemental Table S12. Karyotypic study of all chromosome in male and female of *L.boringii*.

Mitosis	Male			Female		
	Relative Length of Chromosome (RL)	Arm Ratio (AR)	Centromeric Index (CI)	Relative Length of Chromosome (RL)	Arm Ratio (AR)	Centromeric Index (CI)
1	15.15±0.96	1.25±0.06	44.60±1.11	14.61±0.78	1.20±0.08	45.66±1.72
2	12.76±0.74	1.44±0.17	41.17±2.87	12.16±0.50	1.51±0.16	39.98±2.49
3	11.84±0.56	1.61±0.17	38.53±2.52	11.43±0.52	1.75±0.25	36.73±3.44
4	10.84±0.59	1.58±0.20	39.00±2.93	10.51±0.30	1.55±0.10	39.41±1.45
5	9.28±0.28	1.49±0.11	40.20±1.75	8.98±0.41	1.40±0.11	41.75±1.80
6	7.19±0.55	1.28±0.11	44.09±2.09	6.79±0.34	1.29±0.14	43.82±2.63
7	5.76±0.43	1.47±0.23	40.98±3.68	6.11±0.30	1.50±0.22	40.36±3.57
8	5.42±0.30	1.52±0.23	39.99±3.42	5.78±0.30	1.43±0.12	41.39±2.04
9	5.09±0.36	1.43±0.19	41.44±3.35	5.40±0.33	1.42±0.18	41.58±2.86
10	4.65±0.46	1.47±0.21	40.73±3.30	5.06±0.32	1.32±0.15	43.34±2.75
11	4.40±0.43	1.32±0.18	43.37±3.34	4.78±0.37	1.28±0.17	44.02±3.04
12	4.16±0.38	1.43±0.14	41.28±2.39	4.46±0.45	1.32±0.21	43.44±3.50
13	3.64±0.42	1.64±0.26	38.37±3.86	4.01±0.43	1.57±0.27	39.42±3.76

Supplemental Table S13. Statistical results of the Hi-C assisted assembly for two haploid genomes of *L. boringii*, with a chromosome anchoring rate of 97.30% of HapA genome and 97.68% of HapB genome.

Category	Genome Version	Sequence Set	Sequence length (bp)	Sequence number	Contig N50 (bp)	Scaffold N50 (bp)	Contig N90 (bp)	Scaffold N90 (bp)
HaploidA	Before Mounting	Whole Genome	3,923,887,969	950	25,341,697	25,341,697	3,034,138	3,034,138
		Whole Genome	3,923,946,169	370	25,341,697	437,489,029	3,022,311	146,307,837
	After Mounting	Chromosome	3,818,146,437	13	25,647,232	437,489,029	3,735,721	146,307,837
		Unanchored Sequences	105,799,732	357	1,201,288	1,443,274	90,428	92,883
HaploidB	Before Mounting	Whole Genome	3,740,238,720	686	28,085,800	28,085,800	3,453,974	3,453,974
		Whole Genome	3,740,294,820	127	28,085,800	428,935,640	3,453,974	138,549,874
	After Mounting	Chromosome	3,653,333,437	13	28,147,371	428,935,640	4,234,898	138,549,874
		Unanchored Sequences	86,961,383	114	1,424,100	3,708,995	190,604	326,356

Supplemental Table S14. BUSCO assessment of the assembly quality for two haploid genomes of *L.boringii* using the BUSCO gene set metazoan_odb9 as reference.

Category	HaploidA		HaploidB	
	Proteins	Percentage (%)	Proteins	Percentage (%)
Complete BUSCOs (C)	5,058	95.25	5,027	94.67
Complete and single-copy BUSCOs (S)	4,975	93.69	4,967	93.54
Complete and duplicated BUSCOs (D)	83	1.56	60	1.13
Fragmented BUSCOs (F)	41	0.77	46	0.87
Missing BUSCOs (M)	211	3.97	237	4.46
Total BUSCO groups searched	5,310	100	5,310	100
Database	tetrapoda_odb10		tetrapoda_odb10	

Supplemental Table S15. Summary of assembled pseudochromosomes in HaploidA and HaploidB.

Chromosome_ID	HaploidA	HaploidB
	Sequence Length (bp)	Sequence Length (bp)
1	630513475	611001509
2	525759985	512142565
3	473326093	453898949
4	437489029	428935640
5	382116861	363536537
6	246698808	225517690
7	219274935	194728218
8	185097934	179020277
9	174088974	172012298
10	155409082	139785352
11	146307837	138549874
12	130607099	128851846
13	111456325	105352682

Supplemental Table S16. Statistical information of unmapped ratio and coverage for male and female short reads when aligned to Haploid A and Haploid B genome of *L. boringii*.

Category	HaploidA		HaploidB	
	Male	Female	Male	Female
unmapped ratio (%)	0.264	0.203	0.374	0.303
coverage (%)	95.89	95.85	96.58	96.44

Supplemental Table S17. Statistical information of unmapped and mapped Y-mer and Female-mer reads when aligned to Haploid A and Haploid B genome of *L. boringii*.

Category	Female-mer		Y-mer	
	Haploid A	Haploid B	Haploid A	Haploid B
unmapped ratio (%)	85.45	91.84	89.36	7.04
unmapped ratio of Chr 1(%)	93.15	99.57	92.95	8.11
mapped ratio of Chr 1(%)	6.85	0.43	7.05	91.89

Supplemental Table S18. Statistical information on the sex-specific genetic profiles of the *L. boringii* for males and females.

Chromosome_ID	Female		Male	
	NumMarkers	chromlength(cM)	NumMarkers	chromlength(cM)
1	762	452.434	910	92.685
2	619	410.555	681	94.594
3	495	377.17	622	72.699
4	531	358.594	561	94.587
5	357	227.343	516	119.242
6	194	194.909	268	70.797
7	190	196.631	234	77.108
8	138	139.324	290	109.297
9	219	176.737	171	95.537
10	165	165.137	208	98.558
11	189	157.555	151	85.316
12	167	122.6	150	108.893
13	68	67.024	121	73.196

Supplemental Table S19. Details of the sex-linked SNPs used for Sanger sequencing to identify the genotypic sex of *L.boringii*.

SNP_id	CHROM: pos	Gene	REF	ALT	Primer sequences(5'–3')	Female genotype	Male genotype
SNP1	chr1:115680776	<i>Fem1C</i>	T	A	F: 5' TAATCAACGCTTGTGGCA 3' R: 5' GCTCCTGTCGGCTTCTGT 3'	TT	AT
SNP2	chr1:128933609	<i>Slc12a2</i>	A	G	F: 5' TTCACAAGGCGAAATCCA 3' R: 5' CAAGGTGTTGCCAATCTG 3'	GG	GA
SNP5	chr1:246117642	<i>Dmrt1</i>	A	C	F: 5' AGTCACCCTGAGCACCAT 3' R: 5' TAGGCAGAATCCATAACCAA 3'	CC	CA
SNP6	chr1:246263378	<i>Dmrt3</i>	T	A	F: 5' AGAGGCAGAGGGTGATGG 3' R: 5' TTGAGTGATGCTGTATAGGGA 3'	AA	AT

Supplemental Table S20. Details of the PCR conditions and procedures for Sanger sequencing.

System	Volume (μL)	Reaction conditions	Time	Number of cycles
2×EasyTaq PCR SuperMix	10.00	Pre-denaturation at 95°C	5min	1 cycle
DNA of <i>L.boringii</i>	1.00	Denaturation at 95°C	30s	35 cycles
ddH2O	7.00	Annealing at 55°C	30s	
Forward primer	1.00	Extension at 72°C	45s	
Reverse primer	1.00	Final extension at 72°C	5min	
Overall system	20.00	Storage at 4°C	Holding	1 cycle

Supplemental Table S21. Correlation between coexpressed modules and sample sex. Correlation r values calculated using Kendall's coefficient, and p values based on Fisher's asymptotic.

Traits	Modules	r	p value
Sex	Me01	0.361157559	0.037666922
Sex	Me02	0.401286177	0.020921335
Sex	Me03	0.34109325	0.04964723
Sex	Me04	0.351125405	0.043308143
Sex	Me05	0.662122192	5.31E-05
Sex	Me06	0.471511258	0.006656727
Sex	Me07	0.712282964	8.64E-05
Sex	Me08	0.692186501	4.15E-05
Sex	Me09	0.70225081	1.39E-04
Sex	Me10	-0.220707397	0.20402387
Sex	Me11	0.662122192	1.39E-04
Sex	Me12	0.652090038	1.75E-04
Sex	Me13	-0.100321544	0.563702862
Sex	Me14	0.571832802	9.99E-04
Sex	Me15	0.692218655	6.78E-05
Sex	Me16	-0.050160772	0.772829993
Sex	Me17	-0.060192927	0.72903449
Sex	Me18	-0.030096463	0.86249023
Sex	Me19	-0.080257235	0.644167223