A High-quality Draft Genome Assembly of *Sinella curviseta*: A Soil Model Organism (Collembola)

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Abstract

*Sinella curviseta*, among the most widespread springtails (Collembola) in Northern Hemisphere, has often been treated as a model organism in soil ecology and environmental toxicology. However, little information on its genetic knowledge severely hinders our understanding of its adaptations to the soil habitat. We present the largest genome assembly within Collembola using 44.86 Gb (118X) of single-molecule real-time Pacific Bioscience Sequel sequencing. The final assembly of 599 scaffolds was ~381.46 Mb with a N50 length of 3.28 Mb, which captured 95.3% complete and 1.5% partial arthropod Benchmarking Universal Single-Copy Orthologs (n = 1066). Transcripts and circularized mitochondrial genome were also assembled. We predicted 23,943 protein-coding genes, of which 83.88% were supported by transcriptome-based evidence and 82.49% matched protein records in UniProt. In addition, we also identified 222,501 repeats and 881 noncoding RNAs. Phylogenetic reconstructions for Collembola support Tomoceridae sistered to the remaining Entomobryomorpha with the position of Symphypleona not fully resolved. Gene family evolution analyses identified 9,898 gene families, of which 156 experienced significant expansions or contractions. Our high-quality reference genome of *S. curviseta* provides the genetic basis for future investigations in evolutionary biology, soil ecology, and ecotoxicology.

Key words: PacBio sequencing, Entomobryidae, comparative genomics, phylogenomics, gene family.

Introduction

Soil invertebrates, as well as soil microbes, contribute to essential soil functions and ecosystem services through trophic and nontrophic effects, such as organic matter degradation, nutrient cycling, pest control, human health (Wall et al. 2012, 2015). However, our knowledge on soil biodiversity and their function remains limited. As one of the oldest hexapod clade (Hirst and Maulik 1926), springtails (Collembola) are among the most dominant soil arthropods, predominantly living in almost all terrestrial ecosystems (Christiansen 1992). Besides the great values in evolutionary biology, collembolan species are often selected as model organisms for soil ecology especially ecotoxicology, such as “standard” parthenogenetic *Folsomia candida* Willem (ISO 1999; Organisation for Economic Co-operation and Development 2009). Bisexual reproducing species *Sinella curviseta* Brook (fig. 1) is an alternative model listed in OECD. It belongs to the largest collembolan family Entomobryidae (Entomobryomorpha) and is among the most widespread springtails in Northern Hemisphere (Bellinger et al. 1996–2018). Easy morphological identification and a high rate of reproduction make it suitable for various laboratory experiments. Reproduction, development and life history of *S. curviseta* have been well documented (Waldorf 1971; Nijima 1973; Gist et al. 1974; Zhang et al. 2011). However, limited genetic data, usually presented by sequences of barcoding and rRNA (Zhang...
et al. 2014), severely hinders its further application in a wider scope. A high-quality reference genome will provide a solid genetic basis for the understanding of molecular mechanisms and physiological functions in adaptations to environmental change, as well as evolutionary biology. To date, only three genome assemblies have been published for Collembola: Orchesella cincta Linnaeus (Faddeeva-Vakhrusheva et al. 2016), F. candida (Faddeeva-Vakhrusheva et al. 2017), and Holocanthella duospinosa Salmon (Wu et al. 2017). Here, we present a de novo genome assembly of S. curviseta using single-molecule real-time (SMRT) Pacific Bioscience (PacBio) long reads. We annotated the essential genomic elements, repeats, protein-coding genes, and noncoding RNAs (ncRNAs), and further compared gene family evolution across main arthropod lineages. Phylogeny of Collembola was also investigated using genomic data for the first time.

Materials and Methods

Sample Collection and Sequencing

The culture of S. curviseta used in this study was collected from Purple Mountain (32.056°N, 118.83°E, Nanjing, China) in April 2015 and was maintained for three years in our laboratory. Animals were collected with aspirator, washed with ddH2O, and crushed with liquid nitrogen. A total of 500, 10, 200 individuals were prepared for PacBio, Illumina whole genome, and Illumina transcriptome sequencing, respectively. Genomic DNA/RNA extraction, library preparation and sequencing were carried out at Novogene Co. Ltd. (Beijing, China). For long-read sequencing, a library was constructed with an insert size of 20 kb and sequenced using P6-C4 chemistry on the PacBio Sequel platform. For short-read sequencing, paired-end libraries were constructed with an insert size of 300 bp and sequenced (2 × 150 bp) on the Illumina HiSeq X Ten platform. Raw Illumina short reads were compressed into clumps and duplicates were removed with clumpify.sh (one of the BBTools suite v37.93, Bushnell). Quality control was performed with bbduk.sh (BBTools): Both sides were trimmed to Q20 using the Phred algorithm, reads shorter than 15 bp or with >5 Ns were discarded, poly-A or poly-T tails of at least 10 bp were trimmed, and overlapping paired reads were corrected. Assembly and annotation pipelines are shown in figure 2.

Genome Size Estimation

We employ the strategy of short-read k-mer distributions to estimate the genome size. K-mer length and maximum k-mer coverage cutoffs may have impacts on estimated genome size. The histogram of k-mer frequencies was computed with 21-mers and 27-mers using Jellyfish v2.2.7 (Marçais and Kingsford 2011). Genome size was estimated with a maximum k-mer coverage of 1,000 and 10,000 using GenomeScope v1.0.0 (Vurture et al. 2017).

De novo genome assembly with long reads were performed using two pipelines, Canu and Minimap2/Miniasm. Because of high heterozygosity for S. curviseta, we used the parameters ‘corOutCoverage = 200 “batOptions = -dg 3 -db 3 -dr 1 -ca 500 -cp 50”’ with Canu v1.7.1 (Koren et al. 2017) to output more corrected reads and be more conservative at picking the error rate for the assembly to try to maintain haplotype separation. For Minimap2/Miniasm pipeline, overlaps between long reads were generated with Minimap2 v2.9 (Li 2018). We employed Miniasm v0.3 (Li 2016) to assemble contigs and three rounds of Racon v1.3.1 (Vaser et al. 2017) to generate consensus and correct errors. To improve genome contiguity, two assemblies generated from Canu and Minimap2/Miniasm pipelines were merged with three rounds of quickmerge (Chakraborty et al. 2016) following USAGE 2 (https://github.com/mahulchak/quickmerge/wiki; last accessed September 1, 2018). Redundant heterozygous sequences were removed from merged assembly with Purge Haplotigs v20180917 (Roach et al. 2018); percent cutoff for identifying a contig as a haplotype was set as 60 (-a 60) with other parameters as the default. The resulting contigs were polished with PacBio long reads using two rounds of Arrow mode in GenomicConsensus v2.3.2 (Chin et al. 2013). Furthermore, assembly was polished with Illumina short reads using two rounds of Pilon v1.22 (Walker et al. 2014). We again removed redundant sequences with Purge Haplotigs. Contaminants were examined using PhyLOdigo v0.9-alpha (Mallet et al. 2017). Potential untargeted sequences were identified by exploring compositional similarity on a tree (phyloselect.R) and hierarchical DBSCAN clustering (phyloselect.py), and inspected with BlastN v2.7.1 (Camacho et al. 2009) against the NCBI nucleotide database. Finally, vector contamination was checked using VecScreen against the UniVec database. The mitochondrial genome of S. curviseta was assembled based on Illumina short reads with NOVOPlasy v2.7.0 (Dierckxsens et al. 2017) using COI sequence.

Fig. 1.—An adult of Sinella curviseta. It has a pale orange body and two longitudinally arranged eye spots on each side.
(KM978373) as the initial seed. Transcriptome assembly was performed with a genome-guided method. RNA-seq reads were mapped to assembled genome with HISAT2 v2.1.0 (Kim et al. 2015) and assembled with StringTie v1.3.4 (Pertea et al. 2015). Redundant isoforms were removed with Redundans v0.13c (Pryszcz and Gabaldón 2016) with the defaults. To assess the completeness of assemblies, we applied Benchmarking Universal Single-Copy Orthologs (BUSCO, Waterhouse et al. 2018) analyses against arthropod data set (n = 1066). In addition, we also mapped PacBio long reads and Illumina short reads to the final genome assembly with Minimap2.

**Genome Annotation**

A de novo species specific repeat library was constructed using RepeatModeler v1.0.11 (Smit and Hubley 2008–2015), and was then combined with Dfam_2.0, Dfam_Consensus-20170127 (Hubley et al. 2016) and RepBase-20170127 databases (Bao et al. 2015) to generate a custom library. We then used RepeatMasker v4.0.7 (Smit et al. 2013–2015) with the custom library to identify and mask repeats in the genome assembly.

Gene prediction was conducted with the MAKER v2.31.10 pipeline (Holt and Yandell 2011) by integrating ab initio, transcriptome-based and protein homology-based evidence. Ab initio gene predictions were performed with Augustus v3.3 (Stanke et al. 2004) and GeneMark-ET v4.33 (Lomsadze et al. 2005). Two predictors were trained using BRAKER v2.1.0 (Hoff et al. 2016) with RNA-seq data. Previously assembled genome-guided transcripts were used as transcriptome-based evidence. Protein sequences of Daphnia pulex, Acrithosiphon pisum, and Drosophila melanogaster were downloaded from Ensembl (Flicek et al. 2014) as protein homology-based evidence.

Homology-based gene functions were assigned using Diamond v0.9.18 (Buchfink et al. 2015) against UniProtKB (SwissProt + TrEMBL) database with a sensitive mode and an e-value threshold of 1e–5 (–sensitive -e 1e–5). Protein domains, as well as Gene Ontology (GO) and pathway annotation, were searched with InterProScan 5.30-69.0 (Finn et al. 2017) against Pfam (Finn et al. 2014), PANTHER (Mi et al. 2017), Gene3D (Lewis et al. 2018), Superfamily (Wilson et al. 2009), and CDD (Marchler-Bauer et al. 2017) databases (-dp -f TSV, GFF3 -goterms -iprlookup -pa -t p -appl Pfam, PANTHER, Gene3D, Superfamily, CDD).
ncRNAs were identified with Infernal v1.1.2 (Nawrocki and Eddy 2013) against Rfam v14.0 (Kalvari et al. 2018) database. Transfer RNAs were further refined with tRNAscan-SE v2.0 (Lowe and Eddy 1997).

Phylogenomic Analyses

We conducted a phylogeny of Collembola using public genomic data (three genomes and four transcriptome assemblies): O. cincta (GCA_001718145.1), F. candida (GCA_002217175.1), H. duospinosa (GCA_002738285.1), Anurida maritima (GAUE00000000.2), Tetrodontophora bielanensis (GAXI00000000.2), Pogonognathellus sp. (GATD00000000.2), Sminthurus viridis (GAT00000000.2), and One Protura (Acrcentomon sp., GAXE00000000.2) and one Diplura (Catajapyx aquilonaris, GCA_000934665.2) species were selected as the outgroup. Transcriptomic assemblies were reported in Misof et al. (2014) and Diplura genome was from Thomas et al. (2018). Complete single-copy genes were generated with BUSCO assessments against arthropod data set. Gene training set constructed by BRAKER was used for Augustus species gene-finding parameters. Shared single-copy genes were aligned using MAFFT v7.394 (Katoh and Standley 2013) with the LINS1 strategy, trimmed using trimAl v1.4.1 (Capella-Gutierrez et al. 2009) with the heuristic method automated1, and concatenated using FASconCAT-G v1.04 (Kuck and Longo 2014). We constructed the phylogenetic trees using maximum likelihood (ML) and coalescent-based species tree (ASTRAL) methods. ML reconstructions were performed using IQ-TREE v1.6.3 (Nguyen et al. 2015) with 1,000 ultrafast bootstrap (UFBoot, Hoang et al. 2018) and 1,000 SH-aLRT replicates (Guindon et al. 2010) estimated. Partitioning schemes and substitution models were estimated with ModelFinder (Kalyaanamoorthy et al. 2017) built-in in IQ-TREE. We used a subset of substitution models with the options “-mset” (HKY and GTR for nucleotides, WAG and LG for proteins), and implemented the relaxed hierarchical clustering algorithm (Lanfear et al. 2014) with the setting “-rc luster 10.” Species trees were estimated using ASTRAL-III v5.6.1 (Zhang et al. 2018) based on gene trees generated with IQ-TREE on individual gene alignments. Local branch supports were estimated from quartet frequencies (Sayyari and Mirarab 2016).

Gene Family Identification and Evolution

We identified gene families among 12 arthropod species, including four collembolans, four insects (A. pisum, D. melanogaster, Tribolium castaneum, Zootermopsis nevadensis), three other nonhexapods (Liodes scapularis, Strigamia maritima, D. pulex). OrthoFinder v2.2.7 (Emms and Kelly 2015) was used to infer orthologs with Diamond as the sequence aligner. Gene family evolution (gain and loss) was analyzed using CAFE v4.2 (Han et al. 2013) with lambda parameter to calculate birth and death rates. Species tree and divergence time were generated from TimeTree database (Kumar et al. 2017).

Results and Discussion

Genome Sequencing and Assembly

We generated 4,196,991 subreads of 44.86 Gb (118X) on the PacBio Sequel platform. The mean and N50 length of long subreads reached 9.80 kb and 14.67 kb. A total of 37.95 Gb (99X) and 7.86 Gb clean data were produced on the Illumina HiSeq X Ten platform for whole genome and transcriptome sequencing, respectively.

We estimated the genome size with GenomeScope under the four parameter combinations, ranging from 327.12 Mb to 340.37 Mb (Table 1). Genome repetitive length estimates increased with the larger maximum k-mer coverage cutoff, ranging from 19.96 Mb to 32.65 Mb. Unique (nonrepetitive) length estimates were more consistent among analyses, ranging from 304.71 Mb to 311.28 Mb. Overall rate of heterozygosity (0.746–0.886) and the distinct first peak at a mean kmer coverage of 30.7–32.5 in the k-mer plots (Supplementary fig. S1, Supplementary Material online) indicated that this genome may have a high rate of heterozygous regions, which should be carefully considered in the subsequent assembly processes.

A Canu assembly of 633.80 Mb and 5,606 contigs (Table 2) was generated with most haplotypes kept for diploid populations. Minimap2/Miniasm pipeline resulted in an assembly of 549.01 Mb and 3,288 contigs. Size of both assemblies was much larger than estimated due to the presence of a great number of heterozygous sequences. Both assemblies were merged into 5,437 contigs (L50 708 kb) with quickmerge. A total of 4,779 (246.11 Mb) and 58 (1.37 Mb) redundant heterozygous sequences were respectively removed with two rounds of Purge Haplotigs, resulting a great improvement in N50 length (3.28 Mb). No evident contaminants were found using PhyloOligo (Supplementary fig. S2, Supplementary Material online). A vector sequence was excluded. Final draft assembly of S. curviseta has 599 contigs/scaffolds (no gaps), total length of 381.46 Mb, N50 length of 3.28 Mb, a maximum scaffold length of 12.99 Mb, and 37.51% GC content. Our assembly has a largest genome size among four collembolan species, a much higher assembly quality than O. cincta and H. duospinosa, but is slightly more fragmented than F. candida (Table 3). With the genome-guided strategy, a total of 27,976 transcripts were assembled with a mean and N50 length of 2.26 kb and 3.50 kb.

We generated a circularized mitochondrial genome of 14,840 bp. It consists of 13 protein-coding genes, 2 rRNA genes and 22 tRNA genes. The mitochondrial gene number and order are similar to most collembolan species. The A+T
content (69.8%) in S. curviseta is slightly smaller than those in known Entomobryomorpha species.

Assembly completeness were assessed with BUSCO analyses against arthropod data set \( (n = 1066) \). We identified 92.8–95.4% complete, 1.5–3.5% fragmented, and 2.9–3.7 missing BUSCOs for all versions of genome assemblies (table 2). Comparable results to other collembolan genomes indicated the high completeness of our assembly. Genome-guided transcriptome assembly also showed similar completeness to the genome assembly. The tremendous decreasing of duplicated BUSCOs indicated that Purge Haplotigs and Redundans could be highly efficient for reducing heterozygous regions. In addition, we mapped 93.22% and 96.07% of PacBio long reads and Illumina short reads to the final genome assembly. Also, 27,956 (99.93%) assembled transcripts were aligned to the genome using BlastN with an identity value of 0.99.

**Genome Annotation**

RepeatMasker identified 222,501 repeats which masked 9.79% of the genome assembly. The top five abundant repeat types were simple repeats, unclassified repeats, low complexity repeats, Helitron transposable elements, and Gypsy LTR retrotransposons (supplementary table S1, Supplementary Material online). We compared repeat components among four collembolan species (table 3). Three species (S. curviseta, O. cincta, F. candida) belonging to Entomobryomorpha have similar repeat compositions, but sharply differ from H. duospinosa in DNA and unclassified repeats. High similarity between S. curviseta and O. cincta is also consistent with their systematic positions (within the same family Entomobryidae).

**Table 1**

| K-mer Coverage | Heterozygosity (%) | Repeat Length (Mb) | Unique Length (Mb) | Genome Size (Mb) |
|----------------|-------------------|--------------------|--------------------|------------------|
|                | Mix               | Max                | Mix                | Max              | Mix              | Max              |
| 21             | 1,000             | 0.851              | 0.886              | 22.42            | 22.53            | 304.71           | 306.22           | 327.12           | 328.85           |
| 21             | 10,000            | 0.863              | 0.874              | 32.60            | 32.65            | 305.22           | 307.50            | 337.62           | 338.36           |
| 27             | 1,000             | 0.746              | 0.772              | 19.96            | 20.05            | 309.83           | 311.28           | 329.79           | 331.33           |
| 27             | 10,000            | 0.755              | 0.763              | 29.54            | 29.59            | 310.32           | 310.79           | 339.87           | 340.37           |

**Table 2**

| Assembly | Total Length (Mb) | No. Scaffolds | N50 Length (kb) | Longest Scaffold (Mb) | GC (%) | BUSCO \( (n = 1066) \) (%) |
|----------|------------------|---------------|-----------------|----------------------|--------|-----------------------------|
|          |                  |               |                 |                      |        | C, D, F, M                  |
| Canu     | 633.80           | 5,606         | 521             | 8.89                 | 37.55  | 95.4, 40.5, 1.7             | 2.9              |
| Minimap2/Miniasm | 549.01 | 3,288 | 309             | 7.249               | 37.60  | 92.8, 15.8, 3.5             | 3.7              |
| quickmerge | 628.88 | 5,437 | 708             | 12.98               | 37.55  | 95.0, 39.5, 1.8             | 3.2              |
| purge_haplotigs_1 | 382.77 | 658 | 3288            | 12.98              | 37.53  | 94.2, 5.2, 2.4              | 3.4              |
| Arrow    | 383.93           | 658           | 3290            | 12.99               | 37.53  | 95.3, 4.9, 1.7              | 3.0              |
| Pilon    | 382.83           | 658           | 3284            | 12.90               | 37.52  | 95.3, 5.6, 1.6              | 3.1              |
| purge_haplotigs_2 | 381.46 | 600 | 3284            | 12.99              | 37.51  | 95.3, 5.4, 1.5              | 3.2              |
| Final genome assembly | 381.46 | 599 | 3284            | 12.99              | 37.51  | 95.3, 5.4, 1.5              | 3.2              |
| Transcript assembly | 63.34 | 27,976 | 3.50           | 0.056               | 40.68  | 94.6, 8.4, 2.3              | 3.1              |

Notes.—Reduction of heterozygous regions was carried out twice \(_1, _2\) with purge_haplotigs. Values of final assemblies are bold. C, complete BUSCOs; D, complete and duplicated BUSCOs; F, fragmented BUSCOs; M, missing BUSCOs.
elements (3 families), and 1 other (Metazoa_SR) ncRNAs (supplementary table S2, Supplementary Material online). snRNAs were classified into six spliceosomal RNAs (U1, U2, U4, U5, U6, U11), three minor spliceosomal RNAs (U12, U4atac, U6atac), two H/ACA box and 12 C/D box snoRNAs (small nucleolar RNA), and one (SCARNA8) scaRNA (small Cajal body-specific RNA). A total of 21 tRNA isotypes were identified except for Supres-isotype missing.

**Phylogenomic Analyses**

Nucleotide and protein matrices comprising 229 shared single-copy genes had 250,783 and 81,557 sites, and were divided with ModelFinder into 32 and 39 partitions, respectively. ML trees from nucleotide and protein matrices generated similar topologies except for the position of Symphypleona S. viridis. Species trees generated with ASTRAL-III had the same performance as the ML trees. All support values were absolutely high for most nodes (fig. 3). The phylogeny of Collembola at high levels were far from resolved in previous studies (D’Haese 2002, 2003; Xiong et al. 2008; Yu et al. 2016). Topological hypothesis of Symphypleona sistered to Entomobryomorpha based on nucleotide matrix agreed with morphologically cladistic analyses (D’Haese 2003), but alternative hypothesis of Symphypleona sistered to the remaining collembolan taxa were usually consistent with molecular phylogenies, as well as our results from the nucleotide matrix. Interestingly, our trees provided robust evidence supporting the sister relationship between Tomoceridae (Pogonognathellus sp.) and the remaining Entomobryomorpha, as indicated by evidence from the first instar larvae (Yu et al. 2016). Wider taxa sampling with more families included may help to achieve the ultimate phylogeny of Collembola.

**Gene Family Evolution**

Gene families were identified among 12 arthropod species with OrthoFinder. A total 67.03% (166,850) genes were assigned into 14,387 gene families with a mean orthogroups size of 11.6. Among 2,396 families shared by all species, 207 are single-copy orthogroups. In S. curviseta, 18,590 (77.64%) genes were clustered into 9,898 gene families, and 66 families and 487 genes were species-specific (supplementary table S3, Supplementary Material online). We analyzed gene family evolution (gain and loss) using CAFE. Estimated gene birth rate (lambda) was 0.00165, accounting for
duplications/gene/Mya. Expansions and contractions of gene families for 12 species are shown in figure 4. A total of 445 gene families experienced significant expansion or contraction events across the tree with a family-wide $P$-value $< 0.05$ (supplementary table S4, Supplementary Material online). Sinella curviseta showed 156 (131 expansions, 25 contractions) rapidly evolving families among 445 ones previously detected. The top five of the largest expanded families included Zinc-finger proteins (392), Ribonuclease H-like proteins (139), C-type lectin proteins (104), Carboxylesterase proteins (83), and F-box proteins (81) (supplementary table S5, Supplementary Material online). Zinc-finger proteins are transcription factors serving a wide variety of biological functions by binding DNA, RNA, proteins, or small molecules (Laity et al. 2001). Ribonuclease H-like superfamily is involved with nucleic acid metabolism, including DNA replication and repair, homologous recombination, transposition and RNA interference. C-type lectins have functions in innate and adaptive antimicrobial immune responses (Brown et al. 2018). Carboxylesterase enzymes participate in phase I xenobiotic metabolism. F-box proteins are associated with cellular functions such as signal transduction and regulation of the cell cycle (Craig and Tyers 1999). The top expanded gene families of three Entomobryomorpha species (S. curviseta, O. cincta, F. candida) have great similarities, participating in detoxication and xenobiotic metabolism, nucleic acid metabolism, immune system progress, signaling, etc. Expansion of these families are essential for adaptations to the complicated soil environment. This partly explains the reasons why the three species can be widespread in the Northern Hemisphere. It is not clear that whether these expansions are Entomobryomorpha-specific because genomic data are still lacking for the indigenous species.
Conclusion

With PacBio long reads, we report the largest collembolan draft assembly of *S. curviseta*, a soil model organism. Our high-quality genome assembly comprises 599 contigs of 381.46 Mb (N50 length of 3.28 Mb), covering over 95% the arthropod universal BUSCO sets. We also predict 23,943 protein-coding genes. Phylogenomic analysis support Tomoceridae closer to Entomobryomorpha than Poduromorpha. The genomic data produced in this study will provide a valuable resource for future studies in evolutionary biology, soil ecology, and ecotoxicology.

Supplementary Material

Supplementary data are available at Genome Biology and Evolution online.

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Author Contributions

F.Z., A.L., and C.Z. designed the study. F.Z., Y.D., J.W., and Q.Z. collected the samples and performed the analyses. F.Z. and A.L. wrote the paper. All authors edited and approved the final manuscript.

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Fig. 4.—Expansions (gain) and contractions (loss) of 14,387 gene families on a species tree. Gain and loss are indicated with symbol + and –. Numbers of gene families for each species are shown following species name. Tree topology and divergence time were generated from TimeTree database.
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