




Whole-Genome Sequencing and *De Novo* Assembly of 61 *Staphylococcus pseudintermedius* Isolates from Healthy Dogs and Dogs with Pyoderma

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ABSTRACT We have *de novo* assembled and polished 61 *Staphylococcus pseudintermedius* genome sequences with Nanopore-only long reads. Completeness was 99.25%. The average genome size was 2.70 Mbp, comprising 2,506 coding sequences, 19 complete rRNAs, 56 to 59 tRNAs, and 4 noncoding RNAs (ncRNAs), as well as CRISPR arrays.

Staphylococcus pseudintermedius is a common microorganism of canine skin (1, 2) and the leading cause of pyoderma in dogs (3, 4). In this study, we aimed to retrieve *S. pseudintermedius* high-quality genome sequences from healthy dogs and dogs with pyoderma using a *de novo* assembly and polishing strategy with Nanopore-only long reads.

Samples were obtained by rubbing a sterile swab on the skin of healthy dogs ($n = 22$; H) and the nonlesional skin of a dog with pyoderma ($n = 6$; DH) or from the pustules of dogs with pyoderma ($n = 33$; D). After culture in blood agar at 37°C for 24 hours, colonies were seeded in 3 ml of brain heart infusion (BHI) broth at 37°C for 16 hours. DNA was extracted with a ZymoBIOMICS DNA miniprep kit (Zymo Research, Irvine, CA, USA). DNA quality and quantity were determined using a Nanodrop 2000 spectrophotometer and Qubit double-stranded DNA (dsDNA) broad-range (BR) assay kit (Fisher Scientific SL, Madrid, Spain). The sequencing libraries were prepared with the rapid barcoding sequencing kit (SQK-RBK004; Oxford Nanopore Technologies, UK). Twelve barcoded samples were loaded into a MinION FLO-MIN106 v9.4.1 flow cell (Oxford Nanopore Technologies Ltd.) and sequenced into a MinION Mk1B instrument. The fast5 files were basecalled and demultiplexed and adapters trimmed with Guppy 4.0.11 (Oxford Nanopore Technologies) (--config dna_r9.4.1_450bp-s_hac.cfg) (--config configuration.cfg --barcode_kits SQK-RBK004 --trim_barcode; min_score threshold default 60). Reads with a quality score lower than 7 were discarded. Run summary statistics were obtained with Nanoplot 1.27 (5) (--N50 --fastq).

Samples assigned to *S. pseudintermedius* by WIMP (6) were *de novo* assembled using Flye 2.7.1 (7) (--nano-raw --genome-size 2.6m --plasmids --trestle). After minimap 2.17 alignment (8), the resulting contigs were polished with Racon 1.4.13 (<https://github.com/lbcb-sci/racon>) and Medaka 1.0.3 (<https://nanoporetech.github.io/medaka/>) (medaka_consensus; -m r941_min_high_g360). Genome completeness was assessed with CheckM 1.1.1 (lineage_wf) (9). Circlator 1.5.5 was used to identify the origin (10) (fixstart --min_id 70). Genomes were annotated with NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (11).

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TABLE 1 Characteristics and accession numbers for high-quality genome assemblies from 61 *Staphylococcus pseudintermedius* isolates^a

Isolate ID ^b	MLST ^c	Source ^d	Yr of isolation	Country of isolation	Assembly ID (accession version no.)	Assembly level ^e	Genome accession no.	Genome assembly	No. of contigs	N ₅₀ (contigs) (bp)	Genome size (all contigs) (bp)	No. of CDS (total)	COV ^f (x)	Comp ^f (%)	Cont ^f (%)
DG040	Unknown	Cf, A, SP	2017	Italy	GCF_016482145.1	Contig	JAENDF0000000000	Circular	3	2,527,299	2,564,892	2,369	664	99.43	0.00
DG050	Unknown	Cf, A, SP	2016	Italy	GCF_016482445.1	Contig	JAENDE0000000000	Circular	4	2,594,061	2,637,713	2,429	326	99.43	0.57
DG059	Unknown	Cf, A, SP	2017	Italy	GCF_016481825.1	Contig	JAENDD0000000000	Circular	2	2,568,059	2,573,568	2,364	223	99.29	0.00
DG062	ST71	Cf, A, SP	2016	Italy	GCF_016482085.1	Contig	JAENDC0000000000	Linear	10	2,096,046	2,991,046	2,901	253	99.43	2.84
DG063	ST71	Cf, A, SP	2016	Italy	GCF_016482045.1	Contig	JAENDB0000000000	Linear	10	2,099,753	2,981,523	2,891	256	99.43	0.57
DG064	ST71	Cf, A, SP	2017	Italy	GCF_016455205.1	Contig	CP066718	Circular	1	2,895,060	2,895,060	2,764	361	99.43	0.57
DG066	ST71	Cf, A, SP	2017	Italy	GCF_016482585.1	Contig	JAENDA0000000000	Circular	3	2,804,246	2,808,032	2,639	213	99.43	0.57
DG067	ST71	Cf, A, SP	2017	Italy	GCF_016482535.1	Contig	JAENCZ0000000000	Linear	3	2,890,387	2,896,399	2,776	533	99.43	0.57
DG071	ST71	Cf, A, SP	2012	Italy	GCF_016482425.1	Contig	JAENCY0000000000	Linear	3	2,800,617	2,807,986	2,644	85	99.43	0.57
DG072	ST71	Cf, A, SP	2012	Italy	GCF_016455165.1	Contig	CP066717	Circular	1	2,837,133	2,837,133	2,690	304	99.43	0.57
DG076	ST258	Cf, A, SP	2012	Italy	GCF_016482525.1	Contig	JAENCX0000000000	Circular	5	2,732,273	2,780,144	2,573	323	99.43	0.85
DG077	ST71	Cf, A, SP	2016	Italy	GCF_016481985.1	Contig	JAENCW0000000000	Circular	4	2,789,419	2,791,496	2,624	479	99.43	0.57
DG078	ST71	Cf, A, SP	2012	Italy	GCF_016481935.1	Contig	JAENCV0000000000	Circular	3	2,792,180	2,799,396	2,637	208	99.43	0.57
DG081	ST301	Cf, A, SP	2012	Italy	GCF_016482005.1	Contig	JAENCU0000000000	Circular	3	2,676,449	2,694,747	2,519	353	99.43	0.00
DG082	ST258	Cf, A, SP	2017	Italy	GCF_016481945.1	Contig	JAENCT0000000000	Circular	2	2,623,528	2,626,557	2,396	259	99.43	0.00
DG089	ST71	Cf, A, SP	2012	Italy	GCF_016481925.1	Contig	JAENCS0000000000	Circular	2	2,793,862	2,797,937	2,628	281	99.43	0.57
DG093	ST258	Cf, A, SP	2017	Italy	GCF_016481905.1	Contig	JAENCR0000000000	Circular	2	2,640,063	2,648,891	2,433	39	99.43	0.00
DG094	ST71	Cf, A, SP	2017	Italy	GCF_016481855.1	Contig	JAENCQ0000000000	Circular	2	2,840,672	2,849,103	2,675	191	99.43	0.57
DG099	Unknown	Cf, A, SP	2017	Italy	GCF_016455185.1	Chrom	CP066716	Linear	1	2,623,014	2,623,014	2,416	168	99.43	0.57
DSP020	ST71	Cf, A, SP	2013	Spain	GCF_016455145.1	Contig	CP066715	Circular	1	2,793,830	2,793,830	2,637	213	99.15	0.57
DSP021	ST71	Cf, A, SP	2013	Spain	GCF_016455225.1	Contig	CP066714	Circular	1	2,795,724	2,795,724	2,637	197	99.43	0.57
DSP022	Unknown	Cf, A, SP	2014	Spain	GCF_016481865.1	Contig	JAENCP0000000000	Circular	3	2,718,542	2,767,901	2,555	174	98.86	0.28
DSP024	ST611	Cf, A, SP	2014	Spain	GCF_016482205.1	Contig	JAENCO0000000000	Circular	2	2,758,929	2,762,026	2,595	289	99.43	0.57
DSP025	ST71	Cf, A, SP	2014	Spain	GCF_016482155.1	Contig	JAENCN0000000000	Circular	6	2,801,923	2,805,515	2,641	295	99.43	0.57
DSP026	ST503	Cf, A, SP	2014	Spain	GCF_016455085.1	Contig	CP066713	Circular	1	2,567,628	2,567,628	2,387	303	99.43	0.00
DSP027	Unknown	Cf, A, SP	2014	Spain	GCA_016455285.1	Contig	CP066712	Circular	1	2,717,194	2,717,194	2,537	352	99.43	0.28
DSP028	Unknown	Cf, A, SP	2019	Spain	GCF_016482125.1	Contig	JAENCM0000000000	Circular	2	2,569,147	2,575,420	2,367	238	98.86	0.00
DSP029	ST258	Cf, A, SP	2019	Spain	GCF_016481265.1	Contig	JAENBQ0000000000	Linear	8	2,413,604	2,723,805	2,517	270	99.43	0.00
DSP030	Unknown	Cf, A, SP	2018	Argentina	GCF_016455265.1	Contig	CP066711	Circular	1	2,612,059	2,612,059	2,386	164	99.43	0.00
DSP032	ST1631	Cf, A, SP	2019	Argentina	GCA_016482035.1	Contig	JAENCL0000000000	Linear	3	1,369,210	2,670,199	2,449	119	98.66	0.57
DSP034	ST1827	Cf, A, SP	2018	Argentina	GCF_016455025.1	Contig	CP066710	Circular	1	2,550,368	2,550,368	2,319	204	99.43	0.00
DSP035	Unknown	Cf, A, SP	2019	Argentina	GCF_016481845.1	Contig	JAENCK0000000000	Linear	2	2,640,488	2,642,865	2,489	117	99.43	0.00
DSP036	ST71	Cf, A, SP	2019	Spain	GCF_016482105.1	Contig	JAENCJ0000000000	Circular	7	2,849,760	2,927,015	2,755	129	99.43	0.57
DSP041	ST71	Cf, A, H	2019	Spain	GCF_016482265.1	Contig	JAENBW0000000000	Linear	3	2,849,717	2,909,751	2,748	123	99.41	0.57
DSP042	ST71	Cf, A, H	2019	Spain	GCF_016482345.1	Contig	JAENBV0000000000	Linear	6	2,822,064	2,930,017	2,768	72	99.43	0.57
DSP043	ST71	Cf, A, H	2019	Spain	GCF_016482305.1	Contig	JAENBU0000000000	Linear	12	1,649,358	2,931,289	2,776	66	99.43	0.57
DSP044	ST71	Cf, A, H	2019	Spain	GCF_016482225.1	Contig	JAENBT0000000000	Circular	4	2,849,602	2,907,542	2,748	132	99.43	0.57
DSP045	ST71	Cf, A, H	2019	Spain	GCF_016482025.1	Contig	JAENBS0000000000	Circular	5	2,849,736	2,915,461	2,742	127	99.41	0.57
DSP046	ST496	Cf, P, H	2019	Spain	GCF_016481295.1	Contig	JAENBR0000000000	Circular	2	2,695,721	2,710,903	2,475	100	99.43	0.00
HSP079	Unknown	Cf, P, H	2019	Spain	GCF_016598895.1	Contig	CP066885	Circular	1	2,585,691	2,587,693	2,337	301	99.43	0.00
HSP080	Unknown	Cf, P, H	2019	Spain	GCF_016598915.1	Contig	CP066884	Circular	1	2,585,570	2,587,506	2,336	199	99.41	0.00
HSP081	Unknown	Cf, P, H	2019	Spain	GCA_016482135.1	Contig	JAENCI0000000000	Circular	4	2,617,897	2,621,254	2,467	94	98.58	1.14

(Continued on next page)

TABLE 1 (Continued)

Isolate ID ^b	MLST ^c	Source ^d	Yr of isolation	Country of isolation	Assembly ID (accession version no.)	Assembly level ^e	Genome accession no.	Genome assembly	No. of contigs	N ₅₀ (contigs) (bp)	Genome size (all contigs) (bp)	No. of CDS (total)	COV ^f (x)	Comp ^f (%)	Cont ^f (%)
HSP082	Unknown	Cf, P, H	2019	Spain	GCA_016481725.1	Contig	JAENCH0000000000	Circular	2	2,617,857	2,620,663	2,466	149	96.73	1.14
HSP093	Unknown	Cf, A, H	2019	Spain	GCF_016481735.1	Contig	JAENCG0000000000	Circular	3	2,571,836	2,590,335	2,361	274	99.43	0.57
HSP094	Unknown	Cf, A, H	2019	Spain	GCF_016481805.1	Contig	JAENCF0000000000	Circular	2	2,567,494	2,570,595	2,337	550	99.43	0.57
HSP095	Unknown	Cf, A, H	2019	Spain	GCF_016482325.1	Contig	JAENCE0000000000	Circular	2	2,570,328	2,575,879	2,360	341	99.43	0.57
HSP096	Unknown	Cf, A, H	2019	Spain	GCF_016482245.1	Contig	JAENCD0000000000	Circular	3	2,570,206	2,578,330	2,359	89	99.43	0.57
HSP097	Unknown	Cf, A, H	2019	Spain	GCF_016481665.1	Contig	JAENCC0000000000	Circular	3	2,570,223	2,575,223	2,356	136	99.43	0.57
HSP118	Unknown	Cf, A, H	2019	Spain	GCF_016455005.1	Compl	CP066709	Circular	1	2,512,855	2,512,855	2,277	313	99.43	0.00
HSP125	ST1248	Cf, A, H	2019	Spain	GCF_016455245.1	Compl	CP066708	Circular	1	2,551,473	2,551,473	2,330	327	98.86	0.00
HSP127	ST1061	Cf, P, H	2019	Spain	GCF_016481685.1	Contig	JAENCB0000000000	Circular	2	2,660,509	2,690,618	2,507	162	98.86	1.14
HSP132	Unknown	Cf, P, H	2019	Spain	GCA_016455125.1	Chrom	CP066707	Linear	1	2,515,164	2,515,164	2,275	132	98.72	0.00
HSP134	Unknown	Cf, P, H	2019	Spain	GCA_016455105.1	Compl	CP066706	Circular	1	2,514,594	2,514,594	2,274	146	98.86	0.00
HSP135	Unknown	Cf, A, H	2019	Spain	GCF_016455065.1	Compl	CP066705	Circular	1	2,512,727	2,512,727	2,277	154	98.86	0.00
HSP136	Unknown	Cf, A, H	2019	Spain	GCA_016455045.1	Chrom	CP066704	Linear	1	2,512,726	2,512,726	2,274	104	98.86	0.00
HSP137	Unknown	Cf, A, H	2019	Spain	GCF_016454985.1	Compl	CP066703	Circular	1	2,512,757	2,512,757	2,271	224	99.43	0.00
HSP138	Unknown	Cf, A, H	2019	Spain	GCF_016454965.1	Compl	CP066702	Circular	1	2,512,830	2,512,830	2,277	227	98.86	0.00
HSP140	Unknown	Cf, P, H	2019	Spain	GCF_016482365.1	Contig	JAENCA0000000000	Circular	2	2,594,004	2,597,272	2,387	296	99.24	0.00
HSP141	ST257	Cf, P, H	2019	Spain	GCF_016482355.1	Contig	JAENBZ0000000000	Circular	4	2,615,633	2,622,529	2,450	150	99.43	0.57
HSP142	Unknown	Cf, P, H	2019	Spain	GCF_016482405.1	Contig	JAENBY0000000000	Circular	2	2,594,059	2,597,098	2,393	461	99.43	0.00
HSP143	Unknown	Cf, P, H	2019	Spain	GCF_016482235.1	Contig	JAENBX0000000000	Linear	6	2,537,079	2,779,670	2,589	293	98.86	0.57

^a Obtained from the lesional skin of 33 dogs with pyoderma (33 D), the nonlesional skin of a dog with pyoderma (6 DH), and 6 healthy dogs (22 H).

^b ID, identifier.

^c MLST, multilocus sequence type; ST, sequence type.

^d Cf, *Canis lupus familiaris*; A, abdominal skin swab; P, perioral skin swab; SP, superficial pyoderma; H, healthy.

^e Compl, complete genome; Chrom, chromosome.

^f COV, coverage; Comp, completeness; Cont, contamination.

Multilocus sequence types (MLSTs) were assigned with software MLST 2.0. and database 2.0.0 (<https://cge.cbs.dtu.dk/services/MLST-2.0/>) (12).

Nanopore sequencing allowed successful *de novo* assembly and polishing of 61 *S. pseudintermedius* isolates (Table 1). The average read N_{50} value was 4,223.38 bp for 2,848,339.50 reads per flow cell (237,361.63 per barcoded sample). The mean genome coverage was $235\times$ ($39\times$ to $664\times$), with an average contig N_{50} value of 2.6 Mbp (1.6 Mbp to 2.9 Mbp). The average genome completeness was 99.25% (98.6% to 99.4%, except for HSP082), which is close to previous results with hybrid assemblies (13). The number of contigs per isolate ranged from 1 to 12 (median, 2). The main contig was circular for 47 isolates. The average genome size of *S. pseudintermedius* was 2.70 Mbp (2.51 to 2.99 Mb), comprising 2,506 coding sequences (CDS; 2,271 to 2,901), 19 complete rRNAs (6 to 7 5S, 6 16S, and 6 23S rRNA genes), 56 to 59 tRNAs, and 4 noncoding RNAs (ncRNAs), as well as CRISPR arrays (0.5; range from 0 to 2). Pangenome analyses of isolates from healthy and diseased individuals will help unravel the differences, if any, that exist between commensal and pathogenic *S. pseudintermedius* populations.

Data availability. The standardized strain descriptions and accession numbers are presented in Table 1; the genome sequence assemblies and genomic data are publicly available in DDBJ/ENA/GenBank under BioProject no. [PRJNA685966](#) with the accession numbers [CP066702](#) to [CP066718](#), [CP066884](#), [CP066885](#), and [JAENBQ000000000](#) to [JAENDF000000000](#). The versions described in this paper are the first versions. The raw data are available from the Sequence Read Archive (SRA) under the same BioProject no., [PRJNA685966](#).

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