The diet of red-throated divers (Gavia stellata) overwintering in the German Bight (North Sea) analysed using molecular diagnostics

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Supplementary material:

Specific information: Primer design

Primer to amplify fish were modified from Chord_16S_F1/ modified Chord_16S_R1 (Waap et al. unpubl. data) to match the range of potential prey species of red-throated divers occurring in the study site.

Primer to amplify cephalopods were modified from modified Ceph _16S_F1/ modified Ceph _16S_R1 (Waap et al. unpubl. data) to match the range of potential prey species of red-throated divers occurring in the study site.

PCR amplification of fish and cephalopod prey DNA from faeces

PCR amplifications of fish and cephalopod prey were performed in single reactions using Multiplex PCR Kits (Qiagen). Each 20 µL PCR reaction volume contained 10 µl Multiplex PCR Master Mix, 1.25 µL of each primer (4µM), 1.25 µL blocking probe (40µM) if present and 4 µL template DNA. For amplification of fish prey DNA, 2 µL of Q solution per sample were added to the reaction mix and for amplification of cephalopod prey DNA 0.2 µL BSA per sample was added to the reaction mix.

PCR amplification of crustacean prey DNA from faeces

PCR amplifications of crustacean prey DNA were performed in separate reactions, using Multiplex PCR Kits (Qiagen). Each 20 µl PCR reaction volume contained 10 µl Multiplex PCR Master Mix, 2 µL of each primer (2 µM) and 4 µL template DNA. For amplification of crustacean prey DNA 0.2 µL BSA per sample was added to the reaction mix.

Specific information on bioinformatic analyses

We received the sequences in Ilumina 1.8 Phred format + 33 format and for further analysis we processed the data as follows, see also Table 1, 2. First we used Trimmomatic v0.36 (Bolger et al. 2014) to trim out low quality sequences and Illumina adapter sequences in the

raw data set. Then we used FLASH v1.2.11 to align paired reads (Magoc et al. 2011), and converted the fastq output to fasta format using FASTX Toolkit v0.0.13.2 (Gordon & Hannon, 2010). Mother v1.37.1 (Schloss et al. 2009) was used to identify sequence reads with an exact match to the primers & MID-tags, to avoid any spurious results due to sequencing errors. As the sequencing library was prepared using blunt-end ligation, each primer & MID combination was checked in both possible orientations. Sequencing reads matching with primers and MID-tags were then demultiplexed by pulling out the fasta sequence IDs for each sample from the mothur 'groups' file and using this list to extract the corresponding sequences from the mothur 'trim' file, giving a new fasta file with sequences specific to each sample for each gene. The mothur 'trim' sequences file also has the primer and MID-tag sequences removed from each sequence, as just the amplified gene region is wanted for the following clustering step. To condense large numbers of sequences and therefore to define molecular operational taxonomic units (MOTUs) we first we dereplicated the sequence file to remove identical replicates using usearch v7.0.1090 -derep-fulllength, then removed any potential chimeric sequences using usearch-uchime2_denovo, and finally clustered the sequences based on 97% identity into MOTUs (Clare et al. 2016, Elbrecht et al. 2016) using usearch -cluster_fast. Taxonomic information on the sequences was assigned using BLASTN against the nucleotide database and a cut-off of 90% sequence identity and an e value of 1e-10.

48 Table 1 Working steps, commands and References performed during bioinformatics analysis

Job to do	Software	Command	Reference
Check and summarise raw data quality	FastQC	fastqc BK-Poolx_R1_trimmed_paired.fq --outdir=./ fastqc BK-Poolx_R2_trimmed_paired.fq --outdir=./	Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Step1: Trimming raw data of any sequencing adapters and low quality sequence	Trimmomatic	trimmomatic PE -phred33 BK-Poolx_S5_L001_R1_001.fastq.gz BK-Poolx_S5_L001_R2_001.fastq.gz BK-Poolx_R1_trimmed_paired.fq BK-Poolx_R1_trimmed_unpaired.fq BK-Poolx_R2_trimmed_paired.fq BK-Poolx_R2_trimmed_unpaired.fq \ ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20 MINLEN:135	Bolger AM, Lohse M, Usadel B (2014): Trimmomatic a flexible trimmer for Illumina sequence data. Bioinformatics, 30, 2114-2120.
Step2: Aligning paired reads and convert fastq to fasta	Flash	flash BK-Poolx_R1_trimmed_paired.fq BK-Poolx_R2_trimmed_paired.fq -M 250 > flash_out	Magoc T & Salzberg SL (2011): FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics, 27 2957-2963.
	FASTX-	fastq_to_fasta -i out.extendedFrags.fastq -Q 33 >	Gordon, A., & Hannon, G. J. (2010). FASTX-Toolkit. Short-reads pre-processing tools. http://hannonlab.cshl.edu/ fastx_toolkit/index.html

	Toolkit	BK-Poolx_aligned.fa	
Step 3a: Identification and selection for sequences with exact matches to oligos & MIDs used, trimming MIDs and primer sequences	Mothur	mothur "#trim.seqs(fasta=BK-Poolx_aligned.fa,oligos=oligos_Poolx.txt,checkorient=T)"	Schloss, P.D., et al., Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol, 2009. 75(23):7537-41.
Step3b:_Demultiplex the sequences into a file for each sample	Deplex – custom perl script	perl deplex_v2.pl SampleList	
		while (<INLIST>) { $lib = $_; chomp($lib);	
		$readids1 = $lib . "_ids.txt"; $fa1 = $lib . ".fasta"; $readidsa = $lib . "a_ids.txt"; $readidsb = $lib . "b_ids.txt"; $readids2 = $lib . "_ab_ids.txt";	
		grep -w $lib $indir/BK-Poolx_aligned.groups | awk '{print \$1}' > $outdir/$readids1"	
		perl deplex_v2b.pl SampleListB	
		while (<INLIST>) {$lib = $_;chomp($lib);	
		$readids1 = $lib . "_ids.txt"; $fa1 = $lib . ".fasta"; $readidsa = $lib . "a_ids.txt"; $readidsb = $lib . "b_ids.txt"; $readids2 = $lib . "_ab_ids.txt";	
		system("cat $outdir/$readidsa $outdir/$readidsb >> $outdir/$readids2");	

		perl - ne'."'".'if(/^>(\S+)/){$c=$i{$1}}$c?print:chomp; $i{$_}=1 if'." @ARGV"'." $outdir/$readids2 $indir/BK-Poolx_aligned.trim.fasta > $outdir/$fa1"	
Step 4:	usearch, custom perl script	perl usearchPoolA.pl SampleListB while (<INLIST>) {$lib = $_;chomp($lib); $fa = $lib . ".fasta"; $usout1 = $lib . "_rc_uniques.fasta"; $usout2 = $lib . "_rc_uniques.out"; $usout3 = $lib . "_rc_uniques_results.uchime"; $usout4 = $lib . "_chimeras.fasta"; $usout5 = $lib . "_nonchimeras.fasta"; $usout6 = $lib . "_uchimealns"; $cent = $lib . "_centroids.fa"; $uc = $lib . "_clusters.uc"; $cons = $lib . "_consout.fa"; $msa = $lib . "_msa.fa";	

Step 4: Removal of identical replicates	usearch v7.0.1090	usearch -derep_fulllength $indir/$fa -output $outdir/$usout1 -sizeout -minseqlength 187 -minuniquesize 5 -strand both -uc $outdir/$usout2	USEARCH and UCLUST algorithms: Edgar,RC (2010) Search and clustering orders of magnitude faster than BLAST, Bioinformatics 26(19), 2460-2461. doi: 10.1093/bioinformatics/btq461
Step 4: Chimera detection		usearch -uchime_denovo $outdir/$usout1 -uchimeout $outdir/$usout3 -uchimealns $outdir/usout6 -chimeras $outdir/$usout4 -nonchimeras $outdir/$usout5	Edgar,RC, Haas,BJ, Clemente,JC, Quince,C, Knight,R (2011) UCHIME improves sensitivity and speed of chimera detection, Bioinformatics doi: 10.1093/bioinformatics/btr381 [PMID 21700674].; UCHIME2 algorithm Edgar, R.C. (2016), UCHIME2: Improved chimera detection for amplicon sequences, http://dx.doi.org/10.1101/074252.
Step 4: Clustering sequences 97%; final dereplication		usearch -cluster_fast $outdir/$usout5 -id 0.97 -centroids $outdir2/$cent -uc $outdir/$uc -sizeout -consout $outdir/$cons -msaout $outdir/$msa");	USEARCH and UCLUST algorithms: Edgar,RC (2010) Search and clustering orders of magnitude faster than BLAST, Bioinformatics 26(19), 2460-2461. doi: 10.1093/bioinformatics/btq461
Step 4b: Connecting each sample to the corresponding sequence/MOTU;		sed 's/^>/>sample_/g' sample_centroids.fa > 146443_centroids_edited.fa cat *_centroids_edited.fa > allsequencesPoolx.fa	
Step 4c: Final dereplication		usearch -derep_fulllength allsequencesPoolx.fa -output allsequencesPoolx_uniques.fasta -sizeout -minseqlength 135 -strand both -uc allsequnences_rc_uniques.out	USEARCH and UCLUST algorithms: Edgar,RC (2010) Search and clustering orders of magnitude faster than BLAST, Bioinformatics 26(19), 2460-2461. doi: 10.1093/bioinformatics/btq461

Step 5: Blast	blastn	export BLASTDB=/usr/local/extras/Genomics/db/ncbi_nt/current blastn -query $outdir/$cent -db nt -num_threads 4 -evalue 1e-10 -outfmt 6 -perc_identity 90 -out Poolx_blast.txt	Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215:403-410

Table 2 Output of Illumina MiSeq sequencing and bioinformatic analysis.

	PoolA		PoolB		Comment
	No sequences	Per cent	No sequences	Per cent	
Read pairs MiSeq - paired-end reads	1162800	100%	916245	100%	
Step1: Trimming and quality filtering with Average quality score ≥ 20 over a 4-base sliding window, min length 135 bp					
Dropped	146385	12.59%	105547	11.52%	
Both surviving	897,964	77.22%	724,779	79%	> 70% % survived
For only	100633	8.65%	72624	7.93%	
Rev only	17818	1.53%	13295	1.45%	
Step2: Aligning both reads					
Total pairs	897,964	100%	724,779	100%	
Combined pairs/aligned paired reads	864,845	96.31	717,735	99.03	> 90% of pairs aligned
Not combined pairs	33119	3.69	7044	0.97	
Step3: Demultiplexing into a sample specific files and removal of MIDs,primer sequences and sequences without exact match to primer sequence					
Group count_number of sequences assigned to each	549,782	63.6 % of	421,457	58.7 % of	

sample		combined pairs		combined pairs	
Aligned_groups list of sequence names and their assigned sample-ID	549,782	/	421,457	/	
Aligned_scrap sequences without matching primers	315,063	/	296,278	/	
Aligned_trim sequences with matching primers	549,782	63.6 % of combined pairs	421,457	58.7 % of combined pairs	
Demultiplexed sequences in a file for each sample	549,782	/	421,457	/	
Step 4: Removal of sequences with fewer than 5 copies and chimeric sequences, clustering at 97% and final dereplication					
Unique sequences/MOTU/cluster sequence	392	0.05 % of combined pairs	287	0.04 % of combined pairs	
Step 5: Blast the representative cluster sequences against NCBI database					
Blast output	386	0.04 % of combined pairs	195	0.03 % of combined pairs	Loss of 1.5 % and 32% respectively due to blast criteria

52 Table 3 Quality criteria of MOTUs that were used for taxonomic assignment

No of MOTUs	q-seq-id	S-seq-id	p-ident	length	e-value	Bit-score
1	146437_M00969_273_000000000-AY5TW_1_1102_14305_3883	AF315121	99	211	1.18E-101	379
2	146437_M00969_273_000000000-AY5TW_1_1102_19526_12384	AF315121	100	211	2.53E-103	385
3	146438_M00969_273_000000000-AY5TW_1_1101_27261_9100	EF042208	100	211	2.53E-103	385
4	146438_M00969_273_000000000-AY5TW_1_1101_13444_19038	EF042208	99	211	1.18E-101	379
5	146439_M00969_273_000000000-AY5TW_1_1102_15025_5592	AF315121	100	211	5.43E-105	390
6	146439_M00969_277_000000000-AYLGV_1_1101_12953_5382	KJ128795	100	211	2.76E-103	385
7	146440_M00969_273_000000000-AY5TW_1_1104_23620_22458	KJ128795	100	211	2.53E-103	385
8	146440_M00969_277_000000000-AYLGV_1_1101_19073_27985	AF315121	99	211	1.28E-101	379
9	146441_M00969_273_000000000-AY5TW_1_1102_7930_5591	KJ128795	100	211	5.43E-105	390
10	146441_M00969_277_000000000-AYLGV_1_1102_15894_4292	KJ128795	99	211	1.28E-101	379
11	146449_M00969_277_000000000-AYLGV_1_1101_12703_21299	AF315121	100	211	2.76E-103	385
12	146450_M00969_273_000000000-AY5TW_1_1103_9519_12161	AF315121	100	211	2.53E-103	385
13	146450_M00969_273_000000000-AY5TW_1_1104_24499_8839	KJ128795	100	211	2.53E-103	385
14	146437_2_M00969_277_000000000-AYLGV_1_1101_18349_9237	KJ128795	100	211	5.93E-105	390
15	146437_M00969_273_000000000-AY5TW_1_1103_20135_15217	KJ128826	100	208	2.49E-103	385
16	146438_M00969_273_000000000-AY5TW_1_1102_19858_13124	KJ128826	99.519	208	1.16E-101	379
17	146438_M00969_273_000000000-	KJ128826	99.519	208	1.16E-101	379

	AY5TW_1_2103_17482_23139					
18	146440_M00969_273_000000000-AY5TW_1_1104_19252_3931	KJ128827	100	208	2.49E-103	385
19	146440_M00969_273_000000000-AY5TW_1_1101_8169_10679	KJ128826	100	206	3.22E-102	381
20	146437_M00969_273_000000000-AY5TW_1_1101_9887_10271	AP017650	100	208	2.49E-103	385
21	146437_M00969_273_000000000-AY5TW_1_1101_27425_16396	KJ128822	100	208	2.49E-103	385
22	146437_M00969_273_000000000-AY5TW_1_1103_19670_3702	KJ128822	99.519	208	1.16E-101	379
23	146438_M00969_273_000000000-AY5TW_1_1101_20098_5621	KJ128822	99.519	208	1.16E-101	379
24	146438_M00969_273_000000000-AY5TW_1_1104_6282_12086	KJ128822	98.558	208	2.50E-98	368
25	146438_M00969_273_000000000-AY5TW_1_1104_14160_17658	AP017650	99.519	208	1.16E-101	379
26	146438_M00969_273_000000000-AY5TW_1_1102_15665_10237	AP017650	99.038	208	5.38E-100	374
27	146438_M00969_273_000000000-AY5TW_1_1101_12786_9136	FR751400	98.558	208	2.50E-98	368
28	146440_M00969_273_000000000-AY5TW_1_1106_23026_14975	FR751400	99.519	208	1.16E-101	379
29	146440_M00969_273_000000000-AY5TW_1_1101_16295_9201	AP017650	99.519	208	1.16E-101	379
30	146440_M00969_273_000000000-AY5TW_1_1103_6716_5247	AP017650	99.519	208	1.16E-101	379
31	146444_M00969_273_000000000-AY5TW_1_2102_28368_19489	AP017650	99.519	208	1.16E-101	379
32	146437_2_M00969_277_000000000-AYLGV_1_1102_14038_27379	AP017650	99.038	208	5.87E-100	374
33	146437_M00969_273_000000000-AY5TW_1_1101_24055_10171	FR849599	100	205	1.14E-101	379
34	146437_M00969_273_000000000-AY5TW_1_1101_17362_10139	KJ128741	100	210	1.94E-104	388
35	146437_M00969_273_000000000-	KJ128910	99.524	210	9.04E-103	383

	AY5TW_1_1102_26108_9370					
36	146437_M00969_273_000000000-AY5TW_1_1105_9560_15855	FR849599	97.561	205	2.48E-93	351
37	146437_M00969_273_000000000-AY5TW_1_1101_18313_16770	KC193756	100	210	1.94E-104	388
38	146437_M00969_273_000000000-AY5TW_1_1104_20457_22121	KJ128910	99.048	210	4.21E-101	377
39	146438_M00969_273_000000000-AY5TW_1_1101_4468_10820	KJ128910	99.048	210	4.21E-101	377
40	146438_M00969_273_000000000-AY5TW_1_1101_14598_7864	KJ128740	100	210	1.94E-104	388
41	146438_M00969_273_000000000-AY5TW_1_1104_16340_20725	KJ128741	99.524	210	9.04E-103	383
42	146438_M00969_273_000000000-AY5TW_1_1101_15625_23263	KJ128910	99.524	210	9.04E-103	383
43	146438_M00969_273_000000000-AY5TW_1_1101_14475_25427	DQ912088	99.024	205	2.46E-98	368
44	146438_M00969_273_000000000-AY5TW_1_1101_8549_4540	FR849599	99.512	205	5.29E-100	374
45	146439_M00969_273_000000000-AY5TW_1_1101_15967_4762	FR849561	99.048	210	4.21E-101	377
46	146439_M00969_277_000000000-AYLGV_1_1101_11296_2925	KC193720	98.571	210	2.14E-99	372
47	146440_M00969_273_000000000-AY5TW_1_1101_21176_21035	FR849599	100	202	5.29E-100	374
48	146440_M00969_273_000000000-AY5TW_1_1103_14268_9831	FR849561	98.571	210	1.96E-99	372
49	146440_M00969_273_000000000-AY5TW_1_1101_3603_13495	FR849599	99.024	205	2.46E-98	368
50	146442_M00969_277_000000000-AYLGV_1_1101_19724_5258	KJ128910	99.524	210	9.87E-103	383
51	146442_M00969_273_000000000-AY5TW_1_1101_20475_24539	KJ128740	99.048	210	4.21E-101	377
52	146442_M00969_273_000000000-AY5TW_1_1102_15042_17323	KJ128741	99.048	210	4.21E-101	377
53	146444_M00969_273_000000000-	KC193777	98.095	210	9.11E-98	366

	AY5TW_1_1102_13813_8004					
54	146444_M00969_273_000000000-AY5TW_1_1101_4546_23469	KJ128910	98.095	210	9.11E-98	366
55	146444_M00969_273_000000000-AY5TW_1_1104_13834_23205	FR849561	97.619	210	4.24E-96	361
56	146450_M00969_273_000000000-AY5TW_1_1101_16275_28455	KJ128910	98.571	210	1.96E-99	372
57	146450_M00969_273_000000000-AY5TW_1_1106_16262_11916	FR849561	99.043	209	1.51E-100	375
58	146450_M00969_273_000000000-AY5TW_1_1101_6461_11968	KC193768	98.571	210	1.96E-99	372
59	146450_M00969_273_000000000-AY5TW_1_1106_22895_25788	KJ128910	98.095	210	9.11E-98	366
60	146452_M00969_277_000000000-AYLGV_1_1101_5272_7177	KJ128910	98.571	210	7.59E-99	370
61	146437_2_M00969_277_000000000-AYLGV_1_1101_18100_2367	KJ128910	100	210	2.12E-104	388
62	146437_2_M00969_277_000000000-AYLGV_1_1101_18439_3172	KJ128741	100	210	2.12E-104	388
63	146437_2_M00969_277_000000000-AYLGV_1_1102_5114_15375	KJ128910	99.048	210	4.59E-101	377
64	146439_M00969_277_000000000-AYLGV_1_1101_14720_9732	KJ128765	100	211	5.93E-105	390
65	146437_M00969_277_000000000-AYLGV_1_1101_17036_4544	KJ128862	100	224	3.78E-112	414
66	146438_M00969_273_000000000-AY5TW_1_1102_15507_15905	KJ128862	99.554	224	1.61E-110	409
67	146439_M00969_277_000000000-AYLGV_1_1101_25155_5000	KU510499	100	224	3.78E-112	414
68	146439_M00969_277_000000000-AYLGV_1_1101_9391_7521	FJ870412	99.107	224	8.17E-109	403
69	146439_M00969_277_000000000-AYLGV_1_1103_6393_5494	KJ128862	99.554	224	1.76E-110	409
70	146440_M00969_273_000000000-AY5TW_1_1101_3584_9022	KU936350	99.107	224	7.49E-109	403
71	146440_M00969_277_000000000-	KJ128862	99.554	224	1.76E-110	409

	AYLGV_1_1102_22459_12199					
72	146441_M00969_277_000000000-AYLGV_1_1101_20701_8232	KJ128862	99.554	224	1.76E-110	409
73	146443_M00969_277_000000000-AYLGV_1_1102_5525_24499	KJ128862	98.661	224	3.8E-107	398
74	146445_M00969_277_000000000-AYLGV_1_1101_12011_8918	KJ128862	99.554	224	1.76E-110	409
75	146437_2_M00969_273_000000000-AY5TW_1_1102_24621_21708	KJ128862	100	224	3.46E-112	414
76	146438_M00969_273_000000000-AY5TW_1_1107_19768_20694	AF122825	99.554	224	1.61E-110	409
77	146438_M00969_273_000000000-AY5TW_1_1101_23705_8805	EU410416	100	217	2.60E-108	401
78	146438_M00969_273_000000000-AY5TW_1_1101_9046_9345	KJ128906	99.107	224	7.49E-109	403
79	146438_M00969_273_000000000-AY5TW_1_1106_5566_12823	EU419747	98.198	222	2.64E-103	385
80	146438_M00969_273_000000000-AY5TW_1_1102_14365_5613	KJ168065	99.526	211	2.53E-103	385
81	146438_M00969_273_000000000-AY5TW_1_1101_20270_19169	KJ128870	100	207	8.89E-103	383
82	146437_2_M00969_277_000000000-AYLGV_1_1101_25250_15535	KJ128871	99.034	207	2.1E-99	372
83	146437_M00969_273_000000000-AY5TW_1_1101_15433_7149	KT633607	100	213	4.25E-106	394
84	146438_M00969_273_000000000-AY5TW_1_1102_10784_2931	KT633607	99.531	213	1.98E-104	388
85	146438_M00969_273_000000000-AY5TW_1_1103_11713_13769	KT633607	100	212	1.53E-105	392
86	146439_M00969_273_000000000-AY5TW_1_1101_4637_21155	KT633607	99.531	213	1.98E-104	388
87	146440_M00969_273_000000000-AY5TW_1_1107_19000_23334	KT633607	99.531	213	1.98E-104	388
88	146437_M00969_273_000000000-AY5TW_1_1101_23206_24521	KJ128898	99.539	217	1.21E-106	396
89	146437_M00969_273_000000000-	KJ128898	99.539	217	1.21E-106	396

	AY5TW_1_1101_19358_7121					
90	146438_M00969_273_000000000-AY5TW_1_1106_14903_24911	KJ128898	99.078	217	5.62E-105	390
91	146438_M00969_273_000000000-AY5TW_1_1101_23851_8482	KJ128898	99.539	217	1.21E-106	396
92	146439_M00969_273_000000000-AY5TW_1_1102_21124_23388	KJ128898	100	217	2.60E-108	401
93	146440_M00969_273_000000000-AY5TW_1_1101_11208_7721	KJ128898	99.539	217	4.32E-106	394
94	146444_M00969_273_000000000-AY5TW_1_1101_11268_6586	KJ128898	99.539	217	1.21E-106	396
95	146444_M00969_273_000000000-AY5TW_1_1104_19247_28671	KJ128898	99.539	217	1.21E-106	396
96	146445_M00969_273_000000000-AY5TW_1_1108_18728_21966	KJ128898	99.078	217	5.62E-105	390
97	146437_2_M00969_277_000000000-AYLGV_1_1101_10880_6810	KU510503	100	217	2.83E-108	401
98	146437_2_M00969_277_000000000-AYLGV_1_1101_8190_6848	KU510503	99.539	217	1.32E-106	396
99	146437_M00969_273_000000000-AY5TW_1_1101_6839_6659	KJ627974	99.519	208	1.16E-101	379
100	146437_M00969_273_000000000-AY5TW_1_1109_4238_10131	KJ627974	100	205	1.16E-101	379
101	146438_M00969_273_000000000-AY5TW_1_1101_16501_11424	KJ627974	100	208	2.49E-103	385
102	158316_M00969_273_000000000-AY5TW_1_1101_28507_12328	KC193769	100	210	1.94E-104	388
103	158318_M00969_277_000000000-AYLGV_1_1101_15095_4934	KC193720	99.524	210	9.87E-103	383
104	158326_M00969_273_000000000-AY5TW_1_1101_4831_14636	KC193777	99.048	210	4.21E-101	377
105	158326_M00969_273_000000000-AY5TW_1_1103_2468_15724	KJ128741	99.048	210	4.21E-101	377
106	158327_M00969_273_000000000-AY5TW_1_1101_19137_7485	KC193732	100	210	1.94E-104	388
107	158328_M00969_273_000000000-	KJ128741	99.524	210	9.04E-103	383

	AY5TW_1_1102_3284_15746					
108	158328_M00969_273_000000000-AY5TW_1_1105_10438_14160	KJ128910	97.619	210	4.24E-96	361
109	158328_M00969_273_000000000-AY5TW_1_1107_16399_4897	KJ128741	99.524	210	9.04E-103	383
110	158329_M00969_273_000000000-AY5TW_1_1106_26173_15504	KJ128740	99.524	210	9.04E-103	383
111	158329_M00969_273_000000000-AY5TW_1_1104_21243_11764	KC193720	98.095	210	9.11E-98	366
112	158331_M00969_273_000000000-AY5TW_1_1104_15579_2627	KJ128741	99.524	210	9.04E-103	383
113	158331_M00969_273_000000000-AY5TW_1_1103_14814_8734	KC193777	97.619	210	4.24E-96	361
114	158332_M00969_273_000000000-AY5TW_1_1103_5388_13732	KJ128741	99.048	210	4.21E-101	377
115	158332_M00969_273_000000000-AY5TW_1_1102_24809_24690	KJ128740	97.619	210	4.24E-96	361
116	158333_M00969_273_000000000-AY5TW_1_1101_23775_21597	KC193768	98.571	210	1.96E-99	372
117	158316_M00969_273_000000000-AY5TW_1_1106_3159_18568	KJ128910	99.524	210	9.04E-103	383
118	158317_M00969_277_000000000-AYLGV_1_1101_9295_7591	KJ128910	99.524	210	9.87E-103	383
119	158326_M00969_273_000000000-AY5TW_1_1103_18938_15756	KJ128910	99.524	210	9.04E-103	383
120	158328_M00969_273_000000000-AY5TW_1_1101_21109_18807	KJ128910	99.524	210	9.04E-103	383
121	158328_M00969_273_000000000-AY5TW_1_1105_3153_13664	KJ128910	98.571	210	1.96E-99	372
122	158329_M00969_273_000000000-AY5TW_1_1103_23245_10923	KJ128910	99.524	210	9.04E-103	383
123	158329_M00969_273_000000000-AY5TW_1_1103_11912_22204	KJ128910	99.524	210	9.04E-103	383
124	158331_M00969_273_000000000-AY5TW_1_1101_13010_19728	KJ128910	99.524	210	9.04E-103	383
125	158332_M00969_273_000000000-	KJ128910	99.524	210	9.04E-103	383

	AY5TW_1_1105_27871_11343					
126	158332_M00969_273_000000000-AY5TW_1_1102_23450_20124	KJ128910	99.524	210	9.04E-103	383
127	158333_M00969_273_000000000-AY5TW_1_1107_24795_16622	KJ128910	99.524	210	9.04E-103	383
128	158333_M00969_273_000000000-AY5TW_1_1109_21956_25566	KJ128910	99.524	210	9.04E-103	383
129	158316_M00969_273_000000000-AY5TW_1_1102_17972_18839	FR849599	99.512	205	5.29E-100	374
130	158316_M00969_273_000000000-AY5TW_1_1101_14384_8657	FR849599	99.024	205	2.46E-98	368
131	158327_M00969_273_000000000-AY5TW_1_1101_8872_10503	FR849599	99.512	205	5.29E-100	374
132	158329_M00969_273_000000000-AY5TW_1_1106_5076_21928	FR849599	99.512	205	5.29E-100	374
133	158327_M00969_273_000000000-AY5TW_1_1101_14191_13106	KJ128827	99.038	208	5.38E-100	374
134	158327_M00969_273_000000000-AY5TW_1_1101_18211_28305	KJ128826	99.519	208	1.16E-101	379
135	158333_M00969_273_000000000-AY5TW_1_1102_4609_20805	KJ128795	99.526	211	2.53E-103	385
136	158333_M00969_273_000000000-AY5TW_1_1103_21347_5449	KJ128795	99.052	211	1.18E-101	379
137	158316_M00969_273_000000000-AY5TW_1_1104_8166_5628	AP017650	99.519	208	1.16E-101	379
138	158316_M00969_273_000000000-AY5TW_1_1101_26101_9817	AP017650	99.519	208	1.16E-101	379
139	158318_M00969_277_000000000-AYLGV_1_1101_10887_15643	AP017650	99.519	208	1.26E-101	379
140	158319_M00969_273_000000000-AY5TW_1_1102_15859_8335	AP017650	99.519	208	1.16E-101	379
141	158327_M00969_273_000000000-AY5TW_1_1102_8194_14530	KJ128822	99.519	208	1.16E-101	379
142	158327_M00969_273_000000000-AY5TW_1_1107_25257_10792	AP017650	99.038	208	5.38E-100	374
143	158327_M00969_273_000000000-	AP017650	99.519	208	1.16E-101	379

	AY5TW_1_1101_20620_19170					
144	158333_M00969_273_000000000-AY5TW_1_1103_15530_21664	KJ128825	100	208	2.49E-103	385
145	158317_M00969_277_000000000-AYLGV_1_1101_8195_6365	KJ128862	99.554	224	1.76E-110	409
146	158318_M00969_277_000000000-AYLGV_1_1101_10365_8785	KU510499	99.554	224	1.76E-110	409
147	158321_M00969_277_000000000-AYLGV_1_1101_21608_2864	KJ128862	99.554	224	1.76E-110	409
148	158327_M00969_273_000000000-AY5TW_1_1103_21252_10689	KJ128862	100	223	1.24E-111	412
149	158327_M00969_273_000000000-AY5TW_1_1103_5622_6457	KJ128862	97.788	226	2.12E-104	388
150	158327_M00969_273_000000000-AY5TW_1_1102_6728_13312	KJ128862	99.554	224	1.61E-110	409
151	158328_M00969_277_000000000-AYLGV_1_1101_14974_8593	KJ128862	99.554	224	1.76E-110	409
152	158330_M00969_273_000000000-AY5TW_1_1102_18265_2051	KJ128862	99.554	224	1.61E-110	409
153	158322_M00969_277_000000000-AYLGV_1_1101_23960_7813	KJ128906	99.107	224	8.17E-109	403
154	158322_M00969_277_000000000-AYLGV_1_1101_20149_5105	KJ128906	98.661	224	3.8E-107	398
155	158327_M00969_273_000000000-AY5TW_1_1102_9564_24989	KJ128870	99.517	207	4.14E-101	377
156	158329_M00969_273_000000000-AY5TW_1_1102_20113_13469	KT633607	99.531	213	1.98E-104	388
157	158329_M00969_277_000000000-AYLGV_1_1101_17608_18399	KU510503	99.078	217	6.13E-105	390
158	158317_M00969_277_000000000-AYLGV_1_1101_12124_6581	KU510503	99.539	217	1.32E-106	396
159	158318_M00969_277_000000000-AYLGV_1_1101_24645_12079	KU510503	99.078	217	6.13E-105	390
160	158319_M00969_273_000000000-AY5TW_1_1101_4065_16408	KJ128898	99.078	217	5.62E-105	390
161	158319_M00969_273_000000000-	KJ128898	99.078	217	5.62E-105	390

	AY5TW_1_1101_27338_12669					
162	158322_M00969_277_000000000-AYLGV_1_1101_10398_4334	KU510503	99.539	217	1.32E-106	396
163	158325_M00969_277_000000000-AYLGV_1_1101_25631_15275	KU510503	98.157	217	1.33E-101	379
164	158326_M00969_273_000000000-AY5TW_1_1101_10267_13492	KJ128898	99.539	217	1.21E-106	396
165	158327_M00969_273_000000000-AY5TW_1_1101_17516_21993	KJ128898	99.539	217	1.21E-106	396
166	158332_M00969_273_000000000-AY5TW_1_1101_25351_7734	KJ128898	99.539	217	1.21E-106	396
167	158329_M00969_277_000000000-AYLGV_1_1101_24101_11074	KJ627974	100	208	2.71E-103	385
168	158329_M00969_277_000000000-AYLGV_1_1101_10726_4988	KJ128825	100	208	2.71E-103	385
169	158334_M00969_277_000000000-AYLGV_1_1101_7624_6843	KJ128870	99.517	207	4.51E-101	377

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