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Erysipelothrix amsterdamensis sp. nov., associated with mortalities among endangered seabirds

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Abstract

Infectious diseases threaten endangered species, particularly in small isolated populations. Seabird populations on the remote Amsterdam Island in the Indian Ocean have been in decline for the past three decades, with avian cholera caused by *Pasteurella multocida* proposed as the primary driver. However, *Erysipelothrix* species have also been sporadically detected from albatrosses on Amsterdam Island and may be contributing to some of the observed mortality. In this study, we genomically characterized 16 *Erysipelothrix* species isolates obtained from three Indian yellow-nosed albatross (*Thalassarche carteri*) chick carcasses in 2019. Histological analyses suggest that they died of bacterial septicaemia. Two isolates were sequenced using both Illumina short-read and MinION long-read approaches, which – following hybrid assembly – resulted in closed circular genomes. Mapping of Illumina reads from the remaining isolates to one of these new reference genomes revealed that all 16 isolates were closely related, with a maximum of 13 nucleotide differences distinguishing any pair of isolates. The nucleotide diversity of isolates obtained from the same or different carcasses was similar, suggesting all three chicks were likely infected from a common source. These genomes were compared with a global collection of genomes from *Erysipelothrix rhusiopathiae* and other species from the same genus. The isolates from albatrosses were phylogenetically distinct, sharing a most recent common ancestor with *E. rhusiopathiae*. Based on phylogenomic analysis and standard thresholds for average nucleotide identity and digital DNA–DNA hybridization, these isolates represent a novel *Erysipelothrix* species, for which we propose the name *Erysipelothrix amsterdamensis* sp. nov. The type strain is A18Y020d^T (=CIP 112216^T=DSM 115297^T). The implications of this bacterium for albatross conservation will require further study.

INTRODUCTION

Infectious diseases are among the major threats to the conservation of endangered species, especially at local scales [1]. This is particularly true for populations in remote areas, where anthropogenic activity has historically been limited but is increasing, such as with commercial tourists to the Antarctic [2, 3]. Concerns have been raised about disease-associated declines among albatross populations and how this could influence their long-term sustainability [4]. Avian cholera, caused by the bacterium *Pasteurella multocida*, has been causing recurrent chick mortalities among two endangered albatross species on the remote Amsterdam Island in the southern Indian Ocean since at least the mid-1990s when it was first detected [5–7]. Both the Indian yellow-nosed albatross (*Thalassarche carteri*) and the sooty albatross (*Phoebetria fusca*) have been affected, and the endemic endangered Amsterdam albatross (*Diomedea amsterdamensis*) is also at risk [5]. The bacterium *Erysipelothrix rhusiopathiae* has

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Abbreviations: ANI, average nucleotide identity; CDS, coding sequences; dDDH, digital DNA-DNA hybridization; GWAS, genome-wide association study; kb, kilobases; SNP, single nucleotide polymorphism.
Reads for all samples sequenced in this study are available on European Nucleotide Archive Sequence Read Archive (SRA) under accession number PRJEB50151. Complete annotated genomes for isolates A18Y020d^T (CIP 112216^T=DSM 115297^T) and A18Y016a are available under accession numbers GCA_940143175 and GCA_940143155, respectively. The 16S rRNA sequence for A18Y020d^T is available on GenBank as accession OQ355811.
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Eight supplementary figures and four supplementary tables are available with the online version of this article.

also been detected by PCR in swabs from live birds of these three albatross species and from the endangered northern rockhopper penguin (*Eudyptes moseleyi*) on Amsterdam Island [6], and it has been isolated concomitantly from mortalities among the Indian yellow-nosed albatross [5]. During ongoing population health monitoring conducted to infer transmission dynamics and management opportunities [8–10], our team isolated *Erysipelothrix* species (identified at the time as *E. rhusiopathiae*) in 2019 from the carcasses of three Indian yellow-nosed albatross chicks on the island, strengthening the hypothesis that this bacterium could also be contributing to albatross mortalities and decreased breeding success.

Erysipelothrix rhusiopathiae is a Gram-positive rod-shaped bacterium. While best known as an opportunistic pathogen of pigs and poultry [11, 12], it affects a wide range of species [13] and in recent years it has been implicated in large-scale wildlife mortalities [14]. It was also responsible for jeopardising the translocation success of several critically endangered kakapo – a flightless parrot – between New Zealand and offshore islands [15], and has caused the death of other endangered birds in that area [16]. Erysipelas in wild birds is a septicaemic disease [12], and deaths have been reported in a range of wild terrestrial birds and seabirds worldwide [15]. Mortalities occur sporadically, and tend to be limited to a small number of individuals, although cases are likely under-recognised and under-reported [17]. Rare mass mortality events in wild birds have also been observed [18].

In this study, we sought to genomically characterize the *Erysipelothrix* strains isolated from Indian yellow-nosed albatross on Amsterdam Island to identify potential sources, and to assess the relationship between the multiple observed cases on the island.

METHODS

Between December 2018 and March 2019, 27 chick and two adult carcasses of Indian yellow-nosed albatross were necropsied in the field on Amsterdam Island (−37.797135, 77.571521) as part of long-term population health monitoring (Appendix S1, Fig. S1, available in the online version of this article, Table S1). Samples from the heart, lung, liver, kidney, spleen, brain and bursa of Fabricius in all three birds, of the adrenal glands in one bird (AMS18-NEC-019), and of the thymus in one bird (AMS18-NEC-016) were collected and fixed in 4% neutral buffered formalin at room temperature. Samples were routinely processed as formalin-fixed paraffin-embedded blocks, and sectioned at 4 µm thickness. Tissue sections were routinely stained with haematoxylin and eosin for examination by light microscopy. Samples from multiple organs (liver, lung, brain and heart) were collected from the carcasses for bacteriological analysis (Appendix S1). Isolates of *Erysipelothrix* species were identified from three of the chick carcasses, all sampled between the 13 and 16 January 2019. The bacterial growth in the field (Appendix S1) showed *Erysipelothrix* species exclusively, suggesting that this bacterium could have been the cause of death. Between four and six isolates were obtained from each carcass (n=16 total; Table S1). These were initially identified in the laboratory as *E. rhusiopathiae* based on colony morphology on horse blood agar and MALDI-TOF, with the exception of strain A18Y019b, which was classified as an *Erysipelothrix* species.

The following phenotypic analyses of isolate A18Y020d were carried out by DSMZ Services, Leibniz-Institut (Braunschweig, Germany). Biochemical characterization was performed using the API Rapid ID32 Strep test (bioMérieux) according to instructions of the manufacturer; this was done in two replicates, starting from individual precultures. pH (tested at increments of one) and temperature range (tested at increments of 5°C) were determined.

Genomic DNA was extracted from sub-cultured isolates (loop of colonies) at the University of Glasgow using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) as per the manufacturer's instructions. Extracted DNA was tested with a probe-based qPCR targeting *E. rhusiopathiae* [19], then sequenced at MicrobesNG (Birmingham, UK). Libraries were prepared using the Nextera XT v2 kit (Illumina) and sequenced on the Illumina HiSeq platform, generating 250 base pair paired-end reads. Long-read MinION sequencing (Oxford Nanopore) was performed at the University of Glasgow for two isolates (A18Y016a and A18Y020d), with library preparation done using the rapid barcoding kit (SQK-RBK004).

Illumina reads were adapter and quality trimmed using Trimmomatic version 0.30 [20] and assembled *de novo* using SPAdes version 3.7 [21] by MicrobesNG. Assembled genomes were evaluated using the online version of Quality Assessment Tool for Genome Assemblies (http://cab.cc.spbu.ru) [22]. Unless otherwise indicated, the remaining bioinformatics analyses were performed within the CLIMB computing platform for microbial genomics [23]. To generate circularized (closed) reference genomes, the Illumina and MinION sequence data were jointly assembled by Unicycler version 0.4.4 [24] applying the 'normal' (default) hybrid mode. For isolate A18Y016a, this did not result in a closed genome, so subsequently Canu version 1.7 [25] was used to perform an initial assembly using the long-read MinION data, and Pilon version 1.22 [26] applied to polish the assembly using Illumina reads, including three iterations. Tablet version 1.21.02.08 [27] was used to visually check the distance between forward and reverse reads across the start and end positions of the linearized chromosome to confirm the genomes' circular nature.

Prokka version 1.13 [28] and BLAST2GO [29] were used to predict coding sequences (CDS), tRNA, rRNA and tmRNA. The general feature format (.gff) files obtained by the two programs were merged to build a complete .gff annotation file. The PHASTER server (https://phaster.ca/) [30] was used to identify and annotate prophage sequences. plasmidFinder server version 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) [31] was used to search for plasmid sequence data in the genomes against the replicon database. Antimicrobial resistance genes were searched for in the hybrid assembly of A18Y020d using both CARD (database downloaded on 28 April 2023) [32] and ResFinder-4.4.1 [33], using a detection threshold of 80% over a minimum length of 60%.

BLAST searches for 16 genes that have been putatively linked with virulence in *E. rhusiopathiae* [34] were conducted against the hybrid assembly of A18Y020d.

BWA-MEM [35] with default settings was used to map Illumina reads of each albatross isolate against the A18Y020d closed genome, and the mpileup command in SAMtools version 1.13 [36] was used to generate summary text files of coverages across the reference genome. The pileup2snp command in Varscan version 2.4.4 [37] was used to generate a list of high-quality single nucleotide polymorphisms (SNPs) that were filtered by applying parameters '--min-coverage' of 10, '--min-var-freq' of 0.8 and '--min-avg-qual' of 20. A custom R script was developed to generate a multi-fasta alignment file based on concatenated SNPs. Pairwise SNP differences between isolates were calculated using the snp-dists package version 0.8.2. Roary version 3.12.0 [38], with default parameters, was used to construct a core gene alignment for a wider phylogenetic analysis, i.e. to place the albatross isolates within the context of global *Erysipelothrix* genomes. This included 106 genomes: (a) the 16 isolates from this study; (b) 86 previously sequenced *E. rhusiopathiae* isolates, representing a diversity of geographic and host origins and spanning the clades described for this species [clade 1 (n=7), clade 2 (n=14), intermediate clade (n=8), clade 3 (n=57)] [39]; (c) two genomes of *Erysipelothrix piscisicarius* (formerly referred to as *E.* sp. strain 2) [40, 41]: the type strain 15TAL0474^T (GCA_003931795.1) [42] and EsS2-7-Brazil (GCA_016617655.1)–*E. piscisicarius* is the most closely related species to *E. rhusiopathiae* currently known; (d) a recently deposited *Erysipelothrix* genome, reported as a new species, *Erysipelothrix* sp. Poltava (GCA_023221615.1); and (e) *Erysipelothrix tonsillarum* DSM14972^T (GCA_000373785.1), which was used as an outgroup, since this was previously reported to be ancestral to these other species [39]. The SNP alignment and core gene alignment files were used to estimate phylogenetic trees for the 16 albatross isolates and the full set of *Erysipelothrix* genomes, respectively, using RAxML version 8.2.4 [43] with the maximum-likelihood method implementing a GTR-GAMMA nucleotide substitution model, selected after using MrModeltest version 2.4 [44].

To determine the relatedness of the albatross isolates from this study with other less well-described *Erysipelothrix* species available in the Genome Taxonomy Database (https://gtdb.ecogenomic.org/), a separate phylogenetic tree based on the *rpoB* gene (beta subunit of RNA polymerase) was estimated. This gene has been found to be a more suitable marker for *Erysipelothrix* species delineation than the widely used 16S rRNA gene [40]. Nucleotide sequences of *rpoB* genes were extracted from representative whole-genome assemblies (one per species, n=15 total), and aligned using the L-INS-i method as implemented in MAFFT version 7.313 [45]. Tree topology was then inferred by RAxML version 8.2.4, implementing the GTR model of nucleotide substitution; robustness of the tree was assessed by performing 1000 bootstrap replicates. For completeness, the 16S rRNA gene sequence of the newly sequenced strain A18Y020d was extracted through BLASTn alignment and compared with 16S gene sequences from *E. rhusiopathiae* ATCC 19414^T (NR_040837.1), *E. piscisicarius* 15TAL0474^T (NR_170392.1) and *E. tonsillarum* DSM 14972^T (NR_040871.1).

Two different genome relatedness indices were calculated in order to compare the *Erysipelothrix* species isolated from albatrosses with previously characterized *Erysipelothrix* species and determine the species assignment. Pairwise nucleotide-level comparisons were made using average nucleotide identity (ANI) Calculator for OrthoANIu [46] (www.ezbiocloud.net/tools/ani) and digital DNA–DNA hybridization (dDDH) was performed using GGDC [47, 48] (https://ggdc.dsmz.de), using Formula 2. This formula is independent of genome length and is thus robust to the use of incomplete draft genome assemblies, as well as differences in gene content.

The output of Roary was used in Scoary version 1.6.16 [49] to conduct a pan-GWAS (genome-wide association study) in order to identify any genes that might be unique to the albatross isolates, where the binary trait considered was 'albatross origin' yes/no. NCBI BLASTn searches were conducted on the Scoary results (i.e. genes identified as unique to the albatross isolates that were absent in other *Erysipelothrix* species) to verify their absence in other bacterial species. Primer sets typically used to amplify *Erysipelothrix* species [50] and *E. rhusiopathiae* specifically [19] were evaluated *in silico* against the complete assemblies using Geneious version 11.0.5 [51] to check for any mismatches. Finally, to further characterize the *Erysipelothrix* isolates from albatross, BLASTn searches of the *de novo* Illumina assemblies were conducted to determine *in silico* the serotype [52] and whether any *spa* genes (A, B or C) [53] were present, using methods described previously [54].

RESULTS

Sixteen isolates identified as *Erysipelothrix* species were recovered from the carcasses of three albatross chicks. Fifteen of these were initially identified as *E. rhusiopathiae* based on colony morphology and MALDI-TOF with 99.9% confidence, and the other strain was identified as an *Erysipelothrix* species. Histological analyses revealed intravascular bacteria in various tissues (notably liver and heart; see for instance Figs S2–3) and occasional thrombosis. These bacteria were associated with interstitial pneumonia and pulmonary congestion, multifocal necrotizing hepatitis and/or splenitis, and/or multicentric haemorrhages (liver, heart, kidneys, spleen, meninges) (Appendix S2). Findings are consistent with bacterial septicaemia as the cause of death of these individuals.

Phenotypic characteristics determined during bacterial strain isolation are given in the species description below and in Appendix S3. All 16 isolates were genome sequenced on the Illumina platform, which yielded a high average depth of coverage of at least 58× for all genomes (Table S1). *De novo* genome assemblies comprised between 52 and 58 contigs per genome, with a total assembly length ranging from 1.80 to 1.83 megabases. Complete circular genomes were obtained for isolates A18Y020d^T (Fig. S4; assembly GCA_940143175) – designated the type strain – and A18Y016a (assembly GCA_940143155), with lengths of 1908712 bp and 1910750 bp, respectively. This is approximately 120 kilobases (kb) longer than the Fujisawa *E. rhusiopathiae* reference genome, corresponding to ~100 additional CDS (Table S2). Like the Fujisawa *E. rhusiopathiae* genome, both genomes from albatrosses had a G+C content of 36.6mol%. Preliminary annotation of the two albatross-derived genomes using Prokka showed that this method predicted a large number of hypothetical proteins, amounting to approximately one third of predicted CDS. Blast2GO facilitated the annotation of just over 1500 further CDS in each genome.

Using PHASTER, an intact prophage region comprising 48 genes was detected in both genomes (Fig. S5, Table S2). Based on global alignment using MUSCLE, this showed 43.6% nucleotide identity with the incomplete phage sequence in the *E. rhusiopathiae* Fujisawa reference genome (gene loci ERH_0581 to ERH_0629). No plasmids were detected, nor any resistance genes predicted. BLAST hits were obtained for all 16 putative virulence genes, all with >85% sequence similarity.

Pairwise identity of the 16S rRNA gene sequence from strain A18Y020d^T with that of *E. rhusiopathiae* ATCC 19414^T was 99.9%; only two SNP differences were present across 1479 nucleotides (at positions 472 and 473; TC instead of CT). Only three further nucleotide differences distinguished these sequences from the 16S sequence of *E. tonsillarum* DSM 14972^T (99.8% pairwise identity). Eight gaps were found in the alignment of the 16S sequence from *E. piscisicarius* 15TAL0474^T compared with the others, which also shared one of the SNPs from A18Y020d^T at position 472, and had one additional nucleotide difference. The phylogenetic tree (Fig. 1a), based on 427 conserved core genes, showed that the 16 *Erysipelothrix* isolates from albatrosses form a monophyletic clade distinct from previously characterized *Erysipelothrix* genomes, which shares its most recent common ancestor with *E. rhusiopathiae*. Based on the *rpoB* gene phylogeny, other *Erysipelothrix* species discovered to date (*E. anatis*, *E. aquatica*, *E. inopinata*, *E. larvae*, *E. urinaevulpis* and five other yet-to-be-named species deposited in the Genome Taxonomy Database) are more phylogenetically distant (Fig. 1b), with the exception of the recently deposited

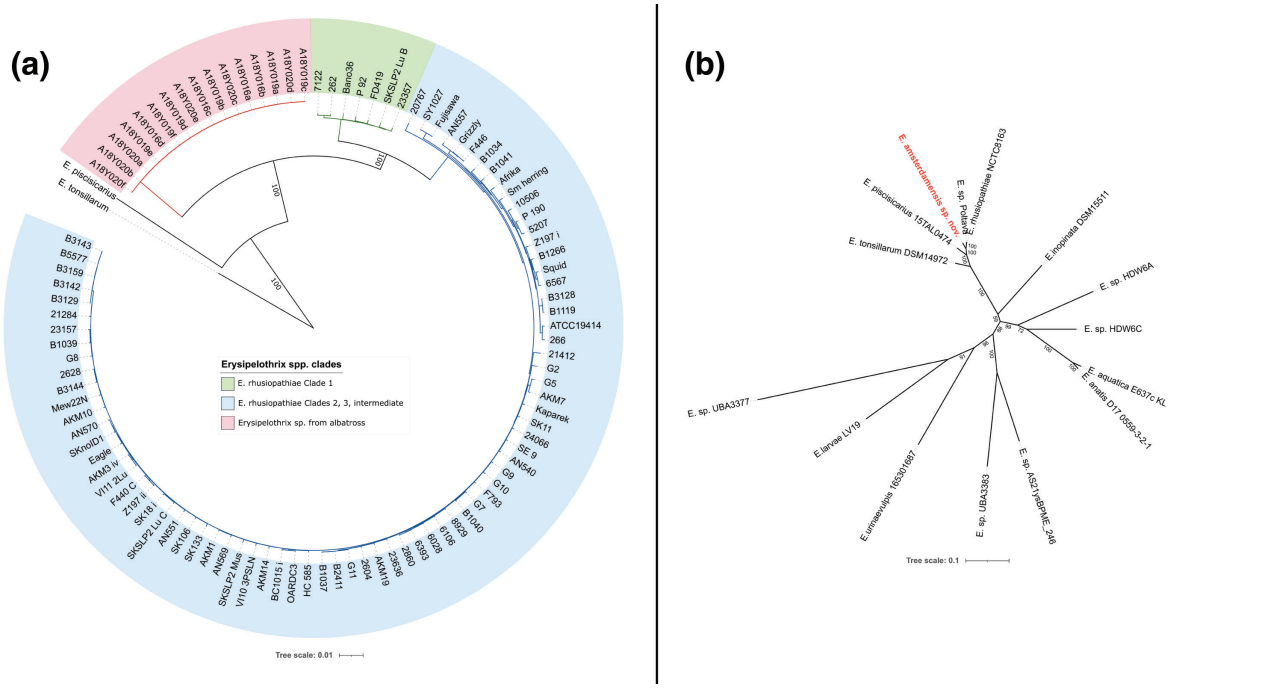

Fig. 1. Phylogenetic relatedness of *Erysipelothrix* species from Indian yellow-nosed albatross to other isolates within the genus. (a) Maximum likelihood phylogenetic tree comparing *Erysipelothrix* isolates (*n*=16) albatross chicks on Amsterdam Island (highlighted in pink) with representative genomes from the most closely related *Erysipelothrix* species. The tree was estimated in RAxML using a multiple sequence alignment of conserved (core) genes identified using Roary, implementing a GTR-GAMMA model. Bootstrap values for all major nodes were 100%. *E. tonsillarum* DSM14972^T was used as an outgroup for rooting. (b) Phylogenetic tree of *Erysipelothrix* species based on *rpoB* gene sequences. This confirms that *E. rhusiopathiae, E. piscisicarius* and *E. tonsillarum* are the most closely related species to the *Erysipelothrix* species isolated from albatrosses in this study. Trees were plotted with Interactive Tree Of Life version 6.3.2 [64] and edited using Inkscape (www.inkscape.org).

sequence for *Erysipelothrix* sp. Poltava; based on *rpoB* analysis, ANI/dDDH (Fig. S6) and phylogenomic analyses (Fig. S7), strain Poltava is concluded to belong to *E. rhusiopathiae* clade 2.

Few SNP differences were observed among the albatross isolates. A total of 53 high-quality unique SNPs were detected across all 16 genomes when mapping against the closed reference genome (A18Y020d^T; Fig. 2). All genomes were unique, including where multiple isolates were sequenced from the same carcass (Table S1). The maximum pairwise SNP difference separating two isolates was 13. *In silico* serotyping based on a genomic polysaccharide biosynthetic locus [49] determined these isolates belong to serotype 1b. All genomes had identical sequences of the *spaA* gene, belonging to Group 2 [54]. These all had one unique amino acid residue at position 288 in comparison with any previously described sequences (serine instead of alanine).

The ANI scores comparing the representative *Erysipelothrix* species type strain from albatross against *E. rhusiopathiae* were 95.0% with *E. rhusiopathiae* clade 1 (Table S3), and just above 94% for representatives from the other clades, whereas they were 86.9% with *E. piscisicarius* and 80.9% with *E. tonsillarum* (Fig. 3). The dDDH scores with *E. rhusiopathiae* clades 1, 2 and 3 were 60.0, 56.3 and 55.5%, respectively, while they were 32.7% with *E. piscisicarius* and 22.8% with *E. tonsillarum*. The accepted values for categorizing isolates as the same species are >95% similarity for ANI [55, 56] and >70% for dDDH [48]. Thus, based on these standard metrics for genomic comparison, and the phylogenomic analysis, we conclude that these isolates belong to a novel species of the genus *Erysipelothrix* – part of the family *Erysipelotrichaceae*.

Two hundred and two genes unique to the albatross-derived *Erysipelothrix* genomes (present in all 16 vs. absent in all 90 other genomes) were found using Scoary. However, after conducting BLASTn searches, the majority of these genes had significant hits to genes in *E. rhusiopathiae* or bacteria within the family *Erysipelotrichaceae*, or more rarely, with other bacteria. Only six genes were found with no hits or hits with low query cover (≤12%); a further six genes had <75% nucleotide identity with the highest scoring BLASTn hit (Table S4).

The *Erysipelothrix* species DNA from all albatross isolates produced typical amplification curves using the probe-based qPCR by Pal *et al*. [19] designed to detect *E. rhusiopathiae*. This is despite the fact that the forward primer and probe sequences had two mismatches each (Fig. S8); based on *in silico* alignment, the 165 bp segment being amplified corresponded to positions 1211721 to 1221885 in the A18Y020d^T genome. The primers designed by Makino *et al*. [50] for *Erysipelothrix* species more broadly were a perfect match, and based on *in silico* alignment would be expected to amplify the targeted 407 bp segment of the 16S rRNA gene (positions 1221445–1221851 in the A18Y020d^T genome).

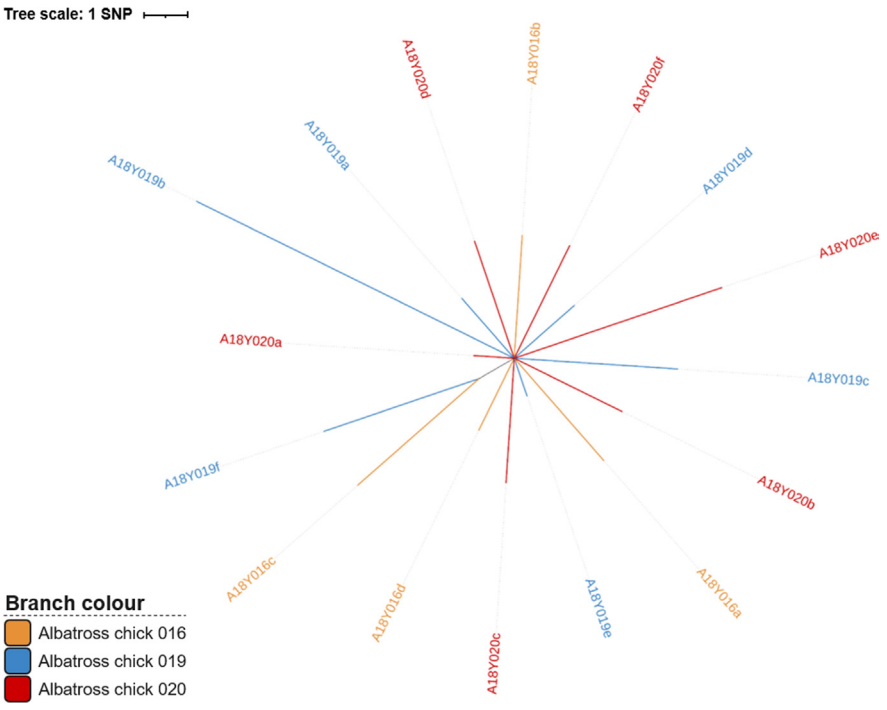

Fig. 2. Unrooted maximum likelihood tree of 16 *Erysipelothrix* isolates from Indian yellow-nosed albatross from Amsterdam Island. The tree was based on high-quality concatenated SNPs detected through mapping to the circularised genome of A18Y020d^T. Isolates originated from three different carcasses, which are distinguished by different branch colours (as shown in the legend). Tree was plotted with Interactive Tree Of Life version 6.3.2 [64].

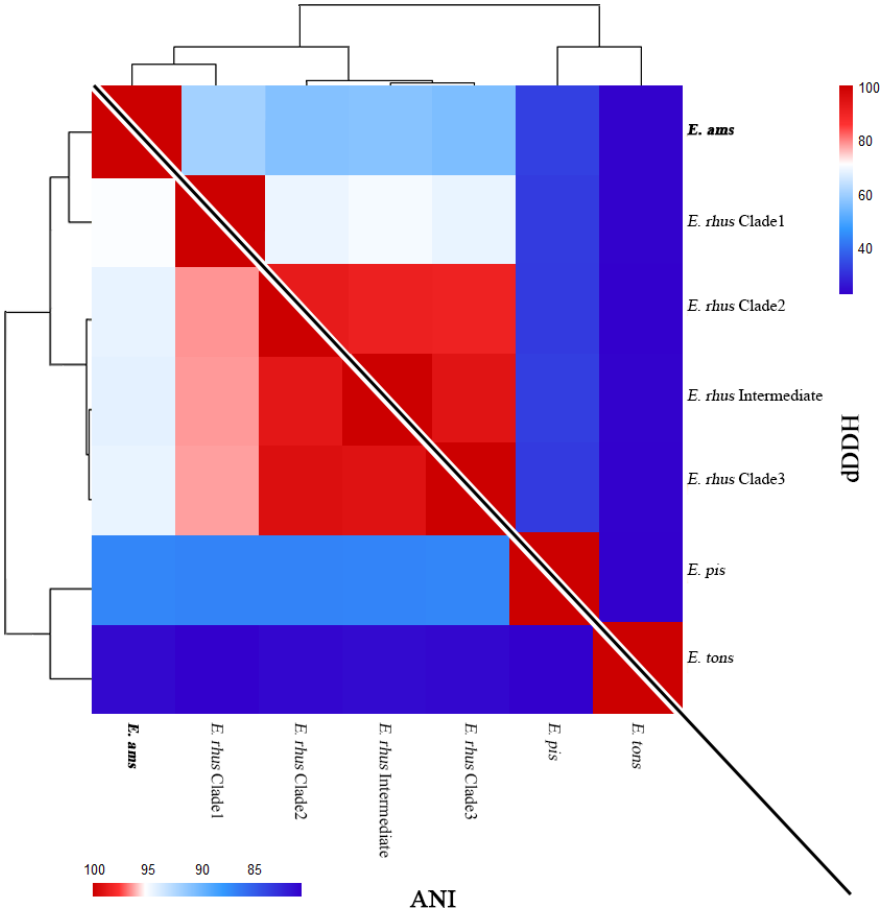

Fig. 3. Genome relatedness of *Erysipelothrix* species from albatross to previously described *Erysipelothrix* species. (a) The ANI scores comparing the *Erysipelothrix* species from albatross (represented by A18Y020d^{T}) against other *Erysipelothrix* species were all below the threshold for being considered a common species (>95%). (b) The dDDH scores comparing the *Erysipelothrix* species from albatross against other *Erysipelothrix* species were also well below the accepted threshold for being considered a single species (>70%). Genomes included for comparison: *E. rhusiopathiae* clade 1 (P-92), clade 2 (P-190), intermediate (Fujisawa) and clade 3 (F793); *E. piscisicarius* (15TAL0474^{T}); and *E. tonsillarum* (DSM14972^{T}).

DISCUSSION

Through genomic comparisons, we have determined that the *Erysipelothrix* strains isolated from Indian yellow-nosed albatross chick carcasses on Amsterdam Island belong to a previously undescribed species, which we propose be named *Erysipelothrix amsterdamensis* sp. nov. This reflects its geographic origin, as well as the co-occurrence of the iconic and endemic Amsterdam albatross, *D. amsterdamensis*. Anthropogenic sources are often suspected when novel pathogens are detected in new locations. While livestock was originally suggested as a possible source of the *Erysipelothrix* species found on Amsterdam Island [5], we were unable to find such a link, since this particular *Erysipelothrix* species has not been previously documented elsewhere. *Erysipelothrix* species have a very wide host range, including marine mammals, fish and seabirds [17, 57, 58]; any number of different hosts could conceivably have roles as vectors and/or reservoirs of this novel *Erysipelothrix* species. While no earlier *Erysipelothrix* isolates from this island were sequenced, using traditional phenotypic methods, initial investigations found that they belonged to serotype 1b [5]. Our finding of the same serotype using *in silico* approaches may suggest that the same species/strain has been in circulation since at least the mid-1990s. Previous PCR amplification of *Erysipelothrix* isolates from Amsterdam Island was performed using the primers by Makino *et al.* [50] targeting the 16S rRNA gene and which should amplify sequence from all members of the genus *Erysipelothrix*. We also found that the primers and probe described by Pal *et al.* [19] targeting a noncoding region 3′ to the 5S rRNA gene for detection of *E. rhusiopathiae* were able to amplify DNA from *E. amsterdamensis*; care should therefore be taken when interpreting results using this primer/probe set on *Erysipelothrix* species isolated from rare sources. Development of a PCR protocol that distinguishes between these two species would be valuable. The genes found to be unique to *E. amsterdamensis* in this study (Table S4) would be good initial candidate targets. Moreover, the two closed genomes generated during this study will facilitate further comparative genomic studies within the genus *Erysipelothrix*.

Our comparisons of the 16S rRNA and *rpoB* gene sequences from different *Erysipelothrix* species highlight the limited nucleotide diversity in the 16S rRNA gene, and confirm previous suggestions that the *rpoB* gene is a more suitable marker for *Erysipelothrix* species delineation [40]. When comparing the genomes of *Erysipelothrix* species from albatross to those of other *Erysipelothrix* species, ANI and dDDH scores were below the accepted thresholds for considering genomes to belong to the same species (95 and 70%, respectively). The one exception was that ANI values of close to, or slightly above 95% were obtained when comparing with genomes belonging to the less common clade 1 of *E. rhusiopathiae* (Table S3). Our choice of a 95% cut-off value for ANI was conservative, given that others have proposed a cut-off of 96% [59]. Taking the combined results of both ANI and dDDH analyses, and the overall differences between *Erysipelothrix* species from albatross and *E. rhusiopathiae*, there is compelling justification for the designation of the new species, *E. amsterdamensis*.

SNP analysis of 16 *E. amsterdamensis* isolates from the three albatross chicks showed that they are highly related. Given that the number of SNPs distinguishing isolates of the same carcass was similar to that among isolates from different carcasses – as well as the close proximity in space and time of the mortality events – this strongly suggests that the three chicks were infected from a common source. Whether this small number of SNPs arose during infection within the host, or was already present in the environment, remains unknown; a molecular clock for *Erysipelothrix* has yet to be established [39]. Similar levels of within-host genomic diversity of *E. rhusiopathiae* have been previously observed in other wildlife species [60]. While we cannot rule out the possibility that mutations occurred while the strains were maintained in conservation medium (several months unfrozen), we feel it is unlikely that sufficient replication occurred to explain the number of variants observed within and among these isolates. Further sampling of the albatross colony would help to determine whether this *Erysipelothrix* species was a new introduction to Amsterdam Island, and whether other strains are in circulation. Moreover, sampling from islands at comparable latitudes would help to elucidate its geographic distribution.

Interestingly, *E. amsterdamensis* harbours a *spaA* gene, which codes for what is commonly described as one of the most critical proteins for immunogenicity and virulence of *E. rhusiopathiae* [61–63]. This gene has been found to occur in all clade 2, clade 3 and intermediate clade *E. rhusiopathiae* genomes described to date [54], whereas *E. rhusiopathiae* clade 1 carries a *spaB* gene [39], and *E. piscisicarius* carries a *spaC* gene [42]. That *E. amsterdamensis* was isolated in association with chick mortalities in the absence of other bacteria suggests it was the likely cause of death, and the presence of the *spaA* gene, in addition to the presence of several putative virulence genes of *E. rhusiopathiae* that are typically among its core genes [34], lends further support to the likely virulence of this species. However, further investigation, including challenge studies, will be necessary for such characterization.

Erysipelothrix rhusiopathiae is typically considered an opportunistic pathogen, often manifesting clinically in stressed individuals or populations, for example following translocation of kakapos [15]. Further studies should explore whether *Erysipelothrix*-associated mortalities detected in other subantarctic islands could have been caused by the new species described here, or other novel taxa, and which set of potential host species of those relatively simple island communities may be involved in the epidemiological dynamics.

DESCRIPTION OF *ERYSIPELOTHRIX AMSTERDAMENSIS* SP. NOV.

Erysipelothrix amsterdamensis (am.ster.dam.en'sis. N.L. fem. adj. *amsterdamensis*, referring to Amsterdam island, from which the first strains to be characterized were isolated).

Cells are Gram-positive, rod-shaped and non-motile. Small pin-point sized, round, flat grey colonies with smooth contours are observed on 5% horse blood agar after 24 h growth at 37°C at normal atmospheric conditions. Presence of alpha haemolysis. Non-spore-forming. Catalase- and oxidase-negative. pH tolerance 6–8, with weak growth at pH 9. Normal growth between 20–40°C. The G+C content of the genomic DNA of the type strain is 36.6mol%. Whole genome sequencing indicates isolates carry the *Erysipelothrix spaA* gene and belong to *Erysipelothrix* serotype 1b. Members of the species can be distinguished from other members of the genus *Erysipelothrix* based on phylogenomic analysis and genomic metrics.

The type strain, A18Y020d^T (=CIP 112216^T=DSM 115297^T), was isolated from an Indian yellow-nosed albatross chick carcass on Amsterdam Island. The whole genome of the type strain A18Y020d^T is available in GenBank (GCA_940143175). The type strain has been deposited with the collections held by Institut Pasteur, France (CIP 112216^T) and with DSMZ, Germany (115297^T). The 16S rRNA sequence for A18Y020d^T is available on GenBank as accession OQ355811. The complete annotated genome for isolate A18Y016a is also available under accession number GCA_940143155. Reads for all 16 samples sequenced in this study are available on European Nucleotide Archive Sequence Read Archive (SRA) under accession number PRJEB50151.

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Author contributions
Formal analysis: J.Z., M.M. Investigation: J.T., A.C., H.G., A.G., T.L.F., T.B. Methodology (Field): T.B., A.C., J.T., A.G.; (Laboratory): H.G., T.B., A.C., J.T., A.G., T.L.F.; (Bioinformatics): J.Z., M.M., T.L.F.; (Histology): R.L.N. Supervision: M.M., T.L.F., T.B. Visualization: J.Z., M.M. Writing – original draft: J.Z., T.L.F. Writing – review and editing: all authors.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The experimental design was approved by the French Regional Animal Experimentation Ethical Committee No. 036 (Ministry of Research permit No. 10257–2018011712301381) and by the Comité de l'Environnement Polaire (A-2018–123 and A 2018–139 for 2018–2019).

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