A bacterium, HKU30T, was isolated from the infected tissue of a patient with wound infection after puncture by a fish fin. Cells are facultative anaerobic, non-spore-forming, non-motile, Gram-positive cocci arranged in chains. Colonies were non-haemolytic. The strain was catalase, oxidase, urease and Voges–Proskauer test negative. It reacted with Lancefield’s group G antisera and was resistant to optochin. It grew on bile aesculin agar and in 5 % NaCl. It was unidentified by three commercial identification systems. 16S rRNA gene sequence analysis indicated that the bacterium shared 98.2, 97.7, 97.4 and 97.1 % nucleotide identities with *Streptococcus iniae*, *Streptococcus pseudoporcinus*, *Streptococcus parauberis* and *Streptococcus uberis*, respectively. The DNA G+C content was 35.6 ± 0.9 mol% (mean ± SD). In view of the occupational exposure of the patient, an epidemiological study was performed to isolate the bacterium from marine fish. Two strains, with similar phenotypic and genotypic characteristics to those of HKU30T, were isolated from a three-lined tongue sole (*Cynoglossus abbreviatus*) and an olive flounder (*Paralichthys olivaceus*) respectively. Phylogenetic analysis of four additional housekeeping genes, *groEL*, *gyrB*, *sodA* and *rpoB*, showed that the three isolates formed a distinct branch among known species of the genus *Streptococcus*, being most closely related to *S. parauberis* (CCUG 39954T). DNA–DNA hybridization demonstrated <53.8 % DNA relatedness between the three isolates and related species of the genus *Streptococcus*. A novel species, *Streptococcus hongkongensis* sp. nov., is proposed. The type strain is HKU30T (=DSM 26014T = CECT 8154T).

*Streptococcus* is an important bacterial genus comprising diverse species of catalase-negative Gram-positive cocci in chains or pairs. They are traditionally classified into three major groups by their pattern of haemolysis into α-haemolytic, β-haemolytic or non(γ)-haemolytic streptococci. Further identification to species level usually relies on Lancefield grouping and/or biochemical tests. Based on 16S rRNA gene sequences, the various species are divided into six groups including anginosus, equinus, mitis, mutans, pyogenes and salivarius (Kawamura et al., 1995). A recent phylogenetic analysis based on partial sequences of recN also supported the existence of six groups though with slightly different groupings (Glazunova et al. 2010). Since conventional phenotypic tests and commercial identification systems often fail to identify certain species of the genus *Streptococcus* such as the viridans group and rarely encountered species, molecular techniques have been employed to allow accurate species identification and discovery of novel species in clinical microbiology laboratories (Glazunova et al., 2006; Kavamura et al., 1998; Lau et al., 2003; Woo et al., 2001, 2002, 2004b; Zbinden et al., 2012). In this report, we describe the isolation and characterization of a novel non-haemolytic, Gram-positive, catalase-negative, coccus-shaped bacterium, initially from a patient with right thumb wound infection after puncture by a fish fin and subsequently from two marine flatfish.

A 44-year-old previously healthy man was admitted to hospital in July 2010 because of increasing right thumb pain.

The GenBank accession numbers for the 16S rRNA gene, *groEL*, *gyrB*, *rpoB* and *sodA* gene sequences of strain HKU30T are HQ335006, JX046531, JX046534, JX046537 and JX120145, respectively.

Six supplementary figures are available with the online version of this paper.
pain and swelling with serous discharge for 3 days. He was a fishmonger who sold fish at a local fish market and had a puncture injury over his right thumb pulp by a fish fin during handling of marine fish one month previously. Examination showed a small wound over the right thumb pulp with surrounding erythema and swelling. X-ray of the right thumb showed the presence of suspected foreign body. The haemoglobin was 13.9 g dl⁻¹, total white cell count 5.0 x 10⁹ l⁻¹, with neutrophil count of 3.5 x 10⁹ l⁻¹, lymphocyte count of 1.0 x 10⁹ l⁻¹, monocyte count of 0.4 x 10⁹ l⁻¹ and platelet count of 180 x 10⁹ l⁻¹. Erythrocyte sedimentation rate was elevated to 17 mm h⁻¹. Emergency removal of foreign body and wound debridement was performed, which revealed a 1 cm-fish fin on the radial side of the right thumb pulp. The patient recovered after treatment with oral amoxicillin-clavulanate for 14 days. Samples of his right thumb tissue and fish fin yielded mixed growth of 

**Streptococcus iniae**
Possessed 1.8, 2.3, 2.6, 2.9, 2.9 and 3 % base differences from **Streptococcus iniae** HKU30ᵀ on 5 % horse blood agar after 24 h incubation.

HKU30ᵀ appeared as Gram-positive, non-spore-forming cocci arranged in chains. It grew on horse blood agar as non-haemolytic, grey colonies of 0.5–1 mm in diameter after 24 h of incubation at 37 °C in ambient air. Enhancement of growth was observed with 5 % CO₂. It also grew in microaerophilic or anaerobic environment and on bile aesculin agar but not MacConkey agar. It grew in 1, 2, 3, 4 and 5 % NaCl but not 6 % NaCl. It reacted with Lancefield’s group G but not group A, B, C or D antiserum (Streptex; Remel). It was resistant to optochin, polymyxin B and bacitracin but sensitive to novobiocin. It was non-motile at both 25 and 37 °C. Vitek-2 GPM, API20 STREP and ATB rapid ID32 STREP (bioMérieux Vitek) showed unidentified or unacceptatable profiles (API20 profile number 6173513, ID32 profile number 74336761150).

Because of the inconclusive phenotypic characteristics, PCR and sequencing of nearly complete 16S rRNA gene was performed, using primers listed in Table S1 (available at IJSEM Online), as described previously (Lau et al., 2003; Woo et al., 2003). Pairwise sequence alignment using EzTaxon (Chun et al., 2007) showed that the 16S rRNA gene of HKU30ᵀ possessed 1.8, 2.3, 2.6, 2.9, 2.9 and 3 % base differences from that of **Streptococcus iniae** ATCC 29178ᵀ, **Streptococcus pseudoporcinus** LQ-940-04ᵀ, **Streptococcus parauberis** DSM 6631ᵀ, **Streptococcus uberis** JCM 5709ᵀ, **Streptococcus dysgalactiae** subsp. **dysgalactiae** ATCC 43078ᵀ and **Streptococcus ictaluri** 707-05ᵀ respectively. Phylogenetic analysis using the maximum-likelihood method in MEGA version 5.01 (Tamura et al. 2011) revealed that HKU30ᵀ formed a distinct branch being most closely related to **Streptococcus iniae** within the pyogenic group of streptococci (Figs 1 and S1). Phylogenetic trees constructed with the neighbour-joining, minimum-evolution and maximum-parsimony methods also displayed similar topology (data not shown). Similar to previous studies (Kawamura et al., 1995; Glazunova et al., 2010), the diverse species of the genus **Streptococcus** formed at least six or seven groups based on 16S rRNA gene analysis (Fig. S1). As 16S rRNA gene sequences among some species of the genus **Streptococcus** may possess low sequence variability and other housekeeping genes, such as groEL, gyrB, rpoB and sodA, have been found to be more discriminative in differentiating members of the genus **Streptococcus** (Glazunova et al., 2009), the partial sequences of groEL, gyrB, rpoB and sodA were determined to further characterize the phylogenetic position of HKU30ᵀ. The partial groEL sequence of HKU30ᵀ exhibited 11.2, 18.2, 18.7, 19.4 and 21.4 % base differences from those of **S. parauberis** CIP 103956ᵀ, **S. urinalis** CIP 106463ᵀ, **S. iberis** CIP 103219ᵀ, **S. porcinus** CIP 103218ᵀ and **S. iniae** CIP 102508ᵀ, respectively.

The partial gyrB gene sequence exhibited 15.2, 21.1, 21.2, 21.6 and 21.8 % base differences from those of **S. parauberis** CIP 103956ᵀ, **S. porcinus** CIP 103218ᵀ, **S. urinalis** CIP 106463ᵀ, **S. iniae** CIP 102508ᵀ and **S. iberis** CIP 103219ᵀ, respectively. The partial rpoB gene sequence exhibited 5.3, 10.2, 10.4, 12.4 and 14.4 % from those of **S. parauberis** CIP 103956ᵀ, **S. gallolyticus** subsp. **pasteurianus** CIP 101722ᵀ, **S. gallolyticus** subsp. *gallolyticus* CIP 105428ᵀ, **S. gallolyticus** subsp. *macedonicus* CIP 105683ᵀ, **S. iniae** CIP 102508ᵀ and **S. iberis** CIP 103219ᵀ, respectively. The partial sodA gene exhibited 13.4, 17.2, 19, 19.3 and 13.1 % base differences from those of **S. parauberis** CIP 103956ᵀ, **S. iniae** CIP 102508ᵀ, **S. iberis** CIP 103219ᵀ and **S. canis** CIP 103223ᵀ, respectively. Phylogenetic analysis based on partial groEL, gyrB, rpoB and sodA gene sequences using maximum-likelihood, neighbour-joining and maximum-parsimony methods revealed that HKU30ᵀ formed a distinct branch, being most closely related to **S. parauberis** in all the trees (maximum-likelihood trees shown in Figs S2 to S5).

Based on the clinical history, an epidemiological study on marine fish was carried out to identify the source of the bacterium. A total of 101 marine fish of 29 species were sampled from local retail markets. Organ tissues and swabs, where available, were obtained as described previously (Lau et al., 2007; Woo et al., 2004a). Homogenized tissue samples and swabs were plated onto horse blood agar containing Streptococcus-selective supplement (Oxoid) for isolation of streptococci (Petts, 1984) and inoculated into isolation of streptococci (Petts, 1984) and inoculated into
(Table 1). Their 16S rRNA gene and partial groEL, gyrB, rpoB and sodA gene sequences were identical to those of strain HKU30T (Figs 1 and S2 to S5). Antimicrobial susceptibility using Kirby Bauer disk diffusion, with results interpreted according to the Clinical and Laboratory Standards Institute for α-haemolytic streptococci (Clinical and Laboratory Standards Institute, 2010), showed that strains HKU30T, FSHK1 and FSHK2 were sensitive to penicillin (MICs 0.032–0.064 μg ml⁻¹ by E-test), clindamycin, ofloxacin, levofloxacin and vancomycin, except for strain HKU30T being resistant to clindamycin. Whole-cell protein content was analysed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as described previously (Lau et al., 2012). The distance dendrogram constructed using MALDI Biotyper 3.0 (Bruker Daltonics) illustrates that strain HKU30T, FSHK1 and FSHK2 were closely related to each other with identical mass spectra, but were distinct from other related species of the genus Streptococcus (Fig. S6).

The G+C content of the genomic DNA of strain HKU30T, as determined by thermal denaturation (Marmur & Doty, 1962) in triplicates and calculated by the formula:

\[
(G+C)_\% = \frac{2.44T_m - 169}{169}
\]

where \(T_m\) is the melting temperature, was 35.6 ± 0.9 mol% (mean ± SD), which lies within the characteristic range of the genus Streptococcus (34–46 mol%) (Spellerberg & Brandt, 2011). To determine the DNA–DNA relatedness of the three isolates and their close phylogenetic neighbours, DNA–DNA hybridization studies were performed using genomic DNA extracted from HKU30T, FSHK1 and FSHK2 and type strains of closely related species, including S. iniae CCUG 27303T, S. parauberis CCUG 39954T, S. uberis CCUG 17930T, S. porcinus CCUG 27628T, Streptococcus dysgalactiae subsp. dysgalactiae CCUG 43079T (AB002516) and S. ictaluri CCUG 52536T, which shared >97% nucleotide identity in their 16S rRNA gene sequences to that of strain HKU30T. Preparation of

Fig. 1. Phylogenetic tree showing the relationship of S. hongkongensis sp. nov. to related species within the S. pyogenes group using 16S rRNA gene sequence analysis. The tree was constructed by the maximum-likelihood method using Kimura’s two-parameter correction (Kimura, 1980) with Streptococcus mutans ATCC 25175T as the outgroup. A total of 1178 nt positions was included in the analysis. Bootstrap values were calculated as percentages from 1000 replicates and only values ≥ 70% were shown. The scale bar indicates the estimated number of substitutions per 100 bases. Names and accession numbers are given as cited in GenBank.
We report the isolation and characterization of a novel species of the genus *Streptococcus* *sp. nov.* from the infected thumb wound of a patient and two marine fish. Its 16S rRNA gene is most closely related to that of *S. iniae*, while sequence analysis of four additional housekeeping genes, *groEL*, *gyrB*, *sodA* and *rpoB*, showed that it is most closely but distantly related to *S. parauberis*. Such an incongruent phylogenetic position has been described in other species of the genus *Streptococcus* and may reflect the different evolutionary rates of the 16S rRNA gene and the housekeeping genes or represent the result of horizontal gene transfer between streptococcal species (Schouls et al., 2003; Zhang et al., 2012). DNA–DNA hybridization studies demonstrated ≤53.8% DNA relatedness between *S. hongkongensis* and closely related species of the genus *Streptococcus*. Based on their unique phylogenetic positions and genetic characteristics, the three isolates should be classified as a separate species among the genus *Streptococcus*.

*S. hongkongensis* also exhibits phenotypic characteristics distinct from related species of the genus *Streptococcus*, which may be useful for species identification in clinical laboratories (Table 1). Most notably, *S. hongkongensis* belongs to Lancefield group G, while *S. iniae*, *S. parauberis* and *S. uberis* are usually non-groupable, although some *S. parauberis* isolates may react with Lancefield group E, C, D, P or U antiserum and some *S. uberis* isolates with Lancefield group E, C, D, P or U antiserum (Bentley et al., 1993; Spellerberg & Brandt, 2011). Moreover, *S. hongkongensis* is non-haemolytic, while *S. iniae* is known to display rainbow or β-haemolysis. It is also different from *S. iniae* in its ability to grow on bile aesculin agar and utilize lactose, but...
inability to utilize melezitose and produce β-glucuronidase. It is different from S. parauberis and S. uberis by its negative Voges–Proskauer reaction and ability to produce β-galactosidase. It is also different from S. parauberis in its ability to utilize glycogen and different from S. uberis in its inability to utilize sorbitol and tagatose. Isolation of non-haemolytic group G streptococci, especially from patients with a history of marine exposure, should raise the suspicion of S. hongkongensis. Analysis 16S rRNA gene or other housekeeping genes, is a reliable approach for definitive identification, which is important in understanding the prevalence and epidemiology.

While S. hongkongensis probably shares similar aquatic habitats to the related species, S. parauberis and S. iniae, its pathogenic potential remains to be elucidated. S. parauberis is an important causative agent of bovine mastitis as well as olive flounder streptococcosis in aquaculture industries (Bentley et al., 1993; Nho et al., 2009). S. iniae, apart from being an important fish pathogen (Pier & Madin, 1976; Shoemaker et al., 2001), can cause invasive infections in humans (Weinstein et al., 1997, Koh et al., 2004, 2007). In contrast to invasive S. iniae infections, the present patient did not have features suggestive of systemic infection, which may be explained by his young age and absence of underlying disease. The two marine fish, from which S. hongkongensis was isolated, also did not exhibit gross abnormalities suggestive of disease. Interestingly, the three related streptococci, S. hongkongensis, S. iniae and S. parauberis, have been isolated from olive flounder, a common flatfish native to the northwestern Pacific Ocean and raised in aquaculture in Asia (Nho et al., 2009). Moreover, both fish positive for S. hongkongensis belonged to the order Pleuromonacoccales (flatfish), suggesting that flatfish may be a common reservoir of these aquatic streptococci. The ability of S. hongkongensis to grow in 5% NaCl is also compatible with its survival in marine environment, in contrast to freshwater-associated bacteria such as Laribacter hongkongensis, which only grew in 2% NaCl (Woo et al., 2004a). Similar to the situation in infections caused by other aquatic bacteria (Woo et al., 2004a), the Asian population may be at higher risk for S. hongkongensis infections because of their cultural preference for freshly killed whole fish for cooking. In our patient, the bacterium had probably gained entry through the wound during injury while he was handling fish for sale. The Asian population should be aware of the chance of acquiring infections from injury during preparation of fish.

### Description of Streptococcus hongkongensis sp. nov.

Streptococcus hongkongensis (hong.kong.en’sis. N.L. masc. adj. hongkongensis of or belonging to Hong Kong, the place where the type strain was isolated).

Cells are facultatively anaerobic, Gram-positive, non-motile, non-spore-forming cocci (0.5–1.0 μm). It grows on blood agar as small (0.5–1.0 mm), opaque, convex, non-haemolytic colonies after 48 h of incubation at 37 °C in ambient air. Enhancement of growth was observed with 5% CO₂. Also grows in microaerophilic or anaerobic environments and on bile esculin agar but not MacConkey agar. Grows in 1, 2, 3, 4 and 5% NaCl but not 6% NaCl. Cells react with Lancefield’s group G antisera. Does not produce catalase. Hydrolyses esculin and arginine but not hippurate. It is negative for Voges-Proskauer, oxidase and urease. Utilizes amygdalin, galactose, glycogen, lactose, maltose, mannotol, mannose, methyl-β-D-glucopyranoside, N-acetyl-D-glucosamine, pullulan, ribose, salicin, sucrose, starch and trehalose. Produces alanine arylami-

<table>
<thead>
<tr>
<th>Source of unlabelled DNA</th>
<th>Results with labelled probe of S. hongkongensis sp. nov. HKU30†</th>
<th>Relative binding ratio%*</th>
<th>sd†</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. hongkongensis HKU30†</td>
<td>100.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. hongkongensis FSHK1</td>
<td>101.1 (98.8)</td>
<td>18.0 (2.1)</td>
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<tr>
<td>S. hongkongensis FSHK2</td>
<td>104.5 (97.7)</td>
<td>8.1 (0.5)</td>
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</tr>
<tr>
<td>S. iniae ATCC 29178†</td>
<td>24.2 (29.0)</td>
<td>6.7 (17.4)</td>
<td></td>
</tr>
<tr>
<td>S. parauberis CCUG 39954†</td>
<td>29.4 (53.8)</td>
<td>8.3 (12.7)</td>
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</tr>
<tr>
<td>S. uberis ATCC 19436†</td>
<td>38.0 (20.3)</td>
<td>17.8 (11.3)</td>
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<tr>
<td>S. porcinus ATCC43138†</td>
<td>49.1 (18.8)</td>
<td>2.0 (2.7)</td>
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<tr>
<td>S. dysgalactiae subsp. dysgalactiae ATCC 43078†</td>
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<td>7.5 (6.2)</td>
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<td>S. ictaluri 707-05†</td>
<td>48.7 (47.0)</td>
<td>5.9 (4.3)</td>
<td></td>
</tr>
</tbody>
</table>

*RBR (relative binding ratio) was determined by the following equation: (dot intensity of labeled DNA probe bound in unlabeled DNA from target strains)/(dot intensity of labeled DNA probe of strain bound with unlabeled DNA of itself) × 100.
††SD calculated from three independent experimental replicates.


