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REVIEW

# *Edwardsiella piscicida*: a significant bacterial pathogen of cultured fish

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ABSTRACT: *Edwardsiella piscicida*, a Gram-negative, facultative aerobic pathogen belonging to the *Enterobacteriaceae* family, is the etiological agent of edwardsiellosis in fish and a significant problem in global aquaculture. *E. piscicida* has been reported from a broad geographical range and has been isolated from more than 20 fish host species to date, but this is likely to be an underestimation, because misidentification of *E. piscicida* as other species within the genus remains to be resolved. Common clinical signs associated with edwardsiellosis include, but are not limited to, exophthalmia, haemorrhages of the skin and in several internal organs, mild to moderate dermal ulcerations, abdominal distension, discoloration in the fish surface, and erratic swimming. Many antibiotics are currently effective against *E. piscicida*, although legal restrictions and the cost of medicated feeds have encouraged significant research investment in vaccination for the management of edwardsiellosis in commercial aquaculture. Here we summarise the current understanding of *E. piscicida* and highlight the difficulties with species assignment and the need for further research on epidemiology and strain variability.

KEY WORDS: *Edwardsiella piscicida* · Aquaculture · Fish disease · Edwardsiellosis

## INTRODUCTION

The genus *Edwardsiella* belongs to the *Enterobacteriaceae* family based on biochemical characteristics (Brenner 1984), the presence of 'Kunin antigen' (Mäkelä & Mayer 1976) and DNA–DNA hybridization with other genera belonging to the family (Brenner 1978). In the early 1960s, *Edwardsiella* was independently reported by several authors as a new group of Gram-negative rods, producing hydrogen sulfide, indole-positive and mannitol-negative. Sakazaki & Murata (1962) described the 'Asakusa Group' of 256 enterobacterial isolates obtained from snakes with similar biochemical characteristics, while King & Adler (1964) isolated a group of bacteria analogous to the 'Asakusa group' from a patient with gastroenteritis assigning the name 'Bartholomew group'. Later, Ewing et al. (1965) described the species *E. tarda* from a new biogroup designated as 'biotype 1483-59', indicating the similarity of this species with the 'Asakusa' and 'Bartholomew' groups. At the same time, Hoshina (1962) described the species *Paracolobactrum anguillimortiferum*, the etiological agent of 'red disease' in eels. Sakazaki & Tamura (1975) suggested the name *Edwardsiella anguillimortifera* to include isolates previously designated as *E. tarda* by Ewing et al. (1965) and as *P. anguillimortiferum* by Hoshina (1962). However, Farmer et al. (1976) detected differences in some phenotypic tests between both descriptions, validating *E. tarda* as a species.

Until 1980, the genus *Edwardsiella* contained only a single species, *Edwardsiella tarda*. *Edwardsiella*

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*hoshinae* (Grimont et al. 1980) isolated from reptiles and birds, and *Edwardsiella ictaluri* (Hawke et al. 1981) isolated from channel and white catfish were then described. Recently, the novel species *Edwardsiella piscicida* (Abayneh et al. 2013) and *Edwardsiella anguillarum* (Shao et al. 2015), both comprising isolates recovered from diseased fish and previously classified as *E. tarda*, were identified. Reclassification of these isolates was based on contemporary genetic methods.

After its first description, identifications of *E. piscicida* were published exponentially. Moreover, genetic surveys of *E. tarda* isolates from historical reports concluded that many isolates previously classified as *E. tarda* actually belong to the species *E. piscicida* (Reichley et al. 2017, Buján et al. 2018b). This recent reclassification and review of archival data suggests *E. piscicida* is more problematic in global finfish aquaculture than *E. tarda*. The aim of this article is to compile the current knowledge of edwardsiellosis caused by *E. piscicida*, focusing on phenotypic, serological, and genetic characters, as well as putative virulence mechanisms of the bacterium. In addition, the geographical distribution, host species affected, diagnostic methods, and potential control or management strategies to prevent the disease are addressed.

## GEOGRAPHICAL DISTRIBUTION AND HOST SPECIES

With the recognition of *E. piscicida* as a discrete taxon (Abayneh et al. 2013, Reichley et al. 2017, Buján et al. 2018b), it has become evident that *E. piscicida* has a global geographic distribution. The first report of mortality in cultured fish caused by *E. piscicida* (identified as *E. tarda* at the time), occurred in Japan in 1979 (Castro et al. 2011a). In recent years, epizootics have been reported in Northern and Southern Europe (the Netherlands, Norway, Greece, France, Spain and Portugal; Castro et al. 2006) as well as China, Japan and the USA (Matsuyama et al. 2005, Griffin et al. 2014, Li et al. 2017), causing enormous economic losses in the fish industry. While many of these descriptions occurred prior to the recognition of *E. piscicida*, recent genetic studies have tied these reports to current *Edwardsiella* systematics (Abayneh et al. 2013, Shao et al. 2015, Reichley et al. 2017, Buján et al. 2018a). At present, *E. piscicida* has been isolated from a wide range of fish species (Table 1). Although *E. tarda* is involved in human clinical infections, until now *E. piscicida* has not been reported as a zoonotic agent. However, Castro et al. (2011a) demonstrated that *E. piscicida* may be virulent for some homoeothermic animals based on pathogenicity assays carried out in mice.

## ISOLATION AND IDENTIFICATION

*E. piscicida* grows on a variety of general growth media including, but not limited to, trypticase soy agar, brain heart infusion agar, Mueller-Hinton agar, Luria broth, and marine agar. Differential media for the *Enterobacteriaceae* such as MacConkey agar, xylose-lysine-desoxycholate agar and *Salmonella-Shigella* agar, as well as *Edwardsiella tarda* agar (Lindquist 1991) which was designed for the specific isolation of *E. tarda*, are unable to differentiate *E. piscicida* from other members of the genus *Edwardsiella* (Castro et al. 2011b). Biochemical test results, especially for carbon utilisation, are variable. Thus, although some phenotypic tests occasionally provide differential results among *E. piscicida* and other members of the genus (Table 2), phenotypic analysis by such methods are not recommended for reliable discrimination between *E. piscicida* and *E. tarda* (Griffin et al. 2013). Similarly, the API 20E and the BBL crystal enteric/nonfermenter identification system codes for *E. piscicida* are similar to those for bona fide *E. tarda* (Reichley et al. 2017).

Fatty acid methyl ether (FAME) analysis was performed in order to determine if the Sherlock Microbial Identification System (MIDI) could be employed for the identification of *E. piscicida* (Castro 2011, Reichley et al. 2017). The MIDI system misidentified *E. piscicida* as *E. tarda* based on species profiles populating the library's database. However, a dendrogram constructed with the MIDI Sherlock software (Fig. 1a) which determines relatedness between species through Euclidean distance, clustered *E. piscicida* separately from *E. tarda* and affords discrimination between species (Castro 2011). Therefore, it is suggested the fatty acid profiles of this species be deposited within the Sherlock Microbial Identification System database to validate its application in the identification of *E. piscicida*. In addition, the 2D plot of principal components (Fig. 1b) revealed 4 groups, 3 of them encompassing European isolates (group I, II and III) while Asian and American strains formed a different group (group IV).

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has also been used for the identification of a collection of *E. piscicida* strains obtained from different host and

Table 1. *Edwardsiella piscicida* infections reported in different fish species

| Species | Disease symptoms | Country | Year | Reference(s) |
|---|---|---|---|---|
| Ayu *Plecoglossus altivelis* | Unknown | Japan | 1995 | Yamada & Wakabayashi (1999[a]) |
| Barramundi *Lates calcarifer* | Congestion of the fins, hemorrhages and erythema in the ventral skin, pallor and mottling of the liver, splenomegaly, granulomas in internal organs | USA | 2017 | Loch et al. (2017) |
| Blackspot seabream *Pagellus bogaraveo* | Exophthalmia, discoloration of the skin, external hemorrhages and petechias in musculature, ascites | Japan | 2002 | Castro et al. (2006[a]) |
| Blotched fantail stingray *Taeniura meyeni* | Ascites, typical symptoms of bacterial infection | USA | 2015 | Camus et al. (2016) |
| Blue catfish *Ictalurus furtatus* | Unknown | USA | 2007 | Griffin et al. (2013[a], 2014) |
| Catfish *Ictalurus punctatus* | Cutaneous lesions, deep abscesses, gastrointestinal septicemia | USA | 2007, 2011 | Tekedar et al. (2013[a]), Griffin et al. (2013[a], 2014) |
| European eel *Anguilla anguilla* | Erratic swimming, bottom-dwelling, ulceration in dorsal surface, discoloration of the skin, red-head disease, exophthalmia, petechias in musculature, granulomas in liver, ascites | Norway<br>Spain | 1989<br>2003 | Abayneh et al. (2013), Buján et al. (2017) |
| Hybrid catfish *Ictalurus punctatus* × *I. furtatus* | Unknown | USA | 2010–2012 | Griffin et al. (2014) |
| Japanese eel *Anguilla japonica* | Exophthalmia, discoloration of the skin, excessive production of mucus, external hemorrhages and petechias in musculature, ascites | Japan | 1972, 1980, 1981, 1989 | Castro et al. (2006[a]), Yamada & Wakabayashi (1999[a]) |
| Japanese flounder *Paralichthys olivaceus* | Erratic swimming, exophthalmia, external hemorrhages, ascites, necrosis in liver and kidney, granulomas in liver | Japan<br>China<br>Korea | 1980, 1982, 1984, 1995, 2002<br>2003, 2005, 2006, 2010<br>1991, 1999, 2000, 2002 | Nakatsugawa (1983[a]), Yamada & Wakabayashi (1999[a]), Castro et al. (2006[a]), Han et al. (2006[a]), Zhu et al. (2006[a]), Takano et al. (2010[a]), Wang et al. (2011[a]), Yang et al. (2013[a]) |
| Koi *Cyprinus carpio* | Unknown | USA | 2000 | Reichley et al. (2017) |
| Korean catfish *Silurus asotus* | Abdominal distention, abscesses and necrosis in organs | South Korea | 2008 | Yu et al. (2009[a]) |
| Largemouth bass *Micropterus salmoides* | White patches of mucus, necrosis of the gills, erosion in fins, granulomas in liver and kidney | USA | 2014 | Fogelson et al. (2016) |
| Rainbow trout *Oncorhynchus mykiss* | Unknown | USA | 2014 | Reichley et al. (2017) |
| Marbled eel *Anguilla marmorata* | Unknown | China | 2008 | Yang et al. (2013[a]) |
| Red seabream *Pagrus major* | Hemorrhagic ulcers in head and fins, procidentia, hydroperitoneum | Japan | 2004 | Matsuyama et al. (2005[a]) |
| Seatrout *Salmo trutta* | Unknown | USA | 1988 | Reichley et al. (2017) |
| Serpae tetra *Hyphessobrycon eques* | Unknown | Singapore | 2000 | Ling et al. (2000[a]) |
| Sharp snout bream *Diplodus puntazzo* | Nodules and abscesses in spleen and kidney | Greece | 2013, 2014 | Katharios et al. (2015) |
| Smallmouth bass *Micropterus dolomieu* | Unknown | USA | 2007, 2009 | Reichley et al. (2017) |

(continued on next page)

Table 1. (continued)

| Species | Disease symptoms | Country | Year | Reference(s) |
|---|---|---|---|---|
| Sole *Solea senegalensis* | Cutaneous lesions in dorsal surface, hemorrhage in ventral surface, exophthalmia, ascites, anaemic liver, petechias in kidney | Spain | 2010 | Castro et al. (2012[a]) |
| Striped bass *Morone saxatilis* | Petechias, skin ulcers, external hemorrhages, opaque corneas, ascites, granulomas in liver and spleen | USA | 1986, 1994, 1996, 1997, 1999, 2000 | Baya et al. (1997[a]), Castro et al. (2006[a]), Van-Soest et al. (2011), Reichley et al. (2017) |
| Striped catfish *Platydoras armatulus* | Discolored areas in the skin, external hemorrhages, a general septicemia in the ventral muscle | India | 2007–2008 | Shetty et al. (2014[a]) |
| Tilapia *Tilapia nilotica* | Exophthalmia, discoloration of the skin, external hemorrhages and petechias in musculature, ascites | Japan | 1979, 1980 | Yamada & Wakabayashi (1999[a]), Castro et al. (2006[a]), Griffin et al. (2013[a]) |
| Turbot *Scophthalmus maximus* | Exophthalmia, loose of pigmentation of the skin, necrotic skin lesions, suppurative abscesses, external hemorrhages, petechias in musculature and ascites | Netherlands | 2004–2006 | Castro et al. (2006[a]), Han et al. (2006[a]), B. Wang et al. (2006[a]), Du et al. (2007[a]), Lan et al. (2008[a]), Bai et al. (2009[a]), Xiao et al. (2008[a]), Y. Wang et al. (2011[a]), Castro et al. (2011c[a], 2012[a]), Yang et al. (2013[a]), Buján et al. (2018a) |
| | | Portugal | 2005, 2006, 2008, 2009 | |
| | | Spain | 2006, 2009 | |
| | | China | 2003, 2006, 2011 | |
| | | UK | 2007 | |
| | | France | 2012 | |
| | | Korea | 2002 | |
| Whitefish *Coregonus lavaretus* | Exophthalmia, external and internal hemorrhages, mottled liver, peritonitis, necrosis in liver and kidney | Finland | 2000 | Shafiei et al. (2016) |

[a]*E. pisicicida* isolates described as *E. tarda*

origins. Although the existing database misclassified the strains of *E. piscicida* as *E. tarda*, specific mass spectra fingerprints were observed for each species due to inherent differences in the cellular proteins expressed (Barja et al. 2008, Fogelson et al. 2016, Reichley et al. 2017). The incorrect assignment of these strains as *E. tarda* is attributed to the fact that the reference protein mass spectra database was compiled prior to the recognition of *E. piscicida* as a species, resulting in outdated identification of the strains included in the database. In fact, the protein profiles included in the database of the strains ACC35.1, ACC36.1 and HL23.1, which have since been identified as *E. piscicida*, are categorized as *E. tarda* in line with their original designations (Buján et al. 2018b).

Lastly, polymerase chain reaction (PCR) protocols were developed for a rapid and specific identification of *Edwardsiella* species from pure cultures and fish tissues. Different pairs of primers designed to target the haemolysin gene (tardaF/tardaR; Chen & Lai 1998), the type 1 fimbrial gene cluster (*etf*A and *etf*D; Sakai et al. 2007) or the *gyr*B gene (*gyr*BF1/*gyr*BR1; Lan et al. 2008) can differentiate *E. tarda* and *E. piscicida* from other members of the genus. Griffin et al. (2014) developed *E. piscicida*-specific PCR primers based on the work of Sakai et al. (2009), and Reichley et al. (2015b) used these species-specific primers in the development of a real-time PCR (qPCR) assay to provide a rapid, quantitative confirmatory test for this microorganism. This qPCR assay has since been used in a multiplex qPCR, which demonstrated the ability to discriminate among *E. piscicida*, *E. ictaluri*, *E. anguillarum*, and *E. tarda* (Reichley et al. 2017).

## PHENOTYPIC AND SEROLOGICAL CHARACTERIZATION

*Edwardsiella piscicida* is a Gram-negative, oxidase-negative, facultative anaerobe, short and rod-shaped microorganism usually motile. The bacterium is positive for lysine and ornithine decarboxylase, produces $H_2S$ and indole from tryptophan, and ferments glucose, mannose and maltose. The phenotypic and physiological properties of *E. piscicida*

Table 2. Differential phenotypic characteristics among *Edwardsiella* species. (+) 90–100% of strains positive; (–) 0–10% of strains positive

| Characteristic | *E. tarda* | *E. hoshinae* | *E. ictaluri* | *E. piscicida* | *E. anguillarum* |
|---|---|---|---|---|---|
| $H_2S$ production | + | +[a] | – | + | + |
| Indole production | + | – | – | + | + |
| Voges-Proskauer | – | – | – | – | + |
| Carbon source | | | | | |
| D-mannitol | – | + | – | – | + |
| L-arabinose | – | – | – | – | + |
| D-mannose | + | + | + | + | – |
| β-methyl-D-glucoside | + | – | – | – | – |
| L-fucose | + | + | + | + | – |
| L-proline | + | – | – | – | – |

[a]Positive on Kligler but negative on TSI

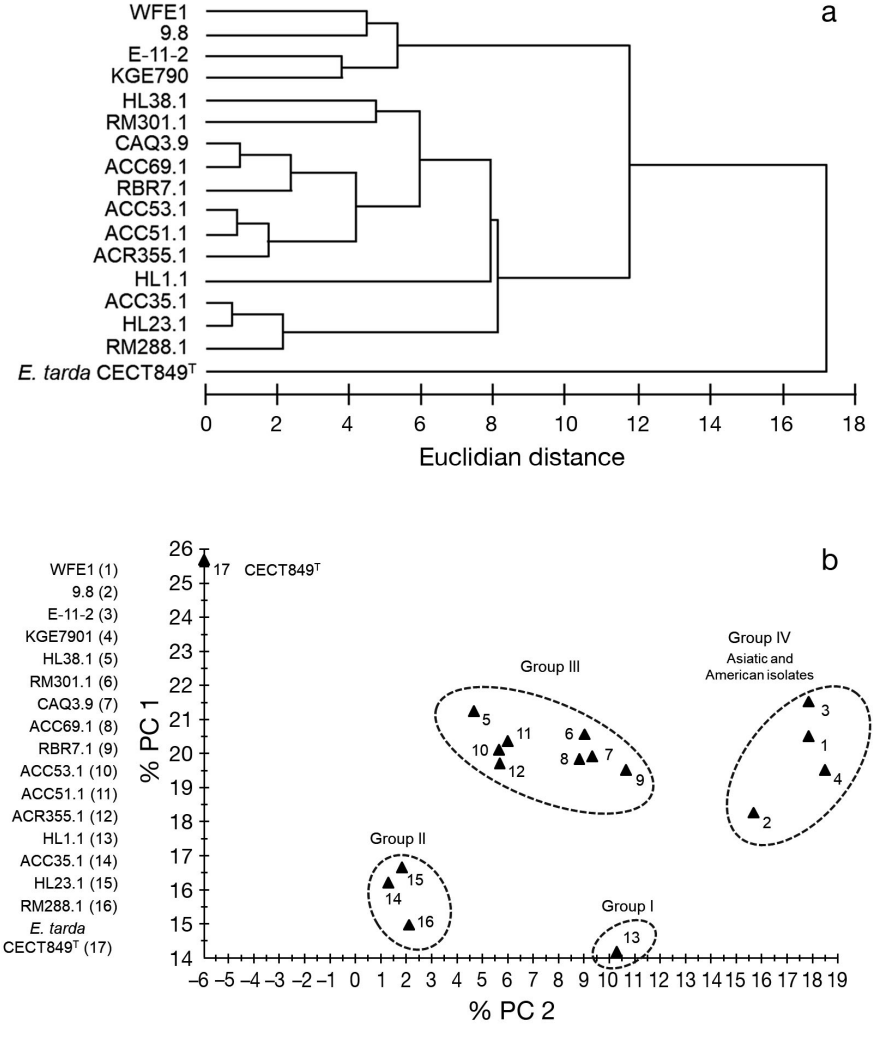



Fig. 1. (a) Dendrogram of Euclidean distance and (b) 2D plot of principal components obtained based on the results of the chromatographic fatty acid profile of different *Edwardsiella piscicida* isolates. PC1: first principal component; PC2: second principal component

assayed during the present study and recovered from other works are listed in Table 3.

The fatty acid profile of this pathogen is composed of 7 principal fatty acids, detected at a level >1%, including saturated, unsaturated and cyclopropane fatty acids. Among them, the most abundant are the 14:0 (tetradecanoic acid or myristate), 16:0 (hexadecanoic acid or palmitate), 17:0Δ (analogous of margaric acid), 19:0Δω8c (analog of lactobacillic acid) and the summed features SF2 (14:0 3-OH, 16:1 iso I), SF3 (16:1ω7c/16:1ω6c, 16:1ω6c/16:1ω7c) and SF8 (18:1ω7c, 18:1ω6c) (Castro 2011, Reichley et al. 2017).

Analyses of the serological relationship among *E. piscicida* strains, (previously designated as *E. tarda*), revealed the existence of at least 2 different serotypes, with all serotype 1 isolates stemming from European hosts (Castro et al. 2006, 2012).

## GENOTYPING, PHYLOGENY AND CLASSIFICATION

While *E. piscicida* and *E. tarda* are difficult to differentiate by phenotype, there are demonstrable genetic differences between the 2 taxa. Applying randomly amplified polymorphic DNA (RAPD), Castro et al. (2006, 2011c) demonstrated that isolates from turbot

Table 3. Phenotypic characteristics of *Edwardsiella piscicida* (Castro et al. 2006, Abayneh et al. 2013, Griffin et al. 2013, Shao et al. 2014, this work). (F) fermentative; (+) 90–100% of strains positive; (–) 0–10% of strains positive; (v) 11–89% of strains positive

| Characteristic | | Carbon source | |
|---|---|---|---|
| Motility | v | D-mannitol | – |
| Oxidase | – | L-arabinose | – |
| Catalase | + | L-rhamnose | – |
| Oxidative/fermentative | F | Maltose | v |
| Arginine descarboxilase | – | D-mannose | + |
| Lysine descarboxilase | + | D-galactose | + |
| Ornithine descarboxilase | + | β-methyl-D-glucoside | – |
| Indole production | + | Tween 80 | v |
| Voges-Proskauer | – | L-fucose | v |
| Methyl red | + | Acetic acid | – |
| Simmons' citrate | v | Citric acid | v |
| Nitrate reduction | + | Formic acid | – |
| Urease | – | D-glucosaminic acid | v |
| Aesculin | – | α-ketobutyric acid | – |
| Tween 80 | – | α-ketovaleric acid | – |
| Amylase | – | Quinic acid | v |
| $H_2S$ production | + | Bromosuccinic acid | v |
| D-glucose gas production | + | Glucuronamide | + |
| | | L-asparagine | + |
| **Growth at** | | L-aspartic acid | v |
| 4°C | – | L-glutamic acid | v |
| 37°C | + | L-proline | – |
| 42°C | v | L-serine | + |
| 0% NaCl | + | Uridine | v |
| 3% NaCl | + | Glycerol | v |
| 6% NaCl | – | D,L-α-glycerol phosphate | v |

and sole, previously identified as *E. tarda* but now reclassified as *E. piscicida*, comprised a unique group with a different fingerprint from those obtained for bona fide *E. tarda* strains. Moreover, 2 different *E. piscicida* clonal lineages coexisting in a single farm but from different outbreaks where identified by RAPD. Repetitive sequence mediated PCR has also been shown to differentiate between different *E. piscicida* strains (Castro et al. 2011c, Griffin et al. 2013, Camus et al. 2016, Shafiei et al. 2016, Reichley et al. 2017). REP-PCR was used to detect different clonal lineages in a single farm even within the same outbreak (Castro et al. 2011c). In contrast, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and BOX-PCR demonstrated a high degree of genetic homogeneity among *E. piscicida* isolates tested, although both techniques generated distinct banding patterns for each species of the genus (Griffin et al. 2014, Reichley et al. 2017). Due to the relative similarity among isolates from different hosts and geographic origins, the lack of resolution provided by ERIC-PCR renders it unsuitable for epidemiological analysis of edwardsiellosis (Shafiei et al. 2016). Genotyping of *E. piscicida* using amplified fragment length polymorphism (AFLP) also separates this species into an independent cluster from other members of the *Edwardsiella* genus, adding to the techniques that may be useful for classification (Buján et al. 2018b). Plasmid profiling of different *E. piscicida* isolates carried out by Reichley et al. (2017) found variability in size, composition and arrangement among the plasmids studied. However, it is noteworthy that the plasmids of turbot isolates used in this study were identical, implying an epidemiological link or host-adaptive factors associated with the plasmids (Reichley et al. 2017).

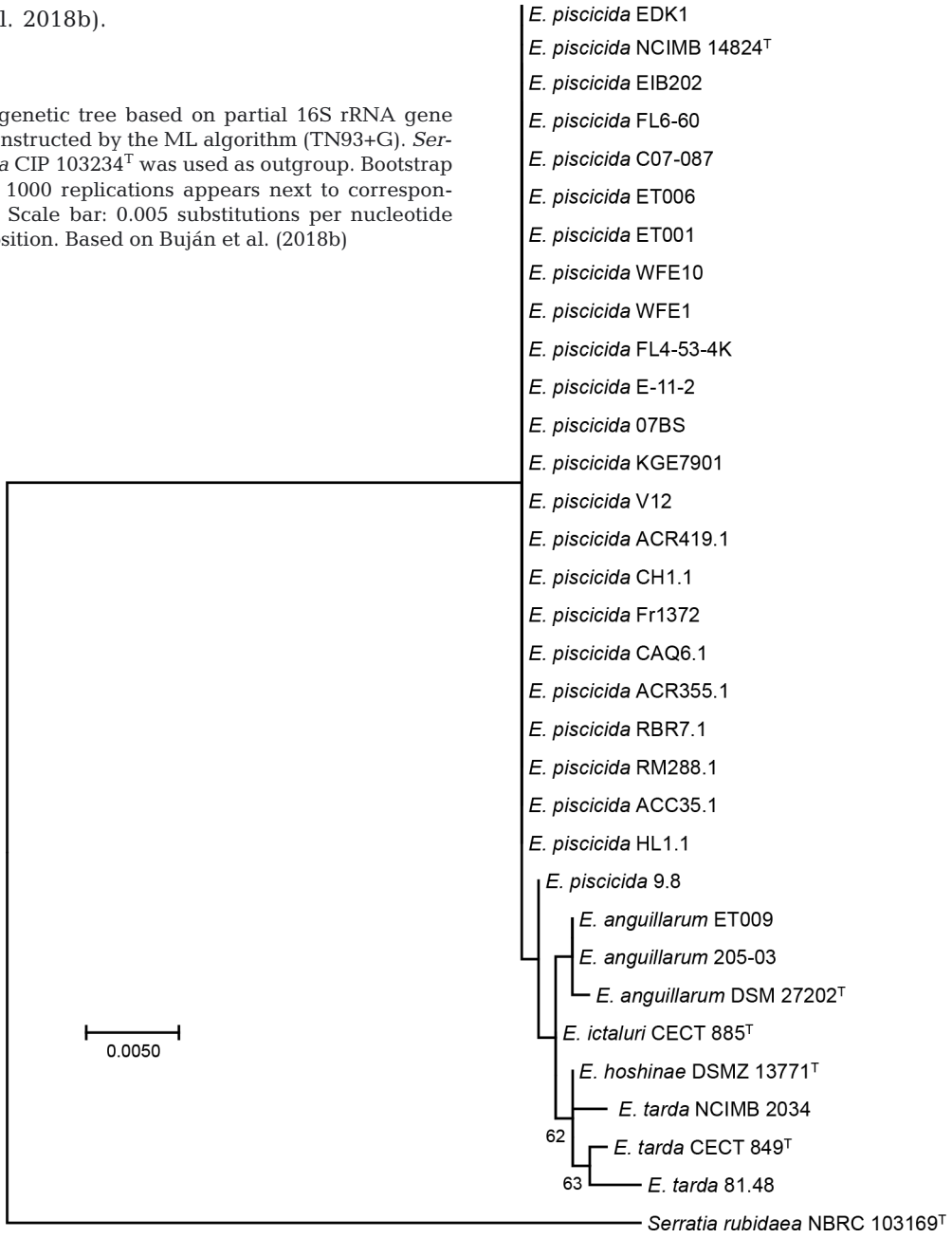
The recognition of *E. piscicida* and *E. anguillarum* as species required a revision of *Edwardsiella* systematics. The tool most used for classification and identification is the sequencing of 16S rRNA. For *Edwardsiella*, phylogenetic analysis based on the 16S rRNA gene has demonstrated low resolving power (Fig. 2), which, coupled with arbitrary similarity cutoffs and misidentification within public nucleotide databases, has led to erroneous identifications due to the high degree of 16S rRNA variability within the genus (Shao et al. 2015, Reichley et al. 2017, Buján et al. 2018b). In comparison, the genes *sod*B and *gyr*B, and *dna*J employed by Reichley et al. (2017) and Buján et al. (2018b) respectively have higher discriminatory power.

Multilocus sequence analysis (MLSA) has grouped *E. piscicida* strains in a robust clade separate from the other members of the genus (Fig. 3) (Abayneh et al. 2012, Griffin et al. 2013, Buján et al. 2018b), suggesting that MLSA is an adequate tool to determine inter- and intra-specific variability within the *Edwardsiella* genus. Furthermore, similar methods indicate genetic discontinuity within *E. piscicida,* reflected by a high number of unique sequence types (Yang et al. 2013). It is interesting to note that all *E. piscicida* isolates from Asian countries are compiled in 2 clonal complexes, while all isolates from European turbot and sole comprise a single separate sequence type (Buján et al. 2018a). This may reflect genetic changes associated with adaption to a new environment through geographical isolation and/or infection of different hosts.

Multilocus variable-number tandem repeat analysis (MLVA) has also been employed successfully with sufficient resolving power for epidemiological and phylogenetic analysis of *E. piscicida* isolates pathogenic to fish (Abayneh et al. 2014).

Advances in complete genome sequencing resulted in the availability of several *E. piscicida* (Oguro et al. 2014, Reichley et al. 2016, Buján et al. 2018b) and *E. tarda* genomes (Reichley et al. 2015a, Buján et al. 2018a). The comparative studies of these and other whole genomes of the genus *Edwardsiella* shows that complete genome sequencing is the most reliable taxonomic tool (Buján et al. 2018b).

Fig. 2. Phylogenetic tree based on partial 16S rRNA gene sequences constructed by the ML algorithm (TN93+G). *Serratia rubidaea* CIP 103234$^T$ was used as outgroup. Bootstrap (≥60%) from 1000 replications appears next to corresponding branch. Scale bar: 0.005 substitutions per nucleotide position. Based on Buján et al. (2018b)




## PATHOGENESIS AND VIRULENCE MECHANISMS

Experimental infection studies have demonstrated that *E. piscicida* is highly pathogenic, not only for the host from which it was isolated but also for other fish species such as zebrafish (Castro et al. 2011a, Abayneh et al. 2012). Therefore, *E. piscicida* does not seem to exhibit host specificity, and edwardsiellosis may be a risk for many marine fish species.

The clinical signs of *E. piscicida* infection are common to all species of fish suffering from the disease.

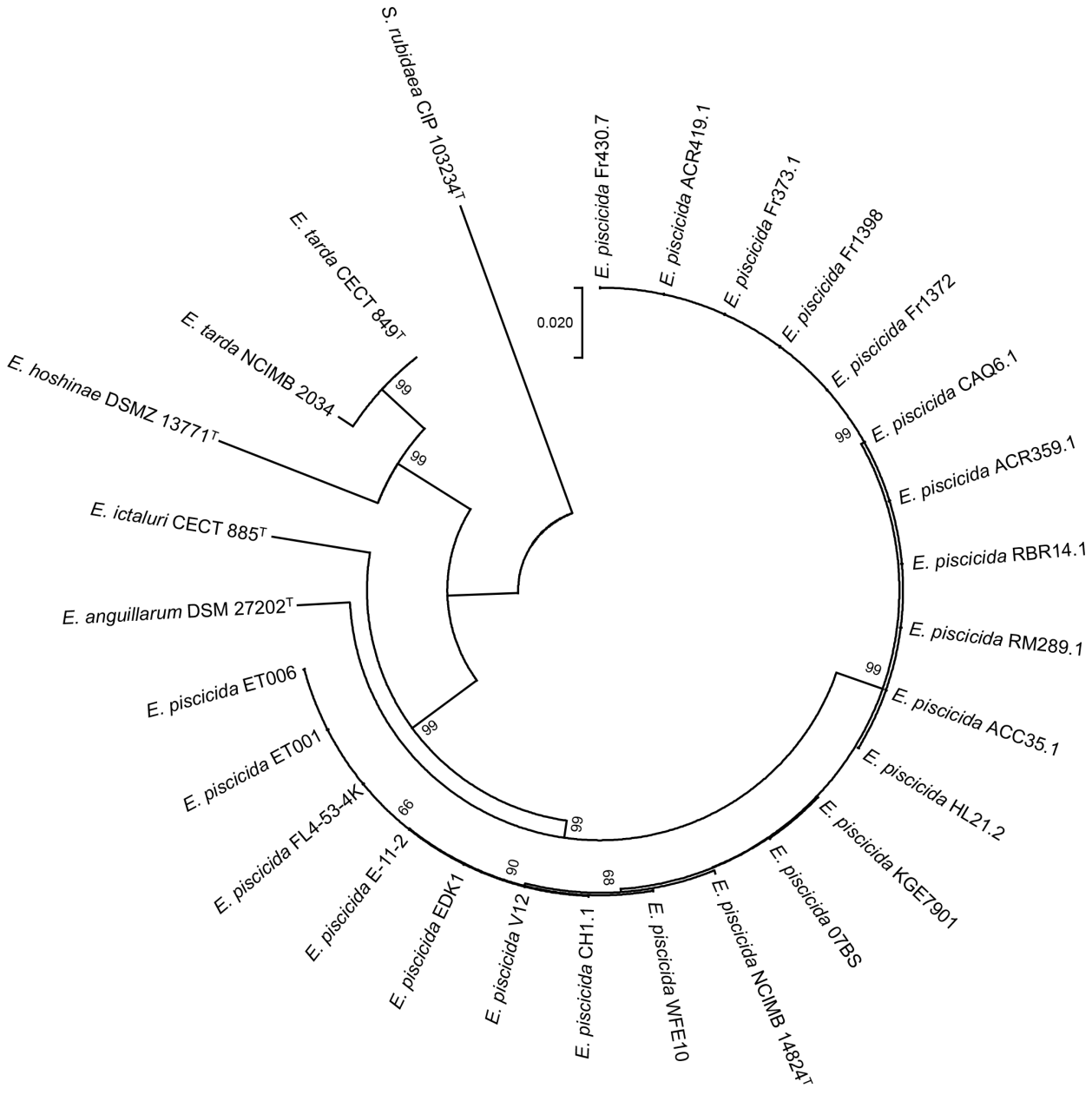



Fig. 3. Phylogenetic tree based on the concatenation of the nucleotide sequences of 6 housekeeping genes (*adk*, *atp*D, *dna*J, *gln*A, *hsp60*, *tuf*) by the NJ method (Kimura 2-parameter model). *Serratia rubidaea* CIP 103234$^{T}$ was used as outgroup. Bootstrap (≥60%) from 1000 replications appears next to corresponding branch. Scale bar: 0.02 substitutions per nucleotide position. Based on Buján et al. (2018b)

Externally, affected fish show discolored areas of the skin with loss of pigmentation, external haemorrhages and a general septicemia in the ventral muscle (Shetty et al. 2014, Griffin et al. 2017). Moreover, exophthalmia, abundant ascitic fluid and general petechiae in the internal organs were also observed in turbot *Scophthalmus maximus* (Castro et al. 2006), and abscesses and nodules in the visceral organs in sharp snout seabream *Diplodus puntazzo* were also described (Katharios et al. 2015). Histological examinations performed in largemouth bass revealed multifocal necrosis scattered throughout the heart, liver, anterior kidney, posterior kidney and spleen (Fogelson et al. 2016).

Several potential virulence factors implicated in the infection process of *E. piscicida* have been proposed. Extracellular products include chondroitinase, related to cartilage degradation (Waltman et al.

1986, Shotts & Cooper 1992), with homologues recently reported in European turbot isolates of *E. piscicida* (Castro et al. 2016) and in an Asian turbot strain of this species (Yang et al. 2012). Different hemolysins and their precursors (EthA, EthB and SlyA) have also been detected (Janda et al. 1991, Kumar et al. 2010, Wang et al. 2010, Xiao et al. 2012).

Contact and adherence to the host often comprise early stages of infection. The invasin Inv1 has been identified in *E. piscicida* (Li et al. 2012), and *E. piscicida* presents different adhesins on the cell surface including flagellins (FliC, FlgD) (Park et al. 2011, He et al. 2012, Liu et al. 2012, Buján et al. 2015a, X. Liu et al. 2017b), fimbrial proteins (FimA) (Srinivasa Rao et al. 2003, Kim et al. 2014) and other adhesins (AIDA) (Sakai et al. 2009). FliC, along with sigma factor RpoS may be involved in the development of biofilms (Xiao et al. 2009, He et al. 2012), although Shafiei et al. (2016) reported the inability of highly virulent *E. piscicida* to form thick biofilms under the conditions tested. Moreover, comparative proteomics of strains with a different degree of virulence implicate flagellin (FliC) in virulence. Indeed, recent unpublished work in our laboratory indicate that flagella-impaired mutants (via *flg*G) are attenuated in turbot and increase biofilm formation (N. Castro unpubl. data).

To survive and subsequently multiply, the microorganisms have a series of mechanisms to resist their host's defenses. Under stress conditions, such as serum resistance or replication in macrophages, *E. piscicida* expresses proteins of the catalase family (KatB and KatG) (Han et al. 2006, Xiao et al. 2012), heat shock proteins (HtpG and Hsp60) (Dang et al. 2011), superoxide dismutase (SodB and SodC) (Han et al. 2006, Gao et al. 2016) and 2-component systems (EsrA-EsrB) (Liu Y et al. 2017, Yin et al. 2017). *E. piscicida* produces the hydroxamate-type siderophore vibrioferrin and can utilize heme groups (hemin or haemoglobin) as an iron source by direct binding (Castro et al. 2016). Under iron limitation this iron uptake system is upregulated along with hemolysin, hydrolases and stress protein Hsp90. Proteins involved in transport, carbohydrate metabolism and amino acid synthesis were also up-regulated (Buján et al. 2015b).

Secretion systems are used by a multitude of microorganisms to release different virulence factors (quorum sensing regulators, exotoxins and exoenzymes) within the host (Tan et al. 2005, Leung et al. 2012). In *E. piscicida*, the proteins EseB, EseC, EseD and EseH belonging to the type III secretion system, are related to the translocation of effector proteins in infected host cells (Srinivasa Rao et al. 2004, Hou et al. 2017). Moreover, proteins of the type VI secretion system, EvpA, EvpB and EvpC (*Edwardsiella* virulence protein) are associated with virulence of *E. piscicida* (Tan et al. 2005, Chakraborty et al. 2011) although the details of their functions are still unknown (Srinivasa Rao et al. 2004). With regard to quorum sensing systems, Romero et al. (2014) reported *in vitro* detection of the N-acyl homoserine lactones (AHLs) C6-HSL and OC6-HSL and Castro et al. (2016) showed the production of these molecules *in vivo* during fish infections. Moreover, they demonstrated a strong increase in AHL production when the fish were infected with low doses of bacteria consistent with AHL production in *E. piscicida* being under density-dependent control in the fish. Castro et al. (2016) also described the presence of genes involved in AHL production (AI1), the AI2 system (*lux*S) and the QscBC system.

## TREATMENT AND PREVENTION

*In vitro* antimicrobial susceptibility testing of *E. piscicida* indicates that, to date, strains isolated from different hosts and geographical regions are susceptible to most commonly used antibiotics for the treatment of edwardsiellosis, including enrofloxacin, oxytetracycline, trimethoprim/sulfamethoxazole or florfenicol among others (Castro et al. 2006, Shafiei et al. 2016, Reichley et al. 2017, Kim et al. 2018).

Prophylactic vaccines are the most cost-effective tools for preventing bacterial infections. Kwon et al. (2006), Castro et al. (2008) and Sun et al. (2011) evaluated the effectiveness of different formalin-killed formulations in turbot, tilapia and flounder respectively. These were found to be generally effective, with the adjuvanted vaccine developed by Castro et al. (2008) providing highest protection. Several live attenuated vaccines were tested in turbot and zebrafish with high relative percent survival (RPS) values (Xiao et al. 2011, 2013, Wang et al. 2013, Yan et al. 2013, Yang et al. 2015) but they are not appropriate for commercial applications due to biosafety and environmental risks. In *E. piscicida* different recombinant proteins were evaluated as potential protective antigens. F. Liu et al. (2016b, 2017) developed various vaccines based on the membrane proteins rOmpI, rOmpX and OmpC obtaining high RPS values (over 80%) in flounder. Flagellar proteins were tested by Zhang et al. (2012) in zebrafish and by X. Liu et al. (2017b) in turbot obtaining the best RPS values with the protein FlgD (76% and 70% res-

pectively). The effectiveness of the FlgD protein as vaccine was supported by X. Liu et al. (2017a), in zebrafish and turbot, using reverse vaccinology approach. On the other hand, the GroEL DNA vaccine tested by Liu et al. (2016a) and the chimeric DNA vaccine encoding the flagellar genes Eta6 fused in-frame to FliC developed by Jiao et al. (2009), were protective in flounder with RPS values of 60% and 72%, respectively. However, better results (RPS over 87%) were obtained in flounder by a bicistronic vaccination using the *flg*D gene, adjuvanted with C5a peptidase protein driven by a modified cytomegalovirus promoter/enhancer to increase gene expression (Liu et al. 2016b). Polyvalent vaccines obtained by shuffling 6 *omp*A genes of 4 bacteria, *V. alginolyticus*, *V. parahaemolyticus*, *E. piscicida* and *E. coli*, were effective in zebrafish assays with values of RPS over 80% (Cheng et al. 2018).

## CONCLUDING REMARKS

In this work, we review the current literature on fish edwardsiellosis caused by *E. piscicida*. Much recent effort has focused on correct identification, classification and phylogenetic positioning of this fish pathogen. As we have seen before, the difficulty in validating and reproducing phenotypic tests to identify species of the *Edwardsiella* genus correctly has necessitated use of specific PCR (Griffin et al. 2014) or sequencing of genes such as *dna*J (Buján et al. 2018b) or *gyr*B (Griffin et al. 2014) for accurate differential identification. Virulence mechanisms seem to be largely aligned with other enteric fish pathogens. However, further information on the epidemiology, strain variation and host–pathogen interactions of this species are required to prevent economic losses in the aquaculture industry through biosecurity other preventative measures. Moreover, whether *E. piscicida* is exclusively a fish pathogen or has zoonotic role, as in the case of *E. tarda*, needs to be elucidated.

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