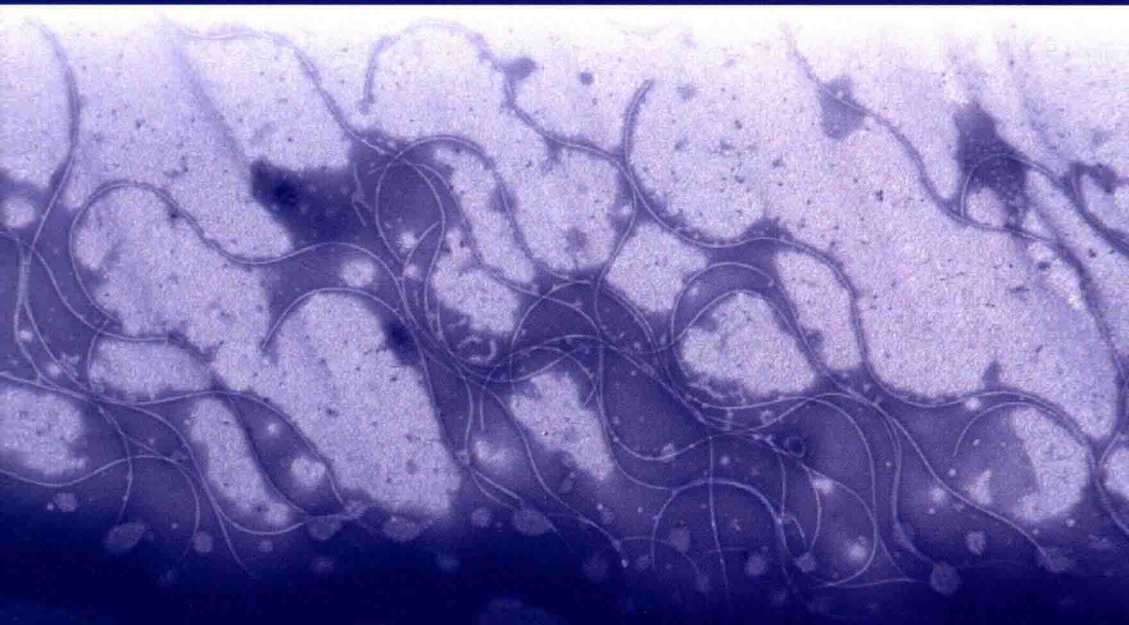
Aeromonas



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Caister Academic Press

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Caister Academic Press

Caister Academic Press
Norfolk, UK

www.caister.com

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN (hardback): 978-1-908230-56-0
ISBN (ebook): 978-1-908230-57-7

Cover design adapted from Figures 8.10 and 9.2.

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Preface

The conception of this book and the groundwork were done by Amy Horneman. Amy has been a driving force in the *Aeromonas* field, which is reflected by her being known to many of us in the community as '*Aeromonas*' Amy. It was natural for her to be the editor of this book. Unfortunately for us in the *Aeromonas* research community, her new position as the Director of Microbiology and Molecular Diagnostics at the Veterans Administration Maryland Health Care System prevented her from editing this book. However, Amy had already convinced Horizon Press of the importance of *Aeromonas*. She laid out a plan identifying the major topics and got leading researchers in this field to commit to write chapters. So I would like to express my thanks to Amy for starting this work.

As the reader will discover in this book about *Aeromonas*, this genus of bacteria encompasses a very interesting group of bacteria that have something of interest to many different microbiologists. These bacteria are capable of causing disease in humans and fish, which can be food borne or acquired from the environment. In contrast, these bacteria are also beneficial symbionts in other animals. In addition, it has proven difficult to identify *Aeromonas* strains at the species level using standard biochemical tests, which complicates many different aspects. The sheer number of species contained within this genus is ever increasing. We now know of more than one dozen *Aeromonas* species and can be confident that more species are yet to be described. Perhaps a better knowledge of the taxonomy will aid in identifying the virulent strains. Over recent years there has been *tremendous* progress in understanding the mechanisms by which *Aeromonas* causes disease and the surface properties that can lay the groundwork for vaccine development.

I would like to thank all of the authors for contributing high-quality manuscripts that cover each of the topic areas in a comprehensive fashion. I would also like to thank Hugh Griffin, Melanie Woodward and other staff members from Horizon Press with editing the manuscripts and helping in the production process.

While science moves at a frantic pace and books are static in time, I hope that this book will provide the reader with an excellent overview of *Aeromonas* that will serve them as a valuable resource for many years.

Joerg Graf
University of Connecticut

Contents

Introduction and Overview

1

Joerg Graf

As the reader will discover, the genus *Aeromonas* is a fascinating group of organisms for a variety of reasons, including the complicated taxonomy, the controversy of causing infections in humans, and the wide range of hosts colonized. The history of bacteria now considered to be *Aeromonas* dates back to 1891, when these bacteria were linked to 'red leg' disease in frogs. It was over 50 years later when *Proteus hydrophila*, which later was reclassified to *Aeromonas hydrophila*, was described by Stanier. During the 1980s DNA–DNA hybridization studies lead to the description of the main hybridization groups that included the species that are the most important fish and human pathogens, *A. salmonicida*, *A. hydrophila*, *A. veronii* and *A. caviae*. The advance of rRNA gene sequencing led to separation of the aeromonads from the Vibrionaceae as their own family. Over the years the interest in *Aeromonas* has increased as reflected by the increase in publications involving *Aeromonas* (Fig. 1.1).

In Chapter 2, Huys presents a brief history of the *Aeromonas* taxonomy followed by a description of how different technologies affected our understanding of the *Aeromonas* taxonomy as the field moved from DNA–DNA hybridization coupled with biochemical tests to more molecular approaches such as amplified fragment length polymorphism and the sequencing of multiple housekeeping genes. He also comments on several taxonomic controversies regarding species identification and makes general recommendations for future species descriptions.

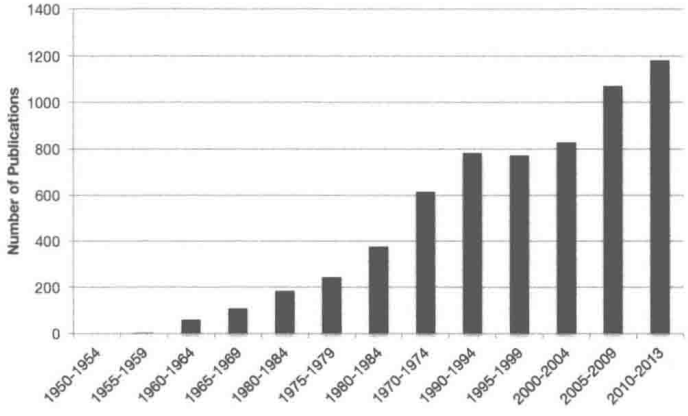

Figure 1.1 Number of *Aeromonas* publications. The number of citations returned with *Aeromonas* as a search term for the indicated time frame is shown.

Perhaps the best-known diseases caused by aeromonads involve fish and are traditionally thought to be caused by non-motile (*A. salmonicida*) or motile (*A. hydrophila*) *Aeromonas* strains. However as Austin describes the current state it becomes clear that an increasing number of other *Aeromonas* spp. that have been shown to be able to cause disease in fish. This chapter covers relevant taxonomy, diagnosis, ecology, virulence factors and disease control.

The role of *Aeromonas* spp. in causing disease in humans is perhaps the most controversial one, especially when it comes to the digestive tract illnesses such as diarrhoea. Figueras and Beaz-Hidalgo present the current evidence for *Aeromonas* being a human pathogen focuses not just on gastrointestinal illnesses but also other infections that *Aeromonas* isolates have been associated with injury or wound infections, nosocomial infections, septicaemia, respiratory tract infections and peritonitis.

While most research focuses on pathogenic associations of *Aeromonas*, some members of this genus are found in a number of different animals that are colonized in a benign or even beneficial manner. Graf describes the best studied example of this is the digestive tract symbiosis of *A. veronii* and the medicinal leech in Chapter 3. In addition, it was shown that *Aeromonas* can induce the normal development of the digestive tract in gnotobiotic zebrafish. In addition, *Aeromonas* spp. have been reported in mosquitoes and vampire bats, but the role in those organisms is less well understood.

Surface structures play an important role in adherence and are also an important target for developing vaccines. Parker and Shaw review *Aeromonas* surface structures such as polar and lateral flagella, pili and fimbriae, lipopolysaccharides, outer membrane proteins, capsule and the S-layer. One interesting feature of *Aeromonas* is that some of the surface proteins are also glycosylated, which is touched upon by the authors.

A description of the virulence factors of *A. hydrophila* is provided by Kozlova, Ponnusamy, and. Chopra. Using molecular genetic investigations of the strain SSU, insight into the molecular requirements for causing disease have been revealed. In this chapter the importance of type III secretion, type VI secretion and quorum sensing is discussed. In addition, the role of the secreted effector molecules or other virulence factors is mentioned.

Finally, but not least, *Aeromonas* in water and food is described in a chapter by Grim. As *Aeromonas* species are wide spread in water understanding the ecology, prevalence and factors influencing their abundance is important. Both results from classical culture-dependent studies and 16S rRNA gene studies are presented.

Taxonomy

Geert Huys

2

Abstract

Since its description by Kluyver and van Niel in 1936, the taxonomic structure of the genus *Aeromonas* has been drastically reshaped each time new technological advances were made in bacterial systematics. Modern *Aeromonas* taxonomy started off at the end of the 1970s essentially relying on physiological and biochemical characterization and DNA–DNA hybridizations, the latter still being considered the 'golden standard' for delineation of bacterial species. The original 'four-species concept' encompassing *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria* and *Aeromonas salmonicida* was soon expanded with multiple DNA hybridization groups (HGs), most of which were later given the species status. The introduction of 16S rRNA gene sequence analysis and amplified fragment length polymorphism (AFLP) fingerprinting in the 1990s allowed to characterize the phylogenetic and genotypic diversity of larger sets of *Aeromonas* isolates, and facilitated the description of several new *Aeromonas* species or the synonymization of existing taxa. Next, the availability of bacterial whole-genome sequences allowed to evaluate single-copy protein-encoding housekeeping genes such as *gyrB* and *rpoD* as alternative molecular markers in *Aeromonas* taxonomy. Compared to the 16S rRNA gene, these markers display a higher taxonomic resolution and can be combined in a multilocus sequence approach to construct a stable phylogenetic framework to rapidly and reliably recognize new *Aeromonas* taxa and thus avoid new nomenclatural problems.

Following the first reference to an organism which would later be recognized as a motile aeromonad (Sanarelli, 1891), the taxonomy of the genus *Aeromonas* has undergone major changes. The main purpose of this chapter is to reconstruct and discuss the major steps in this process. Following a short description of the genus as commonly found in renowned taxonomic manuals, past and current views on the phylogenic position of *Aeromonas* in the Gammaproteobacteria and the case-specific lack of taxonomic discrimination of the 16S rRNA gene at species level are discussed. Next, the gradual expansion of the genus *Aeromonas* with new species is presented in a more or less chronological order that follows the path of the various technological advances witnessed by bacterial systematics since the 1970s. Finally, a critical overview is given of the methods which helped to shape our present view on *Aeromonas* taxonomy and have proven their use for reliable assignment of unknown isolates to known taxa (i.e. identification).

Short description of the genus

This description is based on the *Aeromonadaceae* chapter in the second edition of *Bergey's Manual of Systematic Bacteriology* (Martin-Carnahan and Joseph, 2005). The reader is also referred to this chapter for additional reading on *Aeromonas* taxonomy, together with the taxonomy chapter (Carnahan and Altwegg, 1996) in the previous *Aeromonas* monograph, the review of Janda and Abbott (2010) and the forthcoming chapter on the *Aeromonadaceae* in the fourth edition of *The Prokaryotes*.

Members of the genus *Aeromonas* are facultatively anaerobic, Gram-negative bacteria that are widespread in still and streaming aquatic biotopes. Cells occur as rigid rods with rounded ends but can also have a coccoid shape; most species, except *A. salmonicida* and *A. media*, are motile by a single polar flagellum. Cells exist singly, in pairs, or in short chains. Their size range is 0.3–1.0 μm in diameter and 1.0–3.5 μm in length. *Aeromonas* strains are oxidase- and catalase-positive, and reduce nitrates to nitrites. Metabolism of glucose is both respiratory and fermentative. Carbohydrates are broken down with the production of acid or acid and gas. Aeromonads are chemoorganotrophic, capable of using a wide range of sugars and organic acids as their source of carbon. They do not require Na for growth, and are resistant to the vibriostatic agent 0/129 (2,4-diamino-6,7-diiso-propylpteridine) and to most penicillins. Growth can occur between pH 4.5 and 9.0. Two major groups are known to exist, i.e. the motile, mesophilic aeromonads (optimum growth temperature 28 to 30°C), and the non-motile, psychrophilic aeromonads (optimum growth temperature 22–25°C). The G + C content ranges from 57 to 63 mol%. The type species is *Aeromonas hydrophila*.

Phylogeny

Phylogenetic position of the genus

In the first edition of *Bergey's Manual of Systematic Bacteriology*, the genus *Aeromonas* was assigned to the *Vibrionaceae* family primarily on the basis of phenotypically expressed properties (Popoff, 1984). At the time, this classification contradicted a previous DNA hybridization study by Staley and Colwell (1973) in which the genomic relatedness between selected reference strains of *Aeromonas* and *Vibrio* was reported to be relatively low (i.e. <10%); this important finding thus suggested that a significant evolutionary distance exists between both aforementioned genera. During the 1980s, the influential work of Woese (reviewed in 1987) and De Ley (reviewed in 1992) demonstrated that comparison of ribosomal RNA (rRNA) cistrons is a highly informative method to study phylogenetic relationships within the Proteobacteria. These new insights triggered the generation of 16S rRNA catalogues, 5S rRNA sequences, and DNA–rRNA hybridization data on the basis of which Colwell and co-workers (1986) concluded that the genus *Aeromonas* represents an evolutionary line that is sufficiently different from the *Vibrionaceae* and the *Enterobacteriaceae* to justify its exclusion from these two families. As a result, these authors proposed to assign the genus *Aeromonas* in a new family within rRNA superfamily I *sensu* De Ley (1992), the *Aeromonadaceae*. The allocation to a separate family was supported by subsequent rDNA sequencing (Martínez-Murcia *et al.*, 1992a) and rRNA sequencing (Kita-Tsukamoto *et al.*, 1993; Ruimy *et al.*, 1994) studies.

In the second edition of Bergey's Manual of Systematic Bacteriology, the *Aeromonadaceae* family is phylogenetically placed in the order *Aeromonadales* (Martin-Carnahan & Joseph,

2005) within the phylum Gammaproteobacteria. Since its first description by Colwell and colleagues (1986), several new genera have been situated in the *Aeromonadaceae* (Fig. 2.1). Based on the 16S rRNA gene tree of the All-Species Living Tree Project (Yarza *et al.*, 2008, 2010) and J.P. Euzéby's List of Prokaryotic names with Standing in Nomenclature (http://www.bacterio.cict.fr/), the family currently contains the type genus *Aeromonas* (Stanier, 1943), *Tolumonas* (Fischer-Romero *et al.*, 1996), *Oceanimonas* (Brown *et al.*, 2001), *Oceanisphaera* (Romanenko *et al.*, 2003) and *Zobellella* (Lin and Shieh, 2006).

Inter-specific phylogeny

Martínez-Murcia and colleagues (1992a) reported on the taxonomic value of small-subunit rRNA sequencing in *Aeromonas* beyond the genus level. Their results showed that the 16S rDNA sequences of 10 *Aeromonas* type strains exhibited a very high level of similarity ranging from 98 to 100%. When comparing with previously published DNA reassociation data, the authors found several cases for which the phylogenetic interrelationships of the corresponding species completely disagreed with DNA–DNA hybridization results. For instance, the ribosomal sequences of the type strains of *A. caviae* (HG4) and *A. trota* (HG13) differed by only one nucleotide (i.e. 99.9% sequence similarity) (Martínez-Murcia *et al.*, 1992a), whereas Carnahan and co-workers (1991c) determined that the DNA relatedness between these two strains was as low as 30%. A similar phenomenon was observed with HG2 and HG3, and HG1 and *A. media*, respectively. Conversely, reference strains of *A. veronii* HG8 and HG10 showed identical 16S rDNA sequences in agreement with the very high genotypic similarity between these two taxa (Hickman-Brenner *et al.*, 1987). Not surprisingly, the occasional lack of congruence between 16S rRNA gene sequence phylogeny and species

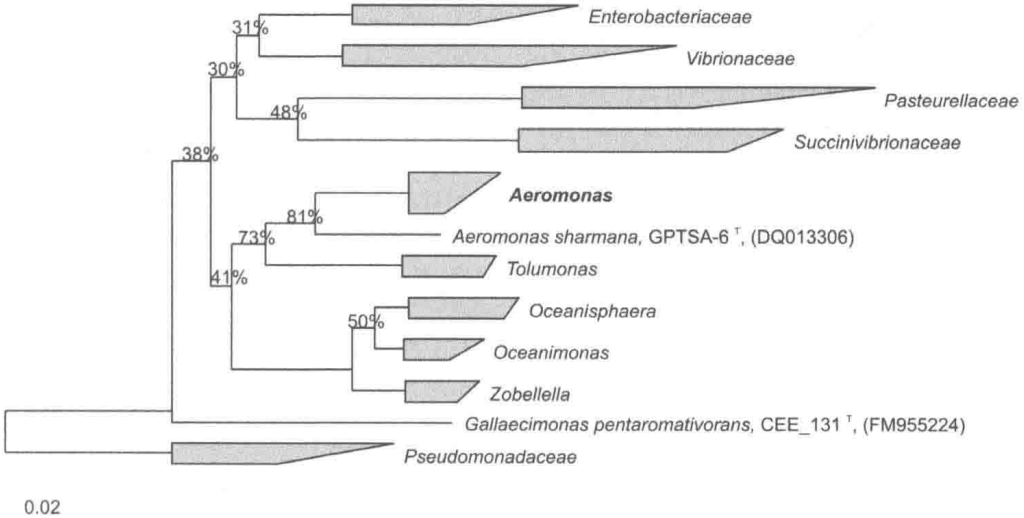

Figure 2.1 Phylogenetic reconstruction of the family Aeromonadaceae based on 16S rRNA and created using the neighbour-joining algorithm with the Jukes-Cantor correction and 100 runs bootstrapping. Percentages on nodes indicate bootstrap values. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza *et al.*, 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related families were used as outgroups. Scale bar indicates estimated sequence divergence.

delineation based on DNA–DNA hybridizations has fuelled several taxonomic discussions. For example, Martínez-Murcia (1999) revealed discrepancies between 16S rDNA sequence analysis and DNA–DNA hybridization results (Huys *et al.*, 1997b) on the taxonomic position of *Aeromonas* HG11. Likewise, genotypic and phylogenetic discrepancies have been reported for the discrimination between *A. salmonicida* and *A. bestiarum* (Martínez-Murcia *et al.*, 2005). Along the same lines, Fox and colleagues (1992) also reported cases of discongruence between 16S rRNA sequences and DNA–DNA hybridization data for *Bacillus* spp. The latter authors concluded that 16S rRNA sequences could be routinely used to establish relationships between genera and well-resolved species, but may fail to recognize very recently diverged species.

In a Letter to the Editor, Sneath (1993) suggested that the observed discrepancies between phylogenetic and DNA–DNA hybridization data in *Aeromonas* may be a typical example of the lack of sensitivity displayed by 16S rRNA sequence analysis at the species level (Fox *et al.*, 1992). In addition, Sneath (1993) also presented evidence for the occurrence of hybrid events in the form of rare recombinations in ribosomal gene sequences of some *Aeromonas* species. In comparison, Eardly and colleagues (1996) reported a similar type of segment-dependent polymorphic site partitioning as described by Sneath (1993) among species of *Rhizobium* and *Agrobacterium*. Further evidence for the occurrence of intragenomic heterogeneity in the 16S rRNA gene sequences of some *Aeromonas* species suggested that this gene should be used with caution for use in *Aeromonas* phylogeny and identification (Morandi *et al.*, 2005). A phylogenetic reconstruction of the genus *Aeromonas* based on 16S rRNA sequences is shown in Fig. 2.2.

Systematics

Historical aspects

In the years following Sanarelli's first description (1891) of *Bacillus hydrophilus fuscus* as what is now considered a motile *Aeromonas* strain, this organism was allocated to many different bacterial genera. As reviewed by Carnahan and Altwegg (1996), early designations of mesophilic *Aeromonas* spp. include members of the genera *Bacillus, Bacterium, Aerobacter, Achromobacter, Pseudomonas, Proteus,* and *Vibrio*. Chester (1901) emended the name *Bacillus hydrophilus fuscus* Sanarelli 1891 and renamed it *Bacterium hydrophilum,* meaning a bacterium that was 'water loving'. The description of the non-motile aeromonads, on the other hand, was initiated by the work of Emmerich and Wiebel (1894). They reported that an organism named *Bacillus der Forellenseuche* was the cause of epizoonosis in trout, for which Chester (1897) proposed the species name *Bacterium salmonicida*. In contrast to the motile aeromonads, the latter bacterium was able to produce a brown diffusible pigment on trypticase soy agar and did not grow at 37°C.

In 1936, Kluyver and van Niel proposed the genus name *Aeromonas*, meaning 'gas-producing unit', in reference to the suspected similarity of the organism's metabolism with the fermentative characteristics of the genera *Aerobacter* and *Aerobacillus*. Originally, the type species and sole member situated in the new genus was *Aeromonas liquefaciens* Beijerinck. In the seventh edition of *Bergey's Manual of Determinative Bacteriology*, Snieszko (1957) described four species in the genus *Aeromonas* which then still resided in the family

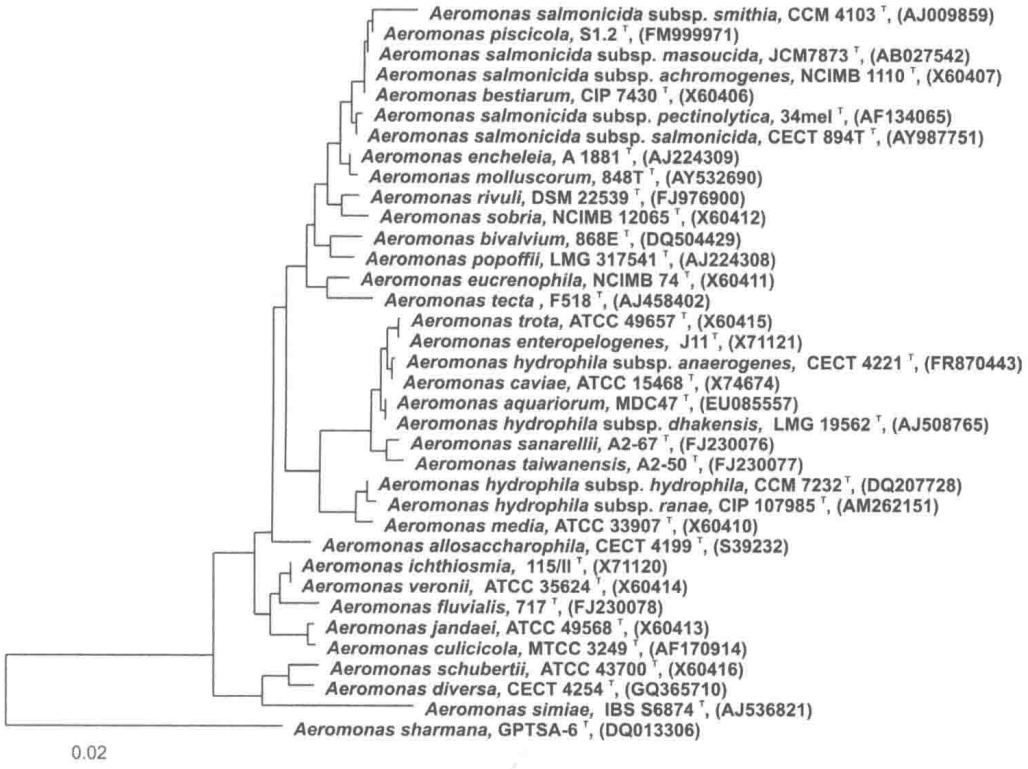

Figure 2.2 Phylogenetic reconstruction of the genus *Aeromonas* and the relative position of *A. sharmana* as extracted from Figure 2.1. The tree is based on 16S rRNA and created using the neighbour-joining algorithm with the Jukes-Cantor correction and 100 runs bootstrapping. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza *et al.*, 2010; http://www.arb-silva.de/projects/living-tree). Scale bar indicates estimated sequence divergence.

Pseudomonadaceae: the three motile species *A. hydrophila, A. punctata,* and *A. liquefaciens,* and the non-motile species *A. salmonicida.*

During the 1960s, the taxonomic structure of the genus *Aeromonas* changed continuously as a result of several phenotypic studies and this was often leading to conflicting conclusions. Eddy (1960) suggested that Snieszko's description of the genus *Aeromonas* in *Bergey's Manual* (1957) should be redefined towards a new, three-species-concept including *A. liquefaciens, A. formicans,* and *A. salmonicida.* Likewise, the proposal of Ewing and colleagues (1961) to classify all motile aeromonads in the species *A. hydrophila* and *A. shigelloides* encountered much resistance. First, Habs and Schubert (1962) suggested to remove the species *A. shigelloides* from the genus *Aeromonas* and reclassify it as *Plesiomonas shigelloides.* Until now, this species remains the only member of the genus *Plesiomonas* (Farmer III *et al.,* 1992). Secondly, Eddy (1962) and Eddy and Carpenter (1964) suggested that the motile *Aeromonas* strains rather belong to the species *A. punctata* and *A. caviae.* These authors argued that *A. punctata,* being a legitimate designation for both *A. liquefaciens* and *A. hydrophila,* should be the type species of the genus *Aeromonas,* and that *A. caviae* was a better name for *A. formicans.* Eventually, Schubert (1968) concluded that *A. liquefaciens* could no

longer serve as the type species of the genus *Aeromonas* and, in agreement with the findings of Eddy and colleagues, conserved this position for *A. punctata*. Smith (1963) proposed to transfer the non-motile *Aeromonas* strains to the new genus *Necromonas* that also comprised the non-pigment-producing species *N. achromogenes*. However, Smith's proposal was not acknowledged by other *Aeromonas* taxonomists.

With the publication of the eighth edition of *Bergey's Manual of Determinative Bacteriology* (1974), another new *Aeromonas* taxonomy was described. In this edition, Schubert compiled all his previously reported findings (1967a,b, 1969), including the division of the genus *Aeromonas* into aerogenic and anaerogenic aeromonads based on their ability to produce gas from glucose. The genus now comprised the motile species *A. hydrophila* (with the subspecies *hydrophila, anaerogenes* and *proteolytica*) and *A. punctata* (with the subspecies *punctata* and *caviae*), and the non-motile species *A. salmonicida* (with the subspecies *salmonicida, achromogenes*, and *masoucida*). In addition, the genus *Aeromonas* was now considered a member of the *Vibrionaceae*, as suggested in an earlier study by Véron (1966).

The foundations of modern *Aeromonas* taxonomy

In 1976, Popoff and Véron performed a taxonomic study on 68 strains of the *A. hydrophila–A. punctata* group in an attempt to improve Schubert's (1974) identification scheme that, according to the former two authors, gave unsatisfactory results with the classification of new motile *Aeromonas* isolates in the *hydrophila–punctata* complex. Based on the numerical analysis of 203 phenotypic characters, the aeromonads under study were classified in two major classes that were actually considered motile *Aeromonas* species. Most strains (62%) belonged to the species *A. hydrophila*, which could be further divided in *A. hydrophila* biovar *hydrophila* and *A. hydrophila* biovar *anaerogenes*, the latter comprising all anaerogenic aeromonads. The remaining 26 strains constituted a second group that did not correspond to any of the previously described species, and these strains were allocated to a new species named *A. sobria*. In the same study, it was argued that *A. punctata* was a later and thus illegitimate synonym for *A. hydrophila*. As a result, the current *Aeromonas* taxonomy still recognizes the latter species as the type species of this genus. Finally, Popoff and Véron also indicated that the halophilic bacterium *A. hydrophila* subsp. *proteolytica* (Schubert, 1974) should be excluded from the genus *Aeromonas*, a suggestion that was readily confirmed at a later stage by transferring this subspecies to the genus *Vibrio* as *V. proteolyticus* (Baumann *et al.*, 1980).

In the years following the phenotypic work of Popoff and Véron, several researchers to further clarified the taxonomic relationships among the existing *Aeromonas* species by means of DNA–DNA hybridizations. McInnes and colleagues (1979) determined in their study that the two main phenotypic groups in the genus *Aeromonas* also corresponded to two legitimate genotypic groups: a diverse group of motile, mesophilic aeromonads and a more homogeneous group of non-motile, psychrophilic aeromonads. Subsequently, Popoff and associates (1981) found at least seven DNA hybridization groups among a collection of 55 motile *Aeromonas* strains. In this respect, the genotypic delineation of three groups in *A. hydrophila* (formerly *A. hydrophila* biovar hydrophila), two groups in the newly proposed *A. caviae* (formerly *A. hydrophila* biovar anaerogenes), and two groups in *A. sobria* by Popoff and co-workers (1981) are now regarded as being the most essential contributions to modern *Aeromonas* taxonomy. These results were adopted by Popoff in the first edition of (1984), in which the author already confronted the reader with two new taxonomic *Bergey's*

Manual of Systematic Bacteriology challenges. First, is was obvious that the individual DNA hybridization groups respectively situated in *A. hydrophila*, *A. caviae*, and *A. sobria* could not be established as new *Aeromonas* species since, at that time, they were phenotypically indistinguishable from one another. A second problem mentioned by Popoff concerned the names of the three *A. salmonicida* subspecies, i.e. subsp. *salmonicida*, *achromogenes*, and *masoucida* (Schubert, 1969, 1974), who were still present in the Approved Lists of Bacterial Names. However, according to the DNA hybridization data of McInnes and colleagues (1979), these three taxa were not sufficiently diverse to warrant their assignation to separate subspecies.

Naming of Popoff's DNA hybridization groups and first emended descriptions and synonyms

Inspired by the pioneering work of Popoff and colleagues (1981), several studies were launched in the 1980s and early 1990s to examine the precise taxonomic position of the various DNA hybridization groups (HGs) in the phenotypically and genotypically heterogeneous species *A. hydrophila*, *A. caviae* and *A. sobria*. In order to find diagnostic markers useful for taxonomic differentiation of HGs in *Aeromonas*, most of these studies relied on a combination of physiological and biochemical characterization with DNA–DNA hybridizations. Based on the guidelines proposed by Wayne and associates (1987), the definition of a HG implies that all constituting strains share ≥7 0% DNA relatedness with ≤ 5% divergence between the related sequences.

According to the chronological order of their first reporting in literature, the HGs originally delineated by Popoff and co-workers (1981) were referred to as HG1, HG2, HG3 (phenotypically resembling *A. hydrophila*), HG4, HG5, HG6 (phenotypically resembling *A. caviae*), HG7 and HG8 (phenotypically resembling *A. sobria*). The pioneering work of Popoff and colleagues resulted in the general usage of the terms phenotypic species or phenospecies (i.e. taxa delineated on the basis of phenotypic characterization) and genospecies, genomospecies, genomic species, or HGs (i.e. taxa delineated by DNA–DNA hybridization) in modern *Aeromonas* taxonomy. As a result, the terms species and phenospecies were long considered interchangeable, whereas HGs were also referred to as geno(mo)(mic)species in *Aeromonas* literature from the 1980s onwards. However, it should be clearly stressed that this apparent duality in *Aeromonas* nomenclature is only relevant in a specific number of cases where referring to only the (pheno)species or the HG designation of a given *Aeromonas* isolate does not fully specify the taxon in question. For example, a group of aeromonads phenotypically identified as belonging to the species *A. hydrophila* may contain members of HG1 and/or HG3. Given the significant difference in the potential clinical relevance of both taxa, an extended identification of *A. hydrophila* isolates down to the HG level may in these cases be an important requirement. Conversely, isolates that were genotypically assigned to HG3 without determining their physiological or biochemical features can belong to either *A. hydrophila* or *A. salmonicida*. Regarding the numbering of *Aeromonas* HGs, a lack of consensus has occasionally been noticed in subsequent species descriptions as one HG designation has been given to several species. For instance, whereas most taxonomists had referred to *A. trota* as *Aeromonas* HG13, researchers at the CDC considered *Aeromonas* Group 501 to be HG13 and have placed *A. trota* in HG14. Alternatively, Esteve and coworkers (1995b) proposed *A. allosaccharophila* to be HG14 although this designation was already assigned to *A. trota* or *Aeromonas* Group 501.