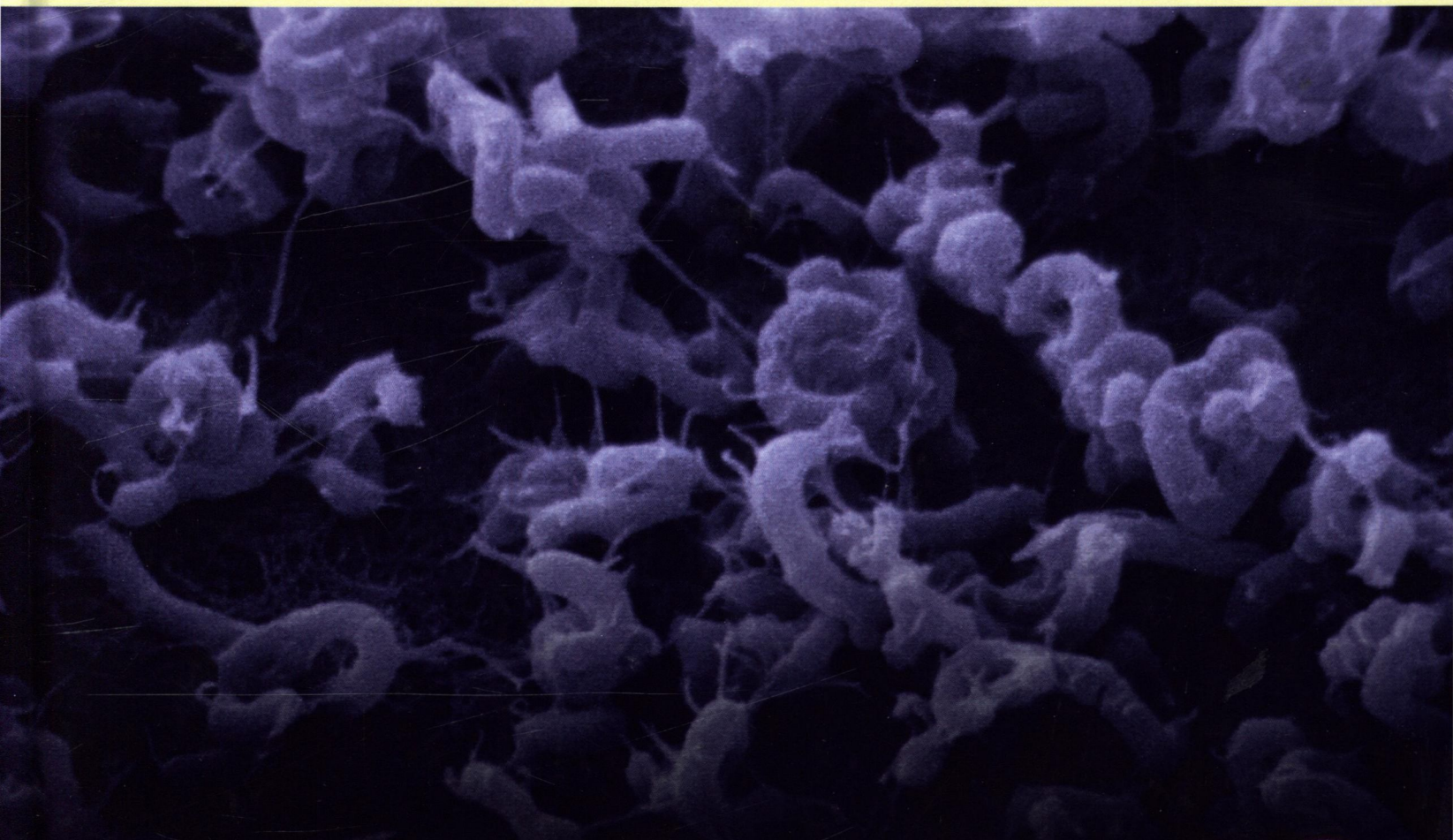


THE BIFIDOBACTERIA AND RELATED ORGANISMS

BIOLOGY, TAXONOMY, APPLICATIONS



Edited by
PAOLA MATTARELLI, BRUNO BIAVATI,
WILHELM H. HOLZAPFEL and BRIAN J.B. WOOD



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BIOLOGY, TAXONOMY, APPLICATIONS

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The Bifidobacteria and Related Organisms: Biology, Taxonomy, Applications presents authoritative reviews covering different aspects of bifidobacteria and other genera classified with them. The book begins with some fundamental aspects of taxonomy underlining the current status of the phylum *Actinobacteria*, genus *Bifidobacterium*, and family *Bifidobacteriaceae*. Also included are lists of *Bifidobacterium* species and subspecies, which have increased greatly in number in recent years. Additionally, detailed advice is supplied for culture media and culture conditions for detection of bifidobacteria in different environments, their cultivation and their storage. The impact of genetics in bifidobacterial studies is also considered. The genus *Bifidobacterium* is being increasingly recognized as particularly friendly for humans, and, increasing evidence suggests, for most, if not all mammals, and possibly for other animals.

Key Features

- Provides an understanding of the major differences between bifidobacteria and lactic acid bacteria
- Provides information on the relationships between bifidobacteria and their host
- Describes 54 species and 10 subspecies of bifidobacteria and highlights the characteristics of the seven new taxa belonging to the so-called scardovial genera, describing the features of genus and species
- Explains the nutritional requirements of bifidobacteria, bifidogenic effect of particular substrates, milk oligosaccharides and carbohydrate metabolism
- Includes applications and technological considerations placed alongside the more academic matters such as nomenclature and phylogeny

About the Editors

Dr. **Paola Mattarelli** has 20-years' experience on the taxonomy, functional properties and microbial ecology of *Bifidobacteriaceae*, especially in human and animal host interactions; other fields of interests are traditional fermented food and interaction between natural substances and microbiota. She is a member of the "Subcommittee on *Bifidobacterium*, *Lactobacillus* and related organisms" (International Committee on Systematic Bacteriology).

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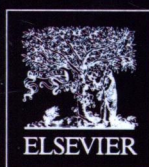
Dr. **Brian J.B. Wood**, although fully retired, has continued his interests in food fermentations and the Lactic Acid Bacteria and cognate genera by writing review articles and editing books with various colleagues, most recently *The Lactic Acid Bacteria: Biodiversity and Taxonomy* with Professor W. H. Holzapfel.

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MATTARRELLI, BIAVATI,
HOLZAPFEL and WOOD



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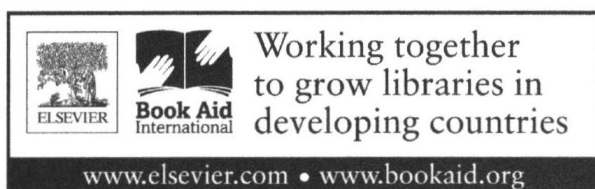
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THE BIFIDOBACTERIA
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Preface

This book is dedicated to *Bifidobacterium*, one of the bacterial genera most agreeable to humans and, as evidence increasingly suggests, most agreeable for the majority, if not all, mammals.

What prompted the editors to submit the proposal for this new book was the awareness, supported by scientific evidence, of a growing interest in the role of beneficial microorganisms and, in particular, of bifidobacteria. This book presents authoritative reviews covering different aspects of bifidobacteria and other genera classified with them.

Chapters 1, 2, and 3 introduce the reader to some fundamental aspects of taxonomy, underlining the current status of the phylum Actinobacteria, genus *Bifidobacterium*, and family Bifidobacteriaceae. Chapter 1 introduces the reader to the great differences between bifidobacteria and lactic acid bacteria. Although long assumed to be closely related, modern evidence shows that they are phylogenetically far removed and, in fact, members of completely different phyla.

In recent years, the number of bifidobacterial species has greatly increased; currently 54 species and 10 subspecies are described. Lists of *Bifidobacterium* species and subspecies are available in Chapter 2. For each one a description and updated information from the literature are presented.

Chapter 3 examines the family Bifidobacteriaceae. It highlights the characteristics of the seven new taxa belonging to the so-called scardovial genera, describing the features of genus and species. The chapter also includes a short remembrance of Professor Vittorio Scardovi, as recognition for the scientific importance of his work.

Chapters 4–11 deal with physiological and biochemical aspects, providing essential information for a better understanding of bifidobacteria. Detailed advice is given for culture media and culture conditions for the detection of bifidobacteria in different environments, their cultivation, and their storage.

Microbial chemistry is an important tool for identifying many major structural components and for understanding their functional role in the physiology of bacterial cells. Bifidobacteria are saccharolytic and derive their energy by fermentation, mainly from carbohydrates, such as lactose, the most abundant solid constituent of breast milk, or from “indigestible” oligosaccharides of plant origin. Multiple bifidobacterial species in the infant gut could be explained by specific human milk oligosaccharide consumption strategies. The number of published bifidobacterial genome sequences continues to grow, bringing a better understanding of the characteristic metabolic traits and key functions of the various species. Three chapters are devoted to the nutritional requirements of bifidobacteria, bifidogenic effect of particular substrates, milk oligosaccharides, and carbohydrate metabolism.

Gut microbiota composition is an important health marker. Bifidobacteria are considered to be beneficial microorganisms. Studies on stress responses to oxygen and bile acid, the two major environmental stresses, provide information on their effects on growth and fermentation reactions of these anaerobes.

Humans depend on externally supplied folate, and folate-producing bifidobacteria can be an important source of this vitamin for the host. A growing use of bifidobacteria for probiotics can also help alleviate the global nutritionally important health problem of folate deficiency.

Exopolysaccharides are present on the surface of many bacteria, including *Bifidobacterium*. Their role in the colonization of their natural habitats and the cross-talk among bifidobacteria and host indicate their importance.

Bifidobacteria are believed to have coevolved through beneficially influencing the health of their human host. Studies on their ecological distribution and genetic adaptation are essential to verify the hypotheses of coevolution. Genetic manipulation technologies in bifidobacteria and applications of currently available systems represent a topic with extraordinary growth potential in the near future. Administration of bifidobacteria in clinical trials for therapeutic purposes points out the variability linked to the strains used, which should stimulate the isolation of new strains with possibly new potential applications. Bifidobacteria are presently applied for therapeutic purposes in treating some pathogenic infections; at the same time, they are considered a hope for the future as an additional resource for human health.

The immunological relevance of bifidobacteria shown by the immunomodulatory ability for the host is opening a new frontier for a rational modification of gut microbiota using specific bifidobacterial strains, to modify the immune responses not only in inflammatory or autoimmune disorders, but in other pathologies, such as cancer.

A growing field is the use of bifidobacteria as health support in animal nutrition. One of the best examples currently available shows that these beneficial symbiotic bacteria (present in the honey stomach) possess antimicrobial characteristics and produce bioactive metabolites that protect honeybees against pathogens and also explain the therapeutically significant properties of honey.

Production of probiotic bifidobacteria is more than just growing biomass. Many parameters affect their growth and stability, and the expression of their desired properties. These properties should therefore be considered early in the development of new probiotic strains.

The term “probiotic” is raising a worldwide debate. A chapter with an overview of the definitions of the three terms: prebiotics, probiotics, and synbiotics, from both a scientific and a regulatory point of view, was therefore considered a necessary part of this book.

As this preface reflects, the most important aspects of bifidobacteria were taken under consideration. The editors thank the publisher, who welcomed our proposal, as well as the authoritative international team of authors who made possible the realization of this book.

The Editors

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The Phylum Actinobacteria

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Ac.ti.no.bac.te'ri.a. Gr. n. *actis* -inos a ray, beam, N.L. n. *bacter* a rod; suff. -ia ending denoting phylum; N.L. pl. neut. n. *Actinobacteria* actinomycete bacteria with diverse morphologies.

1.1 INTRODUCTION

The focus of this book is the genus *Bifidobacterium* and related organisms located within the family *Bifidobacteriaceae* that encompasses the genera: *Aeriscardovia*, *Alloiscardovia*, *Bifidobacterium*, *Bombiscardovia*, *Gardnerella*, *Pseudocardovia*, *Neocardovia*, *Pariscardovia*, and *Scardovia* (Biavati and Mattarelli, 2012; Downes et al., 2011; García-Aljaro et al., 2012; Huys et al., 2007; Jian and Dong, 2002; Killer et al., 2010, 2013; Simpson, 2004). These taxa will be covered in subsequent chapters; this chapter introduces the phylum *Actinobacteria* where the family *Bifidobacteriaceae* is phylogenetically located (Gao and Gupta, 2012; Ventura et al., 2007; Zhang et al., 2016). The phylum is comprised mainly of Gram-positive staining organisms with a high G + C DNA content (>55 mol.%) and constitutes one of the largest phyla within the domain *Bacteria* (Embley et al., 1994). A comprehensive review of all taxa within *Actinobacteria* is beyond the scope of this chapter and the reader is encouraged to review the primary literature where cited.

1.2 HISTORICAL BACKGROUND

Although the focus of this book is the genus *Bifidobacterium* and close relatives contained within the family *Bifidobacteriaceae*, it would be remiss not to first discuss *Lactobacillus* and its history with *Bifidobacterium*. Both genera are routinely recovered from gastrointestinal (GI) and genital tracts of humans and animals, feces, and sewage; but it is their abundance and possible health-promoting effects in the human GI tract where their associations have been most studied (Bondarenko, 2006; Felis and Dellaglio, 2007; Kailasapathy and Chin, 2000; Paliy et al., 2009; Reuter, 2001; Turrone et al., 2008). Superficially, both genera physiologically resemble each other being saccharoclastic and produce lactate and acetate as major end products of fermentation and are generally regarded as lactic acid bacteria (LAB) (Felis and Dellaglio, 2007; Holzapfel and Wood, 2014; Reuter, 2001). Therefore, it is not surprising that the members of these genera have been confused. However, several important traits can be used to differentiate between these taxa, for example, (1) the DNA mol.% G + C of bifidobacteria is much higher than lactobacilli (>60% compared to 33%–39%), (2) the hexose bifidum shunt and the presence of fructose-6-phosphate phosphoketolase (F6PPK), a key enzyme unique to the genus *Bifidobacterium* (Biavati and Mattarelli, 2006; Scardovi and Trovati, 1965). But it is worth noting that in the seventh edition of *Bergey's Manual of Determinative Bacteriology* (Breed et al., 1957) bifidobacteria were included in the genus *Lactobacillus* (Breed et al., 1957). It was not until the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974), that bifidobacteria were classified as a separate genus *Bifidobacterium* (Biavati and Mattarelli, 2012; Sgorbati et al., 1995).

Furthermore, it was not until the application of molecular tools and in particular 16S rRNA gene sequencing that the true relationships were revealed and demonstrated the genera *Lactobacillus* and *Bifidobacterium* were phylogenetically far removed (Fox et al., 1980; Woese, 1987). Lactobacilli were shown to be related to low mol.% G + C Gram-positive bacteria, such as clostridia and relatives while bifidobacteria were shown to be distantly related to this group being related to high mol.% G + C *Actinomyces*.

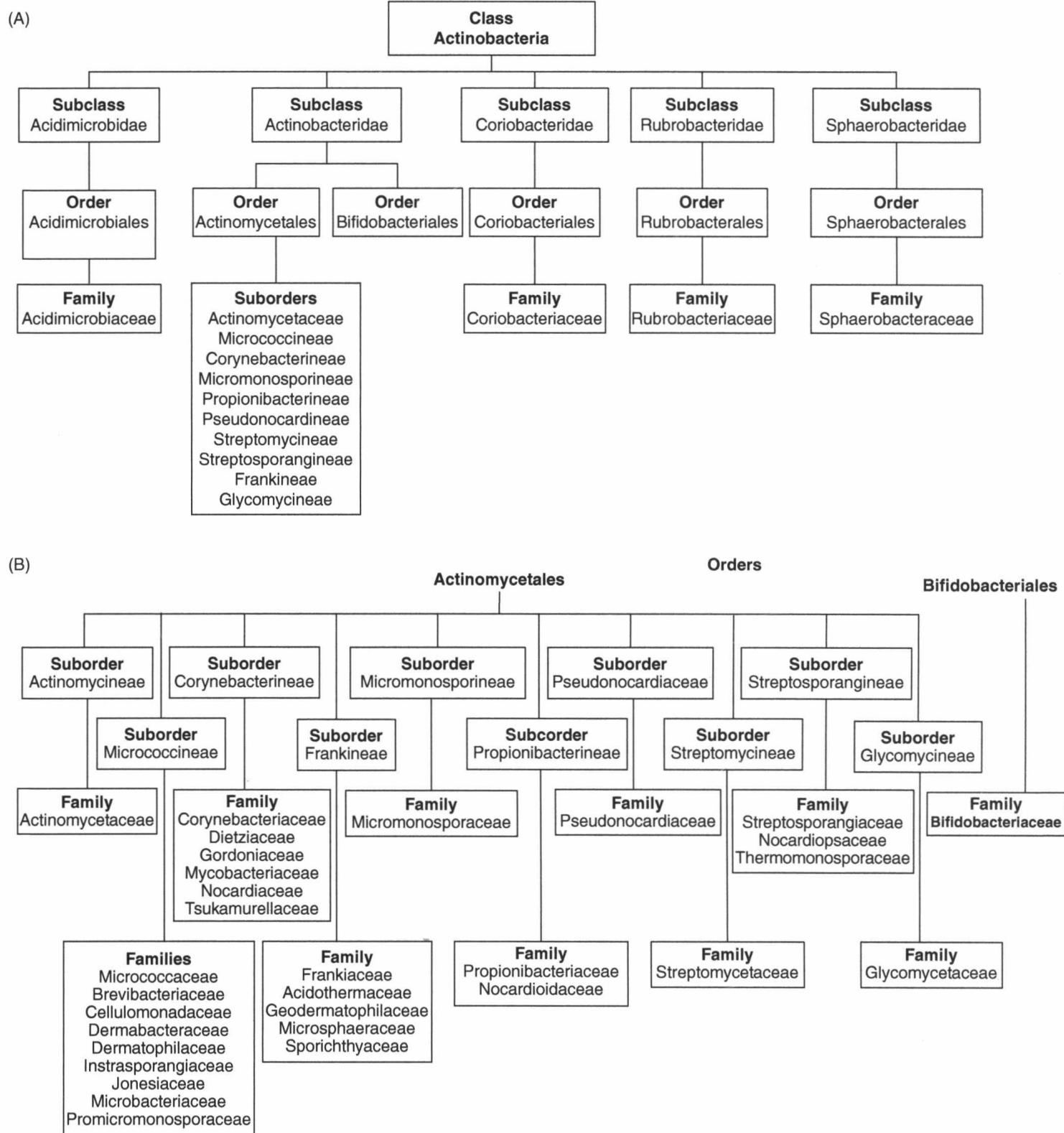


FIGURE 1.1 Phylogenetic classification system for *Actinobacteria* as proposed by Stackebrandt et al. (1997a,b). (A) Assignment of the ranks subclass, order, and family; (B) assignment of suborders and families of the order Actinomycetales.

This revelation resulted in a series of pivotal taxonomic proposals. First, Stackebrandt et al. (1997a) proposed a novel phylogenetic hierarchical system for “*Actinomyces*” solely based on 16S rDNA/rRNA sequences (Fig. 1.1A–B). The proposal included several taxonomic ranks between genus and the class *Actinobacteria*. In addition to the clustering of sequences, the delineation of taxa was accompanied with taxon-specific 16S rDNA/RNA signature nucleotides (Stackebrandt et al., 1997a). This provided a solid foundation for the taxonomy of this group and over the next decade many novel members were assigned to this class, leading Zhi et al. (2009) to update the signature nucleotides. In addition to these nucleotide signatures, several taxonomic ranks were also proposed with suborders