On monospecific genera in prokaryotic taxonomy

Guanghong Zuo, Bailin Hao[*]

T-Life Research Center and Department of Physics, Fudan University, Shanghai 200433, China

ABSTRACT

A monospecific genus contains a single species ever since it was proposed. Though formally more than half of the known prokaryotic genera are monospecific, we pick up those which actually raise taxonomic problems by violating monophyly of the taxon within which it resides. Taking monophyly as a guiding principle, our arguments are based on simultaneous support from 16S rRNA sequence analysis and whole-genome phylogeny of prokaryotes, as provided by the LVTree Viewer and CVTree Web Server, respectively. The main purpose of this study consists in calling attention to this specific way of global taxonomic analysis. Therefore, we refrain from making formal emendations for the time being.

© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Microbiology has entered the era of Big Data. The number of known small-subunit ribosomal RNA (SSU rRNA) sequences reaches several millions, as reflected by the ARB-SILVA [1] and RDP [2] projects. The number of sequenced prokaryotic genomes now exceeds 120,000 and grows daily, see GOLD [3] for a timely summary. As of early January 2017, there were 2552 Archaea and Bacteria genus names and 14,621 species names (without counting subspecies) collected in the List of Prokaryotes with Standing in Nomenclature (LPSN) [4]. These numbers provide a kind of upper bounds as they contain some validly published but defunct names.

Traditionally, prokaryotic taxonomy has been centered around designated species or taxa. Now the availability of these Big Data adds a global angle to look at the issue. It is a common practice that most new genera when first proposed consist of a single or two species. With the advance of microbiology in exploring various ecological niches the monospecific status of many genera would change. However, some genera may remain monospecific over many years. Fig. 1 shows the number of monospecific genera listed in LPSN (July 2017) versus the year of discovery. The leftmost circle in Fig. 1 represents a species *Beggiatoa alba*; it was first described by

J. P. Vaucher in 1803 and assigned to genus *Beggiatoa* by V. Trevisan in 1845. The sharp rising part of the curve in Fig. 1 would keep moving rightwards in forthcoming years and special attention should be put to the flat left part of the curve, i.e., those genera which remain monospecific for a long period of time.

In the present work, we examine some of the monospecific genera in prokaryotic taxonomy which may eventually necessitate taxonomic revisions. A search of LPSN reveals that 1320 genera, i.e. more than half of the total 2552, have only a single species listed. In other words, they seem to be monospecific genera. However, as the concept of prokaryotic species has been subject to long debate, we shall not touch on the definitive aspect of a taxon being monospecific or not. To this end an extreme example is provided by the genus *Brucella* which has been described manifestly as a monospecific genus in a "validly-published" way [5]. Whereas the recognition of this taxon being a monospecific or a multi-specific genus has finally left to individual microbiologists as preferring one or another taxonomic opinion (see related notes in LPSN [4] for details). Instead, we pick up those cases where a seemingly monospecific genus violates the monophyly of a broader genus and thus creates undoubtedly a taxonomic problem.

2. Materials and methods

Methodologically, we start from two distinct and independent kinds of phylogenetic trees, namely, the All-Species Living Tree (LVTree) based on alignment of high-quality 16S rRNA sequences [6–8] and the Composition Vector Tree (CVTree) based on
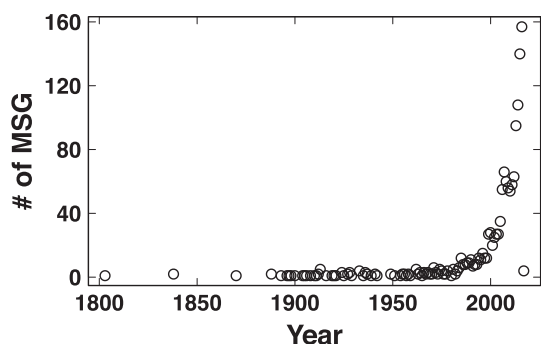
Fig. 1. Number of monospecific genera versus year of discovery according to LPSN (July 2017). The leftmost circle represents *Beggiatoa alba* discovered in 1803, which, however, no longer keeps monospecific status as a new species has been validly published quite recently.

alignment-free comparison of whole prokaryotic genomes [9—12].

CVTrees are constructed by using the latest release of the CVTree Web Server [13]. Instead of the 3000 plus *Bacteria* and *Archaea* genomes provided in the built-in dataset of the publically available CVTree Web Server, we use 8000 to 10,000 genomes with as wide as possible taxonomic distribution as a background in any study of a designated group of species. In this way the overall results appear to be extremely stable and reproducible. As the algorithm behind the CVTree method and the features of the Web Server have been described repeatedly in literature over the years, we refer the readers to the published papers [9—12] and to the online help file that comes with the CVTree3 Web Server [13].

The latest release of LVTree (as of February 2017) is built on 475 *Archaea* and 12,478 *Bacteria* 16S rRNA sequences [14]. In order to compare the branching order of LVTree at all taxonomic ranks from phyla down to species, we have transplanted most of the distinctive features of the CVTree Web Server to a LVTree Viewer [15]. Among the important and convenient features we indicate the possibility of making trial lineage modifications and automatic reporting monophyly/none-monophyly summary of taxa at all ranks of the taxonomic hierarchy. It is desirable to emphasize from the outset that CVTree and LVTree are independent in the input data and in the underlying methodology. There is no a priori reason that the two approaches should yield identical results. The fact that a cluster of leaves in one tree agrees topologically with that in another tree adds weight to the objectiveness of the results. In this study we rely on facts compatible in both kinds of trees. Since LVTree is built with emphasis on type strains while CVTree is based on available sequenced genomes, one cannot expect that one and the same taxon name in both trees corresponds to an identical organism. Nonetheless, this tolerance is understood in taxonomy as classification scheme always concerns populations designated to a taxon, not restricting to individual organisms.

In making comparison of a cluster of leaves in a tree with a taxon in a classification scheme a guiding principle is the notion of monophyly. Coined by Ernst Haeckel in 1866 and stemmed from zoology, the original definition of monophyly requires the knowledge on an ancestor and all its descendants, see, e.g., discussion of James Farris over the years [16,17]. Obviously, this requirement does not apply to prokaryotes which are overwhelmingly reproduced asexually. Therefore, we adopt a pragmatic point of view on monopoly by restricting ourselves to the input dataset with a certain reference taxonomy. If in the reference taxonomy species designated to a taxon appear only in that taxon and do not present in any other taxa, then the taxon is said to be monophyletic. If in a tree branch all leaves come exclusively from a reference taxon and no species from that taxon fall in any other branch, the branch is said

to be monophyletic. In other words, monophyly is a reciprocal notion with respect to both phylogeny and taxonomy. Monophyly of a tree branch or a taxon may change when new species appear in the input dataset. Only monophyletic taxa are considered to be well-defined and acceptable in a flawless taxonomy.

We have mentioned reference taxonomy. For the time being we have in mind the combined use of the following resources:

1. The second edition of Bergey's Manual of Systematic Bacteriology [18] which had been completed in the years 2001—2012. The Bergey's Manual Trust made it clear that further editions of the Manual would be electronic. In fact, the framework of the electronic edition of Bergey's Manual of Systematics of Archaea and Bacteria, abbreviated as BMSAB, appeared in 2015 [19]. For the time being it is only a framework for an electronic edition, as the content of many chapters appears to be identical to their counterparts in Ref. [18] without essential updating.

2. The 4th edition of the multi-volume treatise Prokaryotes [20], especially, the 6 vol on prokaryotic taxonomy published in 2014. These volumes are organized around families and some entries are more updated than the Bergey's Manuals.

3. List of Prokaryote Names with Standing in Nomenclature [4]. This constantly updating list collects names and taxonomic proposals validly published in International Journal of Systematic and Evolutionary Microbiology and a few other periodicals. In using LPSN one should be aware of possible redundancy of the list, as some defunct or erroneous names may be kept until new emendations are officially published.

   Please note that all the above three resources are mainly based on 16S rRNA sequence analysis and thus naturally inherit the limitations of the latter, especially, as compared with the whole-genome-based approaches.

4. Besides the above resources we also make use of the EzBioCloud database maintained by the Chun Lab in Korea [21]. Though still based on 16S rRNA sequence analyses, EzBioCloud pay more attention to genome data. In the latest release of 11 May 2017 [22] it contains 82,605 quality-controlled genome sequences and 62,685 16S rRNA sequences. More importantly, the taxonomic assignments in EzBioCloud are not restricted by published information. In general, these assignments better reflect the actual positions in phylogenetic trees and thus, in most cases, happen to be closer to the CVTree results. This point will be demonstrated by our first example below on monospecific genera within the genus *Pseudomonas*.

3. Monospecific genera that call for taxonomic modifications

3.1. Monospecific genera related to Pseudomonas

It is instructive to commence with *Pseudomonas*. Historically, *Pseudomonas* has caused many taxonomic confusions. The species collected under the genus *Pseudomonas* in the 1st edition of the Bergey's Manual of Systematic Bacteriology [23] were subdivided into more than 10 genera in the following years [24]. Not long ago it was stated in a footnote of a well-known book [25] that members of the *Pseudomonas* genus might be assigned to several different classes (*Alpha-*, *Beta-* or *Gamma-*) within the phylum *Proteobacteria*. However, nowadays the majority of *Pseudomonas* species behaves well by forming monophyletic branches in both LVTree and CVTree mainly due to a number of well-founded taxonomic revisions, among which we make emphasis on those dealing with monospecific genera.

First of all, a "new" genus *Serpens* was suggested in 1977 with a single species *Serpens flexibilis* [26] described. In later years it has

been observed in both 16S rRNA-based and whole-genome-based phylogenetic trees that *Serpens flexibilis* locates deeply inside the genus *Pseudomonas* and thus violating the monophyly of the latter. Eventually, a formal proposal was put forward to reclassify *Serpens flexibilis* as *Pseudomonas flexibilis* comb. nov., with *Pseudomonas tuomuerensis* as a later heterotypic synonym [27]. This proposal is confirmed by Fig. 2, a subtree cut from a big CVTree based on 298 *Archaea* and 7878 *Bacteria* genomes. The neighborhood of *Pseudomonas flexibilis* and *Pseudomonas tuomuerensis* has been expanded within the context of 136 Pseudomonas genomes while all other branches maximally collapsed.

The presence of an *Azotobacter* cluster within *Pseudomonas* in Fig. 2 does not bring about any problem. It simply adds new support to a ten-year-long proposal to consider *Azotobacter* and *Pseudomonas* as synonyms [28]. As a result there is in fact a monophyletic cluster containing 136 genomes with the two *Azotobacter* strains counted as members of *Pseudomonas*.

Anticipating the forthcoming discussion on *Pseudomonas* in LVTree, we note that the genus *Azotobacter*, in fact, should have absorbed the genera *Azomonas*, *Azorhizobium* and *Azomonotrichon*, all located within *Pseudomonas* in some essentially obsolete classification schemes, see, e.g. [29], and related Notes in LPSN [4]. If one examines the neighborhood of *Pseudomonas flexibilis* in LVTree, the situation turns out to be more intricate for several reasons. First, the number of *Pseudomonas*-related organisms represented by 16S rRNA sequences is more abundant than the number of sequenced genomes. Second, in LVTree many entries are not taken into account by design of the project, e.g., the absence of genus name with *Candidatus* status or without standing in nomenclature. Third, some published taxonomic revisions have not been accepted by the LVTree team. Consequently, in the original LVTree, without invoking any lineage modification, the *Pseudomonas* species are far from making a monophyletic cluster (figure not shown). In fact, they scatter into several genera, including:

1. *Pseudomonas geniculate*, *Psudomonas beteli*, *Psudomonas hibiscicola*, and *Psudomonas pictorum* get inserted into the genus *Stenotrophomonas*.
2. The species *Pseudomonas boreopolis* locates in the genus *Xylella*.
3. *Pseudomonas carboxydohydrogena* gets into the genus *Afipia* in the class *Alphaproteobacteria*, a cross-class discrepancy as majority of the *Pseudomonas* strains belongs to the class *Gammaproteobacteria*.
4. Although as early as in 1997 there appeared a suggestion to reclassify *Pseudomonas cissicola* to the genus *Xanthomonas* [30], LVTree and LPSN did not accept this proposal for some reason. Therefore, *Psudomonas cissicola* appears as an outlier in LVTree.
5. *Pseudomonas halophila* clearly does not join the main branch of *Pseudomonas* species, but gets closely to the genus *Halovibrio*.
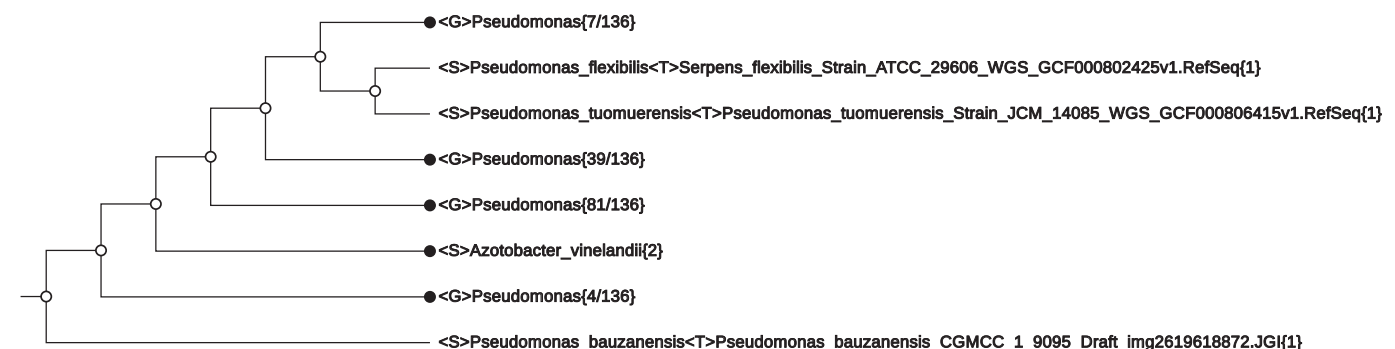
It is remarkable that in the EzBioCloud database [21] all the species listed above have been taken out of *Pseudomonas* and assigned to a corresponding genus such as *Stenotrophomonas*, *Xylella*, *Xanthomonas* or *Halovibrio*, in agreement with branching seen in LVTree. Implementing these assignments using the lineage modification feature of the LVTree Viewer [15], one gets a monophyletic genus *Pseudomonas*{179}, witnessing our earlier statement on *Pseudomonas* taxonomy having reached a well-behaved unification status.

We note that in the latest release of LVTree (February 2017, [22]) one did not see *Pseudomonas tuomuerensis* juxtaposed with *Pseudomonas flexibilis*, because [22] appeared after the publication of [27] so *Pseudomonas tuomuerensis* dropped out from the LVTree dataset as a synonym to *Pseudomonas flexibilis*. However, there is a new monospecific genus *Thiopseudomonas* [31] violating the monophyly of *Pseudomonas*, a situation resembling that of *Serpens flexibilis* a few years ago. We have made a lineage modification that treats *Thiopseudomonas* as a synonym to *Pseudomonas*. Unfortunately, the genomic sequence of the *Thiopseudomonas* strain is unavailable for the time being; otherwise similar lineage modification would be made and checked in CVTree. Therefore, we leave this point as a test case for the approach of this paper.

3.2. Monospecific genera in phylum Actinobacteria

In volume 5 of the 2nd Edition of the Bergey's Manual on Systematic Bacteriology [18], entirely devoted to the phylum *Actinobacteria*, 82 genus names were listed as monospecific. Within a few years after the volume saw the light in 2012, due to discovery of new species, at least 21 genera ceased to be monospecific. From the remaining cases we pick up a few prominent examples that manifestly violate monophyly of other genera in both LVTree and CVTree. An unambiguous example is provided by *Turicella otitidis*, discovered in otitis media and proposed as a new genus in 1994 [32]. In 16S rRNA-based LVTree *Turicella otitidis* clearly gets deeply into the genus *Corynebacterium*, see Fig. 3 below.

Two *Turicella otitidis* genomes have been sequenced so far, enabling the checking of its position in CVTree. Fig. 4 was cut from a big CVTree. The two *Turicella otitidis* strains turn out to locate in the depth of *Corynebacterium* species as well.

Figs. 3 and 4, taken together, suggest strongly that *Turicella otitidis* does not form a separate genus; it is actually a member of the genus *Corynebacterium*. However, given the medical importance and the wide-spread usage of the name, see, e.g. [33], it is inappropriate to change the name now. Nonetheless, in a phylogeny-based taxonomy *Turicella otitidis* should be treated as a synonym to a *Corynebacterium* species without assigning it to a new genus.

Next, we consider a few monospecific "genera" located within

Fig. 2. *Serpens flexibilis* reclassified as *Pseudomonas flexibilis* in consistency with its status being a synonym of *Pseudomonas tuomuerensis*. This figure was cut from a big CVTree based on 298 *Archaea* and 7878 *Bacteria* genomes. For the fact that the *Azotobacter* cluster does not make a problem see the text.
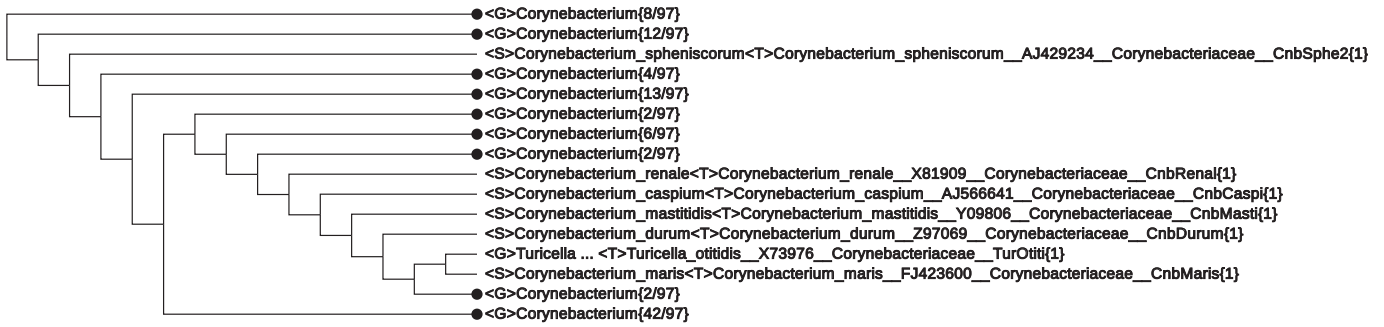
Fig. 3. *Turicella otitidis* locates deeply inside the genus *Corynebacterium* in LVTree, violating monophyly of the latter.
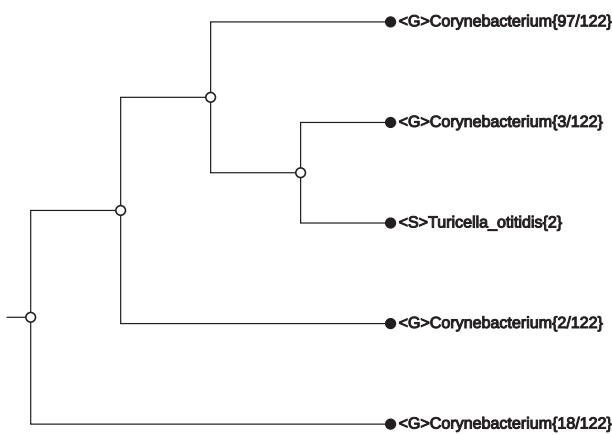

Fig. 4. *Turicella otitidis* strains get into the depth of the genus Corynebacterium in CVTree. The total number 122 includes also the two *Turicella otitidis* genomes.

the genus *Arthrobacter*. Putting aside the taxonomy of *Arthrobacter* as a whole, which is now undergoing essential changes, e.g., 5 new genera dealing with more than 30 species have been reclassified recently [34], we only look at two monospecific genera *Renibacterium* and *Acaricomes*, among many others. The corresponding branch in CVTree is given in Fig. 5:

As shown in Fig. 5, the two monospecific genera locate deeply inside the genus *Arthorbacter*. In order to test the rationality to treat them as members of *Arthrobacter*, let us check the situation in LVTree. Being disturbed by many paraphyletic insertions, the *Arthrobacter* species are far from forming a monophyletic cluster in
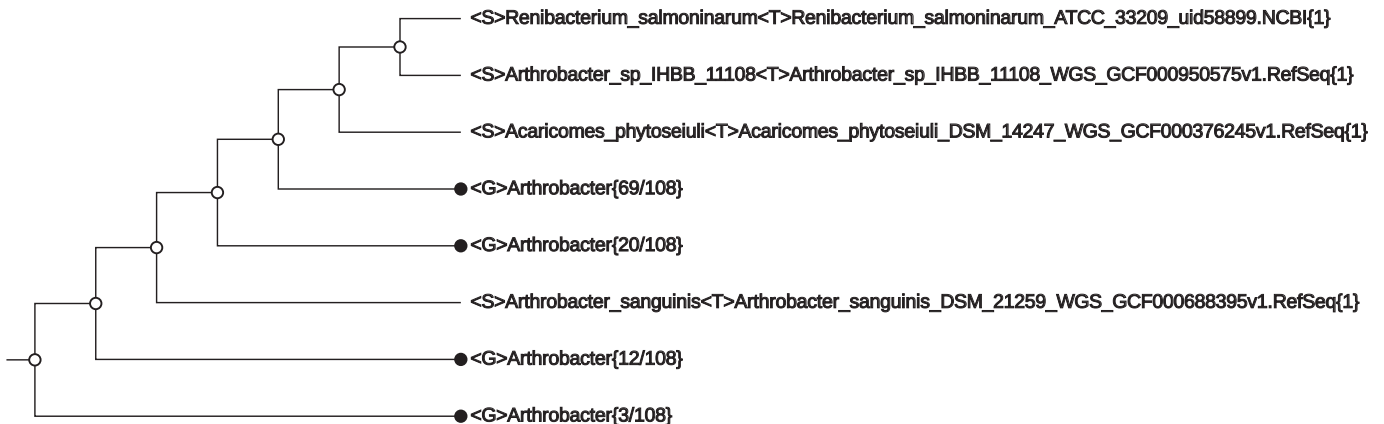
LVTree. Fig. 6 shows a small inner part of the mostly paraphyletic cluster containing the *Arthrobacter* species.

A striking point in Fig. 6 consists in the monophyletic family *Bifidobacteriaceae* getting into the innermost position of the cluster, clearly violating the monophyly of the *Arthrobacter* species. The relative abundance of paraphylies in LVTree as compared to monophylies in CVTree is a manifestation of low resolution power of the 16S rRNA sequence analysis to be elucidated in more details elsewhere. However, it does not invalidate the suggestion to consider *Renibacterium* and *Acaricomes* as species of *Arthrobacter* instead of being monospecific genera themselves.

3.3. Monospecific genera associated with Firmicutes

Historically, the phylum *Firmicutes* has played the role of a huge container, from which many new taxa of different ranks have been taken out. In the 1st edition of the Bergey's Manual of Systematic Bacteriology this phylum comprised three classes: *Bacilli*, *Clostridia*, and *Mollicutes*. In the 2nd edition of The Manual the *Mollicutes* was extracted from *Firmicutes* to become a new phylum *Tenericutes* and a new class *Erysipelotrichia* was added to *Firmicutes*. Soon after another new class *Negativicutes* was proposed [35] to accommodate the Gram-negative strains within the basically Gram-positive *Firmicutes*. From the numerous monospecific genera defined under *Firmicutes* we pick up a few which satisfy our criterion of leading to taxonomic problems by violation monophyly of some otherwise well-behaved taxa.

3.3.1. Monospecific genera within class Clostridia

A substantial majority of taxonomic confusions in phylum *Firmicutes* comes from the class *Clostridia*, in particular, from the
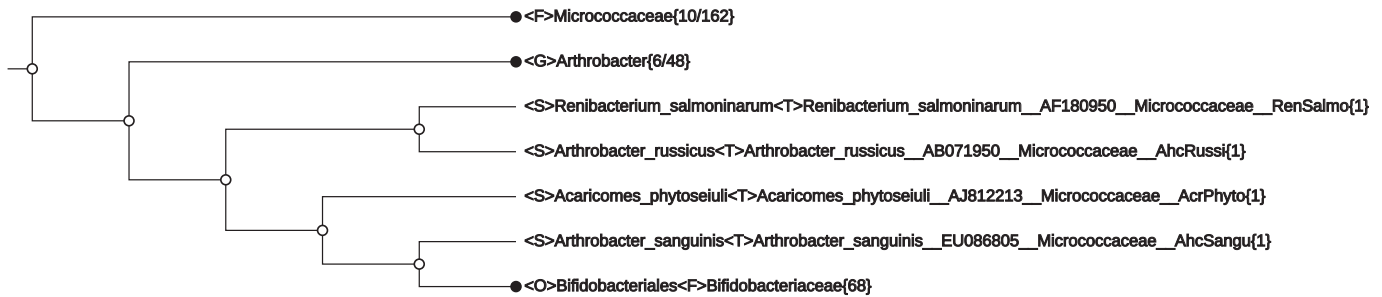

Fig. 5. The genera *Acaricomes* and *Renibacterium* locate in the depth of *Arthrobacter* and violating the monophyly of the latter in CVTree.

Fig. 6. A small inner part of the mostly paraphyletic cluster containing the *Arthrobacter* species in LVTree.

genus *Clostridium*. Though many new genera and species have been taken out from *Clostridium* over the years (see, e.g., [36]), this old (proposed in 1880) and "big" (220 species and 5 subspecies names listed in LPSN [4]) genus is still far from being monophyletic neither in phylogeny nor in taxonomy. Besides a large cluster corresponding to so-called *Clostridium sensu stricto* there are many smaller clusters of various sizes scattered among other more or less established taxa. Reclassification of some genuine monospecific genera may help to bring the taxonomy of *Clostridium* to a better shape.

We start from examining *Thermobrachium*, a monospcific genus since 1996 [37] with a genome sequenced and published later. Fig. 7 shows how it gets into the depth of genus *Caloramator* in LVTree.

We note that in the EzBioCloud taxonomy [21] *Thermobrachium celere* has already been assigned to the genus *Caloramator*. Introducing this lineage modification in LVTree, we get a monophyletic *Caloramator*{10}. It is interesting to extend the observation to the close neighborhood of *Caloramator* in LVTree, as shown below in Fig. 8:

It is worth noting that EzBioCloud database [21] has assigned *Clostridium cylindroporum* to the genus *Caloramator*. This assignment hints on the two monophyletic genera *Fervidicella* and *Fonticella* being members of *Caloramator* as well. Since there is no genome for *Fonticella* at present time, we can only check the position of *Fervidicella* and *Clostridium cylindroporum* in CVTree (Fig. 9).

Taking into account that EzBioCloud database takes *Clostridium cylindroporum* as a member of *Caloramator*, Fig. 9 strongly suggests that *Fervidicella* is also a member of *Caloramator*. Therefore, the whole Fig. 9 may be collapsed to a monophyletic *Caloramator*{8}. We mention in passing that in both the CVTree and LVTree the monophyletic genus *Calomarator* may serve as a pointer to the largest cluster of the *Clostridium* species, namely, the group *Clostridium sensu stricto* in future studies.

Now we examine the genus *Desulfotomaculum* in family *Peptococcaceae* in the order *Clostridiales*, see Figs. 10 and 11 for CVTree
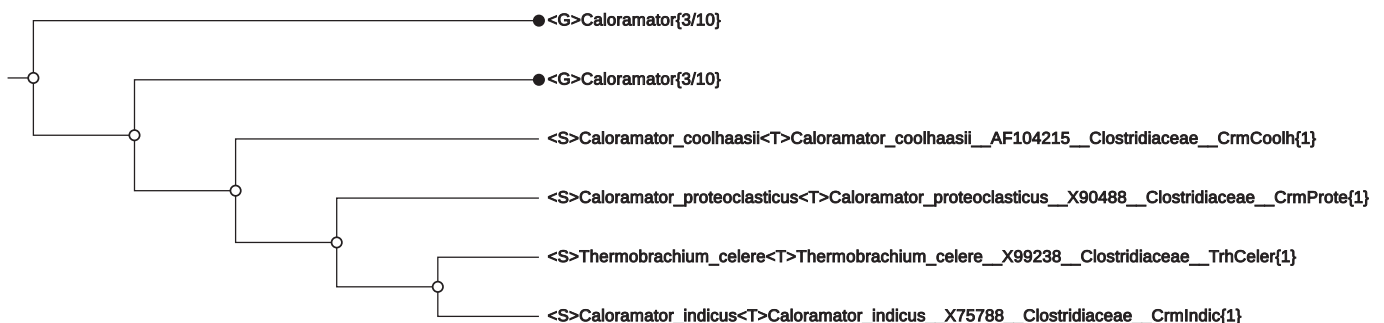
and LVTree, respectively. In both figures, the two monospecific genera, *Desulfovirga* and *Desulfurispora*, definitely get into the depth of *Desulfotomaculum* and violate monophyly of the latter.

Supported by both CVTree and LVTree, one can absorb the two monospecific genera *Desulfovirgula* and *Desulfurispora* into *Desulfotomaculum*. As a long-pointed out misclassification [38] of *Desulfotomaculum guttoideum* [39] remains uncorrected in the original LVTree, we introduce corresponding lineage modification as a remedy. However, even after making all these changes the genus *Desulfotomaculum* does not acquire a fully monophyletic status in both CVTree and LVTree. In CVTree (Fig. 10) the problem is caused by the insertion of genus *Pelotomaculum*. In LVTree (Fig. 11) *Sporotomaculum* and *Cryptoanaerobacter* (a monospecific genus since 2005) are additional intruders. The fact that in both Figs. 10 and 11 the "outermost" branch, i.e., the branch closest to the root, is made of purely *Desulfotomaculum* species (9/26 and 15/32 in number) hints on all intruding species being members of *Desulfotomaculum*. As a discussion of this problem goes beyond the scope of studying monospecific genera, we leave it here.

3.3.2. Monospecific genera within the class Negativicutes

We study the issue step by step. First, the monophyly of the genus *Megasphaera* is violated by an intruding monospecific genus *Anaeroglobus* [40] in both LVTree and CVTree, see Figs. 12 and 13.

In accordance with Figs. 12 and 13 we assign the species *Anaeroglobus geminatus* to the genus *Megasphaera* as did the EzBioCloud database [21] to get monophyletic *Megasphaera*{8} and *Megasphaera*{10} in LVTree and CVTree, respectively.

Close to *Megasphaera* there are two monospecific genera *Negativicoccus* [35] and *Allisonella* [41], the latter violates monophyly of the genus *Dialister* as shown in Figs. 14 and 15:

It is suggestive to include *Allisonella* as a member of the genus *Dialster* as did in the EzBioCloud database [21]. Figs. 14 and 15 are collapsed to yield Figs. 16 and 17 for LVTree and CVTree, respectively.

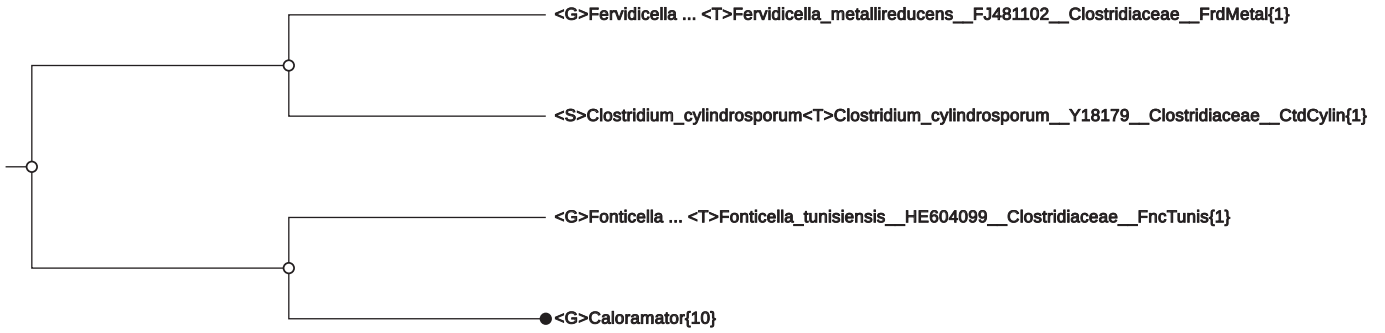The inclusion of *Allisonella* into *Dialister* is well-supported in 16S

Fig. 7. *Thermobrachium celere* gets into the genus Caloramator and violates monophyly of the latter in LVTree.

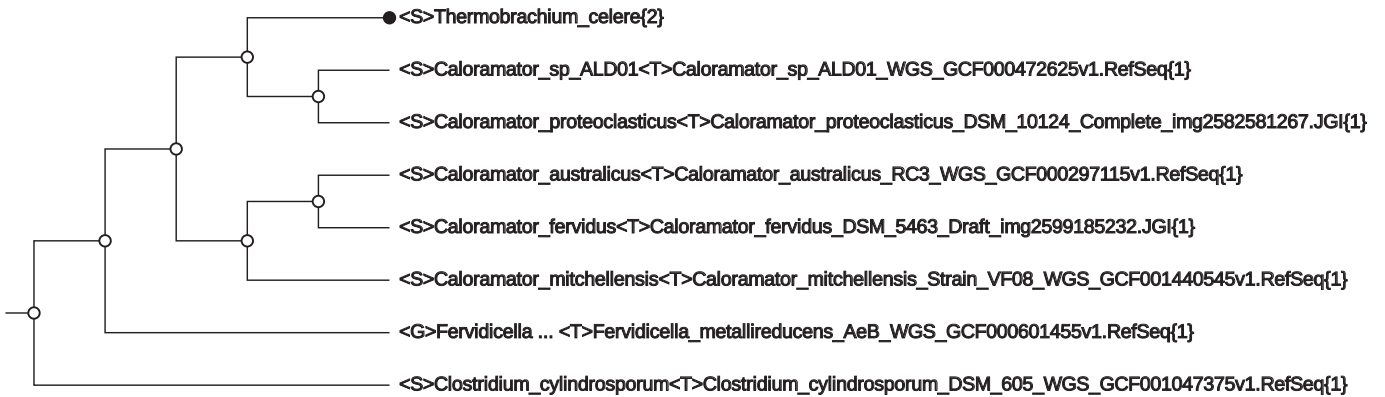**Fig. 8.** The close neighborhood of the genus *Caloramator* in LVTree.

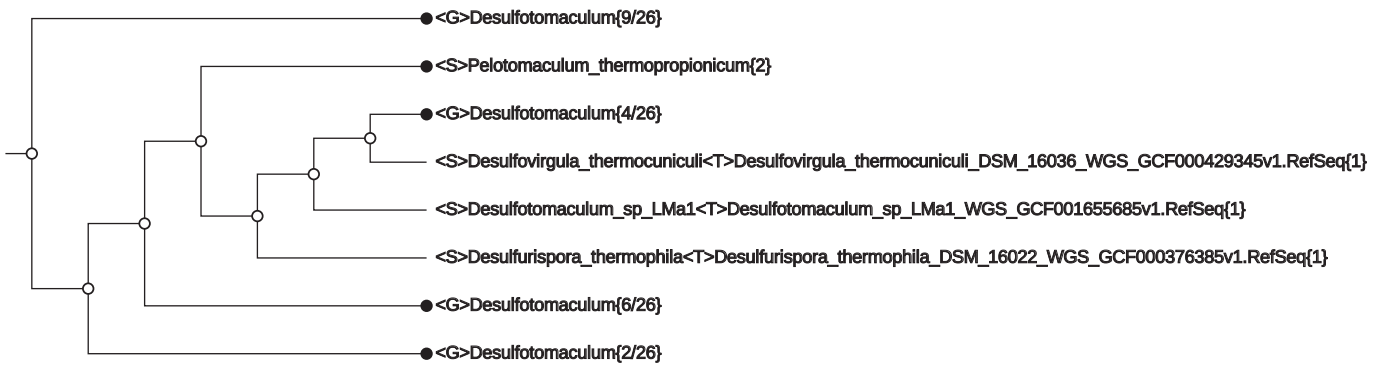**Fig. 9.** *Fervidicella* and *Clostridium cylindrosporum* near *Caloramator* in CVTree.

Fig. 10. The two monospecific genera *Desulfovirgula* and *Desulfurispora* within the genus *Desulfotomaculum* in CVTree.
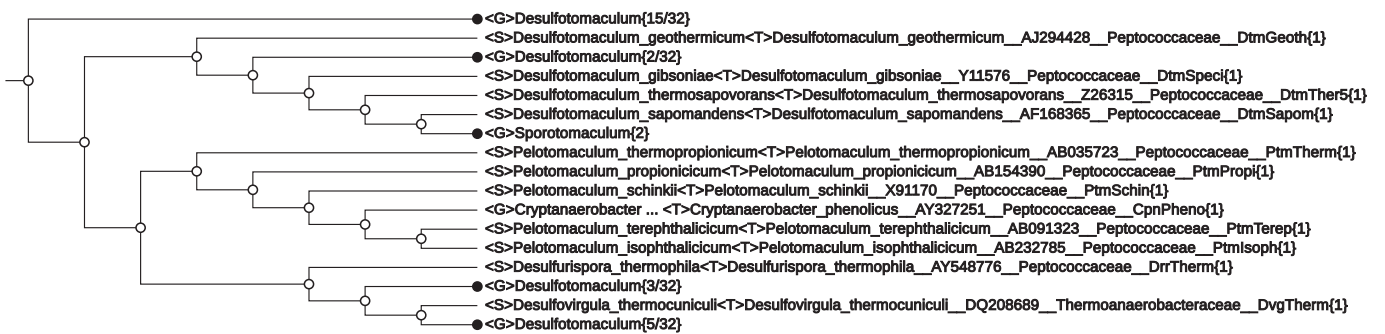

Fig. 11. The two monospecific genera *Desulfovirgula* and *Desulfurispora* within the genus *Desulfotomaculum* in LVTree.
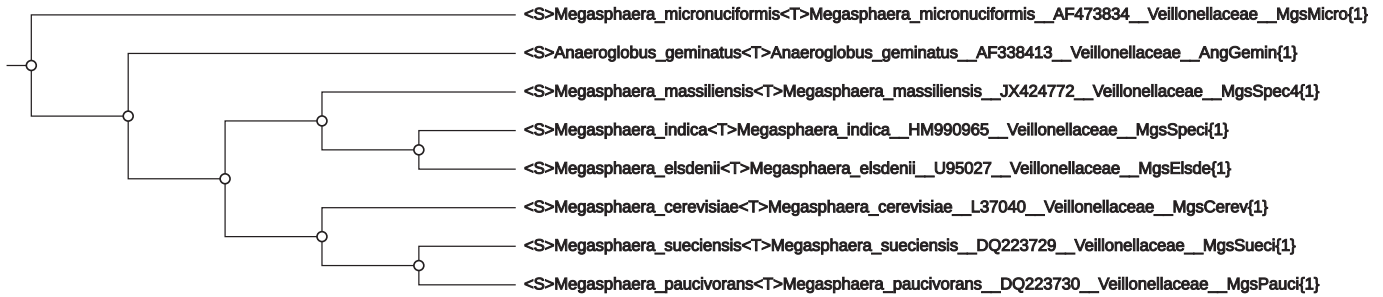
Fig. 12. *Anaeroglobus geminatus* violates the monophyly of the genus Megasphaera in LVTree.
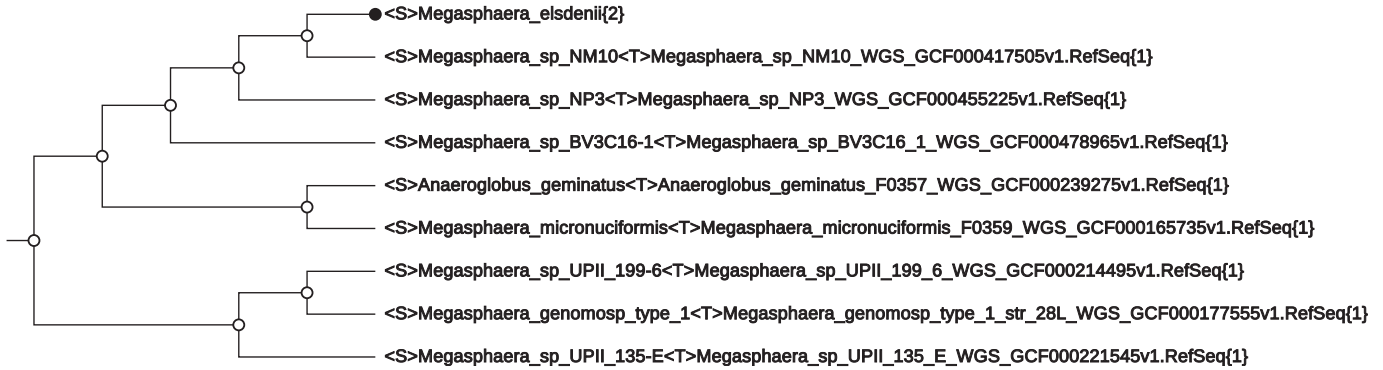

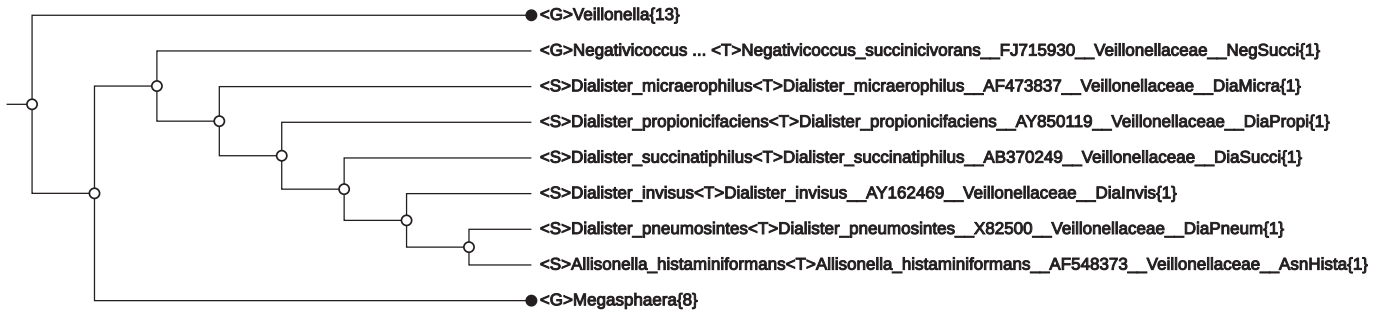**Fig. 13.** *Anaeroglobus geminatus* violates the monophyly of the genus Megasphaera in CVTree.

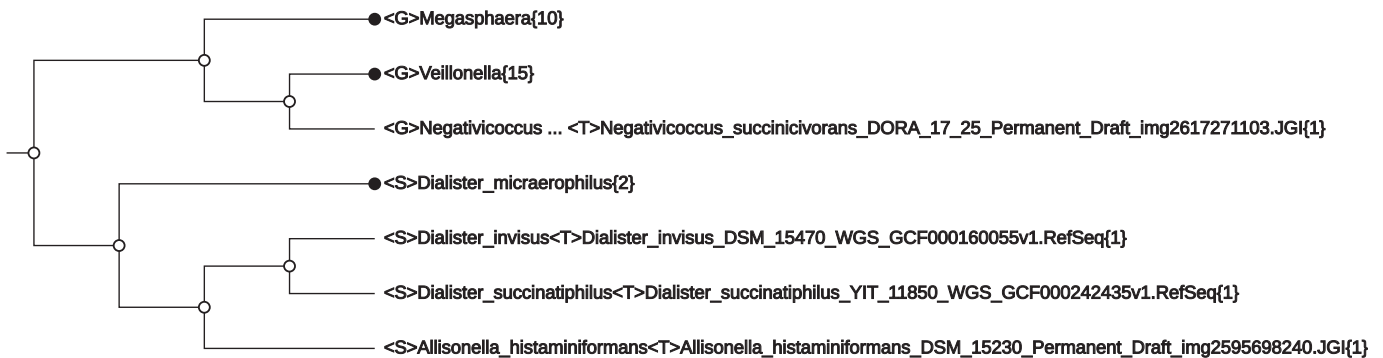**Fig. 14.** *Allisonella* violates monophyly of *Dialister* in LVTree.

Fig. 15. *Allisonella* violates monophyly of *Dialister* in CVTree.

rRNA and whole genome trees. However, in order to judge the relation of *Negativicoccus* to *Dialister* or *Veillonela*, additional data are needed. Therefore, it is better to keep *Negativicoccus* as a monospecific genus for the time being.

3.3.3. Monospecific genera within the class Erysipelotrichia

The new class *Erysipelotrichia*, introduced in 2009 in Vol. 3 of the second edition of the Bergey's Manual, encompasses many species with taxonomic uncertainties. We only single out a species *Sharpea*
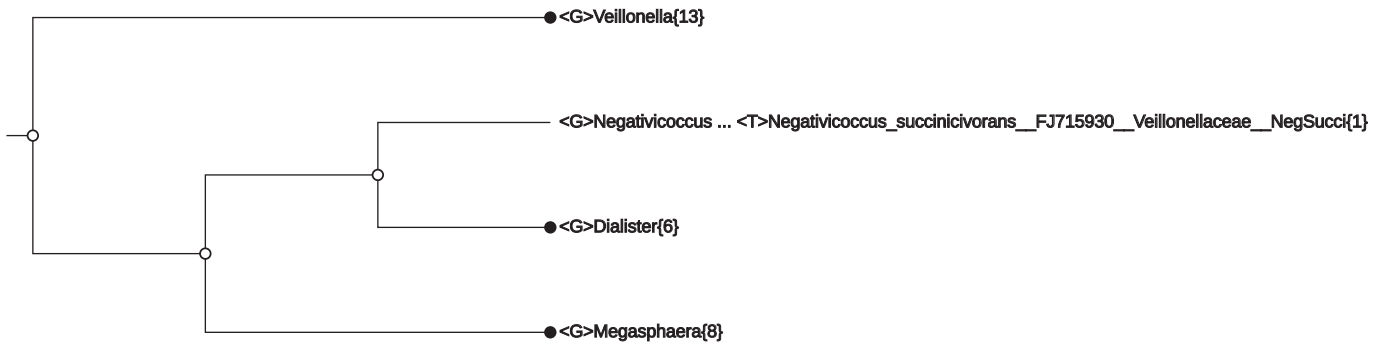
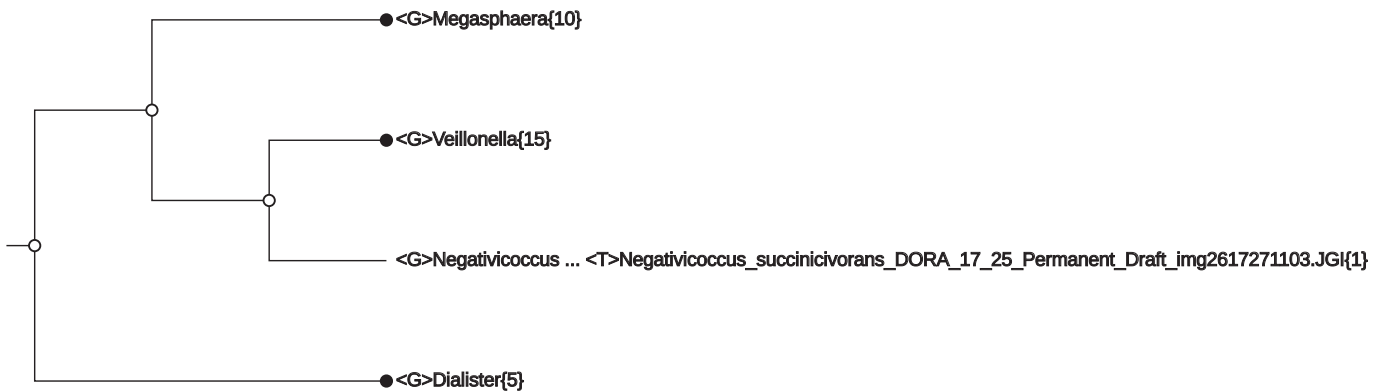**Fig. 16.** Fig. 14 from LVTree collapsed to show the monophyletic taxa.

Fig. 17. Fig. 15 from CVTree collapsed to show the monophyletic taxa.

[42], proposed in 2008 as a new genus within the 16S rRNA group XVII of *Clostridium* [36]. The NCBI Taxonomy [43] assigns it as a genus in family *Lactobacillaceae* in the class *Bacilli*. The original taxonomic information of LVTree follows that of NCBI. However, in both LVTree and CVTree, *Sharpea* gets into the depth of the class *Erysipelotrichia*, definitely not in *Bacilli*, see Figs. 18 and 19:

Therefore, it is reasonable to assign *Sharpea* to family *Erysipelotrichaceae* in the order *Erysipelotrichiales*, in agreement with its close neighbors *Eggerthia*, *Kandleria*, and *Catenibacterium*. In fact, this has been done in the EzBioCloud database [21]. By the way, the last three genera are all monospecific which are represented by one or more strains. There raises a natural question: whether the 4 closely related genera may be combined into one genus? The answer depends on quantitative measures in demarcating species. This is an aspect we did not touch on in this paper. The problem of distances between species will be elucidated in forthcoming

publications.

It is impossible to carry out an exhaustive study of all monospecific genera within the limit of this paper. To conclude this study we return to *Beggiatoa alba* Vaucher 1803, listed as the only species in a monospecific genus since Trevisan 1845 (see LPSN [4] and EzBioCloud [21]). Although a new species *Beggiatoa leptomitiformis* was proposed in 1998 [44] and its genome sequence announced in 2015 [45], the new species name has not appeared in mainstream lists of prokaryotes including LPSN until the time of writing these lines, probably due to the fact that the original description [44] appeared in Russian. The situation in LPSN may change as a valid publication is now available [46]. Anyway, the monospecific status of this genus no longer holds. This example shows that simple comparison of name lists does not always reveal genuine monospecific genera. Checking monophyly of branches simultaneously in both LVTree and CVTree is an effective and reliable way to single out
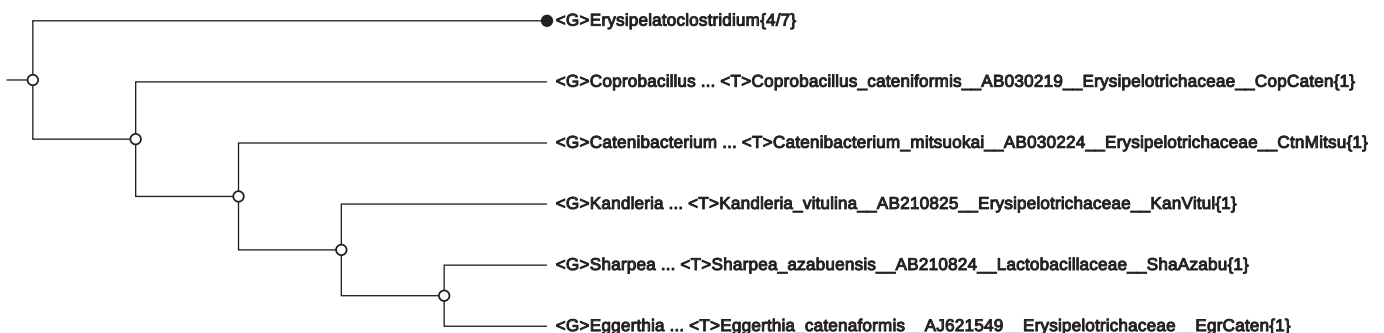

Fig. 18. Monospecific genus *Sharpea* locates in the depth of *Erysipelotrichia* in LVTree.
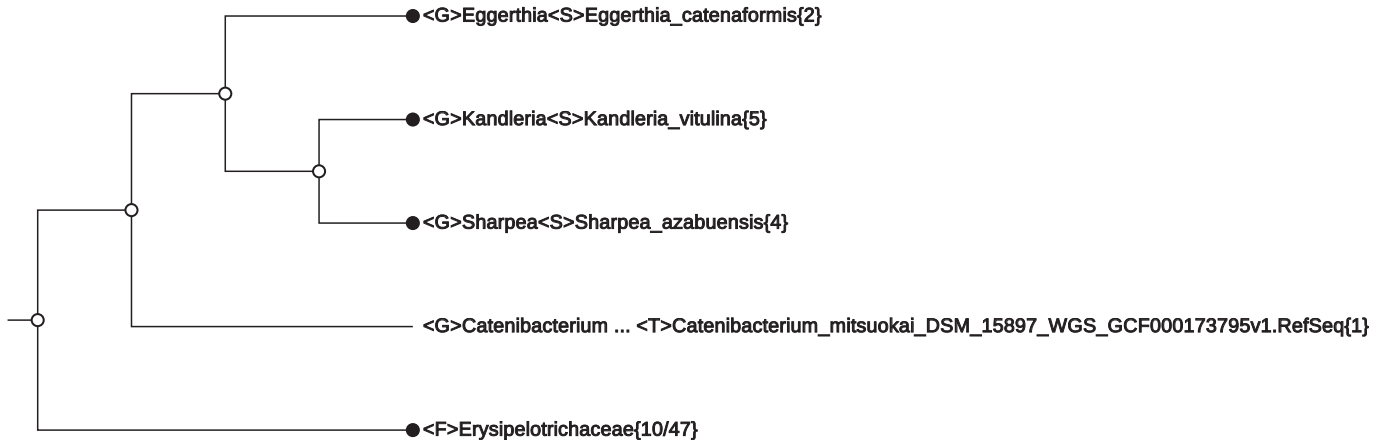
Fig. 19. Monospecific genus *Sharpea* locates in the depth of *Erysipelotrichia* in CVTree. There are 4 genomes from the *Sharpea azabuensis* strains.

monospecific genera.

5. Discussion

Thirty years ago, on the eve of the genome-sequencing era, an Ad Hoc Committee under the International Committee for Systematic Bacteriology wrote in its report [47]: "There was general agreement that the complete DNA sequence would be the reference standard to determine phylogeny and that phylogeny should determine taxonomy. Furthermore, nomenclature should agree with (and reflect) genomic information." This statement, in fact, set a programme for microbiological research.

However, a phylogenetic tree alone can only produce a branching hierarchy of the constituent leaves without bringing about a nomenclature, i.e., a classification and naming scheme. Historically, the nomenclature comes from taxonomy as an independent discipline. Taxonomy comprises a finite number of ranks, say, from phyla down to subspecies. On the other hand, when the number of leaves gets large, the branching hierarchy in a tree may reach significant depth with "binomial" if not "exponential" number of layers. The mapping of a naming scheme onto a branching tree must follow a set of rational guiding principles. Only when both phylogeny and taxonomy have reached a comparatively sophisticated stage, it makes sense connecting the two. With the ever-growing biological Big Data the time is ripe now to carry out the aforementioned programme in a fully fledged manner. The present work just provides an example of such global study in prokaryotic phylogeny and systematics.

Authors' contributions

The two authors together designed this study, collected sequences and taxonomic data, carried out the taxonomic comparison and suggested possible lineage modifications. Zuo maintained the CVTree Web Server and LVTree Viewer. Hao drafted the manuscript. All authors read and approved the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.synbio.2017.08.004.

References

[1] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl Acids Res 2013;41(Database Issue):D590–6.

[2] Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucl Acids Res 2014;42(Database Issue):D633–42.

[3] Mukherjee S, Stamatis D, Bartsch J, Ovchoinikova G, Verenzemska O, Isbandi M, et al. Genomes OnLine Database (GOLD) v.6: data updates and feature enhancements. Nucl Acids Res 2017;45(Database Issue):D446–56.

[4] Parte AC. LPSN — list of prokaryotic names with standing in nomenclature. Nucl Acids Res 2014;42(Database Issue):D613–6.

[5] Verger J-M, Grimont F, Grimont PAD, Grayon M. *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. Int J Syst Bacteriol 1985;35:292–5.

[6] Yarza P, Richter M, Peplies J, Euzeby J, Amann R, Schleifer KH, et al. The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. Syst Appl Microbiol 2008;31:241–50.

[7] Yarza P, Ludwig W, Euzeby J, Amann R, Schleifer KH, Glöckner FO, et al. Update of the all-species living tree project based on 16S and 23S rRNA sequence analyses. Syst Appl Microbiol 2010;33:291–9.

[8] Munoz R, Yarza P, Ludwig W, Euzéby J, Amann R, Schleifer KH, et al. Release LTPs104 of the all-species living tree. Syst Appl Microbiol 2011;34:169–70.

[9] Qi J, Wang B, Hao BL. Whole proteome prokaryote phylogeny without sequence alignment: a K-string composition approach. J Mol Evol 2004;58:1–11.

[10] Li Q, Xu Z, Hao BL. Composition vector approach to whole genome-based prokaryote phylogeny: success and foundations. J Biotechnol 2010;149:115–9.

[11] Zuo GH, Xu Z, Yu HJ, Hao BL. Jackknife and bootstrap tests of the composition vector trees. Genomics Proteomics Bioinforma 2010;8:262–7.

[12] Zuo GH, Li Q, Hao BL. On K-peptide length in composition vector phylogeny of prokaryotes. Comput Biol Chem 2014;53:166–73.

[13] Zuo GH, Hao BL. CVTree3 Web Server for whole-genome-based and alignment-free prokaryotic phylogeny and taxonomy. Genomics Proteomics Bioinforma 2015;13:321–31.

[14] LVTree Web Site. http://www.arb-silva.de/projects/living-tree (last accessed 30 May 2017).

[15] Zuo GH, Zhi XY, Xu Z, Hao BL. LVTree Viewer: an interactive display for the All-Species Living Tree incorporating automatic comparison with prokaryotic systematics. Genomics Proteomics Bioinforma 2016;14:94–102.

[16] Farris JS. Formal definitions of paraphyly and polyphyly. Syst Zool 1974;23(4):548–54.

[17] Farris JS. Haeckel, history, and hull. Syst Zool 1990;39(1):81–8.

[18] Bergey's Manual Trust. Bergey's manual of systematic Bacteriology. second ed., vols. 1–5. New York: Springer-Verlag; 2001–2012.

[19] Whitman WB, editor. Bergey's manual of systematics of Archaea and Bacteria. Wiley Online Library; 2015. http://dx.doi.org/10.1002/9781118960608.

[20] Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The prokaryotes. fourth ed. Heidelberg New York Dordrecht London: Springer; 2014.

[21] Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 2017;67:1613—7.

[22] The EzBioCloud website. http://www.ezbiocloud.net/taxonomy (last accessed 30 June 2017).

[23] Palleroni NJ. Genus *Pseudomonas*. In: Krieg NR, Held JG, editors. Bergey's manual of systematic Bacteriology. first ed.vol. 1. Baltimore: The Williams & Wilkins Co; 1984. p. 141—99.

[24] De Vos P, Yabuuchi EICSB. Subcommittee on the taxonomy of *Pseudomonas* and related organisms Minutes of meeting. Int J Syst Evol Microbiol 2002;52: 2329.

[25] Margulis L, Schwartz KV. Five kingdoms. An illustrated guide to the phyla of life on earth. third ed. New York: W H Freeman; 1998.

[26] Hespell RB. *Serpens flexibilis* gen. nov., sp. nov., an unusually flexible lactate-oxidizing bacterium. Int J Syst Bacteriol 1977;27:371—81.

[27] Shin SK, Hwang CY, Cho YJ, Yi H. Reclassification of *Serpens flexibilis* Hespell 1977 as *Pseudomonas flexibilis* comb. nov., with *Pseudomonas tuomuerensis* Xin et al. 2009 as a later heterotypic synonym. Syst Appl Microbiol 2015;38: 563—6.

[28] Young JM, Park DC. Probable synonymy of the nitrogen-fixing genus *Azotobacter* and the genus *Pseudomonas*. Int J Syst Evol Microbiol 2007;57: 2894—901.

[29] New PB, Tchan YT. *Azomonas macrocytogenes* (*ex* Baillie, Hodgkiss, and Norris 1962, 118) nom. rev. Int J Syst Bacteriol 1982;32:381—2.

[30] Hu FP, Young JM, Stead DE, Goto M. Transfer of *Pseudomonas cissicola* (Takimoto 1939) Burkholder 1948 to the genus *Xanthomonas*. Int J Syst Bacteriol 1997;47:228—30.

[31] Tan WB, Jiang Z, Chen C, Yuan Y, Gao LF, Wang HF, et al. *Thiopseudomonas dentrificans* gen. nov., sp. nov., isolated from anaerobic activated sludge. Int J Syst Evol Bacteriol 2015;65:225—9.

[32] Funke G, Stubbs S, Altwegg M, Carlotti A, Collins MD. *Turicella otitidis* gen. nov., sp. nov., a coryneform bacterium isolated from patients with otitis media. Int J Syst Bacteriol 1994;44:270—3.

[33] von Graevenitz A, Funke G. *Turicella otitidis* and *Corynebacterium auris*: 20 years on. Infection 2014;42:1—4.

[34] Busse H-J. Review of the taxonomy of the genus *Arthrobacter*, emendation of the genus *Arthrobacter sensu lato*, proposal to reclassify selected species of the genus *Arthrobacter* in the novel genera *Glutamicibacter* gen. nov., *Paeniglutamicibacter* gen. nov., *Pseudoglutamicibacter* gen. nov., *Paenarthrobacter* gen. nov. and *Pseudarthrobacter* gen. nov., and emended description of *Arthrobacter roseus*. Int J Syst Evol Microbiol 2016;66:9—37.

[35] Marchandin H, Teyssier C, Campos J, Jean-Pierre H, Roger F, Gay B, et al. *Negativicoccus succinicivorans* gen. nov., sp. nov., isolated from human clinical samples, emended description of the family *Veillonellaceae* and description of *Negativicutes* classis nov., *Selenomonadales* ord. nov. and *Acidaminococcaceae* fam. nov. in the bacterial phylum *Firmicutes*. Int J Syst Evol Microbiol 2010;60: 1271—9.

[36] Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabel J, Garcia P, et al. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int J Syst Bacteriol 1994;44: 812—26.

[37] Engle M, Li Y, Deblois S, Mai V, Reichert A, Mayer F, et al. *Thermobrachium celere* gen. nov., sp. nov., a rapidly growing thermophilic, alkalitolerant, and proteolytic obligate anaerobe. Int J Syst Bacteriol 1996;46:1025—33.

[38] Stackebrandt E, Sproer C, Rainey FA, Burghardt J, Pauker O, Hippe H. Phylogenetic analysis of the genus *Desulfotomaculum*: evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. Int J Syst Bacteriol 1997;47:1134—9.

[39] Gogotova GI, Vainstein MB. The sporogeneous sulfate reducing bacterium *Desulfotomaculum guttoideum* sp. nov. Mikrobiologia 1983;52:789—93 (in Russian).

[40] Carlier JP, Marchandin H, Jumas-Bilak E, Lorin V, Henry C, Carrière C, et al. *Anaeroglobus geminatus* gen. nov., sp. nov., a novel member of the family *Veillonellaceae*. Int J Syst Evol Microbiol 2002;52:983—6.

[41] Garner MR, Flint JF, Russell JB. *Allisonella histaminiformans* gen. nov., sp. nov. A novel bacterium that produces histamine, utilizes histidine as its sole energy source, and could play a role in bovine and equine laminitis. Syst Appl Microbiol 2002;25:498—506.

[42] Morita H, Shiratori C, Murakami M, Takami H, Toh H, Kato Y, et al. *Sharpea azabuensis* gen. nov., sp. nov., a Gram- positive, strictly anaerobic bacterium. Int J Syst Evol Microbiol 2008;58:2682—6.

[43] The NCBI Taxonomy. http://www.ncbi.nlm.nih.gov/taxonomy/ (last accessed on 30 May 2017).

[44] Grabovich MY, Churikova VV, Dubinina GA, Lebedeva VY. Mixotrophic and lithoheterotrophic of the freshwater filamentous sulfur bacterium *Beggiatoa leptomitiformis* D-402. Mikrobiologia 1998;67:383—8 (in Russian).

[45] Fomenkov A, Vincze T, Grabovich MY, Dubinina G, Orlova M, Belousova E. Complete genome sequence of the freshwater colorless sulfur bacterium *Beggiatoa leptomitiformis* neotype strain D-402^T. Genome Announc 2015;3, e01436.

[46] Dubinina G, Savvichev A, Orlova M, Gavrish E, Verbarg S, Grabovich M. *Beggiatoa leptomitoformis* sp. nov., the first freshwater member of the genus capable of chemolithoautotrophic growth. Int J Syst Evol Microbiol 2017;67(2):197—204.

[47] Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, et al. Report of the Ad Hoc committee on reconcilation of approaches to bacterial systematics. Int J Syst Bacteriol 1987;37:463—4.