

## The taxonomy of the genus *Clostridium*: current status and future perspectives

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**Abstract:** The genus *Clostridium* as presently constituted is phylogenetically and phenotypically incoherent. Polyphasic taxonomic data indicate that the genus comprises a collection of very heterogeneous species. Numerous phylogenetic studies, principally based on sequencing of the 16S rRNA gene, indicate that the genus *Clostridium* should be restricted to *Clostridium* cluster I as *Clostridium sensu stricto*. Despite these findings, authors continue to add new species to the genus *Clostridium* that do not fall within the radiation of cluster I and the type species *C. butyricum* thus perpetuating the confusion associated with the taxonomy of this group. Here I formally propose that members of the *Clostridium* (Prazmowski) be restricted to the type species *Clostridium butyricum* and cluster I species. *Eubacterium moniliforme*, *Eubacterium tarantellae*, *Sarcina maxima*, and *Sarcina ventriculi* should be transferred to the genus *Clostridium* as *Clostridium moniliforme* comb. nov., *Clostridium tarantellae* comb. nov., *Clostridium maximum* comb. nov., and *Clostridium ventriculi* comb. nov. A novel genus *Hathewayia* gen. nov. is proposed for the species *Clostridium histolyticum*, *Clostridium limosum* and *Clostridium proteolyticum* as *Hathewayia histolytica* gen. nov. com. nov., *Hathewayia limosa* com. nov. and *Hathewayia proteolytica* comb. nov. The type species of *Hathewayia* is *Hathewayia histolytica*.

**Keywords:** *Clostridium*, Taxonomy, *Clostridium sensu stricto*, *Hathewayia*

## 梭菌属分类研究进展：现状和展望

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**摘要：**目前构成梭菌属的微生物在系统发育和表型特征上不一致。多相分类数据表明，梭菌属物种之间差异大。大量基于 16S rRNA 基因的系统发育研究表明，梭菌属应被限定为梭菌属类群 I，作为狭义梭菌属(*Clostridium sensu stricto*)。尽管有这方面认识，梭菌属新物种仍持续增加，这些新物种并不能与梭菌属类群 I 和标准种丁酸梭菌(*C. butyricum*)形成一致分支，引发梭菌属分类上的混乱。本文明确了梭菌属物种的范围，即只包括模式种和梭菌属类群 I。此外，4 个物种念珠状真杆菌(*Eubacterium moniliforme*)、旋舞真杆菌(*Eubacterium tarantellae*)、最大八叠球菌(*Sarcina maxima*)和胃八叠球菌(*Sarcina ventriculi*)应被调至梭菌属，分别命名为念珠状梭菌(*Clostridium moniliforme*)、旋舞梭菌(*Clostridium tarantellae*)、最大梭菌(*Clostridium maximum*)和胃梭菌(*Clostridium ventriculi*)。一个新属哈撒韦氏菌属(*Hathewayia*)被提议成立，3 个梭菌属物种溶组织梭菌(*Clostridium histolyticum*)、泥渣梭菌(*Clostridium limosum*)和解脲梭菌(*Clostridium proteolyticum*)重新归为溶组织哈撒韦氏菌(*Hathewayia histolytica*)、泥渣哈撒韦氏菌(*Hathewayia*

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*limosa*)和解朊哈撒韦氏菌(*Hatheway proteolytica*), 其中 *Hatheway histolytica* 为模式种。

**关键词:** 梭菌属, 分类学, 狭义梭菌属, 哈撒韦氏菌属

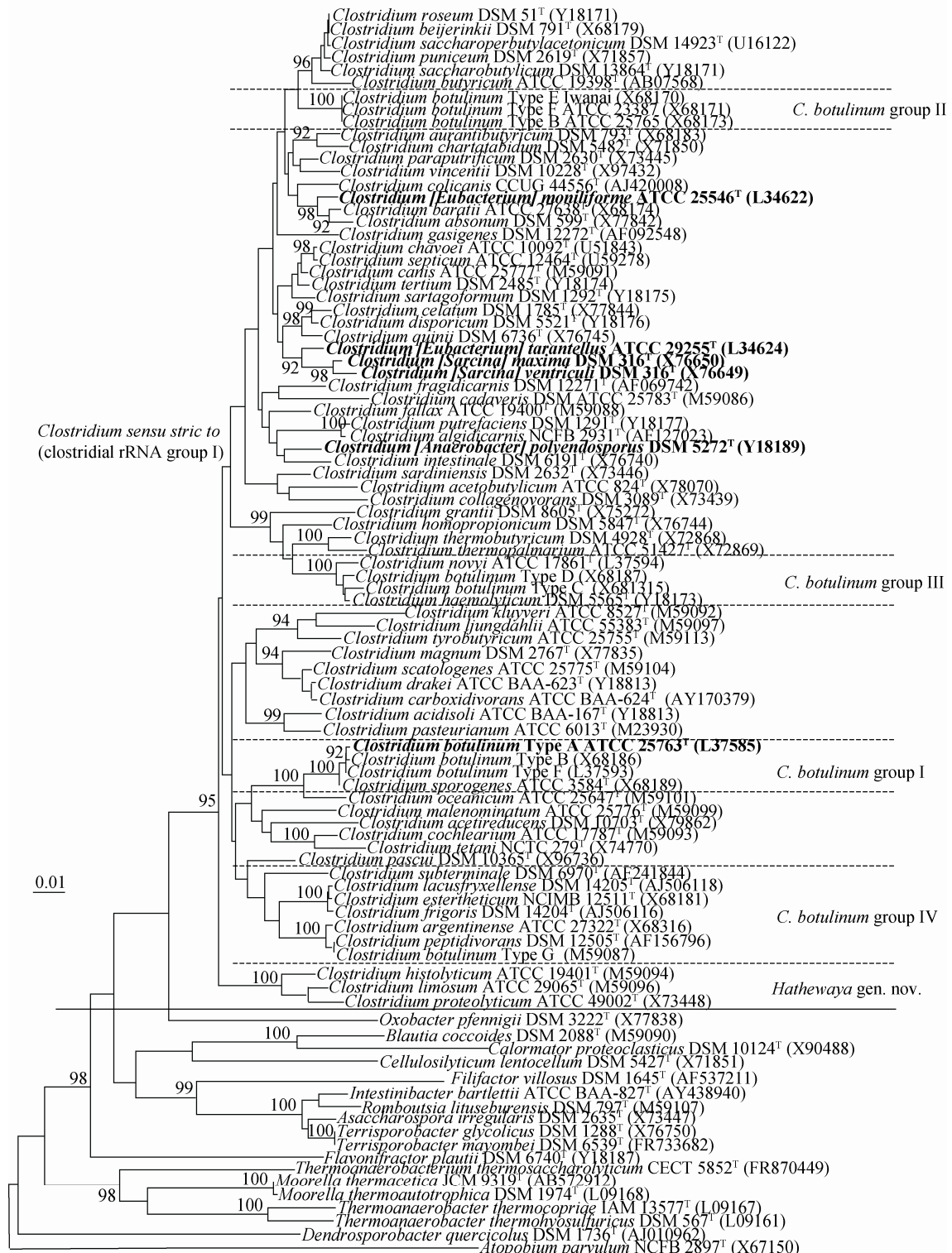
The genus *Clostridium* was proposed in 1880 by Prazmowski with the type species *C. butyricum*<sup>[1]</sup>. Subsequently the genus *Clostridium* became a general depository for Gram-positive staining, sporeforming, anaerobic organisms. There are approximately 228 *Clostridium* species and subspecies with validly published names<sup>[2]</sup> (<http://www.bacterio.net>). The species of the genus *Clostridium* are extremely heterogeneous, with many species displaying a wide range of phenotypes including psychrophiles, thermophiles and acidophiles, organisms that synthesize cytochromes and quinones. In addition, the G+C content of chromosomal DNA ranges from approximately 21% to 54% which is considered to be too extensive a range for a single genus<sup>[3-5]</sup>. Johnson and Francis<sup>[6]</sup> began to pioneer molecular insights into the considerable diversity within the genus using DNA-rRNA pairing, this was later expanded using 16S rRNA oligonucleotide cataloguing studies<sup>[7-9]</sup>. It was not until the advent of the complete (or almost complete) sequencing of the 16S rRNA gene that the true extent of the diversity of the genus and the interrelationships with other taxa was realized. Sequencing studies demonstrated that many *Clostridium* species were phylogenetically distantly related to the type species of the genus, *Clostridium butyricum* and in addition that species of a number of other genera were closely related to this type species<sup>[10-12]</sup>. Collectively, these studies established that the genus *Clostridium* was in need of revision and the first comprehensive effort was attempted by Collins and co-workers<sup>[13]</sup> who proposed a possible hierarchical framework for the classification of clostridia. A total of 19 clusters or groups, (cluster I being recognized as comprising the “true” representatives of the genus *Clostridium sensu stricto* based on it containing the type species of the genus *Clostridium*, *C. butyricum*) were identified leading to the description of five new genera and proposal of eleven new species combinations<sup>[13]</sup>. Successive studies supported and expanded this framework<sup>[5,14-15]</sup> (Figure 1) with many organisms formally recognized as *Clostridium* species being transferred to new or established genera<sup>[3,16-23]</sup>. However, the

reclassification of organisms that fall outside the *Clostridium* cluster I continues and the reader should consult List of Prokaryotic Names with Standing in Nomenclature<sup>[2]</sup> (<http://www.bacterio.cict.fr/>), which is updated regularly on publication of the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM).

In addition to phylogenetic studies based on comparisons of 16S rRNA gene sequences, Gupta and Gao<sup>[24]</sup> identified three conserved indels in three highly conserved proteins (a 4 amino acid insert in DNA gyrase A, a 1 amino acid deletion in ATP synthase beta subunit and a 1 amino acid insert in ribosomal protein S2) that were unique to the species of *Clostridium* cluster I; these conserved indels were not found in any other known bacterial species for which sequences are available<sup>[24]</sup>.

Yutin and Galperin<sup>[25]</sup> provided a potentially important addition to the restructuring of the clostridia using genomic analyses to investigate Gram-negative staining spore formers and other misplaced clostridia. This study, based on a concatenated set of 50 widespread ribosomal proteins with the trees for beta subunits of the RNA polymerase (RpoB) and DNA gyrase (GyrB) and with the 16S rRNA-based phylogeny, resulted in the proposal of a number of novel genera<sup>[25]</sup>. These descriptions do not comply with the Bacteriological Code and therefore the names cannot be validly published in IJSEM

Although it is now generally accepted that *Clostridium* cluster I represents the true genus *Clostridium* and the species included in it should be recognized as *Clostridium sensu stricto*, an anomaly was revealed when a number of species assigned to the genera *Anaerobacter*, *Eubacterium* and *Sarcina* were found to fall within the radiation of *Clostridium* cluster I<sup>[3,5]</sup>. Based on these observations, in his chapter on the *Clostridium* in the *Bergey's Manual of Systematic Bacteriology* Rainey (2009) transferred *Anaerobacter polyendosporus* to the genus *Clostridium*<sup>[3]</sup>. The species *Eubacterium moniliforme* and *Eubacterium tarantellae* are phylogenetically distant to the type species of the genus *Eubacterium* namely, *E. limosum*, and these two species should be



**Figure 1** Neighbor-Joining phylogenetic tree showing the relationship of species with *Clostridium sensu stricto* (rRNA clostridial cluster I) and some other members of the family Clostridiaceae

Note: The tree was constructed using the Neighbor-Joining method based on the pairwise comparison of approximately 1 340 nucleotides. *Atopobium parvulum* was used as the out-group. Bootstrap values (>90%), expressed as a percentage of 1 000 replications. The scale bar indicates 1% sequence divergence.

transferred to *Clostridium*. The situation with the genus *Sarcina* is more complex as this genus was first proposed in 1842 by Goodsir some 38 years prior to the genus *Clostridium*<sup>[1]</sup> giving the genus *Sarcina* nomenclatural priority over the genus *Clostridium*<sup>[1,26]</sup>. Consequently, one could interpret this as meaning all *Clostridium* should be transferred to the genus *Sarcina*. Clearly, with numerous organisms being of clinical significance (*C. botulinum*, *C. perfringens*, *C. novyi*, *C. septicum*) it would cause consternation and confusion amongst the scientific, and especially, the medical community. However, the Bacteriological Code comes to our aid in Rule 24 which states “Priority of publication dates from 1 January 1980. On that date all names published prior to 1 January 1980 and included in the Approved Lists of Bacterial Names of the ICSB are treated for all nomenclatural purposes as though they had been validly published for the first time on that date, the existing types being retained”. Therefore, both genera *Clostridium* and *Sarcina* (the latter with just two species) have equal standing in the nomenclature, and the date of priority of valid publication of both genus names is January 1, 1980. Furthermore, support for retaining the names of medically important species comes from the Code with Rule 56 (5) “perilous names - (*nomina periculosa*). i.e., names whose application is likely to lead to accidents endangering health or life or both or of serious economic consequences”. The transfer of all *Clostridium* species to the genus *Sarcina* would clearly pose a problem and fall into this category.

The taxonomy of the genus *Clostridium* is in a continued state of flux and several important issues remain with medically important species both within (*C. botulinum* complex) and outside rRNA cluster I (*Clostridium difficile*). The *C. botulinum* complex in particular causes a problem with the application of 16S rRNA sequencing revealing at four phylogenetically separate clusters that should represent distinct species or even genera. This highlighted the problems of describing taxa on a single trait such as toxin production especially when said traits are transmissible leading to scientific inconsistencies that are no longer tenable in updated taxonomies<sup>[27]</sup>. Another very important issue centers on the important human pathogen *C. difficile* and the condition known as *Clostridium difficile* associated

diarrhea (CDAD) one of the major scourges of modern health care. A consequence of the publication of Lawson and Rainey<sup>[28]</sup> and the proposal to restrict the genus *Clostridium* to *C. butyricum* and close relatives is that *C. difficile* located in cluster XI and should not be considered as a bonafide member of the genus and should be reclassified. This is likely to cause some consternation among clinical colleagues but change is inevitable, an earlier attempt proposing the name *Peptoclostridium difficile*<sup>[25]</sup> was unsuccessful. This issue must be treated in a sensitive manner to accommodate the medical community and will be the subject of a future paper.

Although *Clostridium* cluster I represents the true genus *Clostridium* and should be recognized as *Clostridium sensu stricto*, unfortunately authors continue to assign organisms to the genus *Clostridium* even when it is obvious using 16S rRNA gene sequence data that these novel organisms fall outside of *Clostridium* cluster I. Therefore, it should be formally proposed that members of the genus *Clostridium* (Prazmowski) be restricted to the type species of the genus, *Clostridium butyricum* and relatives in *Clostridium* cluster I. It is proposed that *Eubacterium moniliforme*, *Eubacterium tarantellae*, *Sarcina maxima*, and *Sarcina ventriculi* be transferred to this genus as *Clostridium moniliforme* comb. nov., *Clostridium tarantellae* comb. nov., *Clostridium maximum* comb. nov., and *Clostridium ventriculi* comb. nov. In addition, it is also recommended that new species that are unrelated to *C. butyricum* and fall outside of *Clostridium* cluster I should not be added to the genus *Clostridium*. A novel genus is also proposed to encompass proteolytic species designated as rRNA cluster II; *Hathewayia* gen. nov. and that *Clostridium histolyticum*, *Clostridium limosum* and *Clostridium proteolyticum* be transferred to this new genus as *Hathewayia histolytica* gen. nov. com. nov., *Hathewayia limosa* com. nov. and *Hathewayia proteolytica* comb. nov. The type species of the genus *Hathewayia* is *Hathewayia histolytica*<sup>[28]</sup>.

Even with these proposed changes, it is acknowledged that future revisions may be necessary due to the internal structure and phylogenetic depth present within clostridia cluster I. However, it is another important step in resolving the taxonomy of the genus *Clostridium* and prevent future assignment

of organisms to the genus that are phylogenetically removed from *C. butyricum* and close relatives.

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