The genus *Listonella* MacDonell and Colwell 1986 is a later heterotypic synonym of the genus *Vibrio* Pacini 1854 (Approved Lists 1980) – a taxonomic opinion

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We analysed the taxonomic position of the genus *Listonella* based on phylogenetic, genomic and phenotypic data. The species of the genus *Listonella* were nested within the genus *Vibrio* according to the 16S rRNA gene sequence-based phylogenetic tree. The closest neighbour of *Vibrio* (*Listonella*) *anguillarum* strains LMG 4437^T and ATCC 68554 (=strain 775) was *Vibrio ordalii* LMG 13544^T, with more than 99.5% 16S rRNA gene sequence similarity. Furthermore, *Vibrio* (*Listonella*) *pelagius* is highly related to *Vibrio splendidus*. According to average amino acid identity (AAI), multilocus sequence analysis (MLSA) and Karlin genome signature, the closest neighbour of *L. anguillarum* ATCC 68554 is *V. ordalii* LMG 13544^T, with 95% AAI, 98% MLSA and 5 in Karlin. *V. anguillarum* ATCC 68554 and *Vibrio cholerae* N16961 had 77% similarity in AAI, 85% in MLSA and 14 in the Karlin signature. Phenotypic analyses of previously published data for *V. (L.) anguillarum* and *V. (L.) pelagius* revealed that the genus *Listonella* is extremely similar to the genus *Vibrio*. *V. ordalii* and *L. anguillarum* strains yielded up to 67% DNA–DNA hybridization. There are only a few phenotypic features that might be used to discriminate these two species: *L. anguillarum* is positive for the Voges–Proskauer reaction, citrate utilization, starch hydrolysis, lipase activity and acid production from glycerol, sorbitol and trehalose, whereas *V. ordalii* is negative for these traits. We suggest that the genus *Listonella* is a later heterotypic synonym of the genus *Vibrio* and propose to use the names *Vibrio anguillarum* and *Vibrio pelagius* in place of *Listonella anguillarum* and *Listonella pelagia*, respectively.

The genus *Listonella* was created in 1985 using 5S rRNA gene sequences to infer phylogenetic relationships among vibrios and related bacteria (MacDonell & Colwell, 1985). This study brought useful information to the taxonomy of superfamily I (*Vibrionaceae* plus *Enterobacteriaceae*), as it correctly concluded that *Vibrio marinus* (now *Moritella marina* in the family *Alteromonadaceae*), ‘*Vibrio psychroerythrus*’ (now *Colwellia psychrerythraea* in the family *Alteromonadaceae*) and *Aeromonas* species (now in the family *Aeromonadaceae*) were not authentic members of the *Vibrionaceae* and should be placed into other families. Another significant outcome of this study was the creation

of the genus *Shewanella* (now in the family *Shewanellaceae*; Ivanova *et al.*, 2004).

According to MacDonell & Colwell (1985), the genus *Listonella* comprised the species *Listonella anguillarum*, *L. damsela* and *L. pelagia*. The species *L. damsela* was subsequently reclassified as *Photobacterium damsela* on the basis of 16S rRNA gene sequence phylogeny (Smith *et al.*, 1991). Although the genus *Listonella* includes the species *L. anguillarum* and *L. pelagia*, the majority of the work on these micro-organisms uses the species names *Vibrio anguillarum* and *V. pelagius*, reflecting the actual preference of the field for the *Vibrio* genus designation. Furthermore, *L. anguillarum*, *L. damsela* and *L. pelagia* were previously classified in the genus *Vibrio* (Baumann *et al.*, 1971; Bergman, 1909; Love *et al.*, 1981). *L. pelagia* and *V. splendidus* show up to 40% DNA–DNA hybridization (DDH) relatedness

Abbreviations: AAI, average amino acid identity; DDH, DNA–DNA hybridization; MLSA, multilocus sequence analysis.

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(Reichelt *et al.*, 1976), whereas the related species *Vibrio ordalii* and *L. anguillarum* have up to 67 % DDH relatedness (Schiewe *et al.*, 1981). Because the known error for DDH experiments is around 5 %, these two species are nearly indistinguishable. *L. anguillarum* and *V. ordalii* appeared nested between the core (Harveyi) and Cholerae clades established by Sawabe *et al.* (2007) using multilocus sequence analysis (MLSA) of nine genes (16S rRNA, *rpoA*, *recA*, *ftsZ*, *mreB*, *topA*, *gyrB*, *pyrH* and *gapA*), indicating that these species are indeed members of the genus *Vibrio*. In agreement with the DDH data, *L. pelagia* appeared within the Splendidus clade (Sawabe *et al.*, 2007).

In this report, we present the results of a taxonomic study based on a larger number of genomic and phenotypic data in order to determine the taxonomic position of *L. anguillarum* and *L. pelagia*. We relied on our recently completed whole-genome sequences of *L. anguillarum* ATCC 68554 (=strain 775) and *V. ordalii* LMG 13544^T. Based on this analysis, we propose to use the names *Vibrio anguillarum* and *Vibrio pelagius* rather than *Listonella anguillarum* and *Listonella pelagia*, respectively.

To determine the exact phylogenetic position of the genus *Listonella* within the family Vibrionaceae, we performed a phylogenetic reconstruction using the complete 16S rRNA gene sequences of several members of this family, as described previously (Thompson *et al.*, 2009). The species of the genus *Listonella* are nested within the genus *Vibrio* according to the 16S rRNA gene and MLSA-based

phylogenetic trees (Figs 1 and 2; sequence alignments included as Supplementary Material). The closest neighbour of *L. anguillarum* strains ATCC 68554 and LMG 4437^T is *V. ordalii* LMG 13544^T, with more than 99.5 % 16S rRNA gene sequence similarity. The 16S rRNA gene sequences obtained from the database (<http://www.taxvibrio.lncc.br>) and from the whole-genome sequences were indistinguishable. Furthermore, *L. pelagia* is highly related to *V. splendidus*. This analysis indicates clearly that the genus *Listonella* is not distinguishable from the genus *Vibrio* by means of 16S rRNA gene sequence analysis. In general, species of the same genus have >95 % 16S rRNA gene sequence similarity and form monophyletic groups (Tindall *et al.*, 2010). The definition of a genus requires a polyphasic approach based not only on 16S rRNA gene sequence similarity, but also on several phenotypic and genomic parameters (Tindall *et al.*, 2010).

In order to evaluate the taxonomic position of *L. anguillarum* in more detail, we next determined the genomic similarity of *L. anguillarum* ATCC 68554 and *V. ordalii* LMG 13544^T with several members of the family Vibrionaceae, as described previously (Thompson *et al.*, 2009). According to average amino acid identity (AAI), MLSA and Karlin genome signature, the closest neighbour of *L. anguillarum* ATCC 68554 is *V. ordalii* LMG 13544^T, with 95 % AAI, 98 % MLSA and 5 in Karlin signature (Table 1). *L. anguillarum* ATCC 68554 and *Vibrio cholerae* N16961 had 77 % similarity in AAI, 85 % in MLSA and 14 in the Karlin signature. In addition, the codon usage bias

Fig. 1. Phylogenetic tree based on complete 16S rRNA gene sequences of several members of the family Vibrionaceae. The tree was reconstructed using the neighbour-joining method and Kimura's two-parameter model. Bar, 1 % sequence difference. Asterisks next to sequences of *L. anguillarum* ATCC 68554 and *V. ordalii* LMG 13544^T indicate 16S rRNA gene sequences obtained from whole genome sequences. The alignment used to reconstruct the tree is available as a supplementary file in IJSEM Online.

Fig. 2. Phylogenetic tree based on sequences of *topA*, *recA*, *pyrH*, *gyrB*, *mreB*, *rpoA* and *ftsZ* genes of several members of the family *Vibrionaceae*. The tree was constructed using the neighbour-joining method and p distances. Bar, 2% sequence difference. The alignment used to reconstruct the tree is available as a supplementary file in IJSEM Online.

(Nc) was 53 for *L. anguillarum* ATCC 68554 and *V. ordalii* LMG 13544^T, 52 for *V. cholerae* N16961, 48 for *Photobacterium angustum* S14, 49 for *Photobacterium damsela* CIP 102761^T and 51 for *Photobacterium profundum* SS9. The mean AAI of *L. anguillarum* towards species of the genera *Vibrio* and *Photobacterium* was 78.1 ± 8.4 ($n=6$) and 67 ± 1.0 ($n=3$), respectively, indicating that *L. anguillarum* is indeed closer to the former genus. The mean MLSA similarity of *L. anguillarum* towards species of the genera *Vibrio* and *Photobacterium* was $86.5 (\pm 5.8)$ and $79.6 (\pm 1.5)$, respectively. These significantly higher values towards the genus *Vibrio* and the phylogenetic allocation of *L. anguillarum* within the genus *Vibrio* reinforce the notion that *L. anguillarum* is not to be considered a member of a separate genus. New genera of vibrios have been described based on a polyphasic approach, including phylogenetic inferences. For instance, the new genus *Aliivibrio* was created based on the argument that several species (*Aliivibrio logei*, *A. salmonicida* and *A. fischeri*), previously classified in the genus *Vibrio*, may form a separate phylogenetic branch on the outskirts of the genus *Vibrio* (Urbanczyk *et al.*, 2007). In addition, the codon usage in *Aliivibrio* appeared to be different from that in the genus *Vibrio* (Thompson *et al.*, 2009). Similar arguments do not apply to *L. anguillarum*, because the genus *Listonella* cannot be differentiated from the genus *Vibrio* in any genomic parameter. The genomic features of *Listonella* and *Vibrio* are indistinguishable. In a previous study, we proposed that a *Vibrio* species is defined as a group of strains that share >96% AAI, >95% DNA identity in

MLSA and <10 in Karlin genome signature (Thompson *et al.*, 2009). Based on this definition, *L. anguillarum* and *V. ordalii* are highly related species. Strains of the same species and species of the same genus will form monophyletic groups on the basis of MLSA. The different genera of the *Vibrionaceae* have less than 70% AAI. These thresholds were proposed based on a limited number of genome sequences, and we highlighted that the analysis of newly sequenced genomes would refine these boundaries for species definition. It would be interesting to determine the taxonomic resolution of AAI, MLSA and Karlin in subsequent studies examining more representative strains of the two species.

Analysis of previously published phenotypic data for *L. anguillarum* and *L. pelagia* revealed that the genus *Listonella* is extremely similar to the genus *Vibrio*. There are only a few phenotypic features that might be used to discriminate *L. anguillarum* and *V. ordalii*. *L. anguillarum* is positive for the Voges-Proskauer reaction, citrate utilization, starch hydrolysis, lipase activity and acid production from glycerol, sorbitol and trehalose, whereas *V. ordalii* is negative for these characteristics (Schiewe *et al.*, 1981). Indeed, *L. anguillarum* and *L. pelagia* have phenotypic features typical of the genus *Vibrio* (Baumann *et al.*, 1984; Thompson & Swings, 2006). The most abundant fatty acids are 18:1 ω 7c, 16:1 ω 7c and/or iso-15:0 2-OH, 16:0, iso-16:0, 14:0, iso-14:0 3-OH, 12:0, 12:0 2-OH and 12:0 3-OH, corresponding to >80% of all fatty acids in most vibrios, including *L. anguillarum* and *L. pelagia* (Austin *et al.*, 1995). The species *L.*

Table 1. Similarity of *L. anguillarum* and *V. ordalii* towards other members of the family Vibrionaceae

Values of AAI (%) / MLSA similarity (%) / Karlin genomic signature are indicated.

Strain	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>L. anguillarum</i> ATCC 68554	–											
2. <i>V. ordalii</i> LMG 13544 ^T	95/98/5	–										
3. <i>V. splendidus</i> 12B01	72/86/56	72/85/61										
4. <i>V. harveyi</i> HY01	73/84/48	73/85/51	73/86/19	–								
5. <i>V. campbellii</i> AND4	75/81/37	75/85/41	73/86/19	90/95/15	–							
6. <i>V. cholerae</i> N16961	77/85/14	78/85/14	71/83/59	72/84/48	74/84/40	–						
7. <i>V. mimicus</i> VM573	77/85/20	78/84/22	70/82/57	72/85/49	74/84/39	91/91/14	–					
8. <i>P. angustum</i> S14	67/81/36	67/80/51	64/82/55	67/82/62	67/81/47	67/81/39	67/80/46	–				
9. <i>P. damsela</i> CIP 102761 ^T	68/80/42	68/70/53	68/81/53	69/80/61	68/80/49	68/70/47	68/80/48	74/75/30	–			
10. <i>P. profundum</i> SS9	66/78/32	66/79/43	65/79/54	66/79/54	67/79/46	67/80/35	66/79/38	73/86/13	72/73/25	–		
11. <i>A. fischeri</i> ES114	70/82/41	70/82/46	67/83/44	68/82/50	69/82/36	69/80/52	68/81/44	68/84/28	71/83/13	68/81/21	–	
12. <i>A. fischeri</i> MJ11	69/82/40	70/82/45	67/83/43	68/82/50	69/82/35	69/81/51	68/81/43	68/84/27	71/83/13	68/81/20	98/99/1	–
13. <i>A. salmonicida</i> FL11238	68/81/35	69/81/40	67/82/47	69/81/54	69/81/39	69/80/43	69/80/41	67/83/34	71/81/29	67/81/25	85/90/27	85/90/26

anguillarum is positive for oxidation of dextrin, glycogen, *N*-acetyl-D-glucosamine, D-fructose, D-glucose and maltose (Austin *et al.*, 1995). Neither phenotypic nor chemotaxonomic data support the separation of the genera *Listonella* and *Vibrio*.

The report of the ad hoc committee for the re-evaluation of the species definition in bacteriology (Stackebrandt *et al.*, 2002) suggested that the introduction of innovative approaches in bacterial systematics provides new opportunities for the development and refinement of taxonomy. According to this committee, evolution underpins taxonomy, and the process of doing taxonomy requires adjustments to scientific developments. Our study brings new genomic taxonomic information for the enhancement of the taxonomy of vibrios. Phenotypic, phylogenetic and genomic data presented in this study clearly show that the genus *Listonella* is indeed nested within the genus *Vibrio*. Thus, it is clear that these two genera are indistinguishable. Because the genus *Vibrio* was described first, the genus *Listonella* is to be considered a later heterotypic synonym of the genus *Vibrio*. This has been going on long enough, and the time is now right for change in order to avoid further confusion and misleading classifications and, thus, based on our analysis, we propose to use the names *Vibrio anguillarum* and *Vibrio pelagius* in place of *Listonella anguillarum* and *Listonella pelagia*, respectively.

Acknowledgements

F. L. T., C. C. T. and G. M. D. thank the CNPq, the US Embassy (Brazil), IFS, CAPES and FAPERJ for support. The contributions of H. N., C. D. and J. H. C. were supported in part by the US Department of Agriculture (#2007-3560018527) and the National Institutes of Health (RO1-AI19018).

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