Whole genome sequences reveal *Vibrio hemicentroti* Kim et al. 2013 as a later heterotypic synonym of *Vibrio splendidus* (Beijerinck 1900) Baumann et al. 1981

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Abstract

The synonymy between *Vibrio hemicentroti* Kim et al. 2013 and *Vibrio splendidus* (Beijerinck 1900) Baumann et al. 1981 was suggested after a recent multilocus sequence analysis of the Splendidus clade, which included the type strains of both species. To clarify their status, we have determined genomic indexes from whole genome sequences of strains *V. hemicentroti* CECT 8714⁴ and *V. splendidus* NCCB 53037⁴. Average Nucleotide Identities of 96.0–96.7 % and an *in silico* DNA–DNA hybridization value of 70.2 %, as well as similarity levels of selected housekeeping gene sequences support the consideration of *V. hemicentroti* as a later heterotypic synonym of *V. splendidus*.

*Vibrio hemicentroti* is the most recent addition to the Splendidus clade within the genus *Vibrio* [1]. This clade encompasses the largest number of species (17) among the different lineages recognized in the genus [2] and contains members involved in fish and shellfish infectious diseases [3]. The type strain of *V. hemicentroti*, AlyHP32⁴, isolated from the gut microflora of sea urchin (*Hemicentrotus pulcherrimus*) is closely related to *Vibrio splendidus* KCTC 12679⁴ based on DNA–DNA hybridization (DDH), DNA G+C content, fatty acid methyl ester analysis and phenotypic tests. The 58 % DDH level obtained was considered differential enough to sustain the status of a novel species for strain AlyHP32⁴ [1].

In a recent update about the species composition of the Splendidus clade, Pérez-Cataluña et al. [4] found it almost impossible to discriminate *V. hemicentroti* from its nearest neighbour, *V. splendidus*, based on a five-gene multilocus sequence analysis (MLSA) study and raised the question of whether there is a case of synonymy between the two species. On the other hand, Oden et al. [5] have suggested that *V. hemicentroti* falls outside the Splendidus clade, which is clearly contradictory to previous observations.

To address the subject, we have obtained the whole genome sequence of *V. hemicentroti* CECT 8714⁴ (FLQQ01) and compared it with the already available genome of *V. splendidus* NCCB 53037⁴ (LNQX01). Three other members of the Splendidus clade were also included for genomic de novo sequencing: *Vibrio atlanticus* CECT 7223⁴ (FLQP01), *Vibrio celticus* CECT 7224⁴ (FLQZ01) and *Vibrio toranzoniae* CECT 7225⁴ (FLDO01). Methods used to obtain, assemble and annotate whole genome sequences have been already described [6]. The available genomes of *Vibrio crassostreae* LGP7⁴ (CCJW01), *Vibrio tasmaniensis* 5 F-79 (AJZP0.1) and *Vibrio kanaloae* 5S-149 (AYX0.1) have been also included in the study.

Genomic relatedness was assessed through the ANIb, ANIm and TETRA [7], OrthoANI [8] and estimated DDH [9] indices (Table 1). In addition, we recovered ten housekeeping genes from the genome of *V. hemicentroti* CECT 8714⁴ and compared them through BLAST searches with their homologues in the genome of *V. splendidus* NCCB 53037⁴. These ten genes (listed in Table 1) were selected to include all genes used by Kim et al. [1], Pérez-Cataluña et al. [4] and Oden et al. [5] and to reproduce their respective MLSA studies. Additionally, a genome comparison showing the percentage of protein sequence similarity between *V. hemicentroti* CECT 8714⁴ and the other reference type strains in this study, based on the RAST server and SEED overview [10], was obtained (Fig. S1, available in the online Supplementary Material).

As it can be observed in Table 1, all indices between the genomes of *V. hemicentroti* CECT 8714⁴ and *V. splendidus* NCCB 53037⁴ are above the thresholds for species definition: 96.0 % for average nucleotide identity (ANI) and 70 % for DDH. In addition to the values shown in Table 1, we...
have determined a TETRA value of 0.9992 between the genomes of the *V. hemicentroti* and *V. splendidus* type strains. All these results clearly indicate that both belong to the same genomic species and should be considered synonyms. As expected, *V. atlanticus* CECT 7223T, *V. celticus* CECT 7224T, *V. toranzoniae* CECT 7225T and other members of the clade yielded values below those thresholds (Table 2).

The high genomic similarity between *V. hemicentroti* CECT 8714T and *V. splendidus* NCCB 53037T is also noticeable based on the graphical representation of protein identity depicted in Fig. S1. The image indicates identities above 99% between *V. splendidus* NCCB 53037T and *V. hemicentroti* CECT 8714T.

The results of the direct genomic comparison between the two type strains should be considered more relevant and accurate than the wet lab DDH determination (58%) that served Kim et al. [1] to substantiate their proposal, as the inaccuracies of the experimental determination of DDH values are well documented [11-13]. In addition, the very high similarities found between the set of individual housekeeping genes commonly used for phylogenetic analysis in the genus *Vibrio* (Table 1) are also supportive of the synonymy and confirm the inability to resolve the two reportedly different species through MLSA [4].

A point of controversy is the recent observation by Oden et al. [5] that *V. hemicentroti* is excluded from the Splendidus clade. These authors based their opinion on the fact that *V. hemicentroti* CECT 8714T, the same collection strain used in this study, failed in the amplification of six, out of seven, housekeeping genes used for their MLSA. They only succeeded in the amplification of the *atpA* gene of *V. hemicentroti* CECT 8714T but its further phylogenetic analysis (not shown in the original publication) apparently placed this strain outside of the Splendidus clade. However, in our hands *V. hemicentroti* CECT 8714T not only yielded genomic indices that prove it represents the same species as *V. splendidus*, but it also showed very high similarities (97.2-100%) when comparing the set of MLSA selected genes extracted from their genomes (Table 1). These values and the one obtained after summing the ten gene sequences together (99.2% similarity over more than 14 000 positions compared) are well within the intraspecific range,
according to Sawabe et al. [2]. As an additional control we have checked that the nine housekeeping genes obtained by Kim et al. [1] for V. hemicentroti AlyHP32® are located in the draft genome of V. hemicentroti CECT 8714T, including the atpA gene with 100 % similarity. Thus, we consider that Oden et al. [5] were probably dealing with an erroneously labelled strain instead of the proper V. hemicentroti CECT 8714T when performing the amplification procedures in their study.

In conclusion, there is no justification to maintain a different species status for V. hemicentroti and V. splendidus. Since the name V. splendidus (Beijerinck 1900) Baumann et al. 1981 has priority of publication over V. hemicentroti Kim et al. 2013, and based on the evidence presented in this study, we propose that V. hemicentroti is considered a later heterotypic synonym of V. splendidus.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

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