

**To:**

Jared Leigh
Principal Biodiversity
BHP Group Ltd.

Memo:

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Author:
Daniel White
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Misclassification of stygofauna oligochaete, *Pristina longiseta*, at East Jimblebar

BHP have requested an explanation as to why a stygofauna oligochaete worm of family Naididae, *Pristina longiseta*, erroneously retained an initial species code assignment of Tubificidae sp. 'BOL074'. The animal was collected in bore HCM0021 at East Jimblebar on the 7th March 2020.

Naming of this animal

After collection in March 2020 by haul net, and post morphological examination, this worm was assigned a higher order taxonomic classification of Tubificidae sp. due to taxonomic uncertainty, which is commonplace with stygofaunal worms due to the physical condition of the animal at time of examination and/or its life stage. To help resolve lower order taxonomic assignment this animal was flagged for DNA analysis and was sequenced for the mitochondrial COI gene on the 21st September 2021. After comparison of this sequence to other tubificid DNA sequences stored in the Bennelongia database by a previous staff member, no match was found. Comparison of this sequence was also made to the non-redundant nucleotide database stored at Genbank, and a species-level match with an uncorrected pairwise genetic distance of 7.3% was returned to *Pristina longiseta* isolate CE1588 (Genbank accession number GU902108), described in Erseus *et al.* (2010). However, for an unknown reason, this result was not recognized/reported at that time. As a result, the animal was assigned a new species code of Tubificidae sp. 'BOL074'. As this was the sole record of this species there was a possibility that it was a restricted species. It was not until recent, further scrutiny of its sequence that this error was revealed, and the correct taxonomic assignment was made to *Pristina longiseta*, which is widespread across Australia. This assignment was confirmed by COI sequence analysis of a subsequent adult *Pristina longiseta* animal, collected in the same hole several months later on the 30th June 2020, which was both morphologically and genetically identified as *Pristina longiseta*.

Source of error

After retrospective review, it appears there may have been two causes of human error in this misclassification. Firstly, the higher level taxonomy of oligochaete worms is flexible, with the families Tubificidae and Naididae being very close. In fact, Tubificidae is often treated as a subfamily Tubificinae within Naididae, and re-named to Tubificinae (Erseus *et al.* 2008). Confusingly, the name Tubificidae is still used by oligochaete researchers rather than Tubificinae or Naididae. It is possible that the staff member was not aware of this situation when analysing the COI sequence when the animal was originally assigned the higher order name of Tubificidae sp., consequently making the initial homology search too narrow by not including all sequences in the Bennelongia database from the family Naididae in the analysis. If this had been done, it is possible that the similarity of the target animal's COI sequence to other members of Naididae would have been recognized. Similarly, when the subsequent *Pristina longiseta* was recovered

from the same bore (HCM0021) in June 2020 and sequenced on the 10th February 2023, knowledge of the amalgamation of Naididae and Tubificidae would have led to re-analysis of the Tubificidae sp. 'BOL074' sequence.

Secondly, it appears that the original Genbank search returned a species-level match to *Pristina longiseta* but this result was not reported. This appears to be an oversight by the ex-staff member who conducted the analysis as less similar matches were reported. Without the opinion of this ex-staff member, it is difficult to understand with confidence the cause of this error.

In conclusion, this case has exposed possible sources of error in the handling and interpretation of molecular data. In response to an increased emphasis on molecular evidence, and to help remove such human-based errors, Bennelongia has recently brought in new protocols and strengthened its molecular expertise, with all analysis done by two staff members with PhDs based mainly on molecular analysis.

References

- Erseus, C., Rota, E., Matamoros, L., and De Wit, P. (2010) Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Molecular Phylogenetics and Evolution* **57**: 849-58.
- Erseus, C., Wetzel, M.J., and Gustavsson, L. (2008) ICZN rules—a farewell to Tubificidae (Annelida, Clitellata). *Zootaxa* **1744**: 66-68.