Teaching driving research: a case study using a novel microsporidium found in western king prawns off the Townsville coast

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In 1988 a prevalence study of the Queensland coast identified Microsporidia infections in five species of prawns. Microsporidia were presumptively identified as being either *Thelohania spp*., which is now *Agmasoma* if found in a marine environment, or *Ameson spp*. (Owens and Glazebrook, 1988). Funding restraints precluded further identification work on these samples at the time. In 2011 Western king prawns were found off the Townsville coast. These presented with signs and histology identical to *Thelohania* spp.-infected prawns found off the Townsville coast 23 years earlier, providing another opportunity to study this infection.

Since the original presumptive identification of these Microsporidia, it has become increasingly apparent that morphological identification of Microsporidia has limitations, such as divergent spore morphologies, that can be overcome by molecular analysis (Stentiford et al., 2013, Vossbrinck and Debrunner-Vossbrinck, 2005). The small subunit ribosomal RNA (ssrRNA) gene has been identified as a useful tool for phylogenetic analysis and identification in Microsporidia (Franzen, 2008). Current identification methods should therefore include molecular analysis for identification and the ssrRNA gene appears to be a good target. However, a lack of immediate external interest in funding further identification and other priorities for research students looking for a project put the samples collected in 2011 into cold storage.

The School of Veterinary and Biomedical Sciences at James Cook University delivers a block mode intensive course to third year and graduate students entitled “Microbial molecular diagnosis and epidemiology”. Good quality, highly infected tissues are desired for the course as it is designed to teach students the basics of extraction, detection and analysis of nucleic acids of microorganisms using authentic infected tissue samples. This requirement has provided an opportunity for researchers to be the beneficiaries of sequence data and phylogenetic analysis produced by the course. In 2012, the above mentioned prawns collected in 2011 were examined. Two published primer sets targeting the conserved ssrRNA gene (Fedorko et al., 1995, Lee et al., 2010) were purchased and briefly tested with RNA extracted from prawn tissue to ensure bands were produced on a gel. One set (Fedorko et al., 1995) was then used by students on the course. Plasmid products produced by students were sequenced and the NCBI database searched using tBlastx.

The closest match to recorded sequence was a *Trichonosema* with a 79% similarity (E values 6e-07). Published phylogenetic trees indicate this genus has a freshwater host (Vossbrinck and Debrunner-Vossbrinck, 2005). This molecular course has provided the first sequence evidence for a novel marine microsporidium. Further bioinformatical analysis is being carried out by students in this course in 2013 to update phylogenetic trees and design new primers to expand the sequence data available for this species. This will aid in our understanding of the relationship of this pathogen to other Microsporidia. In addition, this case has shown the potential of teaching subjects to generate research data.

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