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Short communication

Is *Cryptosporidium* from the common wombat (*Vombatus ursinus*) a new species and distinct from *Cryptosporidium ubiquitum*?

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ABSTRACT

The emerging zoonotic pathogen *Cryptosporidium ubiquitum* has been found in a variety of mammalian hosts, including humans, throughout the world. Advances in the molecular characterization of this parasite using the sequence of the 60 kDa glycoprotein (*gp60*) gene have allowed the classification of "subtypes". Sequences derived from faecal samples from the common wombat (*Vombatus ursinus*) have identified a novel *gp60* subtype designated here as *C. ubiquitum* XIIg. Phylogenetic analysis suggests that subtypes of *C. ubiquitum* can be divided into generalist and specialist groups, which is important when considering the zoonotic potential of *C. ubiquitum* in the context of drinking water safety.

1. Introduction

Currently, 31 species and more than 40 genotypes of *Cryptosporidium* have been recorded (Holubová et al., 2016; Kváč et al., 2016; Zahedi et al., 2016), of which *C. hominis* and *C. parvum* are responsible for the majority of cryptosporidiosis cases in humans (Ryan et al., 2014). The recent recognition of *C. ubiquitum* as an emerging zoonotic pathogen has elevated concerns within the public health community due to its apparently ubiquitous occurrence in an array of mammalian hosts throughout the world (Li et al., 2014). Knowledge of the species and/or genotypes present in natural drinking water catchments is crucial for assessing the possible risk of cryptosporidiosis transmission and for developing management strategies (Nolan et al., 2013; Zahedi et al., 2016).

Cryptosporidium ubiquitum was formerly called *Cryptosporidium* sp. cervine genotype, until it was officially recognised as a species based on morphological and molecular features (Fayer et al., 2010). Originally, the nuclear ribosomal RNA small subunit (*SSU*) gene as well as the *actin* and *Cryptosporidium* oocyst wall protein (*COWP*) genes were employed as markers to identify *C. ubiquitum* by PCR-based tools (reviewed by Fayer et al., 2010). Subsequently, part of the 60 kDa glycoprotein (*gp60*) gene has been used for the classification of subtypes of *C. ubiquitum* (see Li et al., 2014). Based on the sequence of the latter gene, *C. ubiquitum* has been divided into six subtypes: XIIa, Old and New World ruminants; XIIb-XIId, various New World rodents; XIIe and XIIf, field mice from the Slovak Republic (Li et al., 2014; Mi et al., 2014; Wang et al., 2014; Guo et al., 2015; Stenger et al., 2015; Qi et al., 2015). Additionally, all subtypes, except XIIe and XIIf, have been recorded from humans.

Cryptosporidium rarely occurs in wombats. To date, there has been one report of *Cryptosporidium* from the common wombat (*Vombatus ursinus*) detected using an immunomagnetic separation/flow cytometry (IMS/FC) technique (Power, 2002, 2010), and another utilizing PCR-based methods (Koehler et al., 2016). In the past six years of monitoring animals in Melbourne's natural water catchments, 616 faecal samples from wombats were screened using molecular tools for the presence of *Cryptosporidium*; nine of them were test-positive for *SSU*, seven were test-positive for *C. fayeri* and two were inferred to contain *C. ubiquitum* (see Nolan et al., 2013; Koehler et al., 2016). Here, we used *SSU*, *gp60* and *actin* gene regions to further molecularly characterize *C. ubiquitum* from faecal deposits from common wombats.

2. Materials and methods

The *SSU* sequences representing *C. ubiquitum* (GenBank accession nos. KU531665 [546 bp] and KU531681 [245 bp]) derived by PCR-

Abbreviations: *gp60*, 60 kD glycoprotein gene; *SSU*, ribosomal RNA small subunit gene; *COWP*, *Cryptosporidium* oocyst wall protein.

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based sequencing (Koehler et al., 2016) from the two faecal DNA samples (C3604 and OS5267) from common wombats were available for the present study. Sample C3604 originated from the Cardinia water reservoir catchment (37°47′S 145°24′E; 31 July 2013), and sample OS5267 represented the O′Shannassy catchment (37°40′S 145°48′E; 26 November 2014). Sequences KU531665 and KU531681 were identical over 245 bp, and we elected to use the longer (former) sequence for analyses in the present investigation. Here, we sequenced regions of the *gp60* (859 bp) and *actin* (686 bp) genes of *Cryptosporidium* from sample C3604 using an established nested PCR-based approach (cf. Koehler et al., 2016) and deposited them in the GenBank database (accession nos. KX029226 and KX029227, respectively). Then, we used sequence data for all three loci from sample C3604 for phylogenetic analysis using a Bayesian Inference (BI) method, as described previously (Koehler et al., 2016). Other representative sequence data for the *SSU* ($n = 35$), *gp60* ($n = 13$) and *actin* ($n = 33$) genes were extracted from GenBank (Supplementary Table 1) and used for phylogenetic construction (Figs. 1–3).

3. Results and discussion

Koehler et al. (2016) showed that the 245 bp *SSU* region was identical for *Cryptosporidium* from two faecal samples from common wombats (sample C3604 [GenBank accession nos. KU531665] and OS5267 [KU531681]) and that the consensus sequence was consistent with that of *C. ubiquitum* from a goat (accession no. KM199749; 97% similarity); therefore, this particular genotype of *Cryptosporidium*, although rare, has been documented twice in the water catchments (~90 km

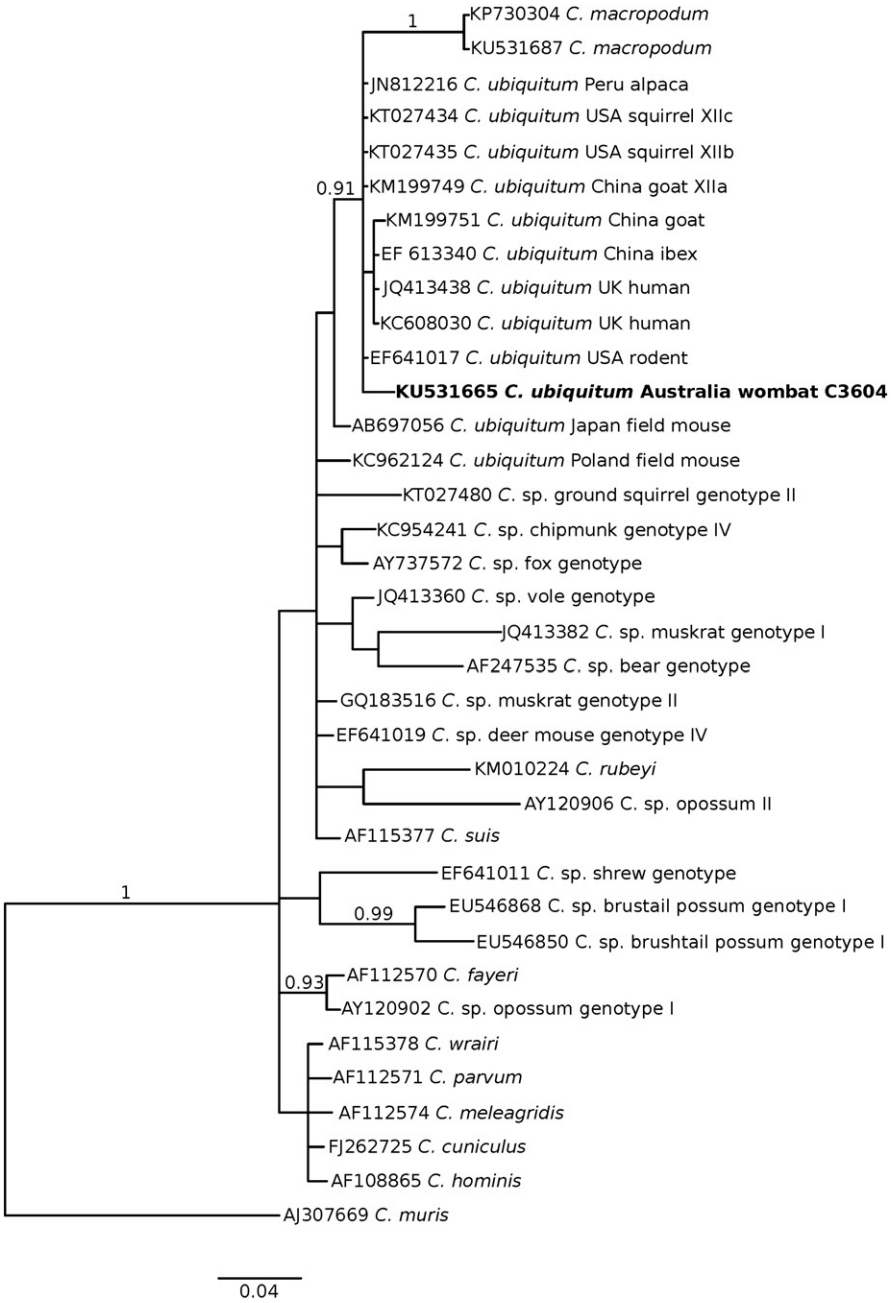

Fig. 1. Phylogenetic relationships of selected *Cryptosporidium* species inferred from partial sequence (475 bp) of the small subunit rRNA (*SSU*) gene using Bayesian inference. Posterior probabilities are indicated for select nodes. *Cryptosporidium muris* was used as an outgroup. GenBank accession numbers precede species names. *Cryptosporidium ubiquitum* from the common wombat (featured in this study) is in bold-type.