

Reviewer Report

Title: Torix Rickettsia are widespread in arthropods and reflect a neglected symbiosis

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Reviewer name: Steve Perlman

Reviewer Comments to Author:

Insects are commonly infected with bacterial symbionts, and these play incredibly important roles in the biology of their hosts. The best known insect symbiont is Wolbachia, which is thought to infect at least 25% of insect species. In this paper, the authors make the surprising discovery that a lineage of Rickettsia, called the Torix Rickettsia group (after one of their leech hosts), is at least as, if not more, widespread, particularly in aquatic hosts. While the best known strains of Rickettsia cause serious human diseases, and are vectored by blood-feeding arthropods, this is just a tiny sliver of Rickettsia diversity. This is an exciting discovery for the insect symbiosis field. The host range of this microbe is quite striking: arthropod, gastropod, and protist.

The authors use three different approaches to document Torix group diversity. They first show that most microbial contaminant sequences in the Barcode of Life database belong to Torix Rickettsia. This is because the commonly used animal barcoding primers, used to amplify mitochondrial COI, may also amplify Rickettsia COI (mitochondria evolved from an Alphaproteobacterial symbiont allied with Rickettsia). They use more primers on material stored at the Barcode of Life center to confirm some of these Rickettsia infections. They also pull out Torix Rickettsia 16S rRNA from many insect SRA datasets. Finally, they screen a large sample of arthropods (and a few gastropods) for Rickettsia, using PCR. They report these bacteria associated with a broad range of hosts, including parasitoids, aquatic insects, blood feeders, sap feeders, and molluscs. This paper will shine a light on a previously unrecognized group of symbionts; the next step will be to begin to understand how these microbes affect host biology. It will be useful for the barcoding community to be reminded of possible symbiont contamination. The approach used for pulling out microbes from SRA datasets will also be of general interest.

My only concern is that Torix group Rickettsia and their relatives have also been identified in protists, such as nucleariid amoebae. So I wonder how many of these Rickettsia, particularly in aquatic hosts, are symbionts of protists residing in animal guts. Have the authors tried to pull out protist 18S sequences from the SRA datasets (or tried to amplify protist genes via PCR, although that would be much more difficult)?

Minor comments:

Line 194 - Psyllidae spelling

Line 242 & Table 2 - Chaoboridae spelling

Line 251 - Simulium spelling

Lines 340 - I would replace refs 49 and 50 with Gehrler & Vorburger, Biol. Lett., 2012

Line 362 - this sentence is confusing because the citations refer to Rickettsia in the belli group

Table 2 - Siphonaptera spelling
Line 819 - Parentheses spelling

Methods

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