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SHORT COMMUNICATION

Endosymbiotic Rickettsiales (Alphaproteobacteria) from the spider genus *Amaurobioides* (Araneae: Anyphaenidae)

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Abstract. Endosymbiotic bacteria are commonly found in terrestrial arthropods and their effects have been studied extensively. Here we present the first recorded case of endosymbiotic bacteria found in the spider family Anyphaenidae. A fragment of the cytochrome oxidase c subunit I "barcoding" region belonging to unidentified Rickettsiales, presumably belonging to the genus *Rickettsia*, was sequenced from six individuals of *Amaurobioides africana* Hewitt, 1917.

Keywords: Bacteria, barcoding, intertidal

The gram-negative proteobacteria are one of the most widespread and diverse groups of bacteria, including medically important pathogens, free-living nitrogen-fixing organisms, and the order Rickettsiales, which contains obligate intracellular bacteria such as *Wolbachia* and *Rickettsia* that can be found living inside the cells of terrestrial arthropods (Ferla et al. 2013). These two genera have been the subject of numerous studies in arachnids, as they represent important pathogens in some cases (e.g., Paddock et al. 2010), while in others they are endosymbionts that manipulate their host's physiology, behavior and/or bias the host's sex ratio to favor their transmission (Rowley et al. 2004; Goodacre et al. 2006; Duron et al. 2008; Gunnarsson et al. 2009; Wang et al 2010; Vanthournout et al. 2011; Goodacre & Martin 2012, 2013). *Rickettsia*-infected spiders have also been shown to display increased long-distance dispersal tendencies (Goodacre et al. 2009). Bacterial endosymbionts are usually transferred vertically in spiders, although there is evidence for horizontal transfer in closely related taxa (Baldo et al. 2008).

Early methods of detection of bacterial endosymbionts in insects and spiders relied on staining techniques (Cowdry 1923). With the advent of PCR-sequencing techniques, molecular detection of specific endosymbionts was made possible with relative ease, in the case of spiders resulting in targeted studies (e.g., Baldo et al. 2008; Jin et al. 2013) or sometimes as a byproduct of a study with a different aim (e.g., Řezáč et al. 2014 in *Dysdera microdonta* Gasparo, 2014). With bacteria-specific primers, amplification of endosymbiont DNA from potential host tissue provides positive results only for infected hosts. On the other hand, more "universal" primers may find the annealing sites in both host and symbiont, if said sites are conserved enough for the genomic region. One such case appears to be the "barcoding" fragment of the Cytochrome Oxidase C subunit I (COI), a protein-coding gene that appears to have its origins deep within the origins of life on the planet (Castresana et al. 1994). The fact that COI has highly conserved regions means that certain primers can be used across a wide range of organisms to amplify the same gene region. This is advantageous if the tissue used for DNA extraction exclusively belongs to one species. However, in the case of organisms hosting endosymbionts, this could be seen as a complication, although for large-scale barcoding studies, it has been shown to be manageable (Smith et al. 2012).

As part of a larger study on the intertidal anyphaenid genus *Amaurobioides* O. Pickard-Cambridge, 1883 (Araneae: Anyphaenidae) (Ceccarelli et al. in prep.), DNA was extracted from leg tissue of 19 individuals of *A. africana* Hewitt, 1917 and approximately 630 base-pairs of the COI gene fragment were amplified and sequenced using the primers LCOI 1490 (Folmer et al. 1994) and HCOoutout (Prendini et al. 2005). For six out of the 19 individuals, the sequenced COI region did not belong to the targeted host species, but to an unknown species of the order Rickettsiales, presumed to be an intracellular symbiont. There was no variation in the nucleotides of the six sequences obtained, indicating that the *A. africana* individuals in this study were all infected with the same bacterial species. The sampling localities of the six infected specimens are shown in Fig. 1a and the COI sequences have been deposited in GenBank (accession numbers KU600819–KU600824).

The identification of the Rickettisales COI sequences amplified in this study was based on comparisons to sequences available in the public databases *International Barcode of Life Database* (BOLD systems; http://www.boldsystems.org/) and *GenBank* (http://www.ncbi.nlm.nih.gov/genbank/). The information available from BOLD was minimal (the level of identification provided was to the order Rickettsiales) and there were cases of misidentification in GenBank, as the *BLAST*-queried sequences from this study were 99% identical to COI sequences labelled as "Hymenoptera sp.", while the correct identification as proteobacteria started at 90% identity.

At this point, questions relating to why non-bacterial primers preferentially amplified COI regions of endosymbionts rather than host DNA in this study—even resulting in clean sequences (rather than a mix of host and symbiont amplicon)—remain largely unanswered. A visual inspection of the priming sites revealed that 9 Rickettsiales COI sequences downloaded from GenBank had 80–90% identity in the last 10 base-pairs towards the 3′ end of the forward and reverse primers. This base-pair identity, coupled with the possibility of a very high number of endosymbiotic Rickettsiales, may have been enough to give initial preference and later exclusivity to the bacterial over the host DNA for primer annealing and DNA amplification during PCR. As mentioned earlier, the presence of endosymbionts is not thought to interfere with DNA barcoding of arthropods when using universal primers (Smith et al. 2012). However, the possibility still exists that COI sequences of endosymbionts are obtained when in fact the target organism is the host, as shown in this study, along with other isolated cases (e.g., Řezáč et al. 2014) and the presence of misidentified Rickettsiales COI sequences in GenBank (where the target organism was the host and thus the identification was placed as Hymenoptera sp.).

Of the COI sequences in GenBank from Rickettsiales (all belonging to the genus *Rickettsia*) with an identity score >75% for the sequences from this study, ten were selected for Bayesian phylogenetic analyses, along with a sequence from a closely related genus (*Orientia*, based on Weinert et al. 2009), four sequences of *Wolbachia* and a sequence belonging to *Anaplasma*, to root the tree. The COI sequences were

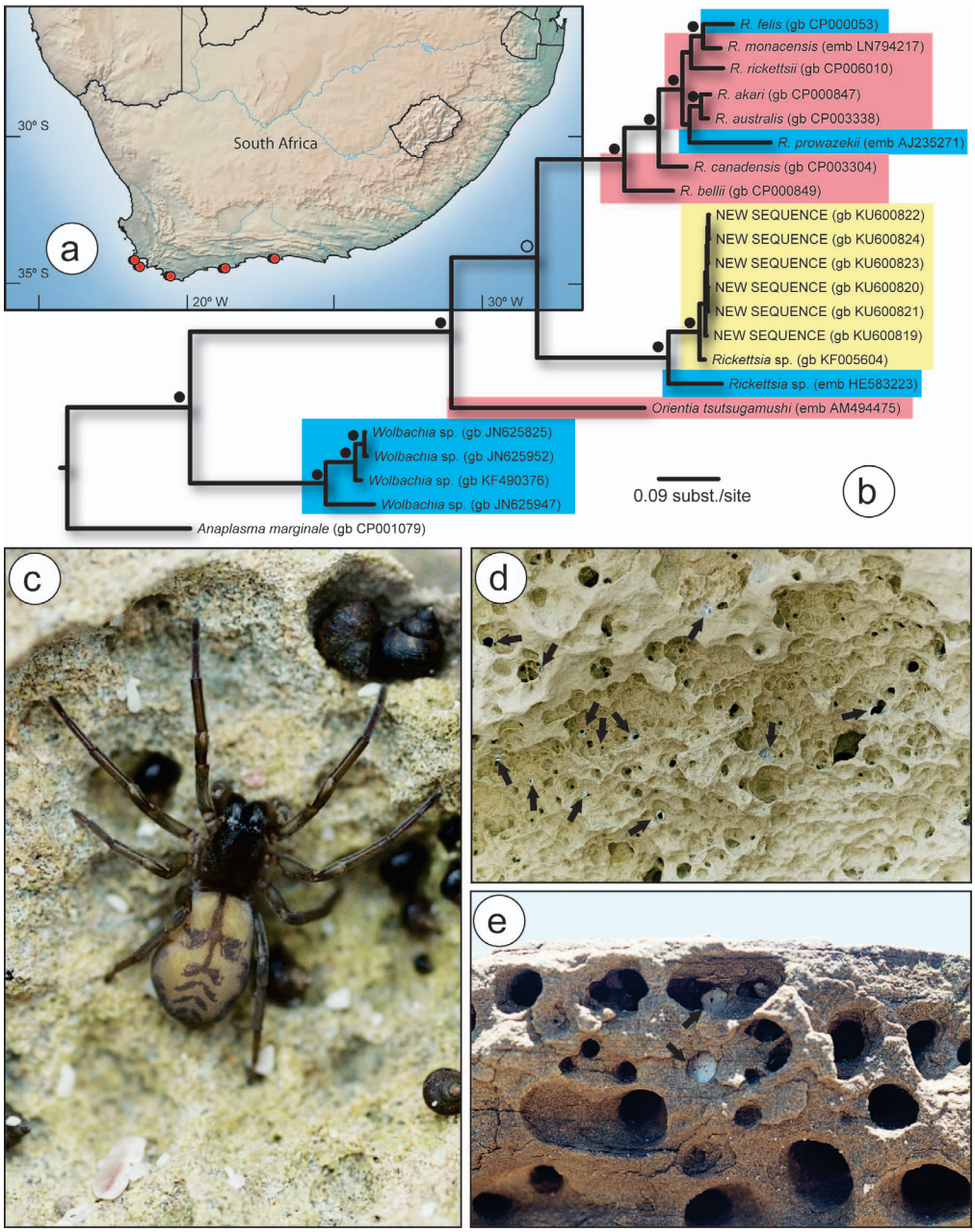

Figure 1.—a. Map showing localities where Rickettsiales-infected specimens of *Amaurobioides africana* were collected; b. Bayesian phylogenetic tree of COI sequences for selected *Rickettsia* species and specimens from closely related genera, obtained from the NCBI database. Nodal support in Bayesian posterior probability (PP) represented by filled ($0.95 < PP <= 1$) and empty ($0.9 < PP <= 1$) circles. GenBank accession numbers are shown after taxon names in brackets. Terminal taxa labelled as NEW SEQUENCE are from this study. Colored boxes around taxon names represent host classes (blue = Insecta; red and yellow = Arachnida; arachnid orders: red = Acari; yellow = Araneae); c. General habitus of *A. africana*; d–e. Rock faces in the intertidal zone at De Hoop Nature Reserve, showing abandoned retreats (arrows) of *A. africana* (d), and at Jeffrey's Bay, showing sealed retreats of *A. africana* (e). Photos: C.R. Haddad.

aligned using TranslatorX (Abascal et al. 2010) and a partitioning strategy along with nucleotide substitution models for each partition (TrNef+I, TrN+G and TrN+I+G for COI 1st, 2nd and 3rd codon positions, respectively) chosen by PartitionFinder v.1.1.1 (Lanfear et al. 2012). A Bayesian phylogenetic tree was obtained by forming a consensus of 20,000 trees (minus 10% burn-in) from 20 million generations of Markov Chain Monte Carlo simulations performed in MrBayes v. 3.2.3 (Ronquist et al. 2012). Based on the phylogenetic tree obtained (Fig. 1b), the sequences from this study belong to an unidentified *Rickettsia* species, closely related to a *Rickettsia* species infecting the spider *Dysdera microdonta*. Apart from being confident that *A. africana* can harbor the endosymbiont *Rickettsia*, a more in-depth study is required to fully understand the distribution, ecology and biology of the endosymbionts detected in this study.

Of particular interest in this relationship is the biology of the host spiders, which are exclusively found in the intertidal zone of rocky shores in marine habitats (Fig. 1c). The spiders regularly construct their silken retreats in rock faces (Fig. 1d, e), which they seal with silk

during high tide to avoid immersion in salt water, emerging at low tide to forage (Lamoral 1968). Therefore, transmission of the symbionts through the water medium in which the spiders occur seems unlikely. Apart from the most likely transmission pathway of the endosymbionts in *A. africana* being vertical transmission, the possibility of horizontal transmission should not be ruled out at this stage; a plausible additional explanation may be the transmission of the endosymbionts during ingestion of prey tissues, such as isopods, amphipods and dipterans that occur in the intertidal zone (Lamoral 1968). Further, the possibility that the same endosymbionts may infect various other spiders and pseudoscorpions occurring in the intertidal zone in South Africa (Lamoral 1968; Haddad & Dippenaar-Schoeman 2009; Larsen 2012; Owen et al. 2014), and the platygastrid wasp egg parasitoid of the only other truly exclusive intertidal spider in South Africa, *Desis formidabilis* (O. Pickard-Cambridge, 1890) (Desidae), viz. *Echthrodesis lamorali* Masner, 1968 (Owen et al. 2014), requires further investigation. Nevertheless, this study represents the first record of Rickettsiales in anyphaenids and is a contribution towards a broader understanding of proteobacteria in spiders.

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