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TOWARDS A *DE NOVO* GENOME ASSEMBLY OF *HYALESTHES OBSOLETUS* (CIXIIDAE) OF THE STINGING NETTLE HOST-RACE

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Next generation sequencing has made *de novo* genome sequencing of non-model organisms possible at comparatively low costs. Genome sequencing of field caught whole specimens represents meta-genomes, which include the genome of the target species as well as the genomes of the associated microorganisms. This poses both a liability and an advantage for research of the target organism. The liability is false inclusion of non-target sequences into the *de novo* assembly of the target species, while the advantage is the additional information obtained on the microorganisms associated therewith. Hemipteran species in particular have a range of endosymbiont bacteria. These include primary, essential symbionts, which typically assist in dietary processes and may determine the diversity of host-plant utilisation, and secondary, facultative symbionts. Facultative symbionts may impact the host negatively, neutrally or positively depending on the associations between them.

The present note reports results of an initial contig assembly based on mate pair reads of the planthopper *Hyalesthes obsoletus*, vector of the Bois noir pathogen *Ca.* Phytoplasma solani (stolbur), and a Blastn search for associated microorganisms. The reference genome will serve to study hereditary (genetic) versus phenotypic (state dependent) components of the ability to acquire and transmit stolbur in *H. obsoletus*. Although it is known that stolbur is acquired by *H. obsoletus* nymphs when feeding on the roots of infected plant hosts, there neither is information about individual variation in the ability to acquire the pathogen nor is there information about interactions between the pathogen and the vector's ability to transmit the pathogen.

MATERIAL AND METHODS

The assembly was performed on DNA obtained from males of the *H. obsoletus* host race associated with *Urtica dioica* (stinging nettle) sampled at Bernkastel-Kues (Moselle), Germany. The *U. dioica* host race is responsible for the majority of Bois noir outbreaks in Germany in recent years. Sequencing was done with Illumina Nextseq 500 technology (sequence length 150bp) and included paired end and mate pair reads (3kb and 8kb inserts). Paired end sequences were obtained from one male. The mate pair reads were obtained from a pool of 10 males. Quality processing was done with the Fastqc and Fastx packages. First, the 5'-ends of raw reads were cut for 8-10bp, adapters clipped, followed by quality trimming and quality filtering. Quality cut off for 3'-trimming was set to 20. Quality filtering was used to discard all reads with less than 80 % bases with quality $\geq$ 20. The presented assembly, based on paired ends only, was done with SOAPdenovo2 (kmer=63) (LOU et al. 2012). The contig assembly was converted to a Blast database for further analysis.

RESULTS AND DISCUSSION

Illumina Nextseq 500 sequencing generated c. 350 Mio. reads. The preliminary de novo assembly based on paired end reads presently has 1.82 Mio contigs of lengths > 500bp and c. 325,000 contigs of lengths of > 1,500bp. The longest contig is 45,000bp. The total number of letters and sequences in the generated database are 3,500 Mio and 22 Mio, respectively. As of writing, an assembly using mate pairs and scaffolding has not been performed. Blastn search for bacterial endosymbionts successfully identified five species known for *H. obsoletus* (BRESSAN et al. 2009; GONELLA et al. 2011) (Table 1). Stolbur was not present in the assembly. This is not surprising as the rate of stolbur infection in German *H. obsoletus* nettle populations typically is 10-15 percent (MAIXNER & JOHANNESEN 2012) and the sequenced individual was assigned for DNA quality rather than stolbur testing prior to sequencing. We blasted 16S of further seven endosymbiotic bacteria and of one spiroplasma known to be associated with Hemiptera. None of these species were found in the assembly (Table 1). It should be noted that

Table 1. Endosymbiont search in contig assembly of *H. obsoletus*. The assembly was based on one male individual. Gene refers to the genes used for search. Insect host is the DNA source of the bacterial endosymbiont used for the search analysis. Sequence length refers to the length of the analysed (published) sequence. Identity value np = not present.

Species	Gene	Insect Host[1]	Present	Sequence length	Identity %	Genbank accession no.
Wolbachia pipientis	wsp	Ho	Yes	501	100	JJ[2]
Cardinium hertigii	16S	Ho	Yes	360	100	JJ[2]
Purcelliella pentastirinorum	16S	Ho	Yes	1415	100	FN428799.1
Vidania fulgoroidae	16S	Ho	Yes	1403	99	FR686932.1
Sulcia muelleri	16S	Ho	Yes	694	100	JJ[2]
Phytoplasma solani	SecY/Stamp	Ho	No	827/489	np	JQ977707.1/ JQ977713.1
Baumannia cicadellinicola	16S	Pm	No	1391	np	AY676896.1
Rickettsia sp.	16S/gltA/Sca1	Mp	No	1422/708/458	np	HE583202.1/ FJ766354.1/ FJ766355.1
Buchnera aphidicola	16S	Ap	No	1460	np	NR074159.1
Arsenophorus sp.	23S	Bm	No	469	np	FJ766372.1
Hamiltonella spp.	GyrB/16S	Bm/Am	No	814/2254	np	FJ766343.1 KF835614.1
Fritschea sp.	16S	Bm	No	1319	np	JQ009299.1
Phlomobacter fragariae	16S	?	No	1462	np	PFU91515
Spiroplasma sp.	16S	Om	No	1439	np	AB775906.1

[1] Ho = *Hyalesthes obsoletus*, Pm = *Paromenia isabellina.* Mp = *Macrolophus pygmaeus*, Ap = *Acyrthosiphon pisum*, Bm = *Bemisia tabaci*, Am = *Aphis mendocina*,? = not reported but *Cixius wagneri* is a known host, Om = *Orius minutus*, [2] JJ = unpublished data from Sanger sequencing of German *H. obsoletus*

environmental 16S sequencing might be preferred to assess the endosymbiont diversity but the initial results presented here were performed to assess data quality for detecting metagenomics sequences.

Although Euhemipteran species are important vectors of a range of plant diseases, there are only eight species with published (NCBI Genbank) genome assemblies, of which only one (*Nilaparvata lugens*) belongs to Archaeorrhyncha. Genome sizes vary considerably, from 500 Mb in *Diaphorina citri* (Asian citrus psyllid) to 2,200 Mb in *Homalodisca vitripennis* (glassy-winged sharpshooter). At present, it is not possible to predict the genome size of *H. obsoletus* from the preliminary contig assembly but it might be around 1 Gb as in the brown planthopper *Nilaparvata lugens* (c. 1,1 Gb). The next steps in this study are inclusion of mate pairs for scaffolding, parameter and re-assembly optimisation followed by filtering of endosymbionts from the assembly.

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