Journal of Invertebrate Pathology 118 (2014) 71–74

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Short Communication

A novel strain of sacbrood virus of interest to world apiculture

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ARTICLE INFO

Article history:
Received 16 January 2014
Accepted 4 March 2014
Available online 17 March 2014

Keywords:
Apis mellifera
Apis cerana
Apis dorsata
Serotype
Recombination
Honeybee

ABSTRACT

This study has characterised a novel serotype of Sacbrood virus (SBV) infecting *Apis mellifera* in New Guinea that has emerged in the presence of the introduced European and Asian serotypes, which infect *A. mellifera* and *Apis cerana*, respectively. The New Guinea serotype appears to have evolved through mutation of the European serotype with no evidence of recombination between known strains, although recombination was detected in other SBV isolates from Asia. SBV was also confirmed for the first time causing disease in *Apis dorsata* (giant Asian honeybee) in Indonesia and found to be infected by the Asian serotype.

1. Introduction

Sacbrood virus (SBV) is a single-stranded RNA virus that causes larval disease in European honeybees (*Apis melllifera*) (Bailey et al., 1964) and consists of two well-characterised serotypes (Ghosh et al., 1999; Zhang et al., 2001). The 'European serotype' infects *A. mellifera* throughout the world, while the 'Asian serotype' infects Asian honeybees (*Apis cerana*) (Bailey et al., 1982; Allen and Ball, 1996). Multiple complete genomes and genetic variation of both serotypes have now been reported including distinct strains from *A. mellifera* in South Africa and Russia that are not yet fully characterised (Grabensteiner et al., 2001; Lomakina and Batuev, 2012). However, during investigations of bee viruses in Australia, Bailey et al. (1979) briefly mentioned another SBV serotype infecting *A. mellifera* in New Guinea, a large island located north of Australia incorporating the country of Papua New Guinea (PNG) in the east and the Indonesian province of Papua in the west. Surprisingly, there has been no further research on this 'New Guinea serotype', despite being a novel pathogen of *A. mellifera*.

Honeybees (*Apis* spp.) are not native to New Guinea, but hived colonies of *A. mellifera* were introduced to the region from Australia and New Zealand since the 1940s (Clinch, 1979) and from the Indonesian island of Java since the 1980s (Anderson, 1994). *A. cerana* was also introduced from Java in the 1970s and is now endemic throughout the island (Delfinado-Baker and Aggarwal, 1987; Anderson, 1994). Thus both European and Asian serotypes of SBV were likely introduced to New Guinea and provide an opportunity to create a novel serotype through cross-infection and recombination between serotypes of the different honeybee hosts. Recombination is an important mechanism in generating viral diversity (Holmes, 2008) and potentially provides an explanation of the origins of the New Guinea serotype.

Here, we confirmed that SBV in New Guinea *A. mellifera* is a unique serotype and provide insight into its evolutionary origins by examining SBV isolates from *A. mellifera* and *A. cerana* in New Guinea, Indonesia, Malaysia and Australia and examined for the first time, SBV isolates infecting *Apis dorsata* (the giant Asian honeybee) in Indonesia.

2. Methods

2.1. Collection and preparation of SBV isolates

Diseased larvae of *A. mellifera*, *A. cerana* and *A. dorsata* showing symptoms of SBV were collected from colonies in Australia, Indonesia, Malaysia and New Guinea and stored at −20 °C. Isolates were prepared for serological testing as described by Anderson (1984) and Bailey (1982) and conventional gel diffusion tests (Mansi, 1958; Anderson, 1984) were used to distinguish between SBV serotypes.

RNA was extracted from SBV-positive isolates, confirmed by gel-diffusion, using TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's standard protocol. Reverse-transcription PCR was conducted using the Phusion two-step RT-PCR kit (Finnzymes,

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http://dx.doi.org/10.1016/j.jip.2014.03.001
0022-2011/

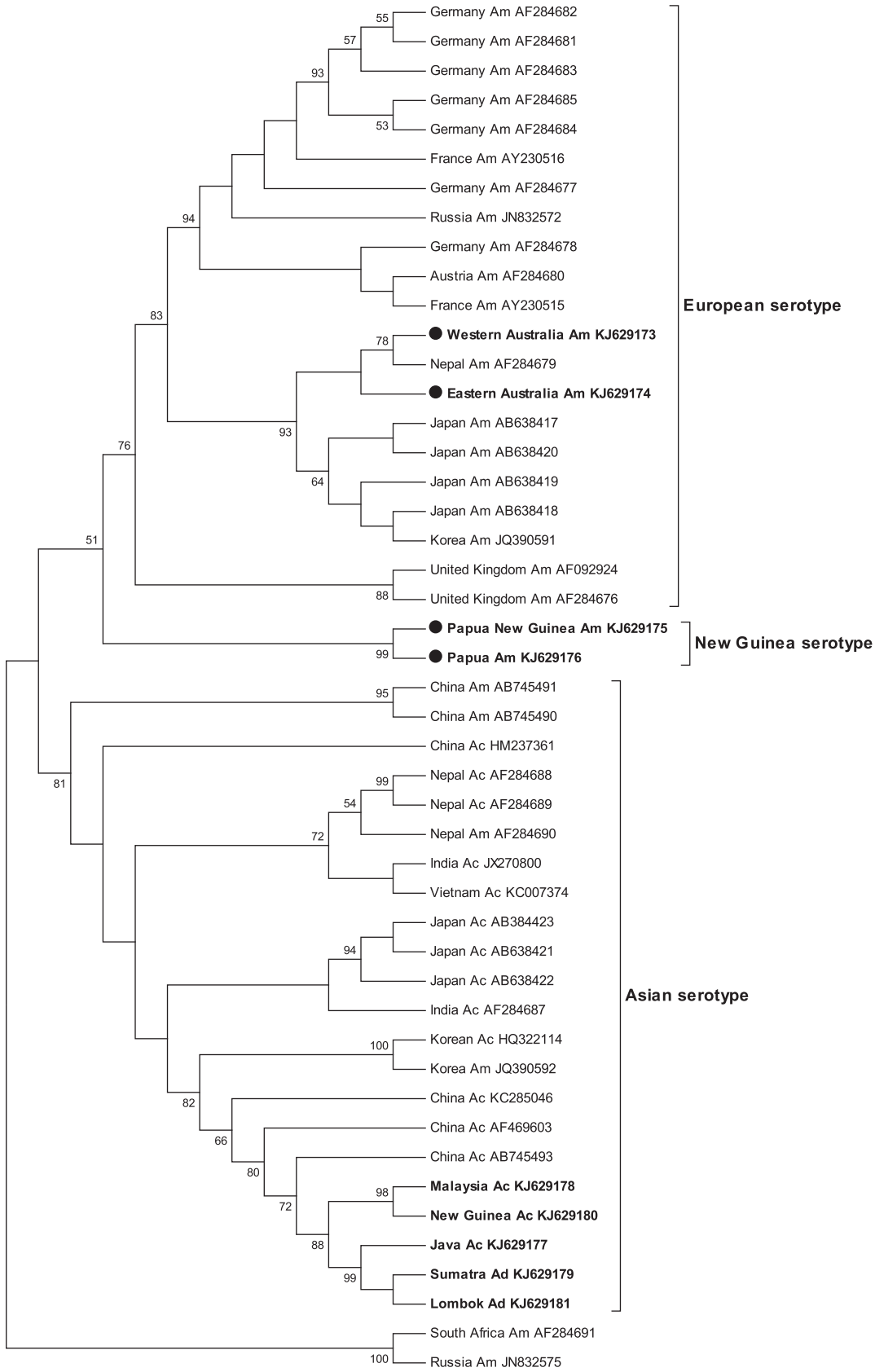

Fig. 1. Maximum likelihood consensus tree using a Tamura 3-parameter distance model for a 386 base pair region of the RNA-dependent RNA polymerase gene from isolates of sacbrood virus. New isolates described in this study are indicated by a closed circle. Virus hosts are *A. mellifera* (Am), *A. cerana* (Ac) and *A. dorsata* (Ad).