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Identification and characterisation of a highly divergent geminivirus: Evolutionary and taxonomic implications[☆]



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ABSTRACT

During a large scale “*non a priori*” survey in 2010 of South African plant-infecting single stranded DNA viruses, a highly divergent geminivirus genome was isolated from a wild spurge, *Euphorbia caput-medusae*. In addition to being infectious in *E. caput-medusae*, the cloned viral genome was also infectious in tomato and *Nicotiana benthamiana*. The virus, named *Euphorbia caput-medusae latent virus* (EcmLV) due to the absence of infection symptoms displayed by its natural host, caused severe symptoms in both tomato and *N. benthamiana*. The genome organisation of EcmLV is unique amongst geminiviruses and it likely expresses at least two proteins without any detectable homologues within public sequence databases. Although clearly a geminivirus, EcmLV is so divergent that we propose its placement within a new genus that we have tentatively named *Capulavirus*. Using a set of highly divergent geminivirus genomes, it is apparent that recombination has likely been a primary process in the genus-level diversification of geminiviruses. It is also demonstrated how this insight, taken together with phylogenetic analyses of predicted coat protein and replication associated protein (Rep) amino acid sequences indicate that the most recent common ancestor of the geminiviruses was likely a dicot-infecting virus that, like modern day mastreviruses and becurtoviruses, expressed its Rep from a spliced complementary strand transcript.

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1. Introduction

Among plants viruses, those of the family *Geminiviridae* are responsible for a disproportionately large number of recently emergent crop diseases worldwide. They have dramatically impacted agricultural yields over the past 50 years (Moffat, 1999), and are a major threat to the food security of developing countries in the tropical and sub-tropical regions of the world (Rey et al., 2012; Rybicki and Pietersen, 1999). Most at risk are countries in sub-Saharan Africa where reports near the beginning of the 1900s of

diseases in exotic introduced cultivated staple food species such as cassava and maize provided the first clear descriptions of geminivirus infections (Fuller, 1901; Warburg, 1894). Caused by at least seven different African geminivirus species, cassava mosaic disease (CMD) is today recognised as the most important biotic constraint of cassava production throughout this region (Legg and Fauquet, 2004; Patil and Fauquet, 2009). For instance, a recent CMD epidemic affected at least nine countries in East and Central Africa (spanning an area of 2.6 million square kilometres) inflicting annual economic losses of US\$1.9–2.7 billion (Patil and Fauquet, 2009). Similarly, throughout sub-Saharan Africa the geminivirus species that causes maize streak disease (MSD) inflicts annual losses averaging approximately US\$120–480 million (Martin and Shepherd, 2009). In addition, a range of other African geminivirus species have been described in the past three decades that, while obviously causing serious yield reductions in tomatoes, beans, and sweet-potatoes, have a currently unquantified impact on African agriculture (Rey et al., 2012).

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All characterised geminivirus genomes are composed of one or two circular single stranded DNA components each of which contains fewer than 3640 nucleotides (Loconsole et al., 2012). Each genomic component is encapsidated in a geminate (twinned) incomplete icosahedral particle. To date, seven genera have been approved within the *Geminiviridae* family: Begomovirus, Curtovirus, Topocovirus, Mastrevirus, Becurtovirus, Turncurtovirus, and Eragrovirus (http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/plant-official/4454.aspx). The criteria for demarcating genera in the family *Geminiviridae* are genome organisation, insect vector, host range and sequence relatedness (Fauquet and Stanley, 2003). It is noteworthy, however, that not all of these criteria are necessary for the approval of new genera as, for example, the genus Topocovirus has been distinguished based only on sequence relatedness and vector species while the genus Mastrevirus includes viruses that have multiple different vector species and infect either monocotyledonous or dicotyledonous hosts. Mastrevirus genomes also have the fewest genes and these viruses express only four different proteins with two of these, the replication associated protein (Rep) and RepA, sharing identical N-termini but distinct C-termini (they are expressed from alternatively spliced versions of the same transcript). By contrast the other six genera have between 5 and 8 genes, with only the coat protein (*cp*) and *rep* genes being detectably homologous across all of the genera.

The high diversity of geminivirus genome sequences is likely facilitated by these viruses having much higher mutation and recombination rates than those seen in many other DNA viruses. Despite geminiviruses utilising host DNA polymerases during their replication, their mutation rates are as high as many RNA viruses that replicate using error prone RNA dependent RNA polymerases (Duffy and Holmes, 2008; Ge et al., 2007; Isnard et al., 1998). Whereas it is most likely that the high recombination rates of geminiviruses occur as a consequence of their replication involving a mixture of rolling circle and recombination dependent mechanisms (Jeske, 2009), the generally broad host ranges of these viruses together with the frequent occurrence in nature of mixed infections (Martin et al., 2011) has resulted in both frequent instances of inter-species recombination (Padidam et al., 1999), and occasional instances of inter-genus recombination (Briddon et al., 1996; Stanley et al., 1986). While recombination events have sometimes yielded new geminivirus species, it is also plausible that past inter-genus recombination events may have yielded new geminivirus genera (Briddon et al., 1996; Stanley et al., 1986).

The development and application of rolling circle amplification (RCA) based approaches to discover novel circular ssDNA viruses from a variety of environmental sources (Delwart, 2012; Ng et al., 2011b) has tremendously accelerated the rate at which such viruses have been discovered (Rosario et al., 2012) and, when applied to the study of plant samples, has revealed that geminivirus diversity likely far exceeds that which is currently known (Haible et al., 2006; Ng et al., 2011a; Schubert et al., 2007). Besides the characterisation of divergent curtoviruses, mastreviruses and begomoviruses, various geminivirus species have been discovered that are so divergent that they cannot be convincingly assigned to any of the four established geminivirus genera (Briddon et al., 2010; Loconsole et al., 2012; Varsani et al., 2009; Yazdi et al., 2008). Both the creation of new genera such as Becurtovirus, Eragrovirus and Turncurtovirus to accommodate some of these divergent species and perpetually growing numbers of new species within the existing “older” genera underline the steadily increasing complexity of geminivirus taxonomy and the need to recurrently re-evaluate the objectivity and meaningfulness of genus and species demarcation criteria that are applied to the members of this family (Muhire et al., 2013).

Amongst the most divergent of these newly discovered geminiviruses is *Eragrostis curvula* streak virus (ECSV) isolated from

an uncultivated African grass species, *Eragrostis curvula* (Varsani et al., 2009). Given both that there exists a tremendous bias favouring the discovery of novel viruses in cultivated species, and that many novel geminivirus species have in the past been discovered in uncultivated hosts (Briddon et al., 2010; Tan et al., 1995; Varsani et al., 2009), it is likely that further attempts to discover divergent geminiviruses in uncultivated hosts will prove successful.

The potential risks to cultivated crops of viruses that predominantly infect only uncultivated plant species has been documented for maize streak virus (MSV) (Varsani et al., 2008), the African Mastrevirus that causes MSD. MSV is the most economically important virus of maize in Africa. Maize was introduced to West Africa by the Portuguese in the early 1500s and to southern Africa by the Dutch in the mid-1600s but probably only began manifesting evidence of severe MSD around the 1860s (Monjane et al., 2011) with the emergence of a maize-adapted recombinant of two *Digitaria*-adapted MSV strains (Varsani et al., 2008). Therefore, besides purely taxonomic reasons for characterising geminiviruses that mainly infect uncultivated species, the ever present risk that such viruses can become adapted to and cause disease in cultivated hosts is a strong incentive for cataloguing the entire range of plant viral species that are found within terrestrial ecosystems.

Here we describe a new highly divergent geminivirus species isolated from the uncultivated South African spurge, *Euphorbia caput-medusae*. This new geminivirus has a unique genome organisation and distant sequence relatedness to other known geminiviruses and likely represents a new genus-level geminivirus lineage. Using an infectious genomic clone, we show that although it causes an asymptomatic infection in its uncultivated natural host, it can cause a severe infection in an important cultivated species such as tomato. The virus was named *Euphorbia caput-medusae* latent virus (EcmLV) and, accordingly, we propose that the new genus within which it should be placed be named *Capulavirus*. Together with a selection of diverse geminivirus sequences we use this new sequence to infer, firstly, previously undetected instances of likely inter-genus recombination in the geminiviruses and, secondly, that the most recent common ancestor of the geminiviruses was possibly a dicot-infecting virus with a *rep* gene that was expressed from a spliced complementary strand transcript.

2. Materials and methods

2.1. Plant sampling

In 2010, samples were collected in the Darling region of the Western Cape from *Euphorbia caput-medusae* plants as part of a large scale survey (for which >800 plants were collected) focusing on viral diversity at the interface between a preserved Cape fynbos ecosystem (Buffelsfontein Game and Nature Reserve) and an intensively cropped agro-ecosystem. Preliminary analysis of the collection of plants that were sampled showed that an unknown geminivirus was detected in an *E. caput-medusae* sample (see below). Nine more *E. caput-medusae* plants were collected in 2011 in the Western Cape region: three from the 2010 sampling site (Buffelsfontein Reserve), and six from coastal fynbos areas, including three near Laaiplek and three in Pater Noster (Supplementary Fig. 1 and Table 1). Whereas samples from the 2010 collection were preserved on dry ice before storage at -80°C , those from 2011 were preserved by drying them with calcium chloride. The botanical identification of the 2010 plants was carried out initially by eye and was later confirmed by sequencing the C-terminal chloroplast *ndhF* gene using the primer pair 972-F (5'-GTC TCA ATT GGG TTA TAT GAT G-3') and 2110-R (5'-CCC CCT AYA TAT TTG ATA CCT TCT CC-3') (Kim and Jansen, 1995).

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